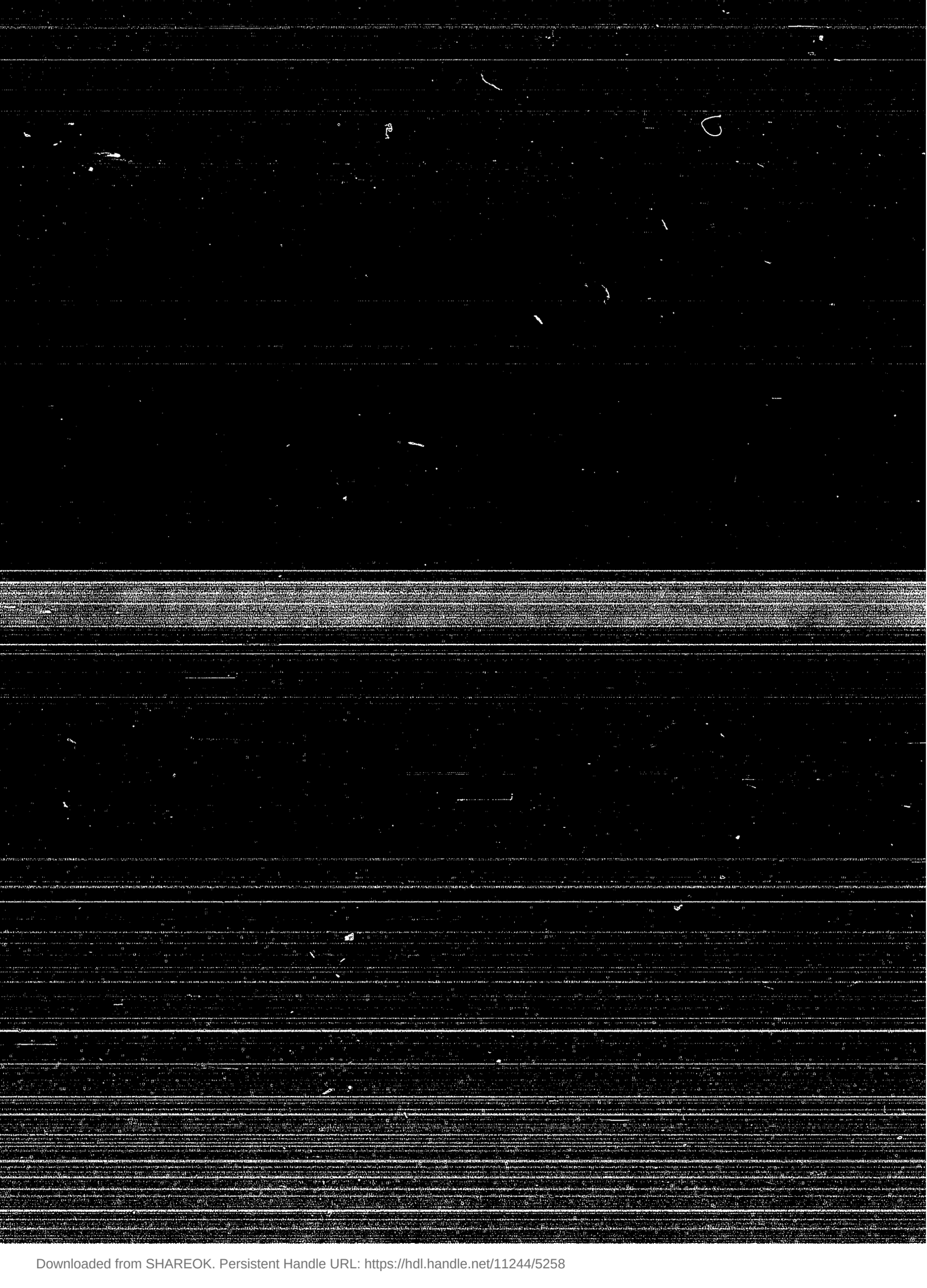


Downloaded from SHAREOK. Persistent Handle URL: https://hdl.handle.net/11244/5258

THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

I. AN INVESTIGATION INTO THE NATURAL PRODUCTS CHEMISTRY OF THE SOFT CORAL *ASTEROSPICULARIA RANDALLI*

II. AN INVESTIGATION INTO THE NATURAL PRODUCTS CHEMISTRY OF THE NUDIBRANCH *TRIDACHIA CRISPATA*

III. STRUCTURE DETERMINATION OF THE 11β,12β-EPOXYPUKALIDE FROM THE GORGONIAN *LEPTOGORGIA SETACEA*

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

DOCTOR OF PHILOSOPHY

BY

MOHAMAD BACHAR KSEBATI

Norman, Oklahoma

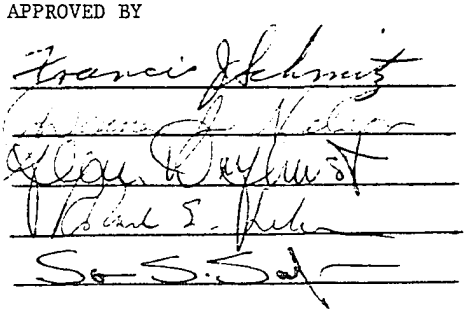
1984

I. AN INVESTIGATION INTO THE NATURAL PRODUCTS CHEMISTRY OF THE SOFT CORAL *ASTEROSPICULARIA RANDALLI*

II. AN INVESTIGATION INTO THE NATURAL PRODUCTS CHEMISTRY OF THE NUDIBRANCH *TRIDACHIA CRISPATA*

III. STRUCTURE DETERMINATION OF THE 11β,12β-EPOXYPUKALIDE FROM THE GORGONIAN *LEPTOGORGIA SETACEA*

APPROVED BY



DISSERTATION COMMITTEE

© 1984
MOHAMAD BACHAR KSEBATI
ALL RIGHTS RESERVED

ACKNOWLEDGEMENTS

I would like to express my deep gratitude to Dr. F. J. Schmitz who suggested these interesting and challenging problems, for his advice and support throughout the course of this study. The author would also like to thank Dr. Leon S. Ciereszko for the isolation of 11β,12β-epoxypukalide, to Mr. Bruce Best for his assistance in collecting *Asterospicularia randalli*, to Dr. Tom Karns for recording low resolution mass spectra, to Dr. Eric Enwall for his counsel in regard to the operation of the 300 MHz NMR spectrometer, and to Dr. Catherine Costello for providing high resolution mass spectral analyses.

Appreciation is also extended to the University of Oklahoma for financial support, and to the Department of Commerce (Grant NO. NA83AA-00011) for support through a research assistantship.

I would also like to thank my mother for her love and support throughout my education.

Very special thanks go to my lovely wife, Hala, for her love, encouragement, and support. Her confidence and understanding were a major element to finishing this study. Therefore, I dedicate this work to her with all my love.

TABLES OF CONTENTS

Page

LIST OF TABLES viii

LIST OF FIGURES xi

CHAPTER 1

I. INTRODUCTION 1

II. RESULTS AND DISCUSSION 11

Identification of 13-Epi-9-O-deacetylxenicin (3) 11

Structure Elucidation of 13-Epi-9-O-deacetyl-7,8-dihydro-7α,8α-epoxyxenicin (40) 20

Identification of Xenialactol (8) 30

Structure Elucidation of Isoxenialactol (42) 35

Xenialactol 9-Acetate (43) 39

Structure Elucidation of 7,8-Dihydro-7α,8α-epoxyxenialactol (44) . . . 42

Identification of Xeniolide-A (7) 47

Structure Elucidation of 9-Deoxyxeniolide-A (47) 51

Structure Elucidation of 9-Deoxy-7,8-dihydro-7α,8α-epoxy-xeniolide-A (48) 53

Structure Elucidation of 15-Dehydroxy-15-acetyldioxyxeniolide-A (49) . 56

Structure Elucidation of 7,8-Dihydro-7α,8α-epoxyxeniolide-A (50) . . . 63

Structure Elucidation of 18-Hydroxyasteroxeniolide-A 9-Acetate (52) . 69

TABLE OF CONTENTS (continued)

CHAPTER 1

		Page
II.	Structure Elucidation of Asteroxeniolide-A (54)	88
	Structure Elucidation of Asteroxeniolide-A 9-Acetate (55)	93
	Identification of Xeniolide-B (13)	98
	Identification of Xeniolide-B 9-Acetate (14)	98
	Identification of 7,8-Dihydro-7α,8α-epoxyxeniolide-B (15)	104
	Structure Elucidation of Asterospicin (56)	106
	Structure Elucidation of 14,15-Dihydro-14ξ,15ξ-epoxyasterospicin-1 (58) and 14,15-Dihydro-14ξ,15ξ-epoxyasterospicin-2 (59)	120
	Structure Elucidation of 14,15-Dihydro-14,15 dihydroxyasterospicin-1 (60) and 61	125
	Structure Elucidation of 3α-Hydroxy-7,8-deepoxy-7,8-dehydro-asterospicin (62)	137
	Structure Elucidation of Asterospiculin (65)	147
	Structure Elucidation of 7,8-Dihydro-7α,8α-epoxyasterospiculin (66)	157
	Structure Elucidation of 4ξ,12ξ:7α,8α-Diepoxy-4,12:7,8-tetra-hydro-3-O-deacetylasterospiculin (69)	162
	Identification of Peridinin (71)	168
	Structure Elucidation of (22R)-24ξ-Methyl-5α-cholestane-3β,-5,6β,22,24-pentaol 6-Acetate (72)	171
III.	SUMMARY	188
IV.	EXPERIMENTAL	194
	Extraction and Partition Procedure of the first Collection	194

TABLE OF CONTENTS (continued)

CHAPTER 1 Page

Extraction and Partition Procedure of the second Collection 195

Isolation of 13-Epi-9-O-deacetylxenicin (3). 196

Isolation of 13-Epi-9-O-deacetyl-7,8-dihydro-7α,8α-epoxyxenicin (40). 196

Acetylation of 13-Epi-9-O-deacetyl-7,8-dihydro-7α,8α-epoxyxenicin (40) 197

Isolation of Xenialactol (8) 197

Isolation of Isoxenialactol (42) 198

Isolation of Xenialactol 9-Acetate (43) 199

Isolation of 7,8-Dihydro-7α,8α-epoxyxenialactol (44) 199

Acetylation of 7,8-Dihydro-7α,8α-epoxyxenialactol (44) 200

Isolation of Xeniolide-A (7) 200

Isolation of 9-Deoxyxeniolide-A (47) 201

Isolation of 9-Deoxy-7,8-dihydro-7α,8α-epoxyxeniolide-A (48) . . . 201

Isolation of 15-Dehydroxy-15-acetyldioxyxeniolide-A (49) 202

Isolation of 7,8-Dihydro-7α,8α-epoxyxeniolide-A (50) 203

Acetylation of 7,8-Dihydro-7α,8α-epoxyxeniolide-A (50) 204

Isolation of 18-Hydroxyasteroxeniolide-A 9-Acetate (52) 204

Acetylation of 18-Hydroxyasteroxeniolide-A 9-Acetate (52). 205

Isolation of Asteroxeniolide-A (54) 205

Isolation of Asteroxeniolide-A 9-Acetate (55) 206

Isolation of Xeniolide-B (13) 207

Isolation of Xeniolide-B 9-Acetate (14) 207

TABLE OF CONTENTS (continued)

CHAPTER 1 Page

Isolation of 7,8-Dihydro-7α,8α-epoxyxeniolide-B (15) 208

Isolation of Asterospicin (56) 208

Acetylation of Asterospicin (56) 209

Dehydration of 7,8-Dihydro-7α,8α-epoxyxenialactol (44) 210

Isolation of 58 and 59 210

Isolation of 60 and 61 211

Epoxidation of Xenialactol (8). 212

Reduction of 45 213

Isolation of 3α-Hydroxy-7,8-deepoxy-7,8-dehydroasterospicin (62) . . 213

Acetylation of 3α-Hydroxy-7,8-deepoxy-7,8-dehydroasterospicin (62) . 214

Isolation of Asterospiculin (65) 215

Isolation of 7,8-Dihydro-7α,8α-epoxyasterospicin (66) 215

Epoxidation of Asterospiculin (65) 216

Isolation of 69 216

Acetylation of 69 217

Isolation of Peridinin (71) 218

Isolation of 72 219

Acetylation of 72 220

Hydrolysis of 72 221

Oxidation of 72 222

Oxidation of 73 223

V. BIBLIOGRAPHY 224

TABLE OF CONTENTS (Continued)

CHAPTER 2		Page
I.	INTRODUCTION	226
II.	RESULTS AND DISCUSSION	227
	Identification of Crispatone (3)	231
	Identification of Crispatene (4)	231
	Structure Elucidation of Tridachiapyrone-B (5) and Isotridachiapyrone-B (6)	240
	Structure Elucidation of Tridachiapyrone-E (7)	245
	Structure Elucidation of Tridachiapyrone-F (8)	248
	Structure Elucidation of Tridachiapyrone-A (9)	250
	Structure Elucidation of Isotridachiapyrone-A (10)	254
	Structure Elucidation of Tridachiapyrone-C (11)	256
	Structure Elucidation of Tridachiapyrone-D (12)	260
	Structure Elucidation of Tridachiapyrone-G (13)	263
III.	SUMMARY	269
IV.	EXPERIMENTAL	271
	Extraction and Partition Procedure	271
	Isolation of Crispatone (3)	272
	Isolation of Crispatene (4)	272
	Isolation of Tridachiapyrone-B (5) and Isotridachiapyrone-B (6)	273
	Isolation of Tridachiapyrone-E (7)	373
	Isolation of Tridachiapyrone-F (8)	274
	Isolation of Tridachiapyrone-A (9)	275

TABLE OF CONTENTS (Continued)

CHAPTER 2 Page

Isolation of Isotridachiapyrone-A (10) 275

Isolation of Tridachiapyrone-C (11) 276

Isolation of Tridachiapyrone-D (12) 276

Isolation of Tridachiapyrone-G (13) 277

V. BIBLIOGRAPHY 278

CHAPTER 3

I. INTRODUCTION 279

II. Structure Elucidation of 11β,12β-Epoxypukalide (8) 283

III. EXPERIMENTAL 297

IV. BIBLIOGRAPHY 299

LIST OF TABLES

CHAPTER 1

Table		Page
1.	^{1}H NMR Data of Xenicins	21
2.	^{13}C NMR Data of Xenicins	21
3.	Results of NOEDS Experiment with 40	31
4.	^{13}C NMR Data of Xenialactols	33
5.	Proton NMR Data of Xenialactols	34
6.	Results of NOEDS Experiment with 44	46
7.	Proton NMR Data of Xeniolide-A and its Derivatives	48
8.	^{13}C NMR Data of Xeniolide-A and its Derivatives	49
9.	Results of NOEDS Experiment with 49	62
10.	Results of NOEDS Experiment with 50	68
11.	Proton NMR Data of Asteroxeniolide-A and its Derivatives	75
12.	^{13}C NMR Data of Asteroxeniolide-A and its Derivatives	76
13.	Results of ^{1}H DDS Experiments with 52	77
14.	Results of NOEDS Experiment with 52	87
15.	Results of NOEDS Experiment with 53	87
16.	Proton NMR Data of Xeniolide-B and its Derivatives	100
17.	^{13}C NMR Data of Xeniolide-B 9-Acetate (14) and 7,8-Dihydro-7α,8α-epoxyxeniolide-B (15)	100
18.	Proton NMR Data of Asterospicins 56 to 64	116
19.	^{13}C NMR Chemical Shifts (ppm) of the Asterospicins	118

LIST OF TABLES (continued)

Table		Page
20.	Results of NOEDS Experiment with 56	119
21.	^{1}H and ^{13}C NMR Chemical Shifts of some signals in 58 and 56	122
22.	^{1}H NMR Chemical Shifts for some signals in 58 and 59	122
23.	^{1}H and ^{13}C NMR Chemical Shifts of some signals in 60 and 61	127
24.	^{1}H and ^{13}C NMR Chemical Shifts for some signals in 56 and 60	127
25.	Results of ^{1}H Difference Decoupling (DDS) Experiments with 62	139
26.	^{1}H and ^{13}C NMR Chemical Shifts Differences between 56 and 62	140
27.	Proton NMR Data of Asterospiculins	150
28.	^{13}C NMR Data of Asterospiculins	151
29.	Results of NOEDS Experiment with Asterospiculin (65)	156
30.	Results of NOEDS Experiment with 7,8-Dihydro-7α,8α-epoxy-asterospiculin (66)	161

CHAPTER 2

1.	^{1}H NMR Data of Tridachione (1), 9,10-Deoxytridachione (2), Tridachiapyrone-E (7), and Tridachiapyrone-F (8)	236
1a.	^{1}H NMR Data of Cispatone (3), Crispatene (4), and Tridachiapyrones	237
2.	^{13}C NMR Data of Tridachione (1) and 9-10-Deoxy-	

LIST OF TABLES (Continued)

Table		Page
	tridachione (2)	238
2a.	^{13}C NMR Data of Crispatone (3), Crispatene (4), and Tridachiapyrones	239

CHAPTER 3

1.	^{1}H and ^{13}C NMR Data of 11β,12β-Epoxypukalide (8)	292
2.	Results of ^{1}H Decoupling ($CDCl_3$) with 8	293
3.	Results of ^{1}H Decoupling (C_6D_6) with 8	294
4.	Results of NOEDS Experiments with 8	295

LIST OF FIGURES

CHAPTER 1

Figure		Page
1.	Diterpenoids Related to Xenicins and their Derivatives . . .	4
2.	300 MHz ^{1}H NMR Spectrum of 13-Epi-9-O-deacetylxenicin ($\underline{3}$) in $CDCl_3$	22
3.	^{13}C NMR Spectra of 13-Epi-9-O-deacetylxenicin ($\underline{3}$) in $CDCl_3$ at 75.4 MHz	23
4.	COSY Spectrum of 13-Epi-9-O-deacetylxenicin ($\underline{3}$) in $CDCl_3$ at 300 MHz	24
5.	Contour Plot of the Heterocorrelated 2-D Spectrum of 13-Epi-9-O-deacetylxenicin ($\underline{3}$) in $CDCl_3$ at 75.4 MHz . .	25
6.	^{13}C NMR Spectra of Compound $\underline{\underline{40}}$ in $CDCl_3$ at 75.4 MHz	27
7.	300 MHz ^{1}H NMR Spectrum of Compound $\underline{\underline{40}}$ in $CDCl_3$	28
8.	COSY Spectrum of Compound $\underline{\underline{40}}$ in $CDCl_3$ at 300 MHz	29
9.	300 MHz ^{1}H NMR Spectrum of Xenialactol ($\underline{8}$) in $CDCl_3$	32
10.	^{13}C NMR Spectra of Isoxenialactol ($\underline{\underline{42}}$) in $CDCl_3$ at 75.4 MHz	37
11.	300 MHz ^{1}H NMR Spectrum of Isoxenialactol ($\underline{\underline{42}}$) in $CDCl_3$.	38
12.	^{13}C NMR Spectra Of Xenialactol 9-Acetate ($\underline{\underline{43}}$) in $CDCl_3$ at 75.4 MHz	40
13.	300 MHz ^{1}H NMR Spectrum of Xenialactol 9-Acetate ($\underline{\underline{43}}$) in $CDCl_3$	41

LIST OF FIGURES (continued)

Figure		Page
14.	^{13}C NMR Spetra of 7,8-Dihydro-7α,8α-epoxyxenialactol (44) in $CDCl_3$ at 75.4 MHz	43
15.	300 MHz ^{1}H NMR Spectrum of 7,8-Dihydro-7α,8α-epoxyxenialactol (44) in $CDCl_3$	45
16.	Possible Mass Spectral Fragmentation Pathways for 44	46
17.	300 MHz ^{1}H NMR Spectrum of Xeniolide-A (7) in $CDCl_3$	50
18.	^{13}C NMR Spectra of 9-Deoxyxeniolide-A (47) in $CDCl_3$ at 75.4 MHz	52
19.	300 MHz ^{1}H NMR Spectrum of 9-Deoxyxeniolide-A (47) in $CDCl_3$	54
20.	^{13}C NMR Spectra of Compound 49 in $CDCl_3$ at 75.4 MHz	58
21.	300 MHz ^{1}H NMR Spectrum of Compound 49 in $CDCl_3$	59
22.	300 MHz ^{1}H NMR Spectrum of Compound 49 in C_6D_6	60
23.	COSY Spectrum of 49 in $CDCl_3$ at 300 MHz	61
24.	^{13}C NMR Spectra of 7,8-Dihydro-7α,8α-epoxyxeniolide-A (50) in $CDCl_3$ at 75.4 MHz	65
25.	300 MHz ^{1}H NMR Spectrum of 7,8-Dihydro-7α,8α-epoxyxeniolide-A (50) in $CDCl_3$	66
26.	COSY Spectrum of 7,8-Dihydro-7α,8α-epoxyxeniolide-A (50) in $CDCl_3$ at 300 MHz	67
27.	^{13}C NMR Spectra of 18-Hydroxyasteroxeniolide-A 9-Acetate (52) in $CDCl_3$ at 75.4 MHz	78
28.	300 MHz ^{1}H NMR Spectrum of 18-Hydroxyasteroxeniolide-A (52) in $CDCl_3$	79

LIST OF FIGURES (continued)

Figure		Page
29.	Spectra from DDS Experiments with 52 at 300 MHZ in $CDCl_3$	80
30.	Stacked Plots of the Homonuclear 2-DJ Spectrum with 52 in $CDCl_3$	81
31.	Contour and Stacked Plots of the COSY Spectra of 52 in $CDCl_3$ at 300 MHz	82-84
32.	Contour Plot of the Heterocorrelated 2-D Spectrum of 52 in $CDCl_3$ at 75.4 MHz	85
33.	^{13}C NMR Spectra of Asteroxeniolide-A (54) in $CDCl_3$ at 75.4 MHz	90
34.	300 MHz ^{1}H NMR Spectrum of Asteroxeniolide-A (54) in $CDCl_3$	91
35.	COSY Spectrum of Asteroxeniolide-A (54) in $CDCl_3$ at 300 MHz	92
36.	^{13}C NMR Spectra of Asteroxeniolide-A (55) in $CDCl_3$ at 75.4 MHz	95
37.	300 MHz ^{1}H NMR Spectrum of Asteroxeniolide-A 9-Acetate (55) in $CDCl_3$	96
38.	COSY Spectrum of Asteroxeniolide-A 9-Acetate (55) in $CDCl_3$ at 300 MHz	97
39.	300 MHz ^{1}H NMR Spectrum of Xeniolide-B (13) in $CDCl_3$	99
40.	300 MHz ^{1}H NMR Spectrum of Xeniolide-B 9-Acetate (14) in $CDCl_3$	101

LIST OF FIGURES (continued)

Figure — Page

41. ^{13}C NMR Spectra of Xeniolide-B 9-Acetate (14) in $CDCl_3$ at 75.4 MHz 102

42. COSY Spectrum of Xeniolide-B 9-Acetate (14) in $CDCl_3$ at 300 MHz 103

43. 300 MHz ^1H NMR Spectrum of 7,8-Dihydro-7α,8α-epoxyxeniolide-B (15) in $CDCl_3$ 105

44. ^{13}C NMR Spectra of Asterospicin (56) in $CDCl_3$ at 75.4 MHz 112

45. 300 MHz ^1H NMR Spectrum of Asterospicin (56) in $CDCl_3$ 113

46. COSY Spectrum of Asterospicin (56) in $CDCl_3$ at 300 MHz 114

47. High-field portion of the 300 MHz COSY Spectrum of 56 in $CDCl_3$ 115

48. 300 MHz ^1H NMR Spectrum of 58 in $CDCl_3$ 123

49. 300 MHz ^1H NMR Spectrum of 59 in $CDCl_3$ 124

50. 300 MHz ^1H NMR Spectrum of 60 129

51. ^{13}C NMR Spectra of 60 at 75.4 MHz 130

52. 300 MHz ^1H NMR Spectrum of 61 131

53. COSY Spectrum of 60 at 300 MHz 132

54. High Field and Low Field Portions of the 300 MHz COSY Spectra of 60 133

55. 300 MHz ^1H NMR Spectrum of 45 in $CDCl_3$ 134

LIST OF FIGURES (continued)

Figure		Page
56.	^{13}C NMR Spectra of 45 in $CDCl_3$ at 75.4 MHz	135
57.	^{13}C NMR Spectra of 3α-Hydroxy-7,8-deepoxy-7,8-dehydroastero-spicin (62) in $CDCl_3$ at 75.4 MHz	141
58.	300 MHz ^1H NMR Spectrum of 62	142
59.	300 MHz ^1H NMR Spectrum of 63 in $CDCl_3$	143
60.	300 MHz ^1H NMR Spectrum of 64 in $CDCl_3$	144
61.	^{13}C NMR Spectra of Asterospiculin (65) in $CDCl_3$ at 75.4 MHz	152
62.	300 MHz ^1H NMR Spectrum of Asterospiculin (65) in $CDCl_3$	153
63.	COSY Spectrum of Asterospiculin (65) in $CDCl_3$ at 300 MHz	154
64.	Contour Plot of the Heterocorrelated 2-D Spectrum of Asterospiculin (65) in $CDCl_3$ at 75.4 MHz	155
65.	300 MHz ^1H NMR Spectrum of 7,8-Dihydro-7α,8α-epoxyastero-spiculin (66) in $CDCl_3$	159
66.	COSY Spectrum of 7,8-Dihydro-7α,8α-epoxyasterospiculin (66) in $CDCl_3$ at 300 MHz	160
67.	300 MHz ^1H NMR Spectrum of 69 in $CDCl_3$	165
68.	^{13}C NMR Spectra of 69 in $CDCl_3$ at 75.4 MHz	166
69.	COSY Spectrum of 69 in $CDCL_3$ at 300 MHz	167
70.	300 MHz ^1H NMR Spectrum of Peridinin (71) in $CDCl_3$ . . .	169
71.	^{13}C NMR Spectra of Peridinin (71) in $CDCl_3$ at 75.4 MHz .	170

LIST OF FIGURES (continued)

Figure		Page
72.	^{13}C NMR Spectra of 72 in CD_3OD at 75.4 MHz	177
73.	300 MHz ^{1}H NMR Spectrum of 72 in CD_3OD	178
73a.	300 MHz ^{1}H NMR Spectrum of 72 in $CDCl_3$	179
74.	300 MHz ^{1}H NMR Spectrum of 75 in $CDCl_3$	180
75.	^{13}C NMR Spectra of 75 in $CDCl_3$	181
76.	Possible Mass Spectral Fragmentation Pathways for 72	182
77.	Possible Mass Spectral Fragmentation Pathways for 74	183
78.	^{13}C NMR Spectra of 73 at 75.4 MHz	184
79.	Possible Mass Spectral Fragmentation Pathways for 73	185
80.	300 MHz ^{1}H NMR Spectrum of 76 in $CDCl_3$	186
81.	Possible Mass Spectral Fragmentation Pathways for 76	187

CHAPTER 2

1.	Metabolites Isolated from *Tridachia crispata, Tridachiella diomelea,* and *Placobranchus ocellatus*	228
2.	Metabolites Isolated from *Tridachia crispata* in this work	229
3.	^{13}C NMR Spectra of Crispatone (3) in $CDCl_3$ at 75.4 MHz	232
4.	300 MHz ^{1}H NMR Spectrum of Crispatone (3) in $CDCl_3$	233
5.	300 MHz ^{1}H NMR Spectrum of Crispatene (4) in $CDCl_3$	234
6.	^{13}C NMR Spectra of Crispatene (4) in $CDCl_3$ at 75.4 MHz	235
7.	300 MHz ^{1}H NMR Spectrum of 5 in $CDCl_3$	243
8.	300 MHz ^{1}H NMR Spectrum of 6 in $CDCl_3$	244
9.	300 MHz ^{1}H NMR Spectrum of 7 in $CDCl_3$	247

LIST OF FIGURES (Continued)

Figure — Page

10. 300 MHz ^{1}H NMR Spectrum of 8 in $CDCl_3$ 249

11. 300 MHz ^{1}H NMR Spectrum of 9 in $CDCl_3$ 253

12. 300 MHz ^{1}H NMR Spectrum of 10 in $CDCl_3$ 255

13. 300 MHz ^{1}H NMR Spectrum of 11 in $CDCl_3$ 259

14. 300 MHz ^{1}H NMR Spectrum of 12 in $CDCl_3$ 262

15. 300 MHz ^{1}H NMR Spectrum of 13 in $CDCl_3$ 268

CHAPTER 3

1. Furanocembranoids and Related Derivatives 280

2. ^{13}C NMR Spectra of 11β,12β-epoxypukalide (8) in $CDCl_3$ at 75.4 MHz 288

3. 300 MHz ^{1}H NMR Spectrum of 8 in $CDCl_3$ 289

4. Partial Structures of 8 290

5. 300 MHz ^{1}H NMR Spectrum of 8 in C_6D_6 291

CHAPTER I

Introduction

The soft coral, *Asterospicularia randalli*,[1] is a new marine species in the family of Asterospiculariidae of the order Alcyonacea. It is common around Guam Is. in shallow water (0-7 m in depth) and occurs as colonies with densities up to 24 colonies per m^2. *Asterospicularia randalli* is beige to light pink, with paler colony bases and is usually attached to solid coral rock substrata, but many are scattered over long upright skeletons of dead *Acropora formosa*. These colonies are under 4 cm tall and most of them have bases under 5 cm in diameter. *Asterospicularia randalli* has a low (5-20 mm) sterile base which gives rise to less than 50 short stalks. Each of these stalks contains a capitulum with 50-100 polyps. These polyps are monomorphic and highly contractile (1.5 mm in height and 1 mm in diameter excluding tentacles).

Soft corals have afforded many unusual and potentially useful diterpenoids.[2] The majority of these diterpenoids are derivatives of the cembrene[3,4] group. Schmitz et al. first isolated a diterpenoid, xenicin (1)[5] (from *Xenia elongata*) which has a nine-membered carbocyclic ring condensed to a dihydropyran ring to give an 2-oxabicyclo [7.4.0] tridecane system. Since that time, many other xenicins,[6] (differing in their

functional groups), xeniolides,[6,7] and the closely related xeniaphyllanes[6,7] have been isolated from different *Xenia* species (*X. macrospiculata, X. obscuronata* and *X. novaebritanniae*). Other representatives of this group have been isolated from other soft corals species (*Nephthea* and *Alcyonium* spp.),[8,9] and from a gorgonian[10] as well.

Recently, Coll et al. have reported the isolation of a modified tricyclic xenicin, 10, from *X. viridis*.[11] Another closely related diterpene, alcyonolide (31), was isolated from the Okinawan soft coral of the genus *Alcyonium*.[9]

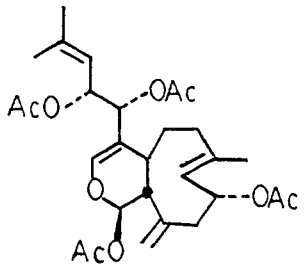
Xenicins are not exclusive to soft corals. Coraxeniolides (34-37) and corabohcin (38) were isolated by Scheuer's group[10] from the Hawiian Pink coral, *Corallium* sp. Finer et al. isolated a cyclononane diterpene, dictyodial (32),[12] from the brown algae *Dictyota crenulata*, and *D. flabellata*, and the closely related dictyolactone (33)[13] from the sea hare *Aplysia depilans* (Opisthobranchia).

In a recent papter,[14] Burns et al. have reported the isolation of a new diterpene carbocyclic ring skeleton (related to xenicin) from an *Efflatounaria* sp of soft coral for which structure 39 was determined.

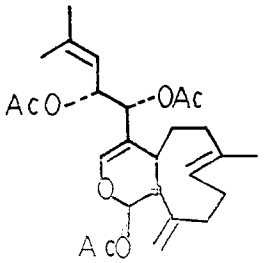
Diterpenoids related to xenicins which have been isolated from soft corals and other marine organisms are displayed in Figure 1.

The soft coral *Asterospicularia randalli*, a close relative to *Xenia*, seems to have received no previous chemical attention. It was the purpose of this work to investigate the chemistry of this new species in the search for biologically active compounds. This research resulted in the isolation of a new steroid, the known carotenoid pigment, peridinin, and 26 diterpenoid compounds. These diterpenoids fall into 7 groups:

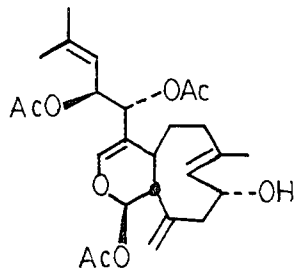
(1) xenicins; (2) xenialactols; (3) xeniolide-A and its derivatives; (4) asteroxeniolide-A and its derivatives; (5) xeniolide-B and its derivatives; (6) asterospicins; and (7) asterospiculins. Twenty out of the 26 isolated compounds are new diterpenoids.



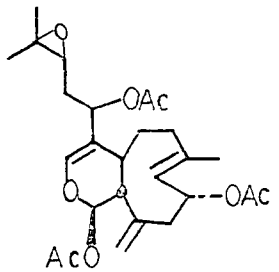
1 xenicin
Xenia elongata[5]



2 9-deacetoxyxenicin
Xenia crassa[11]

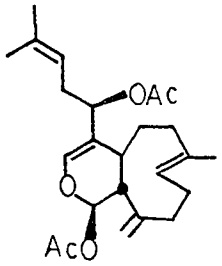


3 13-epi-9-0-deacetylxenicin
Xenia novae-britanniae[15]



4 xeniculin
Xenia macrospiculata[6]

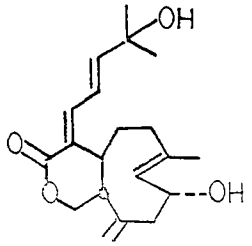
Figure 1. Diterpenoids Related to Xenicins.



5 9-deacetoxy-14,15-deepoxy-xeniculin

6 9-deacetoxy-14,15-deepoxy-7,8-epoxyxeniculin

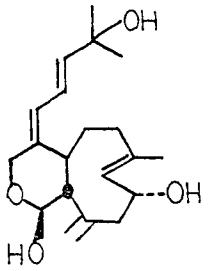
Xenia obscuronata[6]



7 xeniolide-A

Xenia macrospiculata[6]

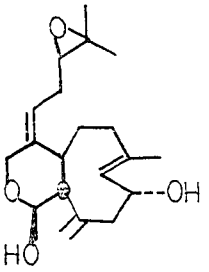
Xenia obscuronata[6]



8 xenialactol

Xenia macrospiculata[6]

Xenia obscuronata[6]

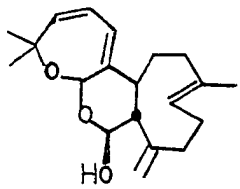


9 xenialactol-D

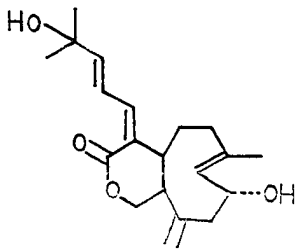
Xenia obscuronata[7]

Xenia lilielae[7]

Figure 1 (continued).



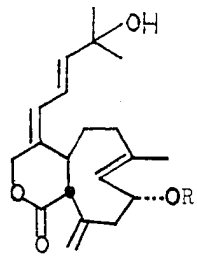
10 *Xenia viridis*[11]



11 4,12Z-isoxeniolide-A

12 4,12Z-7,8-epoxy-isoxeniolide-A

Xenia novae-britanniae[15]



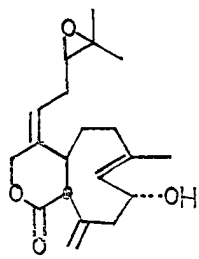
13 R = H, xeniolide-B

14 R = Ac, Xeniolide-B 9-Acetate

15 R = H, 7,8-epoxyxeniolide-B

Xenia macrospiculata[6]

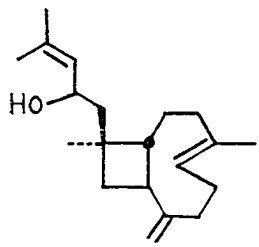
Xenia obscuronata[6]



16 xeniolide-E

Xenia obscuronata[7]

Figure 1 (continued).



17 xeniaphllenol

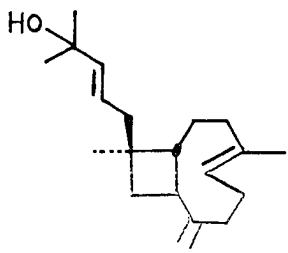
18 4,5-epoxyxeniaphyllenol

19 diepoxyxeniaphyllenol-A

Xenia macrospiculata[6]

Xenia obscuronata[6]

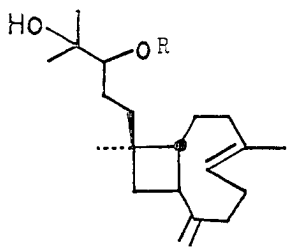
Xenia lilielae[7]



20 isoxeniaphyllenol

21 4,5-epoxyisoxeniaphyllenol

Xenia macrospiculata[6]



22 R = H, xeniaphyllan-14,15-diol

23 R = Ac, 14-acetoxyxeniaphyllandiol

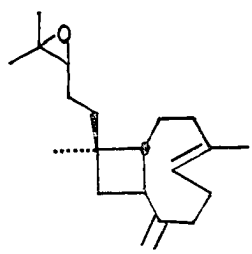
24 R = Ac, 14-acetoxy-4,5-epoxy-xeniaphyllandiol

25 R = H, epoxyxeniaphyllandiol

Xenia macrospiculata[6]

Xenia obscuronata[6]

Xenia lilielae[7]

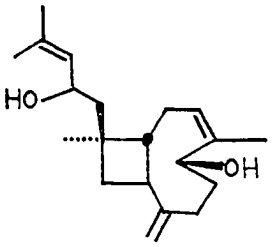


26 xeniaphyllene-14,15-oxide

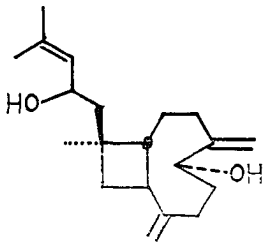
27 xeniaphyllene-dioxide

Xenia lilielae[7]

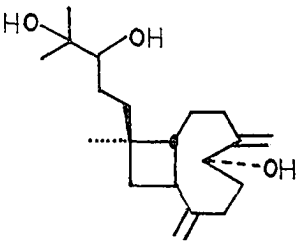
Figure 1 (continued).



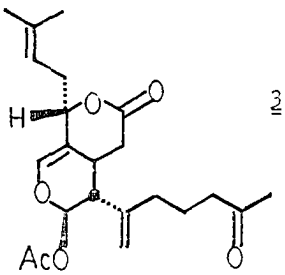
28 xeniaphyllenol-B
Xenia macrospiculata[7]



29 xeniaphyllenol-C
Xenia macrospiculata[7]

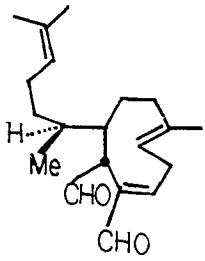


30 xeniaphyllantriol
Xenia obscuronata[7]



31 alcyonolide
Unidentified sp [9]

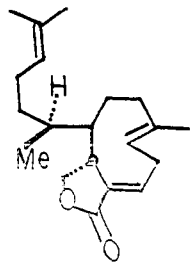
Figure 1 (continued).



32 dictyodial

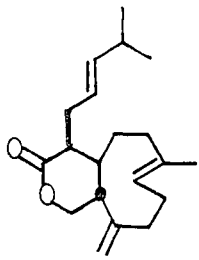
Dictyota crenulata[12]

Dictyota flabellata[12]



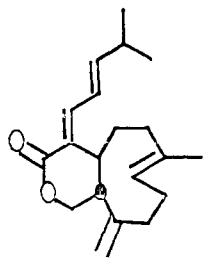
33 dictyolactone

Aplysia depilans[12]



34 coraxenolide-A

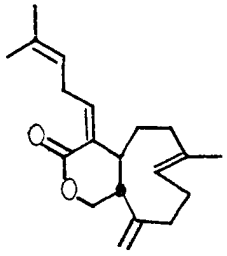
Corallium sp.[10]



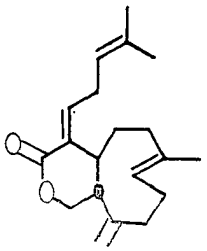
35 coraxenolide-B

Corallium sp[10]

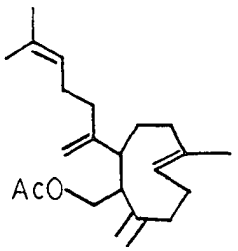
Figure 1 (continued).



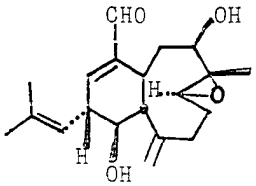
36 coraxenolide-C
Corallium sp.[10]



37 coraxenolide-C'
Corallium sp.[10]



38 corabohcin
Corallium sp.[10]



39 *Efflatounaria sp.*[14]

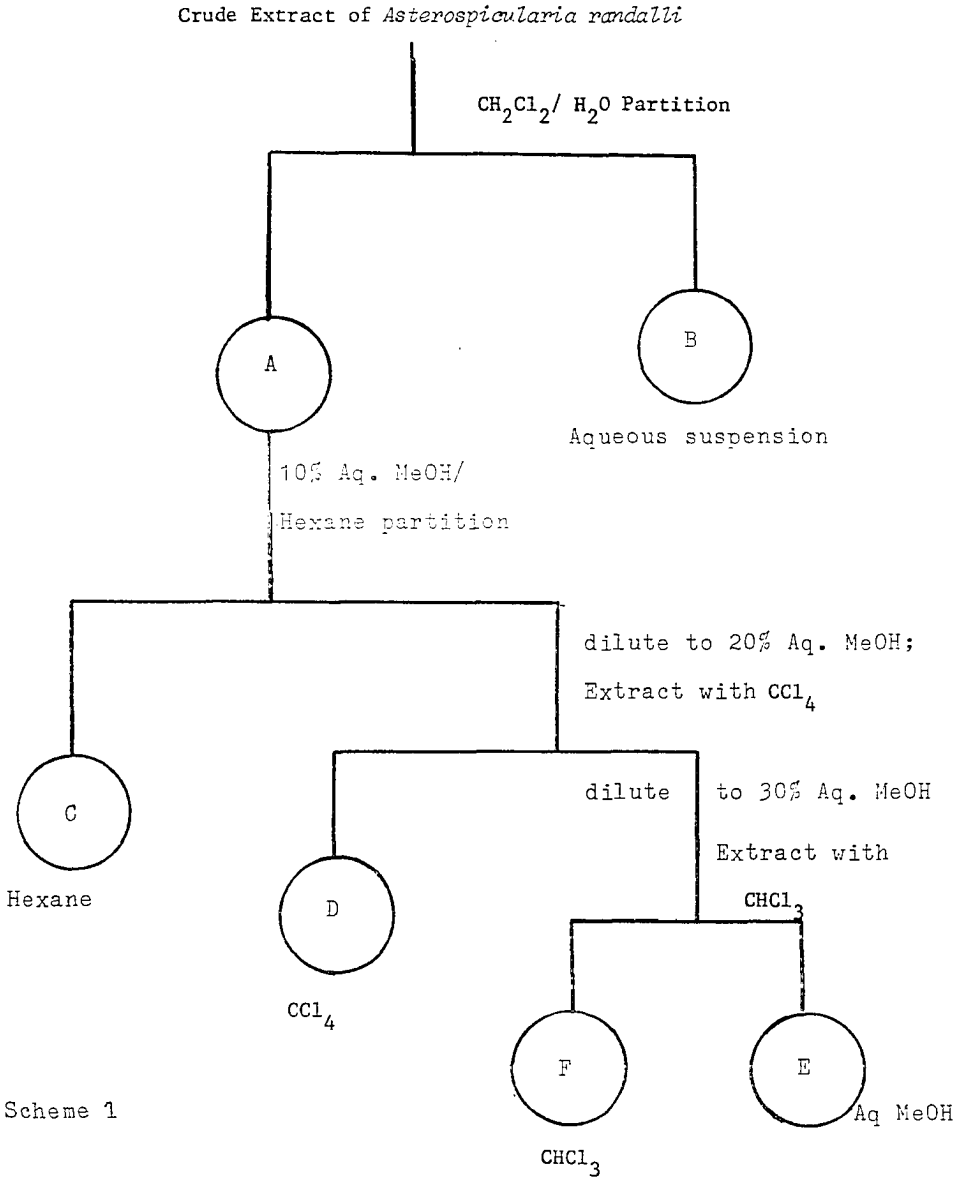
Figure 1 (continued).

Results and Discussion

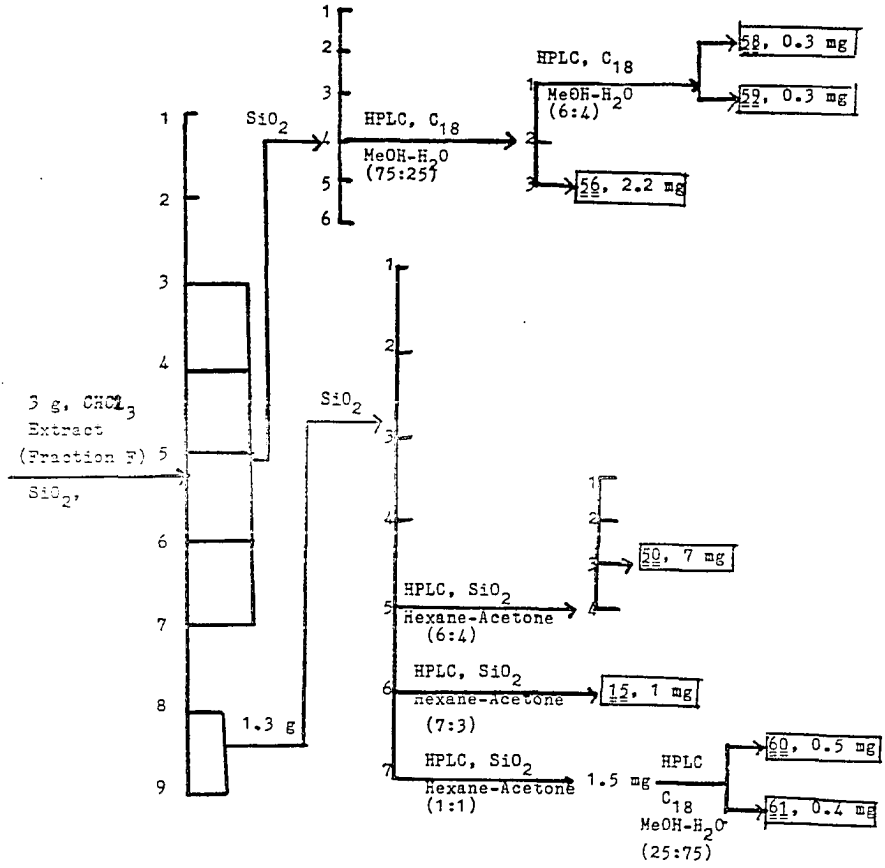
The soft coral, *Asterospicularia randalli*, used in this investigation was collected at two different times from Guam Is. at depths of 3-4 ft. The first collection was in May 1979 and the second in April 1983. Both collections of soft corals were shipped frozen to Oklahoma. The animals from the first collection were allowed to thaw at room temperature for 24 h, soaked in 95% EtOH for a period of 2 days, filtered using cheesecloth, then extracted with MeOH-$CHCL_3$ (1:1), and filtered again. The resulting solutions from both extractions were combined and fractioned according to Scheme 1. The $CHCL_3$ extract (fraction F) and CCL_4 extract (fraction D) were chromatographed extensively according to Scheme 2 to afford eight pure compounds which are described individually below. The second collection was processed using nearly the same procedure as for the first collection (see Scheme 3 for extraction, Scheme 4 for separation). Scheme 4 shows the separation sequence for this second $CHCL_3$ extract (fraction F') to yield 20 pure compounds which are described individually below. Chart 1 displayed the structures of the isolated compounds and their derivatives which will be discussed in this chapter.

Identification of 13-Epi-9-O-deacetylxenicin (3):

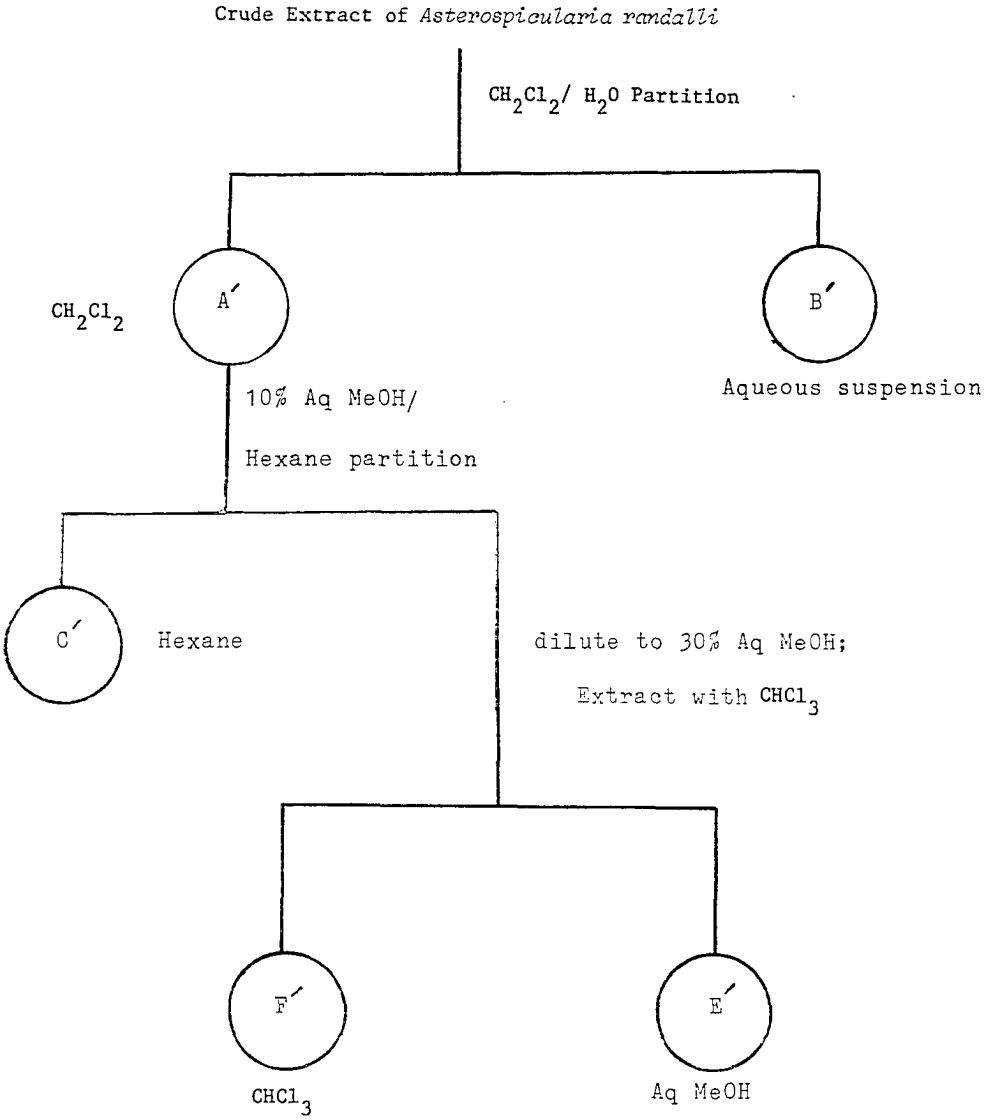
The fourteenth fraction of scheme 4 was chromatographed by HPLC with silica gel to give 9 fractions. The fourth of these fractions was resolved by a reverse-phase HPLC column with MeOH-H_2O (7:3) as eluent to


Crude Extract of *Asterospicularia randalli*
CH_2Cl_2/ H_2O Partition
A
B
Aqueous suspension
10% Aq. MeOH/
Hexane partition
dilute to 20% Aq. MeOH;
Extract with CCl_4
C
Hexane
D
CCl_4
dilute to 30% Aq. MeOH
Extract with
$CHCl_3$
F
$CHCl_3$
E
Aq MeOH

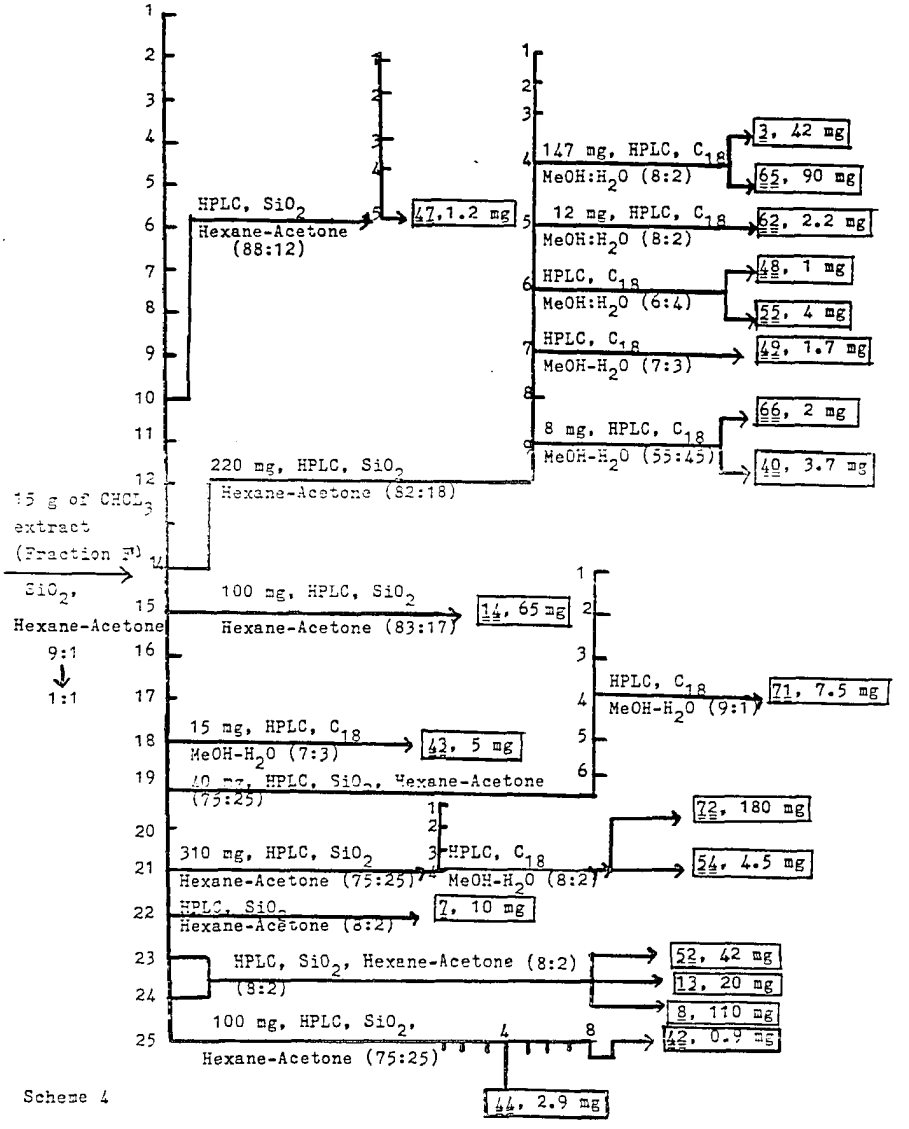
Scheme 1


3 g, CHCl3
Extract
(Fraction F)
SiO2,
SiO2
HPLC, C18
MeOH-H2O
(75:25)
HPLC, C18
MeOH-H2O
(6:4)
58, 0.3 mg
59, 0.3 mg
56, 2.2 mg
SiO2
1.3 g
HPLC, SiO2
Hexane-Acetone
(6:4)
50, 7 mg
HPLC, SiO2
Hexane-Acetone
(7:3)
15, 1 mg
HPLC, SiO2
Hexane-Acetone
(1:1)
1.5 mg
HPLC
C18
MeOH-H2O
(25:75)
60, 0.5 mg
61, 0.4 mg

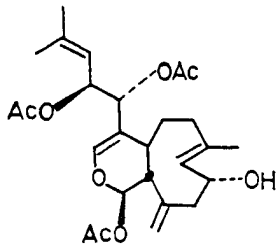
Scheme 2


Crude Extract of *Asterospicularia randalli*
CH_2Cl_2/ H_2O Partition
CH_2Cl_2
A´
B´
Aqueous suspension
10% Aq MeOH/
Hexane partition
C´
Hexane
dilute to 30% Aq MeOH;
Extract with $CHCl_3$
F´
E´
$CHCl_3$
Aq MeOH

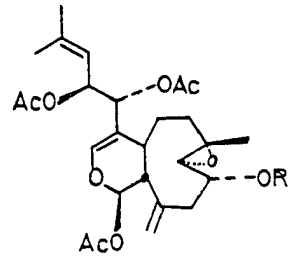
Scheme 3


15 g of CHCl3 extract (Fraction F)
SiO2, Hexane-Acetone 9:1 → 1:1
HPLC, SiO2 Hexane-Acetone (88:12)
47, 1.2 mg
220 mg, HPLC, SiO2 Hexane-Acetone (82:18)
147 mg, HPLC, C18 MeOH:H2O (8:2)
3, 42 mg
65, 90 mg
12 mg, HPLC, C18 MeOH:H2O (8:2)
62, 2.2 mg
HPLC, C18 MeOH:H2O (6:4)
48, 1 mg
55, 4 mg
HPLC, C18 MeOH-H2O (7:3)
49, 1.7 mg
8 mg, HPLC, C18 MeOH-H2O (55:45)
66, 2 mg
40, 3.7 mg
100 mg, HPLC, SiO2 Hexane-Acetone (83:17)
14, 65 mg
HPLC, C18 MeOH-H2O (9:1)
71, 7.5 mg
15 mg, HPLC, C18 MeOH-H2O (7:3)
43, 5 mg
40 mg, HPLC, SiO2, Hexane-Acetone (75:25)
310 mg, HPLC, SiO2 Hexane-Acetone (75:25)
HPLC, C18 MeOH-H2O (8:2)
72, 180 mg
54, 4.5 mg
HPLC, SiO2 Hexane-Acetone (8:2)
7, 10 mg
HPLC, SiO2, Hexane-Acetone (8:2)
(8:2)
52, 42 mg
13, 20 mg
8, 110 mg
42, 0.9 mg
100 mg, HPLC, SiO2, Hexane-Acetone (75:25)
44, 2.9 mg

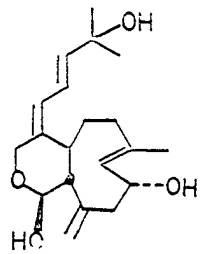
Scheme 4



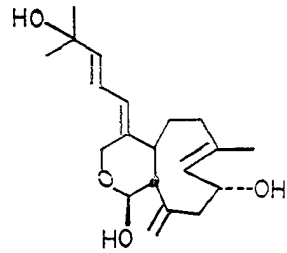
3



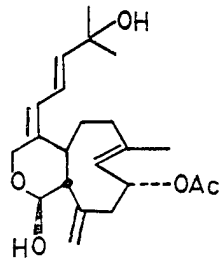
40 R = H
41 R = Ac



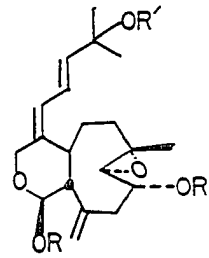
8



42

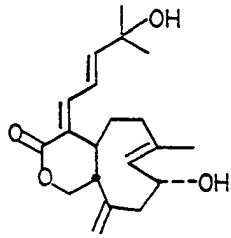


43

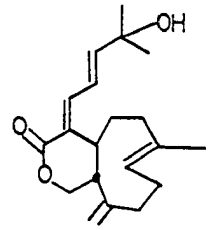


44 R = H, R' = H
45 R = H, R' = OH
46 R = Ac, R' = H

Chart 1

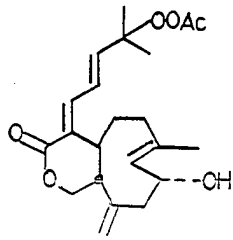


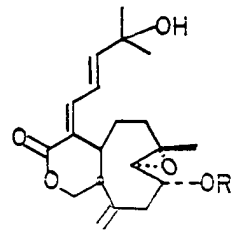
7



47

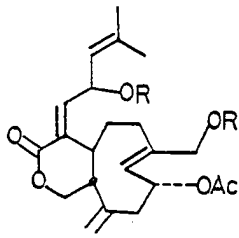
48 7,8-epoxide of 47

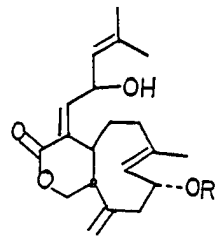




50 R = H

51 R = Ac





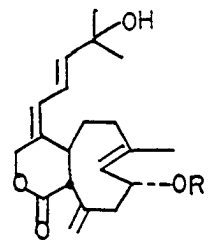
52 R = H

53 R = Ac

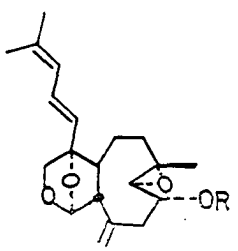
54 R = H

55 R = Ac

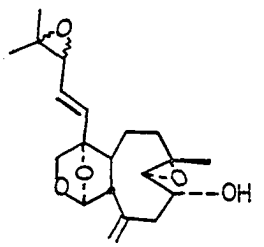
Chart 1 (continued).



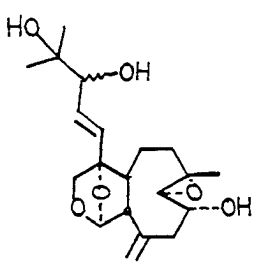
13 R = H
14 R = Ac
15 7,8-epoxide of 13



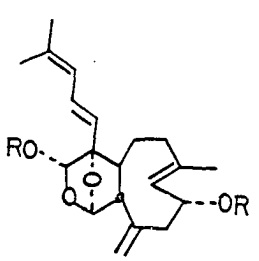
56 R = H
57 R = Ac



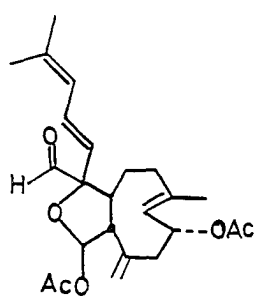
58
59 epimer of 58



60
61 epimer of 60

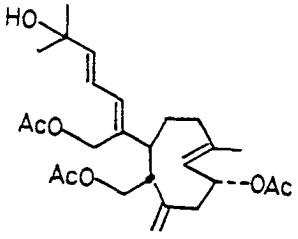


62 R = H
63 R = Ac

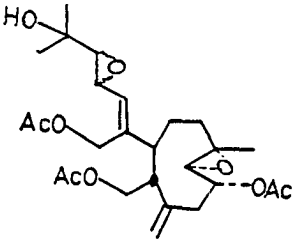


64

Chart 1 (continued).



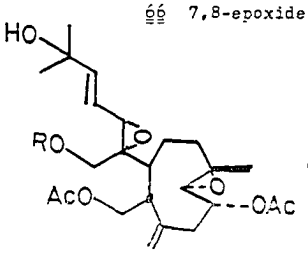
65



67

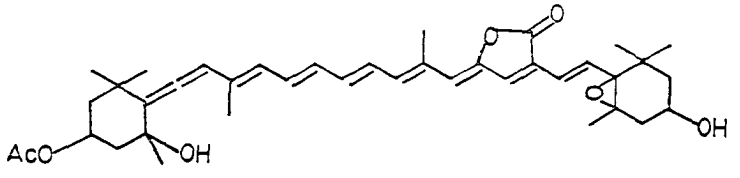
66 7,8-epoxide of 65

68 epimer of 67

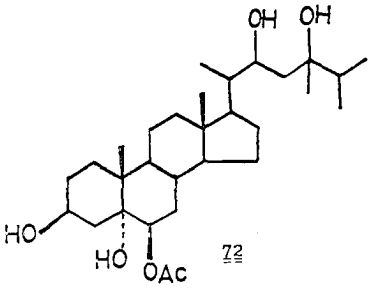


69 R = H

70 R = Ac



71



72

Chart 1 (continued).

yield 2 fractions. The second fraction contained a new compound, asterospiculin (66). The first fraction contained 42 mg of 13-epi-9-O-deacetylxenicin (3) as a colorless oil. The molecular formula $C_{26}H_{36}O_8$ (9 unsaturations) was obtained for 3 from the following spectral data: (a) the low resolution mass spectrum of 3 displayed the molecular ion at 476; (b) the ^1H NMR spectrum (see Figure 2) showed signals for 35 non-exchangeable protons; (c) IR (OH) at 3450 and acetate(s) at 1735 cm^{-1}; (d) the ^{13}C NMR spectra (see Figure 3) indicated the existence of 26 carbons including an acetylated hemiacetal carbon, 91.63 ppm (C-1, d), a carbon deshielded by a hydroxyl group,* 67.64 ppm (C-9, d), two other carbons each deshielded by an acetate group,* 74.87 ppm (C-12, d) and 69.93 (C-13, d). The ^1H and ^{13}C NMR data (see Tables 1 and 2) of 3 were identical to those of 13-epi-9-O-deacetylxenicin isolated earlier by Braekman and Tursch.[15] However, Tursch did not fully assign the ^1H NMR spectrum of 13-epi-9-O-deacetylxenicin, and Kashman[6] was not certain about the assignments of two ^{13}C NMR signals in 13-epi-9-O-deacetylxenicin. For these reasons we performed ^1H difference decoupling,[16,17] ^1H COSY,[18,19,20] and heteronuclear correlated 2-D NMR (HETCOR)[21] experiments and we fully established the ^1H and ^{13}C NMR assignments in 3 (see Figures 4 and 5).

Structure elucidation of 13-Epi-9-O-deacetyl-7,8-dihydro-7α,8α-epoxyxenicin (40):

Silica gel chromatography of fraction F' (see Scheme 4) yielded 25 fractions. Further chromatography of fraction 14 by HPLC gave 9 frac-

*assignments are made by analogy to other compounds in this series.

Table 1 . ^{1}H NMR Data of Xenicins*

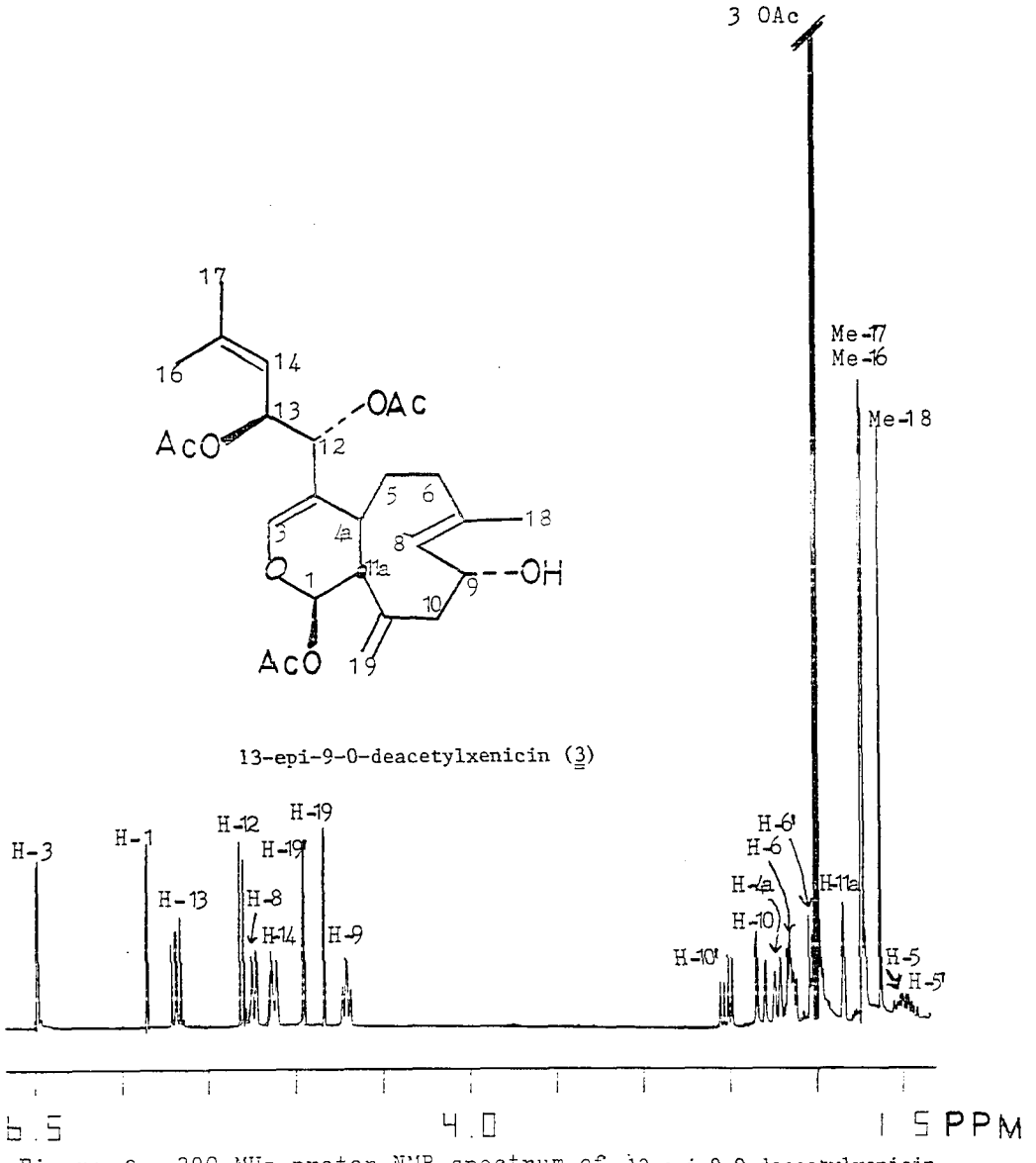
	1	3	40	41
H-1	5.83 d 1.8	5.85 d 1.8	5.92 d 2.6	5.89 d 3
H-3	6.55 d 2.5	6.48 d 2.1	6.52 d 1.5	6.52 d 1.9
H-4a	2.15 br d 9.6	2.23 br d 9.6	2.40 br d 9.5	2.42 br d 9.8
H-5	1.48 m	1.50 m	1.57 m	1.60 m
H-5'	1.53 m	1.55 m	2.08 m	2.13 m
H-6	2.2-2.29 m	2.15 m	2.19 dt 13.5;3.5	2.21 dt 13.2;3.3
H-6'	2.2-2.29 m	2.01 m	1.25 ddd 13.5;12.3;3.5	1.25 ddd 13.2;12.4;4.1
H-8	5.24 br d 8.4	5.23 br d 7.3	3.0 dd 8.5;1	3.14 d 8.7
H-9	5.66 br dd 8.4;6.3	4.71 br dd 7.3;6.3	3.79 ddd 8.5;7.2;2.6	4.81 ddd 8.7;6.3;3
H-10	2.35 br dd 14.7;1.2	2.32 br dd 13.9;1.3	2.42 br dd 14.5;2.6	2.38 br dd 14.6;3
H-10'	2.55 br dd 14.7;6.3	2.52 br dd 13.9;6.3	2.64 ddd 14.5;7.2;2	2.68 br dd 14.6;6.7
H-11a	1.85 br s	1.84 br s	2.32 dt 2.2;2.6	2.33 br t 3
H-12	5.36 d 9	5.31 br d 6.8	5.30 dd 6.2;1.5	5.28 d 6
H-13	5.76 dd 10.5;9	5.68 dd 9.3;6.8	5.69 dd 9.3;6.2	5.69 dd 9.3;6
H-14	5.03 br d 10.5	5.12 d sept 9.3;1.3	5.15 d sept 9.3;1.5	5.17 d sept 9.3;1.4
He-16	1.84 br s	1.72 d 1.3	1.73 d 1.5	1.75 d 1.4
He-17	1.72 br s	1.73 d 1.3	1.74 d 1.5	1.75 d 1.4
He-18	1.70 br s	1.63 br s	1.35 s	1.39 s
H-19	4.78 br s	4.84 br s	4.98 br s	5.02 br s
H-19'	4.92 br s	4.95 br s	5.15 br s	5.13 br s
OAc	2.01 s;2.03 2s 2.07 s	1.99 s;2.01 s 2.05 s	2.0 s;2.01 s; 2.06 s	2.03 s;2.04 s 2.07 s;2.12 s

*Spectra were recorded in $CDCl_3$ at 300 MHz with TMS as internal standard. The values are given in δ units. Assignments were established by ^{1}H difference decoupling (DDS) experiments.

Table 2 . ^{13}C NMR Data of Xenicins[a]

	1	3[b]	40	41
C-1	91.7 d	91.63 d	91.19 d	90.87 d
C-3	142.6 d	141.84 d	142.10 d	141.93 d
C-4	113.5 s	113.50 s	113.61 s	113.61 s
C-4a	37.1 d	36.63 d	36.70 d	36.67 d
C-5	30.5 t	30.48 t	30.0 t	29.69 t
C-6	39.9 t	39.52 t	39.57 t	39.67 t
C-7	134.3 s	132.99 s	59.48 s	59.15 s
C-8	126.2 d	130.97 d	66.83 d	63.31 d
C-9	70.6 d	67.64 d	69.0 d	71.66 d
C-10	42.9 t	46.28 t	43.53 t	41.04 t
C-11	146.5 s	147.28 s	144.81 s	143.96 s
C-11a	49.6 d	49.38 d	48.69 d	48.5 d
C-12	76.4 d	74.87 d	74.53 d	74.45 d
C-13	69.8 d	69.93 d	70.13 d	70.06 d
C-14	119.6 d	119.52 d	119.14 d	118.69 d
C-15	140.7 s	140.15 s	140.42 s	140.36 s
C-16	18.9 q	18.61 q	18.65 q	18.65 q
C-17	25.6 q	25.85 q	25.90 q	25.40 q
C-18	17.5 q	17.78 q	17.64 q	17.2 q
C-19	116.1 t	115.12 t	116.61 t	117.12 t
OAc	169.5 3s	170.1 s,	169.42 s,	170.55 s,
	168.8 s	169.86 s,	169.94 s,	170.15 2s,
	21.0 4q	169.54 s,	169.85 s,	169.65 s,
		20.97 q,	21.19 q,	20.43 q,
		21.17 q,	21.12 q,	20.64 q,
		21.23 q	20.95 q	20.68 2q

[a]Spectra were recorded in $CDCl_3$ at 75.4 MHz. Multiplicities were obtained by DEPT and assignments were made by comparison to 13-epi-9-O-deacetylxenicin (3). [b]Assignments were established by a heterocorrelated 2-D experiment.



13-epi-9-0-deacetylxenicin (3)

Figure 2. 300 MHz proton NMR spectrum of 13-epi-9-0-deacetylxenicin (3) in $CDCl_3$

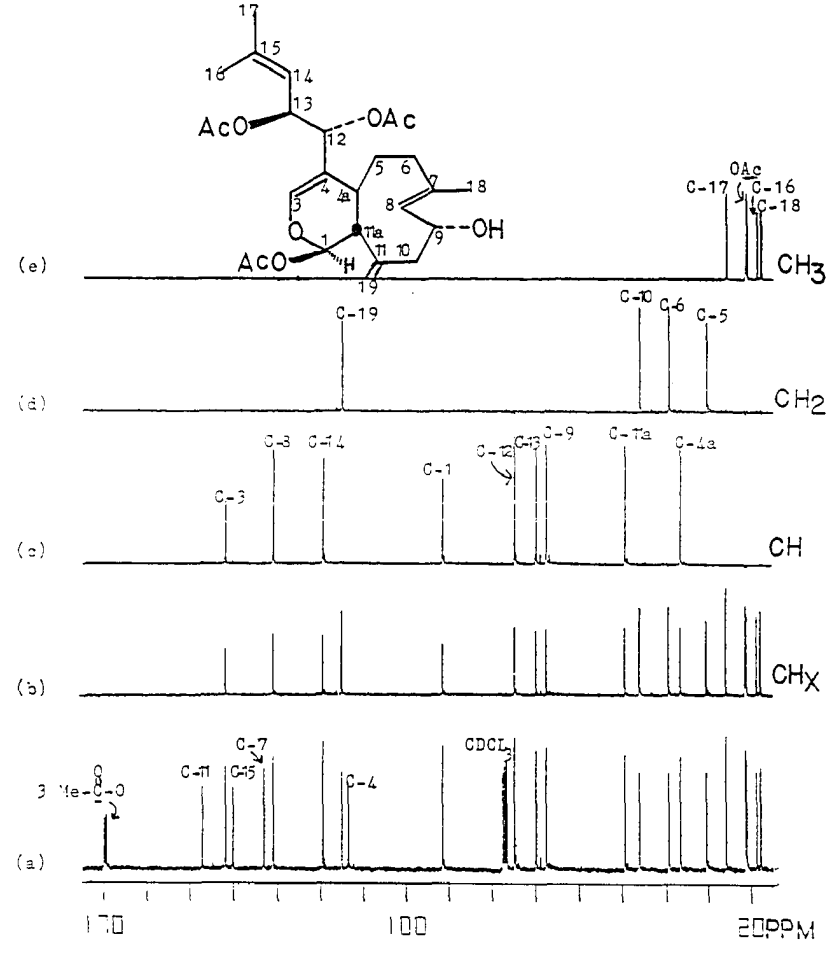


Fig 3. (a) 75.4 MHz broadband ^{1}H decoupled ^{13}C NMR spectrum of 13-epi-9-O-deacetylxenicin (3) in $CDCl_3$. (b) all protonated carbons. (c) methine carbons. (d) methylene carbons. (e) methyl carbons. Spectra b-e were recorded in $CDCL_3$ at 75.4 MHz and resulted from a DEPT[22] experiment.

13-epi-9-0-deacetylxenicin (3)

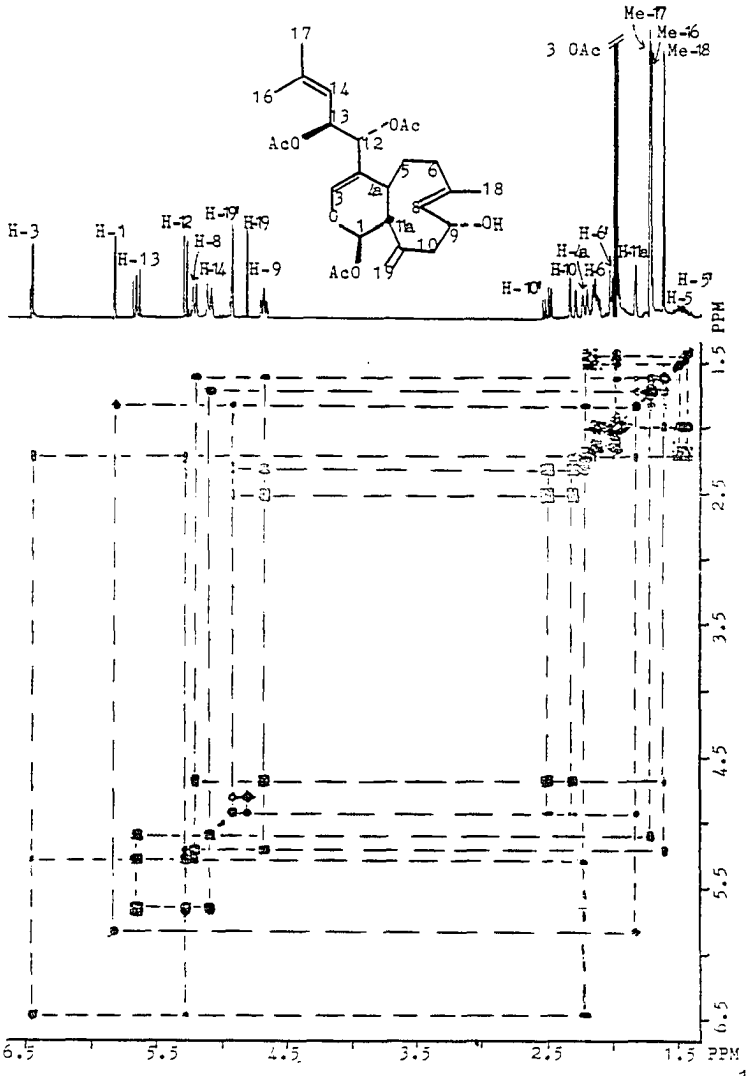


Figure 4. Contour plot of the homonuclear correlated 2-D ^{1}H NMR spectrum of 13-epi-9-0-deacetylxenicin (3) in $CDCl_3$ at 300 MHz. Off-diagonal peaks establish direct proton spin-coupling connectivities.

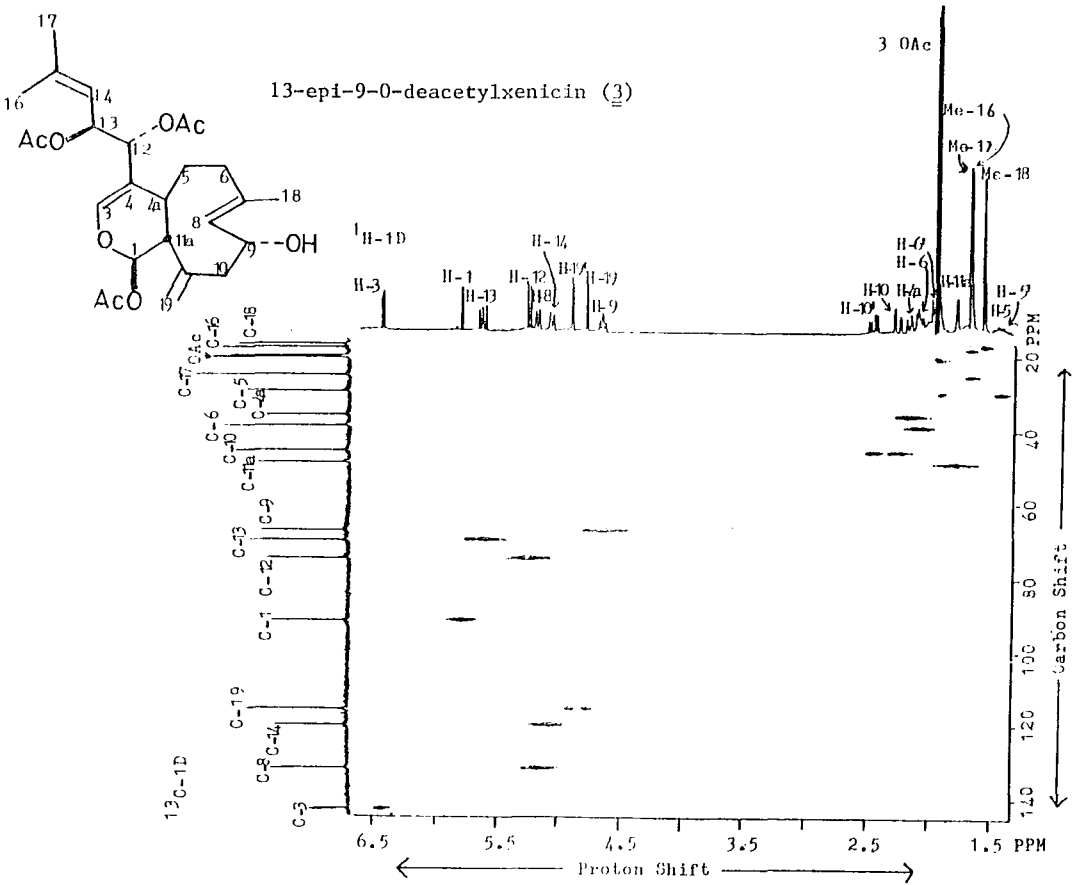


Fig 5.Contour plot of the heteronuclear correlated 2-D spectrum of **13-epi-9-0-deacetyl-xenicin** (3) in $CDCl_3$ at 75.4 MHz. This 2-D plot confirms direct connectivities between bonded nuclei.

tions. Repeated purification of the most polar fraction by HPLC led to the isolation, in trace quantities, of an oil, 13-epi-9-O-deacetyl-7,8-dihydro-7α,8α-epoxyxenicin (40), 3.7 mg, $[\alpha]_D^{25}$ +25.68° (c 0.37, $CHCL_3$). The molecular formula $C_{26}H_{36}O_9$ (9 unsaturations) was established from the following spectral data: (a) an ion in the high resolution mass spectrum which corresponds to M^+-59 was observed at 433.2259 which is in agreement for the formula $C_{24}H_{33}O_7$ (calculated 433.2226); (b) ^1H NMR (35 non-exchangeable protons); (c) IR (OH); (d) ^{13}C NMR [26 carbons with an acetylated hemiacetal carbon, a carbon deshielded by a single oxygen (OH), two carbons deshielded by an oxygen (an epoxide), and 3 acetate carbons]. The IR spectrum of 40 exhibited a broad hydroxyl band at 3500 cm^{-1} and a carbonyl band at 1735 cm^{-1} indicative of acetate group(s). Analysis of the ^{13}C NMR spectra of 40 (Figure 6) revealed the existence of six methyl groups, three sp^3 methylenes and one exocyclic methylene, nine methines, and four quaternary carbons, in addition to the three acetate carbonyl carbons.

Analysis of the ^1H and ^{13}C NMR spectra (Figures 6, 7, and 8) of 40 suggested the presence of three double bonds (>C=CH_2, —CH=C<Me/Me, —C=CH—), an epoxide (—CH—O—C(Me)—), three acetate groups, an acetal, and a secondary hydroxyl group (see Tables 1 and 2). The presence of the latter moiety was unequivocally confirmed by acetylation, which yielded the expected 9-acetate, 41, (H-9 shifts from 3.79 to 4.81 ppm). Comparison of the ^1H and ^{13}C NMR data of 40 with the data of previously isolated 13-epi-9-O-deacetylxenicin[15] (see Tables 1 and 2),

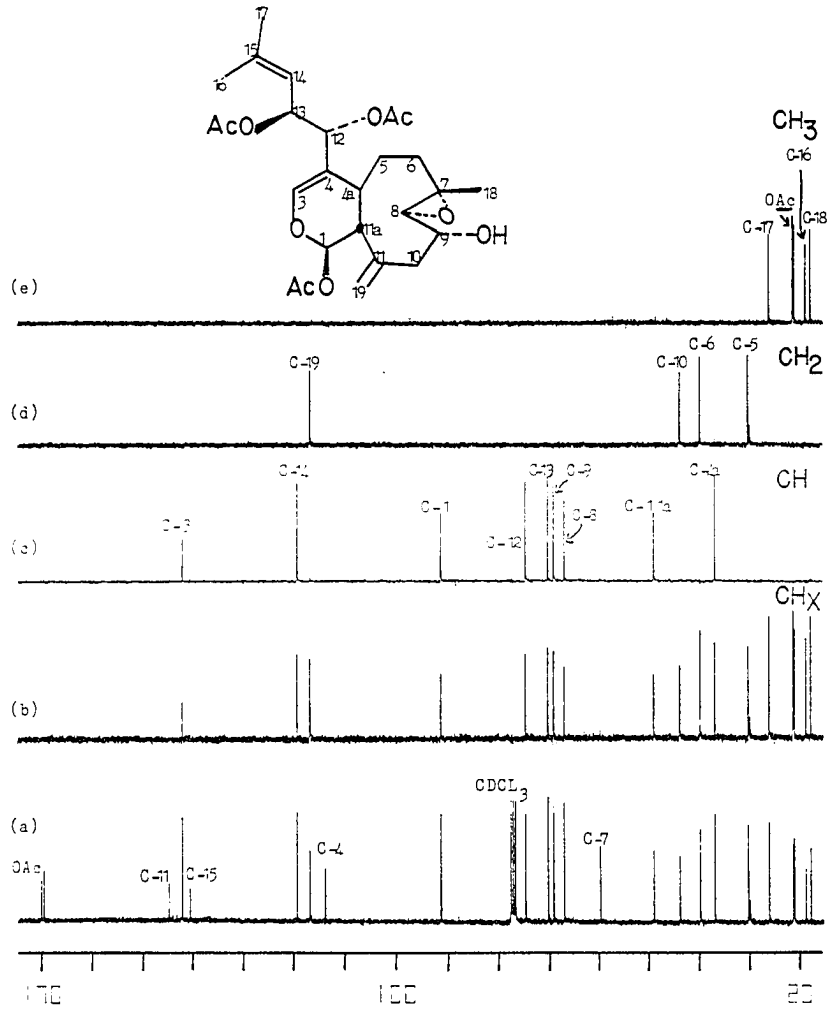


Figure 6. (a) 75.4 MHz broadband proton decoupled ^{13}C NMR spectrum of 40 in $CDCl_3$. (b) all protonated carbons. (c) methine carbons. (d)methylene carbons. (e) methyl carbons. Spectra b-e were recorded at 75.4 MHz in $CDCl_3$ and resulted from the DEPT experiment.

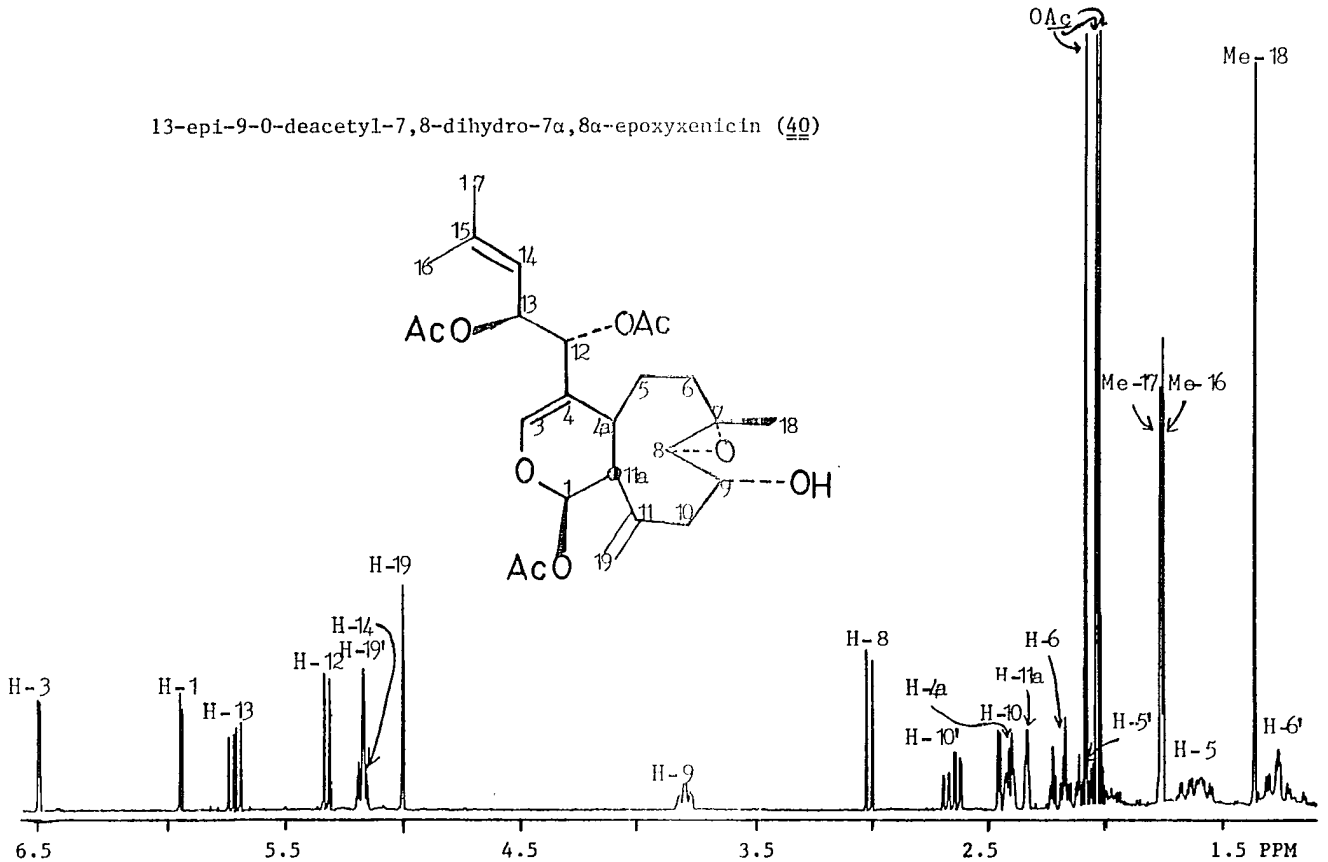


Figure 7. 300 MHz ^{1}H NMR spectrum of 13-epi-9-O-deacetyl-7,8-dihydro-7α,8α-epoxyxenicin (40) in $CDCl_3$.

13-epi-9-O-deacetyl-7,8-dihydro-7α,8α-epoxyxenicin (40)

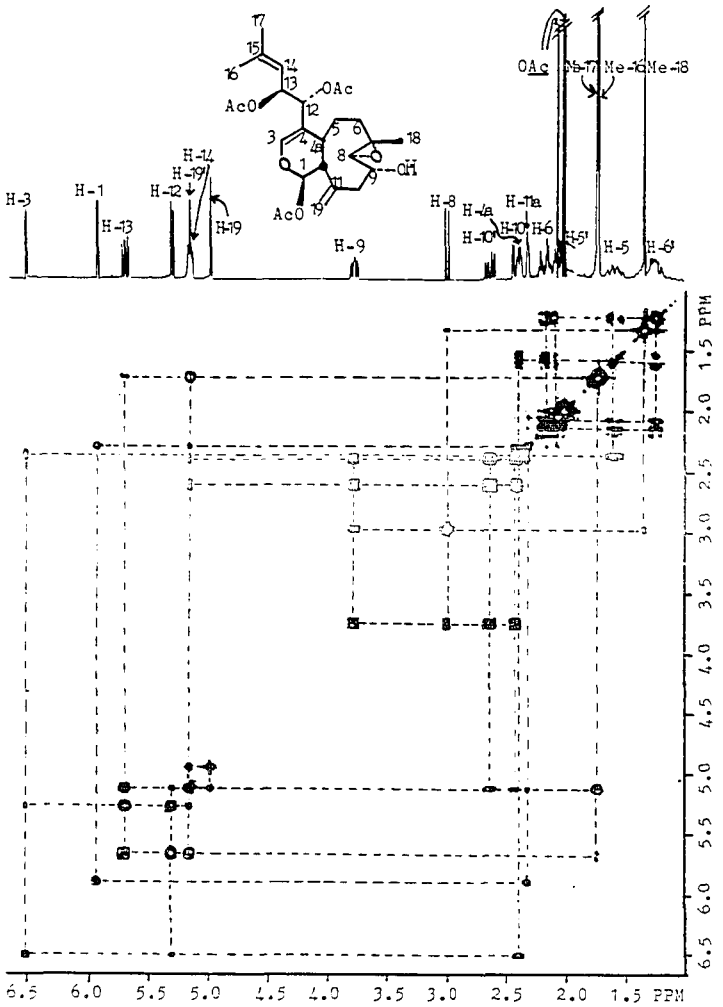


Figure 8. Contour plot of the homocorrelated two-dimensional proton NMR spectrum (symmetrized [20]) of 40 in $CDCl_3$ at 300 MHz. The final $s(F_2,F_1)$ matrix plot consisted of 512 X 512 data points. Off-diagonal contour establish direct proton spin-coupling connectivities.

indicated the bicyclic skeleton of 40 to be identical with that of 13-epi-9-O-deacetylxenicin except that compound 40 possesses an epoxide group at C-7,8.

The proposed structure of 40 was confirmed by double irradiation experiments which established all carbon-carbon connectivities. The ^{1}H assignments were accomplished by ^{1}H difference decoupling spectroscopy (DDS) and homonuclear correlated two-dimensional (COSY) (Figure 8) experiments.

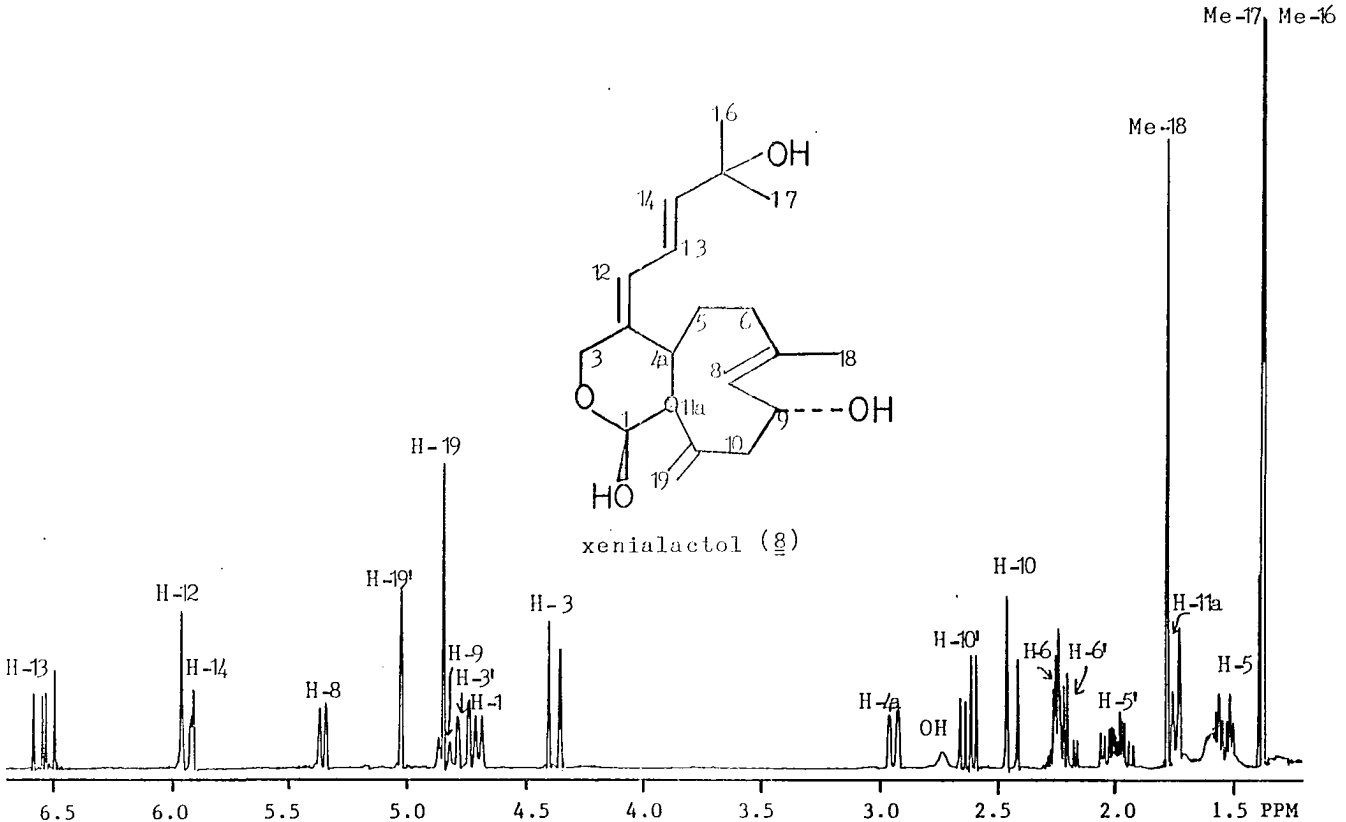
The geometry of the epoxide group at C-7,8 was assigned as trans, on the basis of NOE results (irradiation of Me-18 enhanced H-9) (see Table 3). Since the ^{1}H and ^{13}C NMR data of 40 were very close to that of 13-epi-9-O-deacetylxenicin except as noted, we proposed that 40 possesses the same stereochemistry at C-1, C-11a, C-4a, C-12, C-13, and C-9. NOE experiment results, see Table 3, support these assignments.

Identification of Xenialactol (8):

The $CHCL_3$ fraction, fraction F', was chromatographed over silica gel. Elution with hexane-acetone (1:1) yielded a brown oily material. Addition of a mixture of hexane-acetone (7:3) to the brown oily material caused a light yellow solid (110 mg) to precipitate, but all attempts to crystallize this solid failld. It was identified as xenialactol (8).[6] The molecular formula $C_{20}H_{30}O_4$ (6 unsaturations) of 8 was obtained from the following spectral data: (a) the low resolution mass spectrum exhibited a peak at 316 ($M^{+}-H_2O$); (b) ^{1}H NMR (27 non-exchangable protons and 3 exchangeable protons); (c) ^{13}C NMR (20 carbons including a hemiacetal carbon 99.92 ppm (C-1, d) and an allylic* carbon deshielded by a

Table 3 . Results of a nuclear Overhauser enhancement difference spectroscopy (NOEDS)[23] experiment with 40 in $CDCl_3$ solution.

Proton irradiated, chem shift, ppm	Proton (s) enhanced	% Enhancement
H-3, 6.52	H-12, 5.30	17
H-1, 5.92	H-11a, 2.32	4
	H-10, 2.42	2
H-13, 5.69	H-12, 5.30	13
	H-14, 5.15	15
H-12, 5.30	H-3, 6.52	19
	H-13, 5.69	8
H-8, 3.0	H-4a, 2.40	18
Me-18, 1.35	H-9, 3.79	9
	H-11a, 2.32	9



xenialactol (8)

Figure 9 . 300 MHz proton NMR spectrum of xenialactol in $CDCl_3$.

Table 4 . ^{13}C NMR Data of Xenialactols*

	8[a]	42	43	44	45	46
C-1	99.92 d	100.74	99.80 d	100.10 d	100.10 d	97.24 d
C-3	69.76 t	65.21 t	69.76 t	69.23 t	69.22 t	69.30 t
C-4	139.44 s	139.97 s	139.31 s	138.50 s	138.52 s	137.03 s
C-4a	44.08 d	52.3 d	44.35 d	43.60 d	43. 73	43.19 d
C-5	35.55 t	36.88 t	35.38 t	34.21 t	34.17 t	34.22 t
C-6	40.22 t	40.07 t	40.33 t	40.38 t	40.43 t	40.24 t
C-7	132.99 s	133.17 s	134.05 s	59.03 s	59.60 s	58.90 s
C-8	130.46 d	130.23 d	125.96 d	67.02 d	67.05 d	63.35 d
C-9	67.56 d	67.67 d	70.66 d	68.96 d	68.90 d	71.99 d
C-10	46.86 t	46.88 t	43.69 t	45.18 t	45.19	42.81 t
C-11	151.05 s	150.74 s	150.56 s	149.02 s	149.17 s	146.71 s
C-11a	57.61 d	57.93 d	57.29 d	56.03 d	56.07 d	52.96 d
C-12	122.11 d	122.49 d	122.17 d	122.58 d	123.0 d	123.92 d
C-13	120.81 d	121.49 d	120.74 d	120.40 d	122.69 d	120.10 d
C-14	142.37 d	141.50 d	142.43 d	142.92 d	140.6 d	143.59 d
C-15	70.88 s	71.0 s	70.97 s	70.87 s	74.83 s	70.89 s
C-16	29.99 q	29.89 q	30.0 q	29.96 q	25.96 q	30.0 q
C-17	29.91 q	29.90 q	30.0 q	29.90 q	25.87 q	29.97 q
C-18	17.61 q	17.74 q	17.57 q	17.73 q	17.38 q	17.41 q
C-19	112.35 t	112.43 t	113.14 t	113.76 t	113.59 t	115.86 t
OAc			170.68 s			170.45 s, 21 q
			21.45 q			170.2 s 21.3 q

*Spectra were recorded in $CDCl_3$ at 75.4 MHz. Multiplicities were obtained by DEPT and assignments were made by comparison to xenialactol (8). [a]Assignments were established by a heterocorrelated 2-D experiment.

Table 5 . Proton NMR Data of Xenialactols*

	8	42	43	44	45	46
H-1	4.62 br d 8.2	4.53 br d 8.4	4.61 br d 8.2	4.65 dd 7.8;4.5	4.67 br d 7.4	5.55 d 8.7
H-3	4.30 br d 13.8	4.58 dd 15.7;2.5	4.31 br d 13.7	4.31 br d 13.8	4.33 br d 13.9	4.42 br d 13.9
H-3'	4.68 br d 13.8	4.80 br d 15.7	4.69 dd 13.7;1.3	4.71 dd 13.8;1.5	4.73 br d 13.9	4.73 br d 13.9
H-4a	2.89 br d 10.4	2.29 br d 10.6	2.89 br d 10.5	2.91 br d 10.5	2.91 br d 10.5	2.98 br d 10.5
H-5	1.52 br dt 13.6;3.7	1.57 br dt 13.5;3.7	1.53 br dt 13.5;3.5	1.59 br dt 14;3.5	1.61 br dt 14;3.6	1.60 br dt 14;3.5
H-5'	1.96 tdd 13.6;10.4;3.7	1.95 m	1.95 tdd 13.5;10.5;3.7	2.02 tdd 14;10.5;3.5	2.05 dddd 14;13.3;10.5;3.6	2.04 tdd 14;10.5;3.5
H-6	2.17 m	2.04-2.22 m	2.15-2.22 m	1.22 br td 14;3.5	1.22 br td 13.3;3.6	1.28 br td 14;3.5
H-6'	2.22 m	2.04-2.22 m	2.15-2.22 m	2.24 dt 14;3.5	2.26 dt 13.3;3;6	2.30 dt 14;3.5
H-8	5.27 br d 7.4	5.17 br d 7.4	5.31 br d 7.8	2.99 d 8	3.0 d 8.2	3.17 d 7
H-9	4.76 br dd 7.4;6.5	4.74 br dd 7.4;6.4	5.68 br dd 7.8;7	3.81 br t 8	3.84 br dd 8.2;7.3	4.85 br t 7
H-10	2.40 br d 13.8	2.38 br d 14	2.43 br d 14.3	2.48 br dd 14.3;1.5	2.50 br d 14.3	2.48 br d 13.9
H-10'	2.57 br dd 13.8;6.5	2.55 br dd 14;6.4	2.60 br dd 14.3;7	2.66 br dd 14.3;8	2.69 br dd 14.3;7.3	2.68 br dd 13.9;7
H-11a	1.70 br d 8.2	1.70 br d 8.4	1.72 dt 8.2;1.8	2.08 br d 7.8	2.11 br dt 7.4;1.7	2.09 br d 8.7
H-12	5.82 br d 10.7	5.89 br d 11	5.85 br d 10.8	5.85 br dd 10.8;1.5	5.89 br dd 10.5;1.4	5.95 br d 10.5
H-13	6.42 dd 15.7;10.7	6.22 dd 15;11	6.44 dd 15.5;10.8	6.34 dd 15.2;10.8	6.26 dd 15.4;10.5	6.38 dd 15.7;10.5
H-14	5.83 d 15.7	5.75 d 15	5.83 d 15.5	5.83 d 15.2	5.69 d 15.4	5.90 d 15.7
Me-16	1.35 s	1.34 s	1.36 s	1.32 s	1.28 s	1.38 s
Me-17	1.35 s	1.34 s	1.36 s	1.32 s	1.28 s	1.38 s
Me-18	1.75 br s	1.72 br s	1.81 br s	1.47 s	1.49 s	1.52 s
H-19	4.76 br s	4.86 br s	4.78 br s	4.86 br s	4.87 br s	4.75 br s
H-19'	4.94 br s	4.95 br s	4.92 br s	5.12 br s	5.13 br s	4.93 br s
OAc			2.10 s			2.08 s; 2.16 s

*Spectra were recorded in $CDCl_3$ at 300 MHz with Me_4Si as internal standard. The values are given in δ units. Assignments were established by ^1H difference decoupling (DDS) experiments.

secondary hydroxyl* group 67.56 ppm (C-9, d) and a tertiary carbon deshielded by a tertiary hydroxyl* group 70.88 ppm (C-15, s); (d) IR (OH). The ^{1}H and ^{13}C NMR (see Tables 4 and 5) data of 8 were identical to those of xenialactol which was isolated[6] by Kashman and Groweiss. However, Kashman reported that the chemical shift of the H-9 signal in xenialactol occurred at 4.85 ppm as a multiplet. Our 300 MHz ^{1}H NMR analysis of 8 established the chemical shift of H-9 signal to be at 4.75 ppm as a broad triplet in which the H-19 signal was superimposed (see Table 4 and Figure 9).

Structure elucidation of Isoxenialactol (42):

Silica gel chromatography of fraction F' (see Scheme 4) yielded 25 fractions. The most polar fraction was purified by HPLC and gave a minute amount (0.9 mg) of compound 42, a white powder. The molecular formula $C_{20}H_{30}O_4$ (6 unsaturations) was deduced for 42 from the following data: (a) low resolution mass spectrum (M^+ 334 and 3 consecutive losses of H_2O); (b) ^{1}H NMR (27 non-exchangeable protons); (c) ^{13}C NMR (20 carbons with 3 carbons deshielded by a single oxygen [71.01 ppm (C-15, s), 67.67 (C-9, d), 65.21 (C-3, t)] and one by two oxygens [100.74 ppm (C-1, d)]. The ^{13}C NMR spectra (Figure 10) revealed the existence of 3 methyls, 4 sp^3 methylene and one exocyclic terminal methylene, 4 sp^3 methines and 4 sp^2 methines, and 4 quarternary carbons. The IR spectrum of 42 exhibited a broad hydroxyl band at 3450 cm^{-1}.

*assignments were made by analogy to the known compounds reported in the literature.

Comparison of ^{1}H and ^{13}C NMR spectra (Figures 11 and 10) of compound 42 with the data of previously isolated xenialactol [6] (8) showed close resemblance, except for some minor differences in ^{1}H and ^{13}C NMR spectra associated with the side chain. This strongly indicated that 42 and xenialactol are stereoisomeric with regard to their side chain geometry. The stereochemistry of the side chain in xenialactol was established as E,E on the basis of coupling constants and an NOE effect between H-4a and H-13. In xenialactol, the olefinic proton signals for H-12 (5.82 ppm) and H-14 (5.83 ppm) were very close. However, in compound 42 H-12 and H-14 appeared at 5.89 ppm and 5.75, respectively. Moreover, H-13 in compound 42 appears at 6.22 ppm while in xenialactol it appears at 6.42 ppm. This suggested that these differences in the chemical shifts are due to a different geometry for the diene side chain. Furthermore, the protons giving rise in compound (42) to the AB pattern at 4.58 ppm (H-3) and 4.80 (H-3') are presumably deshielded by the diene moiety, whereas in xenialactol H-3 and H-3' were observed at a higher field (4.30 and 4.68 ppm, respectively). However, H-4a in compound 42 was observed at a higher field (by 0.5 ppm) than H-4a in xenialactol (see Table 4). This indicated that the geometry of the $\Delta^{4,12}$ trisubstituted double bond in 42 is most probably Z. This was confirmed further by comparing (see Table 5) ^{13}C NMR chemical shifts of C-4a observed for the isomeric pair xeniolide-A (7) and isoxeniolide-A[15] (11) with those of xenialactol and 42. Finally, the Z geometry of $\Delta^{4,12}$ in 42 was unequivocally confirmed by an NOE experiment (irradiation of H-13 enhanced H-3).

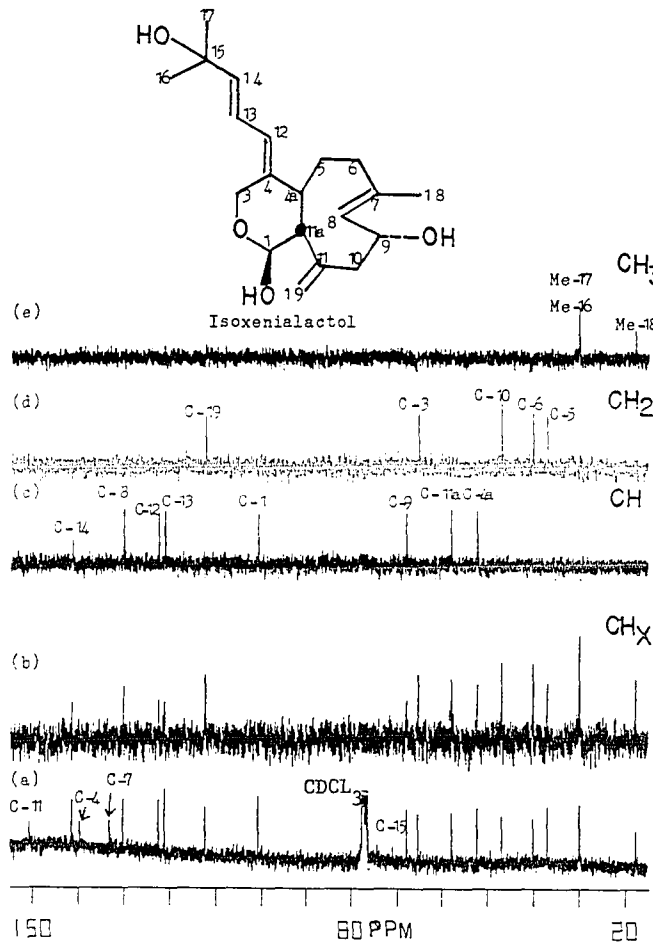


Figure 10. (a) 75.4 MHz broadband ^{1}H decoupled ^{13}C NMR spectrum of 42 in $CDCl_3$ (b) all protonated carbons. (c) methine carbons. (d) methylene carbons. (e) methyl carbons. Spectra b-e were recorded at 75.4 MHz in $CDCl_3$ and resulted from the DEPT experiment.

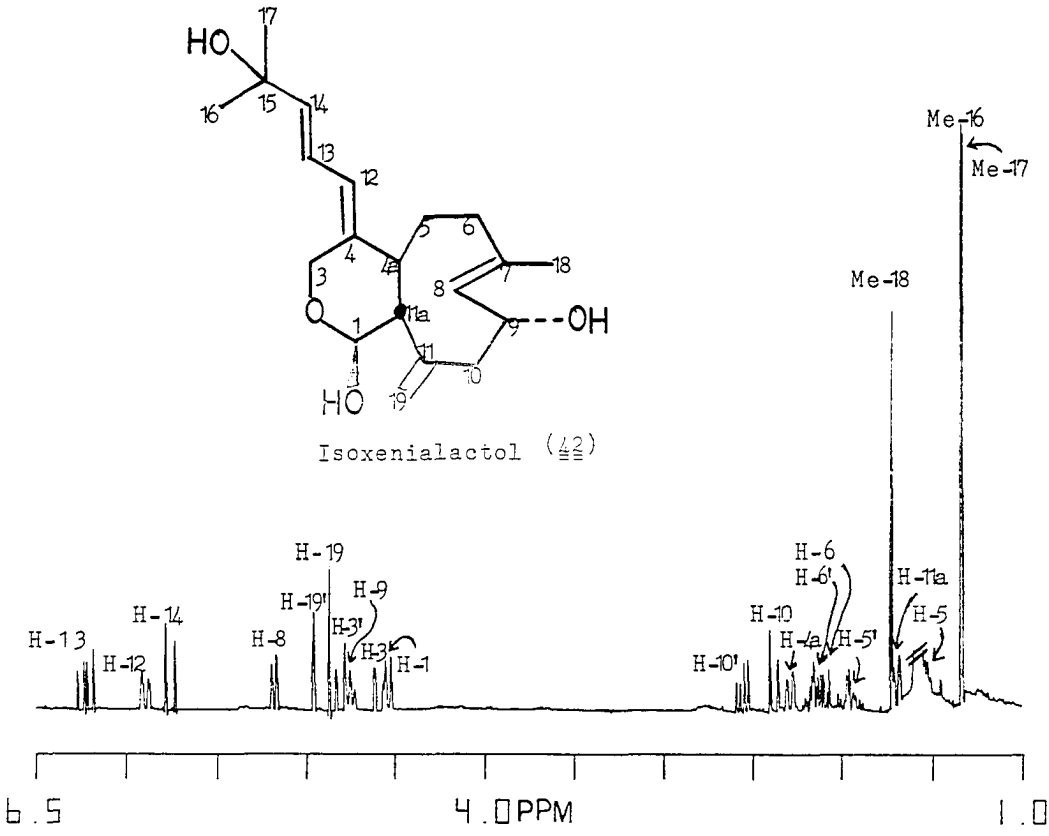


Figure 11. 300 MHz ^{1}H NMR spectrum of Isoxenialactol (42) in $CDCl_3$

The remaining sterochemical features in 42 were judged to be identical to those proposed for xenialactol on the basis of the following similarities in NMR data for the two compounds (Tables 4 and 5). The E geometry for $\Delta^{7,8}$ double bond in 42 was assigned according to the ^{13}C chemical shift of Me-18 appearing at 17-18 ppm (rather than at 22-25 for the Z configuration).[24] Since the chemical shifts of H-9 and C-9 in compound 42 and in xenialactol were identical, the stereochemistry at C-9 in 42 is assumed to be the same as in xenialactol. The trans configuration is assigned to the ring fusion in 42 on the basis of the similarities of the coupling constants ($J_{11a,4a}$ = 2 Hz) observed for 42 and xenialactol. The relative stereochemistry of the hydroxyl group at C-1 in 42 was assigned as β as in xenialactol, because the coupling constant ($J_{1,11a}$ = 8 Hz) was almost identical in xenialactol and in 42. All of the above evidence supports structure 42 for which we propose the name isoxenialactol.

Xenialactol 9-Acetate (43):

The $CHCl_3$ extract of Scheme 4 was chromatographed over silica gel. One of the fractions eluted with hexane-acetone (6:4) was rechromatographed by HPLC to give 5 mg of xenialactol 9-acetate (43) as a colorless oil. The molecular formula $C_{22}H_{32}O_5$ (7 unsaturations) was obtained from the following spectral data: (a) the highest mass peak in the low resolution mass spectrum was detected at 376; (b) the ^1H NMR spectrum (Figure 13) exhibited 26 non-exchangeable protons; (c) the ^{13}C NMR spectra (Figure 12) contained 22 peaks including one for a hemiacetal carbon, 99.8 ppm (C-1, d) and 2 other carbons each deshielded by a

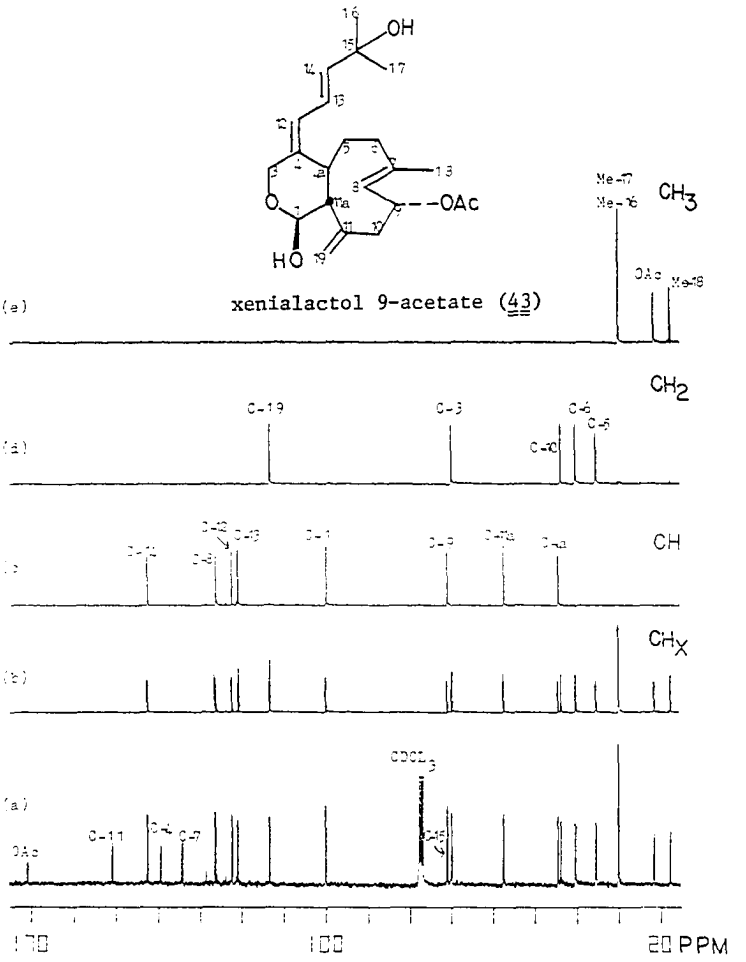
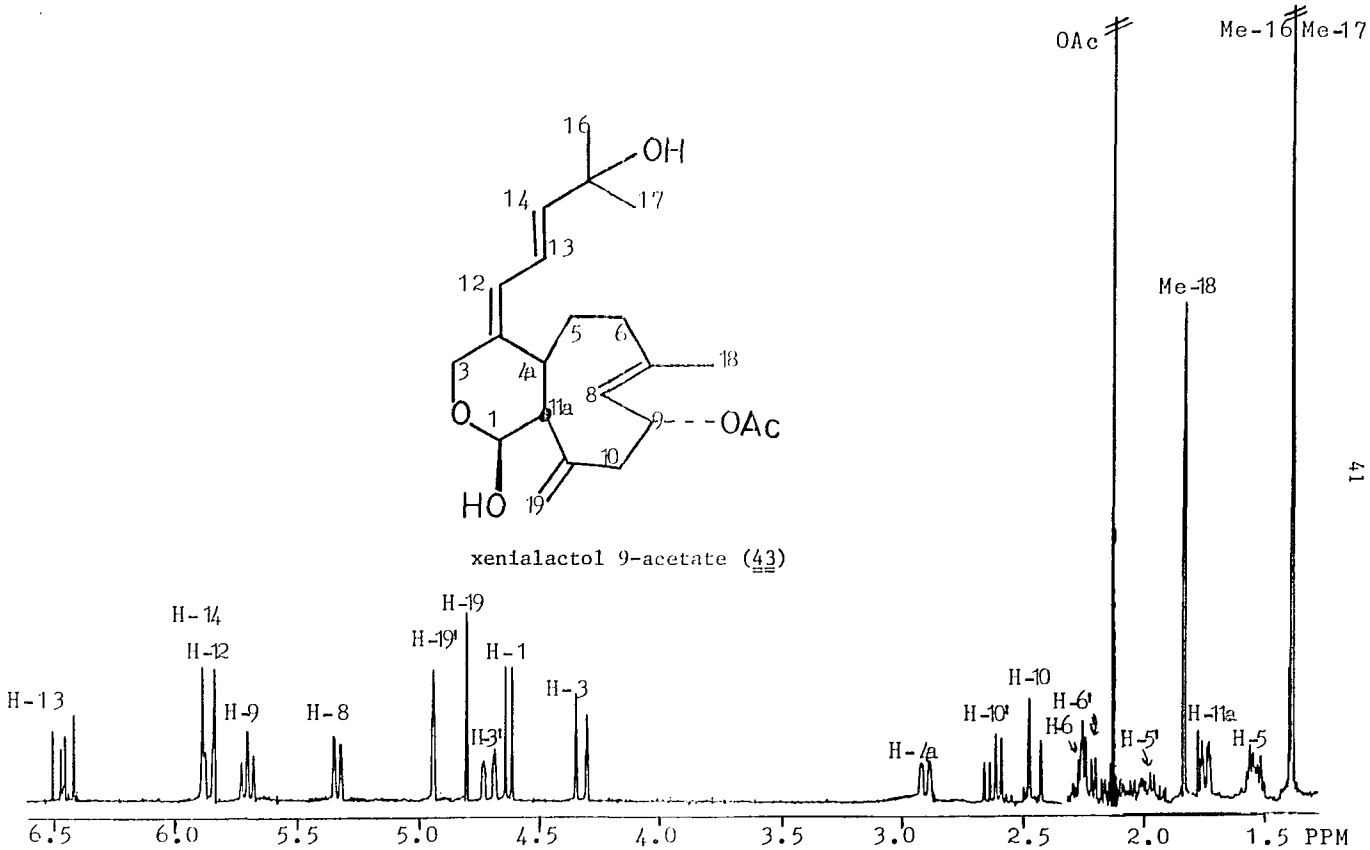


Figure 12. (a) 75.4 MHz broadband proton decoupled ^{13}C NMR spectrum of 43 in $CDCl_3$. (b) all protonated carbons. (c) methine carbons. (d) methylene carbons. (e) methyl carbons. Spectra b-e were recorded at 75.4 MHz in $CDCl_3$ and resulted from a DEPT experiment.



xenialactol 9-acetate (43)

Figure 13. 300 MHz proton NMR spectrum of xenialactol 9-acetate (43) in $CDCl_3$.

single oxygen, 70.66 ppm (C-9, d) and 70.94 ppm (C-15, s) and an acetate carbonyl carbon, 170.68 ppm (o-$\underset{\underset{O}{\|}}{C}$—$CH_3$, s); (d) IR absorptions at 3440 (OH) and 1730 cm^{-1} (acetate group). The ^{13}C NMR (DEPT) spectra (Figure 12) indicated the presence of 4 methyls, 4 sp^3 methylenes and one exocyclic terminal methylene, 4 sp^3 methines and 4 sp^2 methines, and 4 quarternary carbons, in addition to the carbonyl (CH_3-$\underset{\underset{O}{\|}}{C}$-O) mentioned above. The ^1H NMR spectrum (Figure 13) of 43 was almost identical to that of xenialactol[6] (see Table 4) except for the existence of an additional acetate methyl group signal at 2.1 ppm and a downfield shift of the H-9 signal from 4.76 to 5.68 ppm. Moreover, the ^{13}C NMR spectrum of 43 contained two additional carbon signals at 21.45 (q) and 170.68 ppm (s) which corresponded to an acetate group. It was concluded that compound 43 was the xenialactol 9-acetate. The stereochemical features of 43 are assumed to be identical to those proposed for xenialactol, because the ^1H NMR chemical shifts and coupling constants and ^{13}C NMR (see Table 5) data were nearly identical for the two compounds (43 and xenialactol (8)) except as noted.

Structure elucidation of 7,8-Dihydro-7α,8α-epoxyxenialactol (44):

The $CHCl_3$ fraction, fraction F′ of Scheme 3, was chromatographed over a silica gel column using hexane–acetone (9:1) and increasing the amount of acetone. Elution with 1:1 hexane–acetone yielded a fraction which was further chromatographed by HPLC to give 8 fractions. Fraction 8 was rechromatographed by HPLC to give a minute amount of 7,8-dihydro-7α,8α-epoxyxenialactol (44) as a white powder. The molecular ion of 44 was not observed in the high resolution mass spectrum. However, an ion

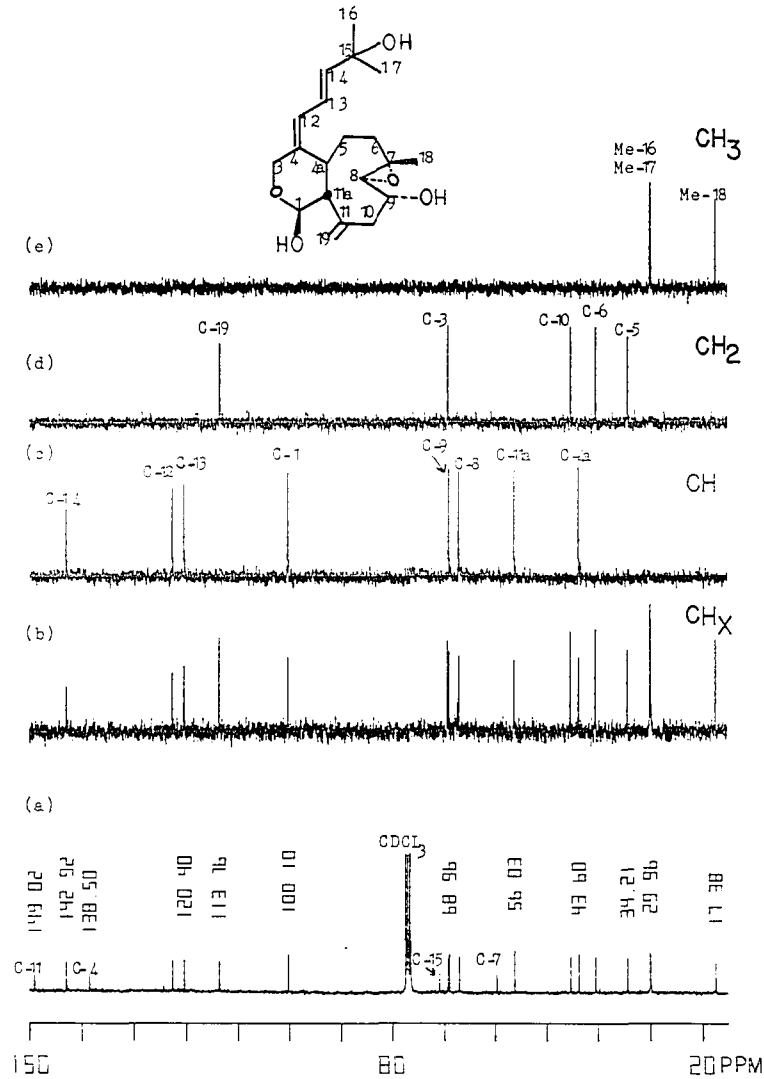


Figure 14. (a) 75.4 MHz broadband proton decoupled ^{13}C NMR spectrum of 44 in $CDCl_3$. (b) all protonated carbons. (c) methine carbons. (d) methylene carbons. (e) methyl carbons. Spectra b-e were recorded at 75.4 MHz in $CDCl_3$ and resulted from a DEPT experiment.

corresponding to M^+-18 was observed at 332.19594 which is in agreement with the formula $C_{20}H_{28}O_4$ (calculated 332.19876). Consideration of both the low resolution (M^+ 350) and high resolution mass spectrometry data established the formula of 44 to be $C_{20}H_{30}O_5$ (6 unsaturations). Analysis of the ^{13}C NMR spectra of 44 (Figure 14) indicated the presence of three methyl groups, four methylenes and one exocyclic terminal methylene, five methines, and four quarternary carbons. The UV spectrum of 44 exhibited a band at 241 nm indicative of a conjugated diene system. Analysis of the ^1H and ^{13}C NMR (Figures 15 and 14) and IR spectra of 44 suggested the existence of three double bonds (>C=CH-CH=CH-, >C=CH_2), an epoxide (-CH(O)-C(Me)-), a hemiacetal, and a secondary OH group (see Tables 4 and 5). The latter two moieties were confirmed by acetylation which gave the expected 1,9-diacetate, 46.

The NMR spectral properties of compound 44 (see Tables 4 and 5) are very similar to those of xenialactol (8), isolated[6] by Kashman and Groweiss, except that the ^{13}C signals for C-7,8, and Me-18 are missing and instead there are ^{13}C signals appropriate for a 7,8-epoxide bearing the 18-methyl (C-18). The ^1H multiplicities and assignments of 44 were accomplished by ^1H decoupling and ^1H difference decoupling spectroscopy (DDS) experiments and these confirm all of the skeletal connectivities presumed by the spectral analogy with xenialactol.

The proposed structure of 44 also received support from NOE experiments (Table 6). The trans geometry of the epoxide at C-7,8 was established by NOE (irradiation of Me-18 enhanced H-9 and no NOE was observed between Me-18 and H-8). The E geometry at the $\Delta^{4,12}$ double bond was also established by NOE experiments (irradiation of H-13 enhanced H-

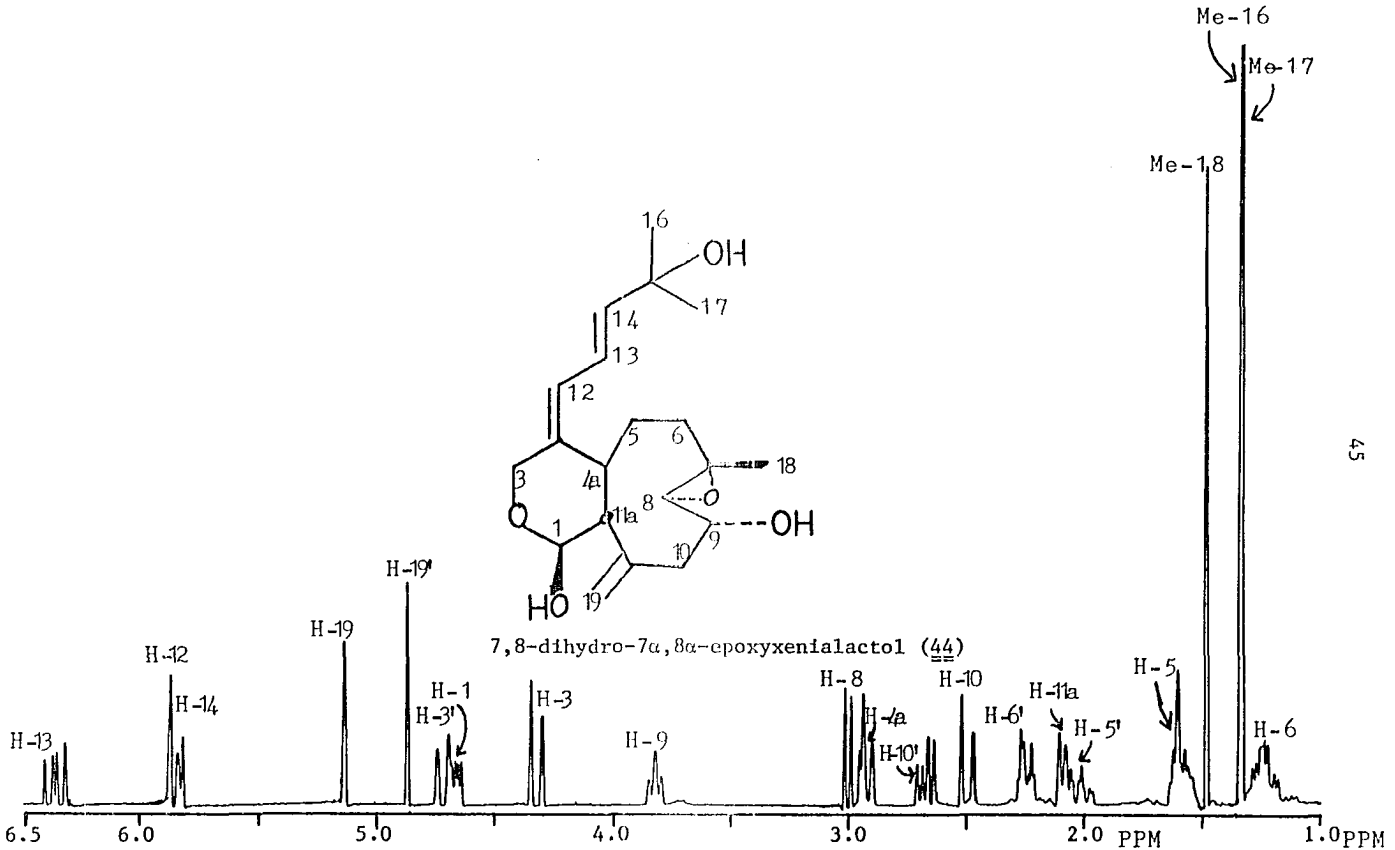


Figure 15. 300 MHz proton NMR spectrum of 7,8-dihydro-7α,8α-epoxyxenialactol (44) in $CDCl_3$.

Table 6. Results of a nuclear Overhauser enhancement difference spectroscopy (NOEDS) experiment with 44 in $CDCl_3$.

Proton irradiated, chem shift, ppm	Proton (s) enhanced	% Enhancement
H-13, 6.34	H-4a, 2.91	9
H-3, 4.31	H-12, 5.85	6
	H-3', 4.71	16
H-4a, 2.91	H-1, 4.65	7
	H-13, 6.34	9
	H-19, 4.85	5
Me-18, 1.47	H-9, 3.81	14
	H-11a, 2.08	3

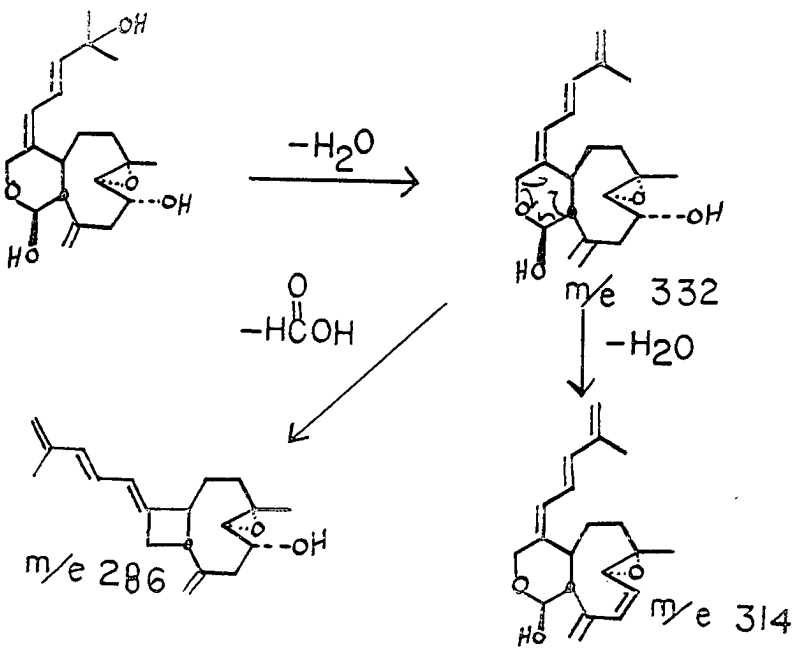


Figure 16. Possible Mass Spectral Fragmentation Pathways for 44

4a) (see Table 6). The relative stereochemistry at C-1, C-4a, C-11a, C-8, and C-9 were established by NOE (see Table 6), independent of the methods used for xenialactol and related compounds. Upon irradiation of H-4a, 4 proton signals (H-13, H-1, H-19 , and H-8) were enhanced (see Table 6). This suggested that these protons are all on the α-face. The lack of any NOE between H-4a and H-11a and the observation of NOE (see Table 6) between Me-18, H-9, and H-11a suggested that Me-18, H-9, and H-11a are on the β-face. The mass spectra data of 44 were consistent with the proposed structure (see Figure 16).

Identification of Xeniolide-A (7):

The $CHCL_3$ extract, fraction F' of Scheme 3 was chromatographed over a column of silica gel. Elution with hexane-acetone (1:1) gave a brown oily fraction. The oily material (fraction 22 of Scheme 4) was resolved by HPLC to yield 4 fractions. The second fraction contained a pure yellow oil which was identified as xeniolide-A (7). The molecular formula $C_{20}H_{28}O_4$ (7 unsaturations) was deduced from the following data: (a) the low resolution mass spectrum exhibited a peak at 314 which was assigned to M^+-H_2O; (b) the ^1H NMR spectrum contained 26 non-exchangeable protons; (c) ^{13}C NMR spectra showed signals for 20 carbons including a carbonyl carbon (171.38 ppm, C-3, s); and 3 carbons that were each deshielded by a single oxygen 71.0 ppm (C-15, s), 67.4 ppm (C-9, d), 71.05 ppm (C-3, t); (d) IR absorptions at 3450 cm^{-1} (OH) and at 1712 (conjugated carbonyl group). The ^1H and ^{13}C NMR data (see Figure 17 and Tables 7 and 8) of 7 were identical to those of xeniolide-A isolated earlier by Kashman and Growiess.[6] However, Kashman reported

Table 7 . Proton NMR Data of Xeniolide-A and its Derivatives*

	7	47	48	49	50	51
H-1	4.09 dd 11.4;5.8	4.07 dd 11.5;5.5	4.16 dd 11.5;5	4.07 dd 11.5;5.5	4.15 dd 11.4;5.5	4.13 dd 11.4;5.3
H-1'	3.60 dd 11.4;12.4	3.57 t 11.5	3.67 t 11.5	3.61 dd 12.5;11.5	3.69 dd 11.4;12	3.64 t 11.4
H-4a	3.11 br dt 9.5;3.5	3.06 br dd 10.6;5.5	3.12 br dd 10.8;5.5	3.06 br dt 10;5.5	3.15 br ddd 9.5;5.5;3	3.11 br dd 9.5;5.5
H-5	1.55-1.65 m	1.50 br dt 13.4;4	1.68 m	1.60 m	1.67 m	1.66 m
H-5'	1.55-1.65 m	1.65 m	1.68 m	1.60 m	1.67 m	1.66 m
H-6	2.17-2.31 m	2.22 m	1.24 m	2.20 m	1.28 m	1.25 m
H-6'	2.17-2.31 m	2.22 m	2.25 m	2.20 m	2.19 dt 13.5;3.5	2.22 dt 13.5;3.5
H-8	5.30 br d 7.3	5.44 br dd 9.2;6.8	3.02 dd 10.3;4	5.30 br dd 7.6;1.2	3.01 d 8.2	3.12 d 8.5
H-9	4.78 ddd 7.3;6.2;2.1	2.10 m	2.34 m	4.78 br ddd 7.6;6.3;2.2	3.79 ddd 8.2;6.3;3.7	4.79 ddd 8.5;6.4;3
H-9'		2.46 m	1.53 m			
H-10	2.37 br dd 13.5;2.1	2.32 m	2.25 m	2.34 br dd 13.5;2.2	2.46 ddd 14;3.7;1.3	2.47 br dd 14.5;3
H-10'	2.48 br dd 13.5;6.2	2.50 m	2.48 m	2.49 br dd 13.5;6.3	2.55 ddd 14;6.3;1.5	2.57 br dd 14.5;6.4
H-11a	2.09 br ddd 12.4;5.8;3.5	2.16 dt 12.5;5.5	2.50 br dt 11.5;5.5	2.09 br dt 12.5;5.5	2.43 ddt 12;1.3;5.5	2.42 br dt 11.4;5.3
H-12	6.91 br d 11.3	6.89 br d 11.3	6.98 br d 11.7	7.01 dd 11;1.5	6.99 dd 11.4;1.3	6.97 br d 11.5
H-13	6.52 dd 15;11.3	6.52 dd 15;11.3	6.45 dd 15;11.7	6.35 dd 15;11	6.46 dd 15;11.4	6.43 dd 15;11.5
H-14	6.25 d 15	6.24 d 15	6.26 d 15	6.29 d 15	6.27 d 15	6.25 d 15
Me-16	1.39 s	1.39 s	1.37 s	1.58 s	1.37 s	1.36 s
Me-17	1.39 s	1.39 s	1.37 s	1.57 s	1.37 s	1.36 s
Me-18	1.71 br s	1.70 br s	1.36 s	1.70 br s	1.39 s	1.42 s
H-19	4.90 br s	4.81 br s	5.08 br s	4.92 br s	5.09 br s	5.09 br s
H-19'	5.08 br s	4.95 br s	5.15 br s	5.08 br s	5.29 br s	5.24 br s
OAc				2.02 s		2.12 s

*Spectra were recorded in $CDCl_3$ at 300 MHz with Me_4Si as internal standard. The values are given in δ units. Assignments were established by ^1H difference decoupling (DDS) experiments.

Table 8 . ^{13}C NMR Data of Xeniolide-A and its Derivatives*

C#	7	47	48	49	50	51
C-1	70.99 t	70.94 t	71.03 t	70.91 t	71.02 t	71.74 t
C-3	171.38 s	171.28 s	171.27 s	170.65 s	170.29 s	170.10 s
C-4	133.11 s	133.20 s	132.35 s	133.18 s	132.06 s	131.87 s
C-4a	42.88 d	43.48 d	42.25 d	42.74 d	41.52 d	40.46 d
C-5	38.0 t	34.45 t	31.79 t	37.93 t	36.39 t	36.37 t
C-6	40.16 t	38.04 t	36.31 t	40.25 t	39.78 t	39.76 t
C-7	133.11 s	135.40 s	58.98 s	133.23 s	59.08 s	58.38 s
C-8	130.59 d	124.64 d	62.64 d	130.45 d	67.27 d	63.81 d
C-9	67.40 d	24.93 t	25.42 t	67.52 d	69.07 d	69.44 d
C-10	44.97 t	40.36 t	39.69 t	44.86 t	42.0 t	42.14 t
C-11	147.56 s	151.85 s	148.83 s	147.28 s	144.57 s	144.44 s
C-11a	49.59 d	49.44 d	49.10 d	49.77 d	49.69 d	47.82 d
C-12	136.14 d	135.74 d	137.23 d	136.0 d	137.57 d	137.47 d
C-13	119.68 d	119.76 d	119.63 d	121.02 d	119.55 d	119.41 d
C-14	150.71 d	150.40 d	151.26 d	146.96 d	151.52 d	151.55 d
C-15	71.14 s	71.17 s	71.67 s	79.82 s	71.02 s	69.96 s
C-16	29.82 q	29.84 q	29.80 q	26.58 q	29.78 q	29.89 q
C-17	29.87 q	29.84 q	29.85 q	26.78 q	29.82 q	29.89 q
C-18	17.46 q	16.46 q	16.73 q	17.55 q	17.74 q	17.63 q
C-19	115.41 t	113.56 t	115.81 t	115.64 t	117.76 t	118.09 t
OAc				169.85 s		170.0 s
				22.14 q		21.25 q

*Spectra were recorded in $CDCl_3$ at 75.4 MHz with TMS as internal standard. The values are given in δ units. Multiplicities were obtained by DEPT and assignments were made by comparison to other compounds in this series.

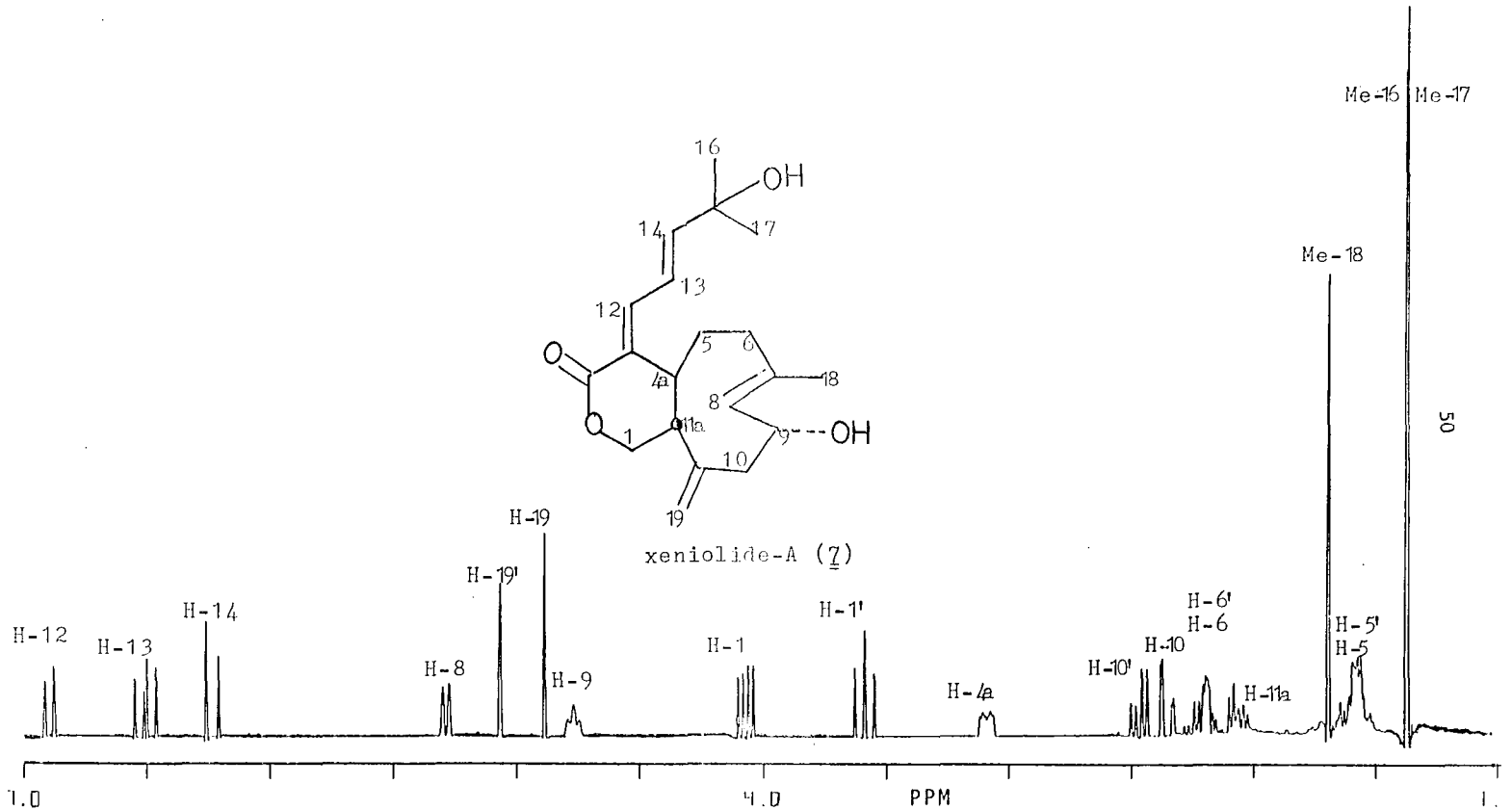


Figure 17. 300 MHz proton NMR spectrum of xeniolide-A (7) in $CDCl_3$

that the chemical shifts of the C-14 and C-12 signals in xeniolide-A were at 136.5 and 151.1 ppm, respectively. Our 75.4 MHz NMR investigation using single-frequency on-resonance decoupling experiments established that the chemical shifts of C-14 and C-12 signals were at 150.71 and 136.14 ppm, respectively. Therefore, compound 7 is xeniolide-A.

Structure elucidation of 9-Deoxyxeniolide-A (47):

Silica gel chromatography of fraction F' of Scheme 3 yielded 25 fractions. One of the fractions eluted with hexane-acetone (7:3) (fraction 10) was rechromatographed by HPLC using silica gel and hexane-acetone (88:12) as eluent to give 5 fractions. Further purification of the fifth fraction led to the isolation, in trace amounts, of 9-deoxyxeniolide-A (47) in ca. 83% purity. The molecular formula $C_{20}H_{28}O_3$ (7 unsaturations) was deduced from the following spectral data: (a) IR 3440 (OH) and 1712 cm^{-1} (conjugated carbonyl group); (b) the ^{13}C NMR spectra (Figure 18) showed signals for 20 carbons: 3 methyls, 6 methylenes, 6 methines, and 4 quaternary carbons and a carbonyl carbon (equals 27 H). Two of these carbons were each deshielded by a single oxygen, 70.94 ppm (C-1, t) and 71.17 (C-15, s). Analysis of the ^1H and ^{13}C NMR data together with the IR data of 47 suggested the existence of 4 double bonds (— CH=CH-CH=C<, CH_2=C<, — CH=C(Me)—), a conjugated carbonyl group, C-3 [171.28 ppm (s)], a tertiary hydroxyl group, C-15 [71.17 ppm (s)]. The ^{13}C NMR spectra (Figure 18) of 47 are identical to those of xeniolide-A[6] (7) except for the presence of an additional methylene group signal at 24.93 ppm, which was assigned to C-9, and the absence of a methine carbon signal at 67.4 ppm which is due to the hydroxylated C-9

9-deoxyxeniolide-A (47)

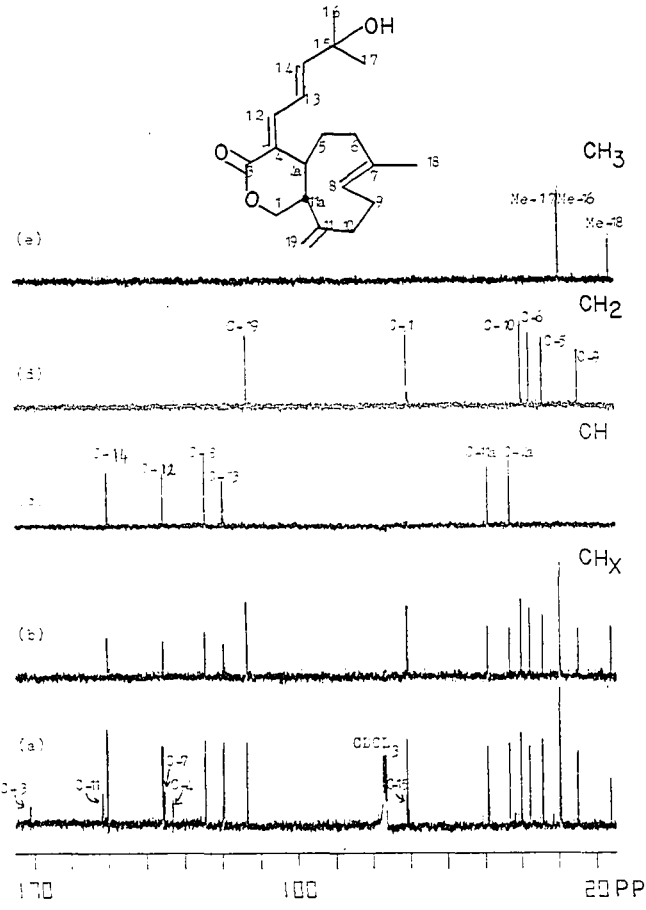


Figure 18. (a) 75.4 MHz broadband proton decoupled ^{13}C NMR spectrum of 47 in $CDCl_3$. (b) all protonated carbons. (c) methine carbons. (d) methylene carbons. (e) methyl carbons. Spectra b-e were recorded at 75.4 MHz in $CDCl_3$ and resulted from a DEPT experiment.

carbon in xeniolide-A. Additionally, the ^{1}H NMR spectrum of 47 lacks the allylic methine proton signal at 4.78 ppm due to H-9 in xeniolide-A. The vinylic proton signal at 5.44 ppm (H-8) in 47 was coupled vicinally to the methylene proton signals at 2.10 (H-9) and 2.46 ppm (H-9'). These data support the conclusion that 47 is the 9-deoxy analog of xeniolide-A (7).

The E configuration of the C-4,12 double bond in 47 was established by the correlation of the ^{1}H and ^{13}C NMR data (see Tables 7 and 8) for 47 and those proposed for xeniolide-A. The E configuration for Δ^{7} was assigned on the basis of the ^{13}C NMR chemical shift of Me-18 appearing at 16.46 ppm (instead of 22-25 for the Z configuration). The trans configuration of the ring fusion in 47 was assigned on the basis of the similarities of the coupling constants ($J_{11a,4a} \approx 5.5$ Hz) and ^{13}C NMR chemical shift signals of C-4a and C-11a observed for 47 and xeniolide-A. Thus, the structure 47 is confirmed for 9-deoxyxeniolide-A (47).

Structure elucidation of 9-Deoxy—7,8-dihydro-7α,8α-epoxyxeniolide-A (48):

Silica gel chromatography of the fraction F' of Scheme 3 afforded 25 fractions. Further chromatography by HPLC of fraction 14 gave 9 fractions, the sixth of which was subjected to HPLC using a reverse-phase C_{18} column to yield, in trace quantities (1 mg), 9-deoxy-7,8-dihydro-7α,8α-epoxyxeniolide-A (48) as a colorless oil; $[\alpha]_{D}^{25}$ +3° (c. 0.1 $CHCL_3$). The high resolution mass spectrum of 48 established a molecular formula of $C_{20}H_{28}O_4$ (7 unsaturations). Analysis of the ^{13}C NMR (DEPT) spectra of 48 revealed the existence of 3 methyls, 5 sp^2

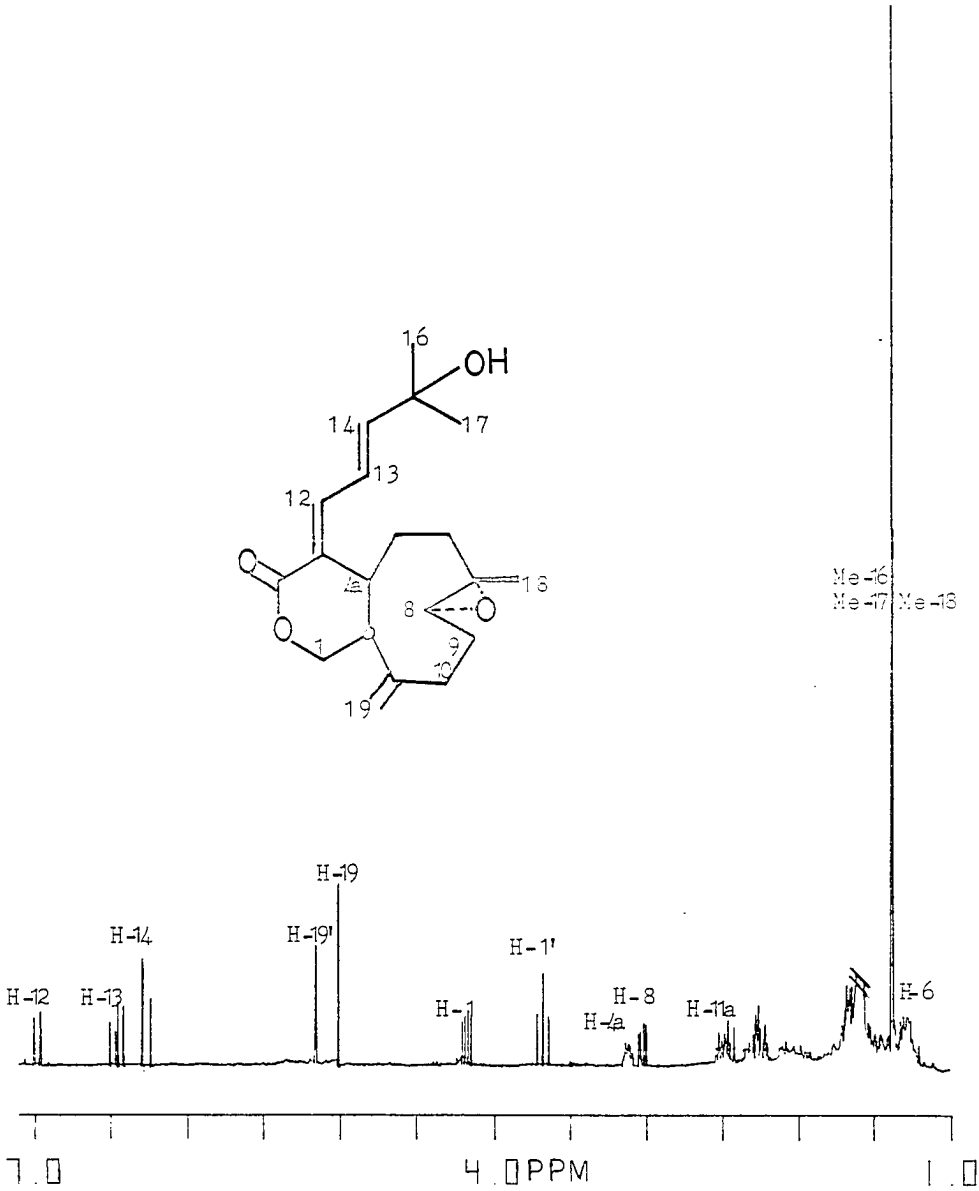


Figure 19. 300 MHz proton NMR spectrum of 48 in $CDCl_3$

methylenes and one exocyclic terminal methylene, 6 methines, and 5 quaternary carbons. Analysis of the ^{1}H and ^{13}C NMR data together with the UV (267 nm) and IR (see Experimental) data indicated the presence of 3 double bonds (— CH=CH-CH=, CH_2=C<), a conjugated carbonyl, an epoxide (—CH—O—C(Me)—), and a tertiary hydroxyl[6] group, [C-15, 71.0 ppm (s)]. The latter moiety was supported by the loss of one molecule of water in the mass spectrum (m/z 314 [$M^+ - H_2O$)]. Comparison of the ^{1}H and ^{13}C NMR data (see Figure 19 and Tables 7 and 8) of 48 with the data of 9-deoxyxeniolide-A (47) showed close resemblance except that 48 exhibited signals corresponding to an epoxide group at C-7,8 [H-8, 3.02 ppm (dd) and H-18 (CH_3), 1.36 (s)]. Therefore, it was concluded that 48 has the same skeleton and functionality as 9-deoxyxeniolide-A except for the 7,8-epoxide. The E configuration of the 7,8-epoxide group was assigned on the basis of the ^{13}C NMR chemical shift similarities observed for 48 and 7,8-dihydro-7α,8α-epoxyxeniolide-A. (Me-18 signal in 48 was observed at 16.73 ppm and Me-18 signal in 7,8-dihydro-7α,8α-epoxyxeniolide-A (50) at 17.74 ppm). The E configuration of the C-4,12 double bond in 48 was established by an NOE experiment (irradiation of H-13 enhanced H-4a). The E configuration of the C-13,14 double bond was assigned on the basis of the magnitude of the coupling constant observed ($J_{13,14} \simeq 15$ Hz). The trans ring fusion was assigned by comparing the coupling constants ($J_{11a,4a} \simeq 5$ Hz) and the ^{13}C NMR chemical shifts of C-4a and C-11a for 48 and 7,8-dihydro-7α,8α-epoxyxeniolide-A (50) (see Tables 7 and 8).

Structure elucidation of 15-Dehydroxy-15-acetyldioxyxeniolide-A (49):

The $CHCL_3$ extract, fraction F', of Scheme 3 was chromatographed over silica gel. One of the fractions eluted with hexane-acetone (7:3) was rechromatographed by HPLC to give 1.7 mg of 15-dehydroxy-15-acetyl-dioxyxeniolide-A (49) as a colorless oil; $[\alpha]_D^{25}$ -12.9° (c. 0.17 $CHCL_3$); UV (EtOH) λ_{max} 265 nm (ε = 14500). The molecular formula $C_{22}H_{30}O_6$ (8 unsaturations) was established from the following spectral data: (a) the highest mass peak in the field desorption spectrum was found at 390 which corresponded to the molecular ion; (b) the ^1H NMR spectrum (Figure 21) exhibited 29 non-exchangeable proton signals; (c) the IR spectrum showed a hydroxyl band at 3440 cm^{-1} and two carbonyl bands at 1730 and 1712 cm^{-1}; (d) ^{13}C NMR spectra (Figure 20) indicated the presence of 22 carbons with an allylic carbon deshielded by a secondary hydroxyl group 67.52 ppm (C-9, d), a carbon deshielded by an oxygen of a lactone group 70.91 ppm (C-1, t), a tertiary carbon deshielded by a peroxide group 79.82 ppm (C-15, s),[25] and two carbonyl carbons 169.85 ppm (s) and 170.65 ppm (s). The ^{13}C NMR spectra of 49 (Figure 20) indicated the existence of 4 methyls, 4 sp^3 methylenes and one exocyclic terminal methylene, 3 sp^3 methines and 4 sp^2 methines, 4 quaternary carbons and a carbonyl carbon. The ^1H NMR spectrum of 49 (Figure 21) was nearly identical to that of xeniolide-A[6] (7) except for the presence of an additional acetate methyl signal at 2.02 ppm, a shift in the H-13 signal to 6.36 from 6.52 ppm, and a shift of the two methyls at C-15 to 1.57 and 1.58 ppm from 1.39 ppm. Hence, a structure like xeniolide-A[6] could be postulated with a minor difference in the side chain. The ^{13}C NMR spectrum of 49 was also very similar to that of xeniolide-A (7) except

for the two extra carbon signals due to the acetate group and a shift in the C-14, C-15, C-16, and C-17 signals to 146.96, 79.82, 26.78, and 26.58 ppm, respectively. These shifts were attributed to the presence of a peroxide groups at C-15 (β effect). The peroxyacetyl group was postulated because of the loss of a AcOO-fragment and the absence of the loss of AcOH in the low resolution mass spectrum (M^+-AcOO, 315). Consequently, the acetyl group was fixed to the peroxide group. Because the H-13 and H-14 signals overlapped in $CDCL_3$ (see Figure 21), the spectrum of 49 was also obtained in C_6D_6 (see Figure 22) and ^1H decoupling and ^1H difference decoupling were performed in both solvents ($CDCL_3$ and C_6D_6) to establish the carbon connectivity in 49. A homocorrelated 2-D (COSY) experiment was performed in $CDCL_3$ which shows direct proton spin-coupling connectivities (see Figure 23).

The stereochemical features of 49 were established by NOE experiments (see Table 9). The E configuration of the C-7,8 double bond was assigned on the basis of the NOE observed between Me-18 and H-9 and the lack of any NOE between Me-18 and H-8. The stereochemistry of the diene at C-4,12,13,14 was confirmed as E,E, on the basis of the magnitude of the coupling constant $J_{13,14}$≈15 Hz and of the observation of an NOE effect between H-4a and H-13. The trans ring fusion was assigned on the basis of the correlations of the coupling constants ($J_{11a,4a}$ = 5 Hz) and the ^{13}C NMR chemical shifts of C-11a and C-4a (see Tables 7 and 8) observed for 49 and xeniolide-A (7). The observation of NOE effects between Me-18, H-11a, and H-9 led to the conclusion that H-9, H-11a, and Me-18 are on the same face (β face). Irradiation of H-4a gave rise to Overhauser enhancements of H-13 and H-8 (see Table 9). This suggested

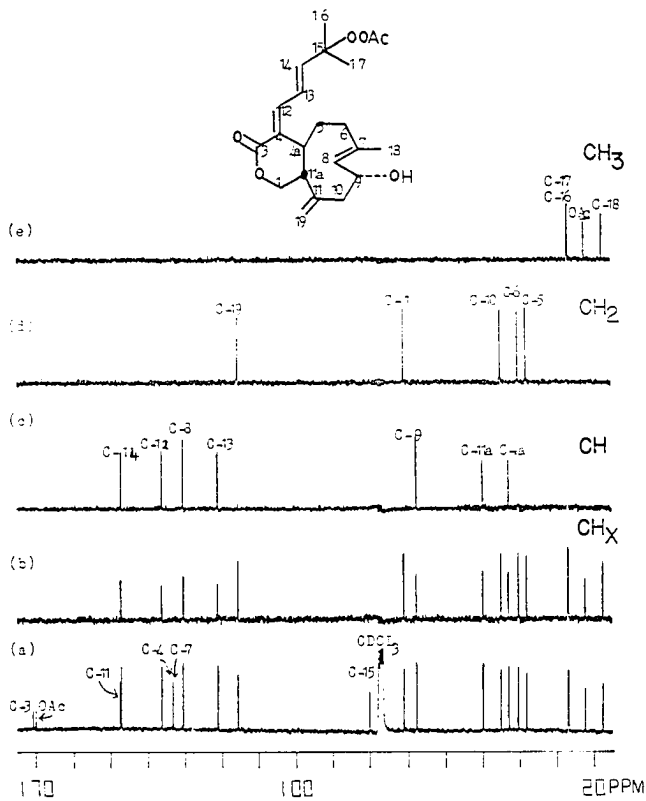


Figure 20. (a) 75.4 MHz broadband proton decoupled ^{13}C NMR spectrum of 49 in $CDCl_3$. (b) all protonated carbons. (c) methine carbons. (d) methylene carbons. (e) methyl carbons. Spectra b-e were recorded at75.4 MHz in $CDCl_3$ and resulted from a DEPT experiment.

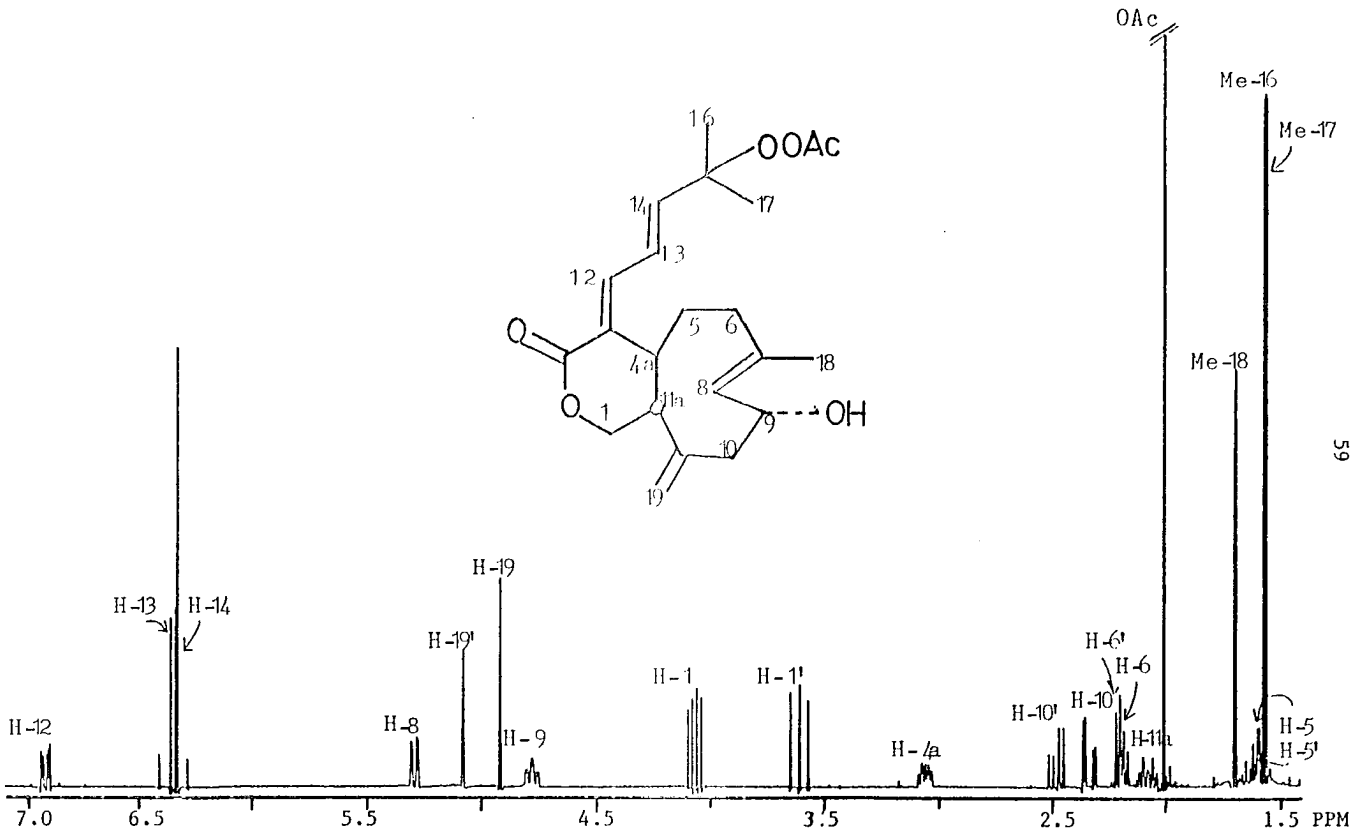


Figure 21. 300 MHz proton NMR spectrum of 42 in $CDCl_3$.

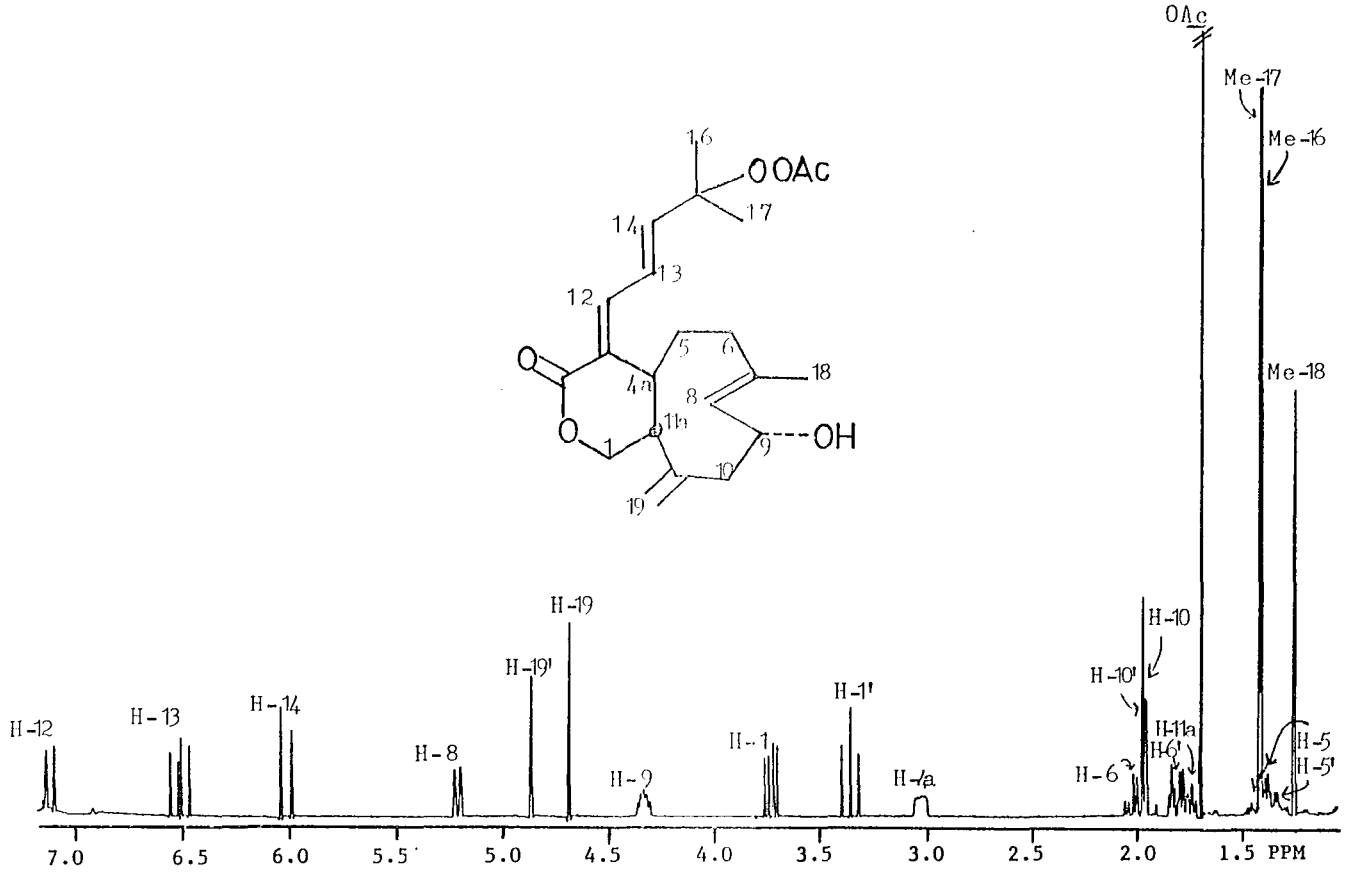


Figure 22. 300 MHz proton NMR spectrum of 49 in C_6D_6 solution.

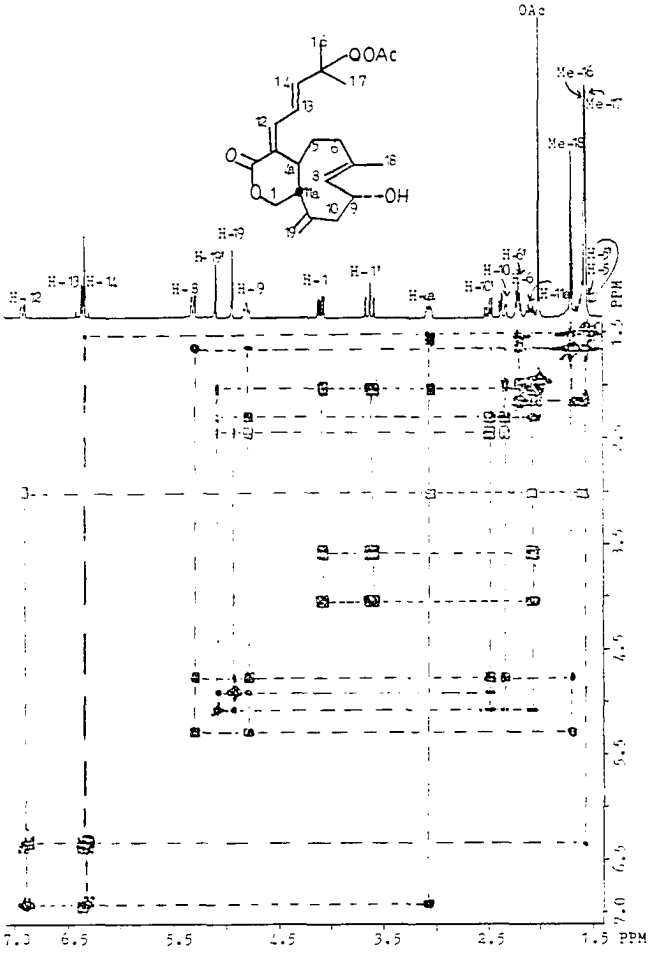


Figure 23. Contour plot of the homocorrelated 2-D proton NMR spectrum of 49 in $CDCl_3$ at 300 MHz. Off-diagonal peaks establish direct proton spin-coupling connectivities.

Table 9. Result of NOE experiments with 49 in C_6D_6 solution at 300 MHz.

Proton (s) irradiated	Proton (s) enhanced	% Enhancement
H-13	H-4a	16
	H-16	1.5
	H-17	1.5
H-9	Me-18	3
H-8	H-4a	5
H-4a	H-19	3
	H-8	8
	H-13	17
Me-16, Me-17	H-14	11
	H-13	9
Me-18	H-9	10
	H-11a	3

that H-4a and H-8 are on the same face (α face). Thus, the structure of 49 is completely elucidated.

Structure elucidation of 7,8-Dihydro-7α,8α-epoxyexniolide-A (50):

Silica gel chromatography of the $CHCL_3$ extract of Scheme 1 gave 9 fractions. Fractions 8 and 9 were combined and rechromatographed over silica gel to afford 7 fractions. The fifth of these fractions was subjected to HPLC to produce pure 7,8-dihydro-7α,8α-epoxyxeniolide-A (50), 7 mg, as a white powder; $[\alpha]_D^{25}$ +22.1° (c. 0.19 $CHCL_3$). The high resolution mass spectrum established a molecular formula of $C_{20}H_{28}O_5$ (7 unsaturations). The ^{13}C NMR spectra (Figure 24) of 50 indicated the presence of 3 methyls, 4 sp^3 methylenes and one exocyclic terminal methylene, 4 sp^3 methines and 3 sp^2 methines, and 3 quaternary carbons, in addition to a carbonyl carbon. The IR spectrum of 50 exhibited a broad hydroxyl band at 3440 cm^{-1} and a band at 1712 cm^{-1} which was assigned to a conjugated carbonyl group. The latter moiety was supported by the UV band at 268 nm.

Analysis of results from ^1H difference decoupling (DDS) experiments, COSY spectrum, and ^{13}C NMR data of 50 (see Figure 25 and Tables 7 and 8) suggested the existence of 3 double bonds (—CH=CH–CH$\lessdot$, CH_2=C$\lessdot$), an epoxide moiety (—CH(O)—C(Me)—), a tertiary hydroxyl group 71.02 ppm (C-15, s), and a secondary hydroxyl group 69.07 ppm (C-9, d). The latter group was confirmed by acetylation of 50 to give 51 in which the H-9 signal was shifted from 3.79 ppm to 5.79. Comparison of the ^1H and ^{13}C NMR spectral data (Figures 25 and 24 and Tables 7 and 8) of 50

with the data of the known lactone xeniolide-A[6] (7) indicated that 50 is the 7,8-dihydro-7α,8α-epoxide of xeniolide-A (7).

The proton NMR data (see Table 7) of 50 were similar to that of xeniolide-A (7) except that the H-8, H-9, and H-B signals in 50 appeared at 3.01 ppm, 3.79, and 1.39, respectively (see Figure 26). Moreover, two signals in the ^{13}C NMR spectrum of 50 were suitable for an epoxide group [59.08 ppm (C-7, s) and 67.27 (C-8, d)].

The carbon connectivity in 50 was confirmed by ^{1}H decoupling, ^{1}H difference decoupling (DDS), and a homocorrelated 2-D NMR spectrum (COSY). Figure 26 shows the contour plot of the COSY spectrum which contains off-diagonal peaks that establish direct proton spin-coupling connectivities.

The stereochemical features of 50 were confirmed by NOE experiments (see Table 10). The trans geometry of the epoxide group at C-7,8 was assigned by an NOE experiment (irradiation of the Me-18 signal enhanced both H-9 and H-11a signals). The stereochemistry of the diene side chain at C-4 was assigned as E,E, on the basis of the value of the coupling constant ($J_{13,14}$ = 15 Hz) and of the observation of Overhauser enhancements between H-4a and H-13. The trans assignment of the ring junction was established on the basis of the similarities of the coupling constants ($J_{11a,4a}$ = 5 Hz) and of the ^{13}C NMR chemical shift signals of C-4a and C-11a observed for 50 and xeniolide-A (7) (see Tables 7 and 8).

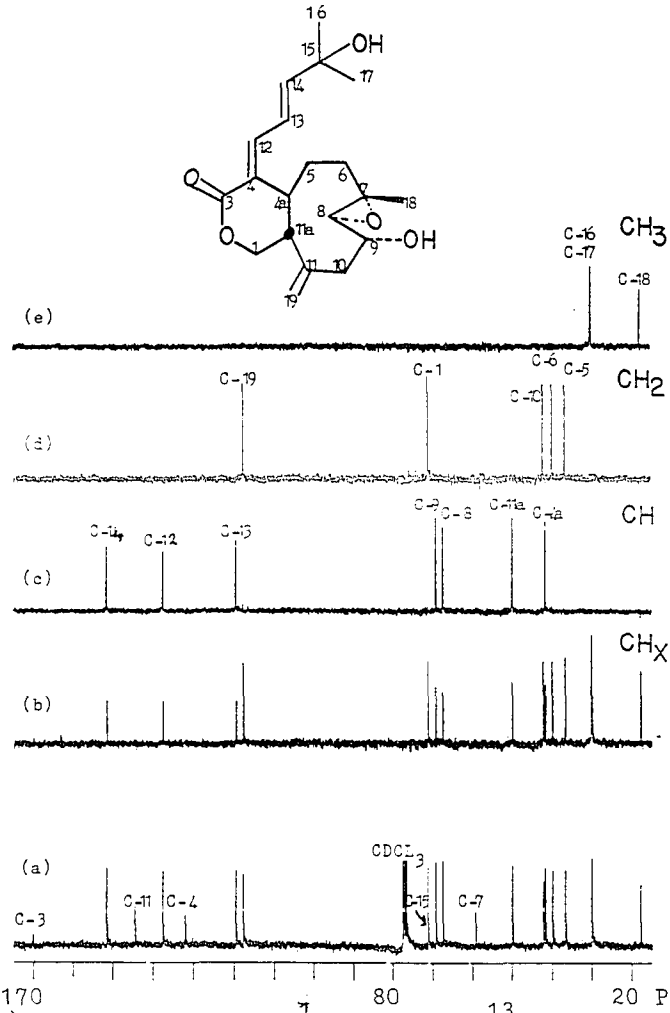


Figure 24. (a) 75.4 MHz broadband ^{1}H decoupled ^{13}C NMR spectrum of 50 in $CDCl_3$. (b) all protonated carbons. (c) methine carbons. (d) methylene carbons. (e) methyl carbons. Spectra b-e were recorded at 75.4 MHz in $CDCl_3$ and resulted from a DEPT experiment.

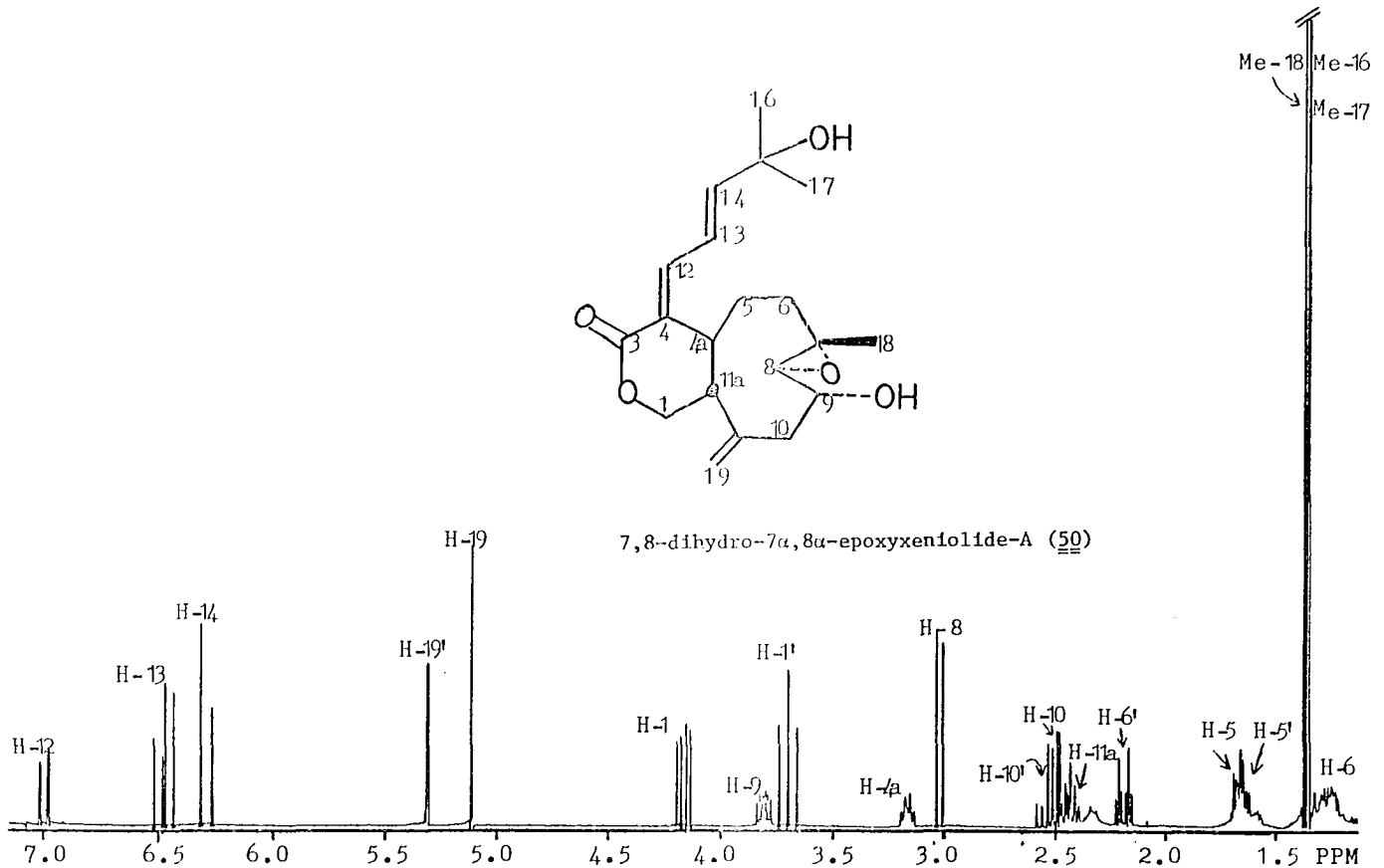


Figure 25. 300 MHz proton NMR spectrum of 7,8-dihydro-7α,8α-epoxyxeniolide-A (50) in $CDCl_3$.

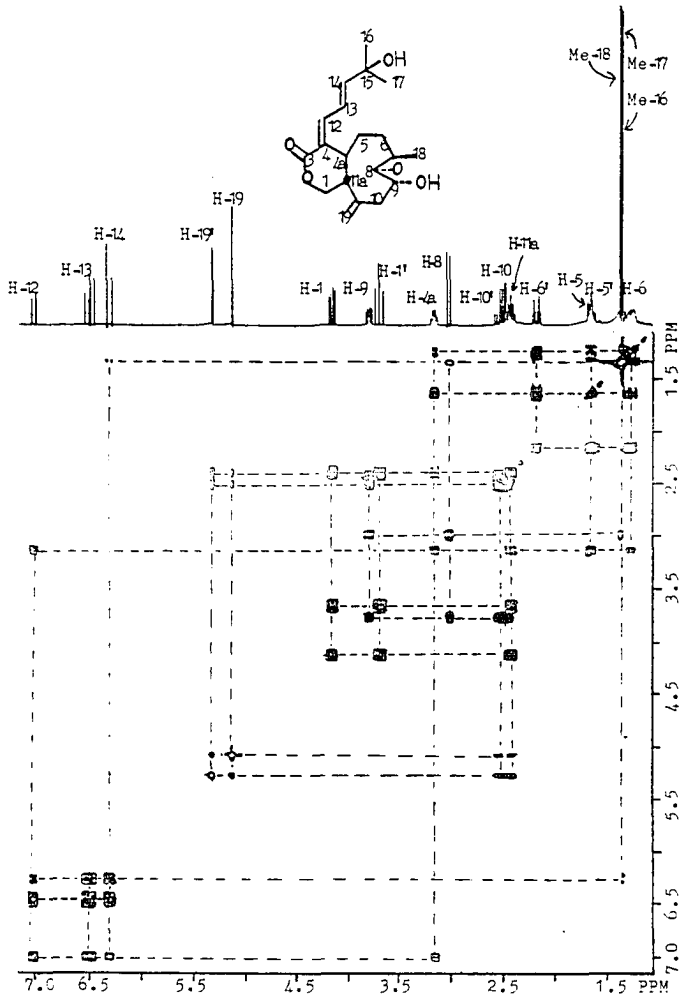


Figure 26. Contour plot of the homocorrelated two-dimensional ^{1}H NMR spectrum (symmetrized) of 50 in $CDCl_3$ at 300 MHz. The final $S(F_2,F_1)$ matrix plot consisted of 512 X 512 data points. Off-diagonal contours establish direct proton spin-coupling connectivities.

Table 10. Results of NOEDS experiment with 50 in $CDCl_3$ at 300 MHz.

Proton(s) irradiated	Proton (s) enhanced	% Enhancement
H-13	H-4a	12
H-14	H-12	17
H-19	H-19'	23
H-19'	H-19	24
H-4a	H-13	13
	H-19'	2
	H-8	7
Me-18, Me-17, Me-16	H-9	9
	H-11a	2
	H-14	11
	H-13	5

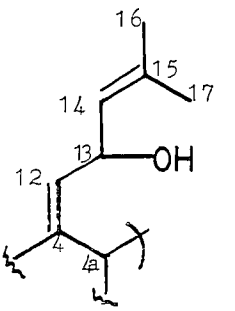
Structure elucidation of 18-Hydroxyasteroxeniolide-A 9-Acetate (52):

The $CHCL_3$ fraction, fraction F' of Scheme 3, was chromatographed over silica gel. The material was eluted using a step gradient beginning with hexane-acetone (9:1) and then increasing the amount of acetone. Two of the fractions eluted with (1:1) hexane-acetone (fractions 23, 24) were purified by HPLC to yield compound 52 as a light yellow oil. The molecular ion (M^+ 390) of 52 was observed in the low resolution mass spectrum. However, in the high resolution mass spectrum the highest mass peak corresponded to M^+-18 (372.19585) which is consistent with the formula $C_{22}H_{28}O_5$ (calculated 372.19368). Consideration of both low and high resolution mass spectrometry data established the formula for 52 to be $C_{22}H_{30}O_6$ (8 unsaturations). The ^{13}C NMR spectra of 52 (Figure 27) revealed the existence of 3 methyl groups, 6 methylenes, 7 methines, and 6 quaternary carbons. The IR spectrum of 52 exhibited a broad hydroxyl band at 3440 cm^{-1} and two carbonyl bands. The first band was at 1735 cm^{-1} (acetate) and the second band was at 1715 cm^{-1} and was assigned to an α,β-unsaturated δ lactone. The latter moiety was supported by the UV absorption at 217 nm.

Analysis of ^1H and ^{13}C NMR spectra (Figures 28 and 27) of 52 indicated the presence of four double bonds (>C=CH_2, —CH=C(Me)(Me), CH=C<, —CH=C<), an acetate, and α,β-unsaturated carbonyl group, a doubly allylic secondary hydroxyl, and a primary allylic hydroxyl group. The ^1H NMR signals due to the 2° and 1° alcohol groups were paramagnetically shifted as expected when 52 was acetylated to give the diacetate 53 (H-13 shifts from 5.20 ppm to 6.15 and H-18 from 4.08 ppm to 4.61 and H-18' from 4.32 ppm to 4.65).

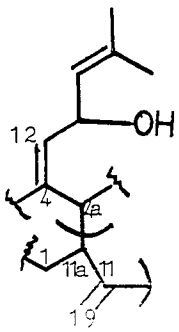
In the ^{13}C NMR spectrum of 52, the resonance at 116.73 ppm (C-19, t) together with the IR band at 890 cm^{-1}, suggested a terminal methylene group. Irradiation of the proton resonance at 5.13 ppm (H-19) (Figure 29a) sharpened the broad resonance at 5.07 ppm (H-19') and caused removal of small allylic couplings (J = 1.3, 1, 1 Hz) from the resonances at 2.16 ppm (H-11a), 2.46 (H-10'), and 2.48 (H-10), respectively. This suggested that (H-10), (H-10'), and (H-11a) were allylic to the terminal methylene protons.

Irradiation of either of the vinylic methyls at 1.76 ppm or 1.73, sharpened the broad doublet at 5.30 ppm (H-14). This suggested that these methyls (Me-16 and Me-17) were vinylic and also geminal. The deshielded proton signal at 6.39 ppm (H-12) was found by a ^{1}H difference decoupling experiment (DDS) (Figures 29b and 29c) to couple vicinally (J = 7.8 Hz) to the proton dd at 5.20 ppm (H-13) and allylically (J = 1.5 Hz) to the signal at 3.02 ppm (H-4a) (see Table 13). The doubled doublet at 5.20 ppm (H-13 was also coupled vicinally (J = 9.1 Hz) to the doubled septet at 5.30 (H-14). These interactions established partial structure A of this molecule.



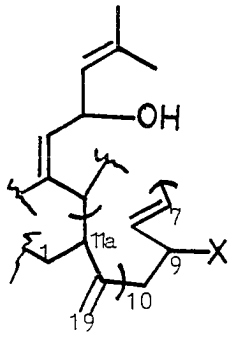
A

An AMX pattern was found (DDS experiments) between the proton resonances at δ 4.09 (H-1), 3.64 (H-1'), and 2.14 (H-11a). The X part (H-11a) was coupled vicinally (J = 3.9 Hz) to the proton resonance at 3.02 ppm (H-4a) and also allylically to the exocyclic methylene proton resonances at 5.13 ppm (H-19) and 5.07 (H-19'), establishing the expanded partial structure B.



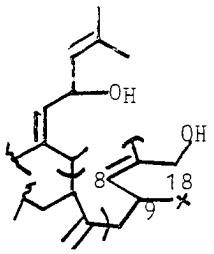
B

An ABX system was found between the proton resonances at 2.48 ppm (H-10), 2.46 (H-10'), and 5.82 (H-9). The AB part of the ABX pattern was found to couple allylically to the terminal methylene proton resonances at 5.13 ppm (H-19) and 5.07 (H-19'). The X part (H-9) of this ABX pattern was coupled vicinally (J = 8.8 Hz) to the olefinic proton resonance at 5.31 ppm (H-8) which was partially obscured by the proton signal of H-14. To unequivocally establish the multiplicities of these two protons (H-14 and H-8) as well as the other protons, a homonuclear two-dimensional J-resolved (2DJ)[26] experiment (Figure 30) was performed. This confirmed a larger partial structure, C.



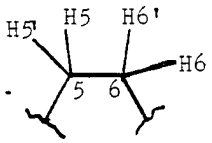
C

The AB protons absorping at 4.08 ppm (H-18') and 4.32 (H-18) were mutually coupled with 13.2 Hz which suggested a geminal coupling. The proton signals of this AB pattern were also coupled allylically to the olefinic proton signal at 5.31 ppm (H-8). Thus, partial structure C could be extended into D.



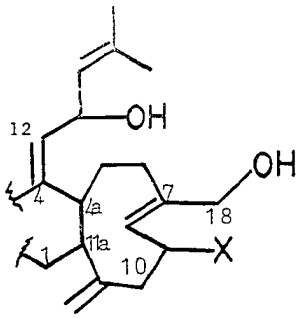
D

In the up-field region (1.3-2.55 ppm) of the ^{1}H NMR spectrum (Figures 31 b-c) of 52, an ABMX pattern was established between the protons absorbing at 1.49 ppm (H-5'), 1.61 (H-5), 2.01 (H-6'), and 2.49 (H-6) and confirmed by homocorrelated 2-D (COSY)[18,19] (see Figures 31a-c) and by heterocorrelated[21] 2-D (see Figure 32) NMR spectroscopy. This unequivocally established the fourth partial structure, E.



E

The proton signal at 1.61 (H-5) was coupled vicinally (J = 10.8 Hz) to the allylic proton signal at 3.02 ppm (H-4a, see partial structure D), linking C-4a to C-5. The connectivity of C-6 to C-7 was established on the basis of the small allylic coupling observed between H-6' and H-8 (see Figures 31a and 28L). Therefore, a carbocyclic ring feature (partial structure F) was established.



F

The double doublet at 6.39 ppm (H-12) is most likely to arise from the β-H and of α,β-unsaturated carbonyl feature. Therefore, the carbonyl group of the δ-lactone (see above) was joined to C-4 and an oxygen link to C-1. The position of the carbonyl lactone was confirmed by selective irradiation of the proton signal at 3.02 ppm (H-4a) in the proton NMR spectrum while observing the ^{13}C fully coupled spectrum. The

result was a removal of 3-bond coupling that sharpened the carbonyl signal (C-3) at 170.48 ppm. An IR band at 1715 cm^{-1}, UV band at 217 nm, the NMR chemical shift similarities of H-1, H-1', and C-1 in 52 and in xeniolide-A[6] (see Tables 11, 12, 7, and 8) all support this assignment. The remaining acetate group was connected to C-9 on the basis of ^{1}H NMR chemical shift observed for H-9 at 5.82 ppm.

The relative stereochemistry of 52 was established from ^{1}H NMR experiments, including nuclear Overhauser enhancement difference spectroscopy (NOEDS) which are summarized in Table 14. The E configuration of $\Delta^{7,8}$ was established by an NOE (irradiation of H-18 enhanced H-9). Moreover, neither H-18 nor H-18' was enhanced when H-8 was irradiated. NOE effects were observed also between H-9, H-11a, H-18, and H-18'. This suggested that H-9 and H-11a are on the same face (β-face). Irradiation of the allylic proton at 3.02 ppm (H-4a) was found (NOEDS experiment) to enhance H-6', H-8, and H-13. This indicated that H-4a, H-6', and H-8 are on the same face (α-face). The E geometry of C-4,12 was assigned on the basis of the observation of NOE effects between H-4a and H-13, and lack of an NOE between H-12 and H-4a. The trans configuration of the ring junction in 52 was established on the basis of the similarities of the coupling constants ($J_{11a,4a} \simeq 4$ Hz) and the ^{13}C NMR chemical shifts of C-11a and C-4a (see Tables 7, 8, 11 and 12) recorded for 52 and xeniolide-A (7).

Because the chemical shifts of H-8 and H-14 are very close (see Table 11) in the proton NMR spectrum of 52 in $CDCL_3$ (Figure 28a), the NOE enhancements observed when H-4a resonance was irradiated, were not certain. In order to confirm the assigned relative stereochemistry, the

Table 11. Proton NMR Data of Asteroxeniolide-A and its Derivatives*

	52[a]	53	54	55
H-1	4.09 dd 11.5;5.8	4.04 dd 11.6;5.5	4.10 dd 11.6;6	4.10 dd 11.5;5.9
H-1'	3.64 dd 12.6;11.5	3.61 t 11.6	3.64 dd 12.3;11.6	3.65 dd 12.5;11.5
H-4a	3.02 dddd 10.8;3.9;1.8;1.5	3.10 br dd 10.3;4.4	2.99 br dd 10.3;3.7	2.99 ddt 10.8;3.8;1.7
H-5	1.61 dddd 14;12.8;10.8;3.5	1.58 dddd 14;13;10.3;4.5	1.57 m	1.59 m
H-5'	1.49 dddd 14;5.2;3.5;1.8	1.45 br ddd 14;4.5;3.5	1.45 br dt 14;3.5	1.45 dddd 14.4;5.2;3.6;1.7
H-6	2.49 dt 12.8;3.5	2.41 dt 13.0;4.5	2.16 m	2.18 br dt 12.6;3.6
H-6'	2.01 ddt 1.3;5.2;12.8	2.07 m	2.07 br dt 12.0;4.0	2.09 ddd 14.0;12.6;5.2
H-8	5.31 ddd 8.8;1.5;1	5.50 br d 8.3	5.22 br d 7.3	5.27 dd 7.8;1.0
H-9	5.82 ddd 8.8;6.4;2.4	5.74 br dd 8.3;6.8	4.78 br dd 7.3;5.8	5.67 ddd 7.8;5.4;2.8
H-10	2.48 ddd 14;2.4;1	2.52 br d 13.2	2.46 br dd 13.7;5.8	2.50 br dd 14.4;5.4
H-10'	2.46 ddd 14.0;6.4;1	2.38 br dd 13.2;6.8	2.40 br dd 13.7;1.6	2.45 br dd 14.4;2.8
H-11a	2.14 dddd 12.6;5.8;3.9;1.3	2.10 br ddd 11.6;5.5;4.4	2.02 br ddd 12.3;6.0;3.7	2.02 dddd 12.5;5.9;3.8;2.0
H-12	6.39 dd 7.8;1.5	6.29 dd 8.8;1.5	6.38 dd 8.0;1.4	6.39 dd 7.7;1.7
H-13	5.20 dd 9.1;7.8	6.15 dd 9.4;8.8	5.22 dd 9.2;8.0	5.23 dd 9.2;7.7
H-14	5.30 d sept 9.1;1.5	5.25 br d 9.4	5.30 d sept 9.2;1.3	5.31 d sept 9.2;1.6
Me-16	1.76 dd 1.5;0.5	1.79 d 1.5	1.79 d 1.3	1.79 d 1.6
Me-17	1.73 dd 1.5;0.5	1.76 d 1.5	1.75 d 1.3	1.75 d 1.6
H-18	4.32 dd 13.2;1.5	$-CH_2$ 4.63 AB quartet 13.4	Me-18, 1.70 br s	Me-18, 1.75 br s
H-18'	4.08 dd 13.2;1.0	13.4		
H-19	5.13 br s	5.29 br s	5.11 br s	5.08 br s
H-19'	5.07 br s	5.17 br s	5.01 br s	5.04 br s
OAc	2.09 s	2.08 s, 2.07 s 2.06 s		2.10 s

*Spectra were recorded in $CDCl_3$ at 300 MHz with Me_4Si as internal standard. The values are given in δ units. Assignments were established by ^1H difference decoupling experiments (DDS). [a]Coupling constants were measured by homonuclear 2-D J-resolved experiment.

Table 12. ^{13}C NMR Data of Asteroxeniolide-A and its Derivatives*

	52[a]	53	54	55
C-1	70.84 t	70.67 t	70.98 t	70.82 t
C-3	170.48 s	170.41 s	170.76 s	170.58 s
C-4	134.14 s	135.17 s	134.26 s	134.28 s
C-4a	43.71 d	43.00 d	44.0 d	44.24 d
C-5	37.92 t	38.06 t	37.32 t	37.28 t
C-6	35.53 t	35.63 t	39.64 t	39.72 t
C-7	136.20 s	131.10 s	132.0 s	133.71 s
C-8	128.68 d	131.81 d	130.66 d	126.32 d
C-9	69.51 d	69.10 d	67.03 d	70.0 d
C-10	43.19 t	43.51 t	45.45 t	42.37 t
C-11	147.25 s	145.77 s	147.0 s	147.69 s
C-11a	49.50 d	49.86 d	49.25 d	49.06 d
C-12	139.10 d	135.88 d	138.87 d	138.86 d
C-13	64.95 d	67.37 d	64.92 d	64.93 d
C-14	124.14 d	119.88 d	124.73 d	124.69 d
C-15	137.36 s	139.57 s	137.11 s	137.27 s
C-16	25.85 q	25.88 q	25.85 q	25.85 q
C-17	18.50 q	18.87 q	18.50 q	18.49 q
C-18	61.34 t	61.77 t	17.32 q	17.30 q
C-19	116.73 t	117.81 t	115.05 t	115.89 t
OAc	170.78 s	170.62 s, 170.35 s,		170.58 s
	21.32 q	170.30 s, 21.19 q,		21.35 q
		21.29 q, 20.86 q		

*Spectra were recorded in $CDCl_3$ at 75.4 MHz. Multiplicities were obtained by DEPT and assignments were made by comparison to 18-Hydroxyasteroxeniolide-A 9-Acetate (52).

[a]Assignments were established by a heterocorrelated 2-D experiment.

Table 13. Results of ^{1}H Difference Decoupling (DDS) Experiments* with (52)

Irradiated proton, chemical shift, ppm, multiplicity (J, Hz)	*effect of ^{1}H decoupling*
H-12, 6.39 dd (7.8, 1.5)	H-13, 5.2 dd (9.1, 7.8) ⟶ d (9.1) H-4a, 3.02 dddd (10.8, 3.9, 1.8, 1.5) ⟶ ddd (10.8, 3.9, 1.8)
H-9, 5.82 ddd (8.8, 6.4, 2.4)	H-8, 5.31 ddd (8.8, 1.5, 1) ⟶ br d (1.5) H-10, 2.48 ddd (14, 2.4, 1) ⟶ dd (14, 1) H-10', 2.46 ddd (14, 6.4, 1) ⟶ dd (14, 1)
H-8, 5.31 ddd (8.8, 1.5, 1)	H-9, 5.82 ddd (8.8, 6.4, 2.4) ⟶ dd (6.4, 2.4) H-18, 4.32 dd (13.2, 1.5) ⟶ d (13.2) H-18', 4.11 dd (13.2, 1) ⟶ d (13.2) H-6', 2.01 ddt (1.3, 5.2, 12.8) ⟶ dt (5.2, 12.8)
H-14, 5.30 d sept (9.1, 1.5)	Me-16, 1.73 d (1.5) ⟶ s Me-17, 1.76 d (1.5) ⟶ s H-13, 5.20 dd (9.1, 7.8) ⟶ d (7.8)
H-19, 5.13 br s	H-10, 2.48 ddd (14, 2.4, 1) ⟶ dd (14, 2.4) H-10', 2.46 ddd (14, 6.4, 1) ⟶ dd (14, 6.4) H-11a, 2.14 dddd (12.6, 5.8, 3.9, 1.3) ⟶ ddd (12.6, 5.8, 3.9) H-19', 5.07 br s ⟶ sharpened
H-18', 4.11 br d (13.2)	H-18, 4.32 br d (13.2) ⟶ br s H-8, 5.31 ddd (8.8, 1.5, 1) ⟶ dd (8.8, 1.5)
H-1, 4.09 dd (11.5, 5.8)	H-1', 3.64 dd (12.6, 11.5) ⟶ d (12.6) H-11a, 2.14 dddd (12.6, 5.8, 3.9, 1.3) ⟶ ddd (12.6, 3.9, 1.3)
H-1', 3.64 dd (12.6, 11.5)	H-1, 4.09 dd (11.5, 5.8) ⟶ d (5.8) H-11a, 2.14 dddd (12.6, 5.8, 3.9, 1.3) ⟶ ddd (5.8, 3.9, 1.3)
H-4a, 3.02 dddd (10.8, 3.9, 1.8,1.5)	H-12 6.39 dd (7.8, 1.5) ⟶ d (7.8) H-11a, 2.14 dddd (12.6, 5.8, 3.9, 1.3) ⟶ ddd (10.8, 5.8, 1.3) H-5, 1.61 dddd (14, 12.8, 10.8, 3.5) ⟶ ddd (14, 12.8, 3.5) H-5', 1.49 dddd (14, 5.2, 3.5, 1.8) ⟶ ddd (14, 5.2, 2.5)
H-11a, 2.14 dddd (12.6, 5.8, 3.9, 1.3)	H-19, 5.13 br s ⟶ sharpened H-1, 4.09 dd (11.5, 5.8) ⟶ d (11.5) H-1', 3.64 dd (12.6, 11.5) ⟶ d (11.5) H-4a, 3.02 dddd(10.8, 3.9, 3.9, 1.8, 1.5) ⟶ ddd(10.8, 1.8, 1.5)
H-5', 2.01 ddt (1.3, 5.2, 12.8)	H-8, 5.31 ddd (8.8, 1.5, 1) ⟶ sharpened H-6 2.49 dt (12.8, 3.5) ⟶ t (3.5) H-5, 1.61 dddd (14, 12.8, 10.8, 3.5) ⟶ ddd (14, 10.8, 3.5) H-5', 1.49 dddd (14, 5.2, 3.5, 1.8) ⟶ ddd (14, 3.5, 1.8)
H-5', 1.49 dddd (14, 5.2, 3.5, 1.8)	H-4a, 3.02 dddd (10.8, 3.9, 1.8, 1.5) ⟶ ddd (10.8, 3.9, 1.5) H-6, 2.49 dt (12.8, 3.5) ⟶ dd (12.8, 3.5) H-6', 2.01 ddt (1.3, 5.2, 12.8) ⟶ dt (1.3, 12.8) H-5, 1.61 dddd (14, 12.8, 10.8, 3.5) ⟶ ddd (12.8, 10.8, 3.5)

*Spectra were recorded in $CDCl_3$ at 300 MHz with Me_4Si as internal standard. The values are given in ppm downfield from TMS.

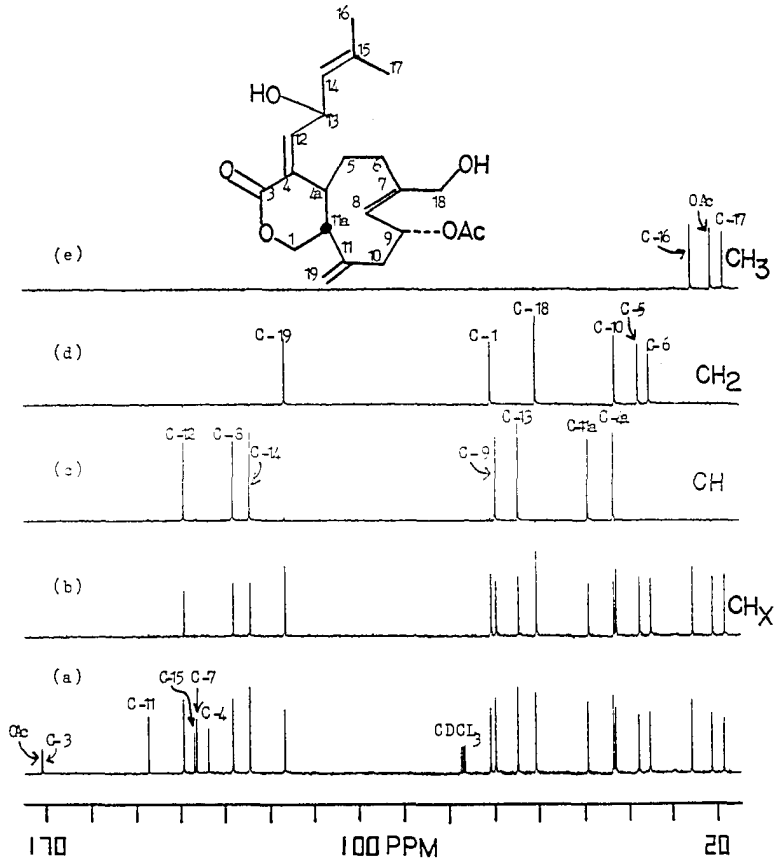


Figure 27. (a) 75.4 MHz broadband proton decoupled ^{13}C NMR spectrum of 52 in $CDCl_3$. (b) all protonated carbons. (c) methine carbons. (d) methylene carbons. (e) methyl carbons. Spectra b-e were recorded at 75.4 MHz in $CDCl_3$ and resulted from a DEPT experiment.

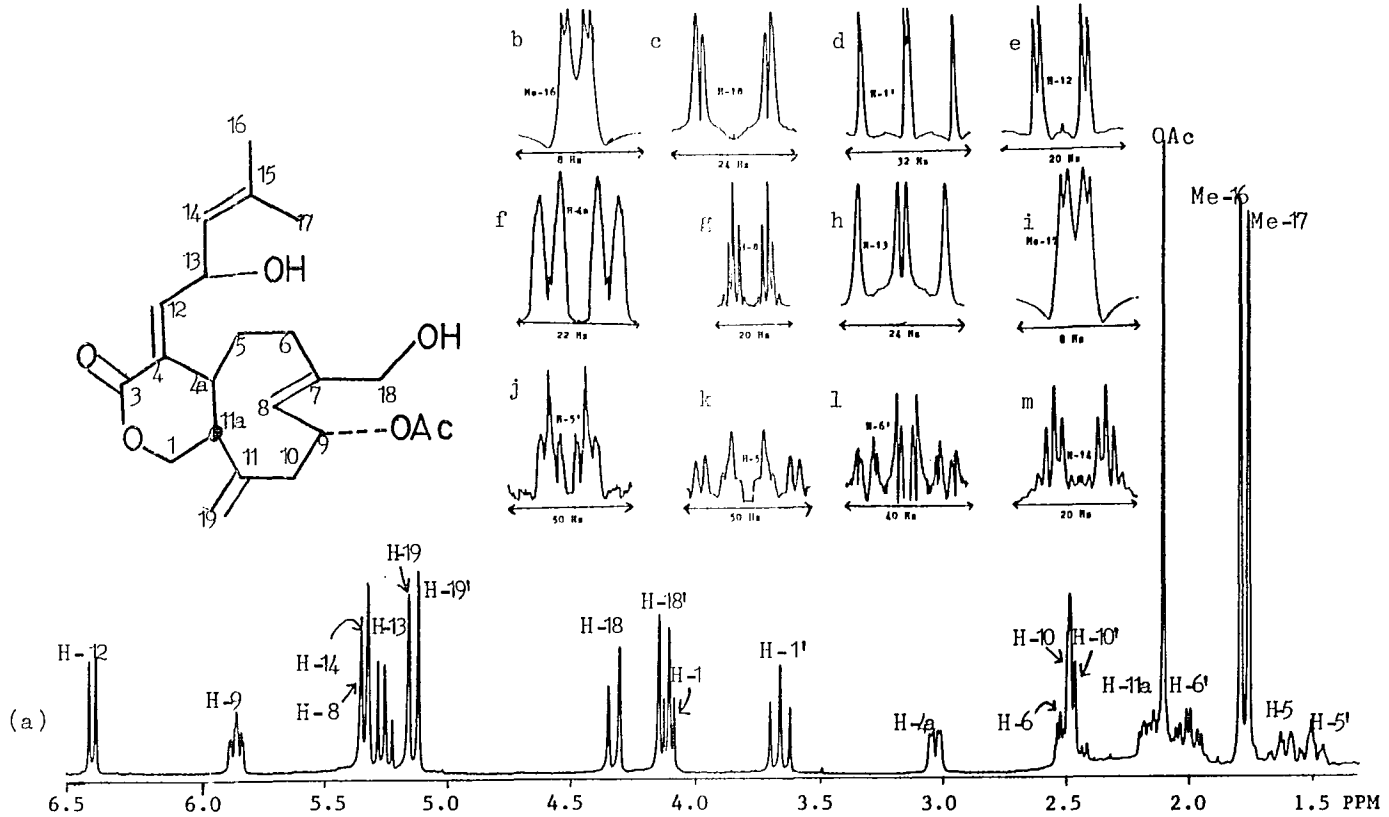


Figure 28. (a) 300 MHz ^{1}H NMR spectrum of 52 in $CDCl_3$. (b-m) Slices from homonuclear two-dimensional J-resolved (2DJ) spectrum in Figure 4.

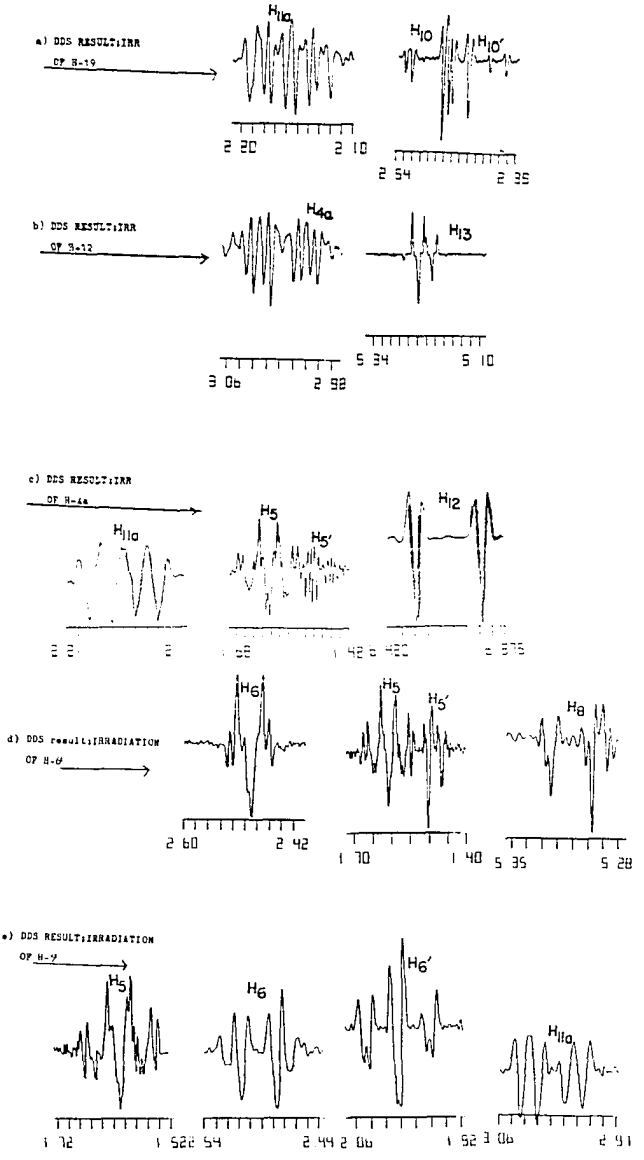


Figure 29. (a-e) Spectra resulted from proton difference decoupling experiments (DDS) with 22 recorded at 300 MHz in $CDCl_3$

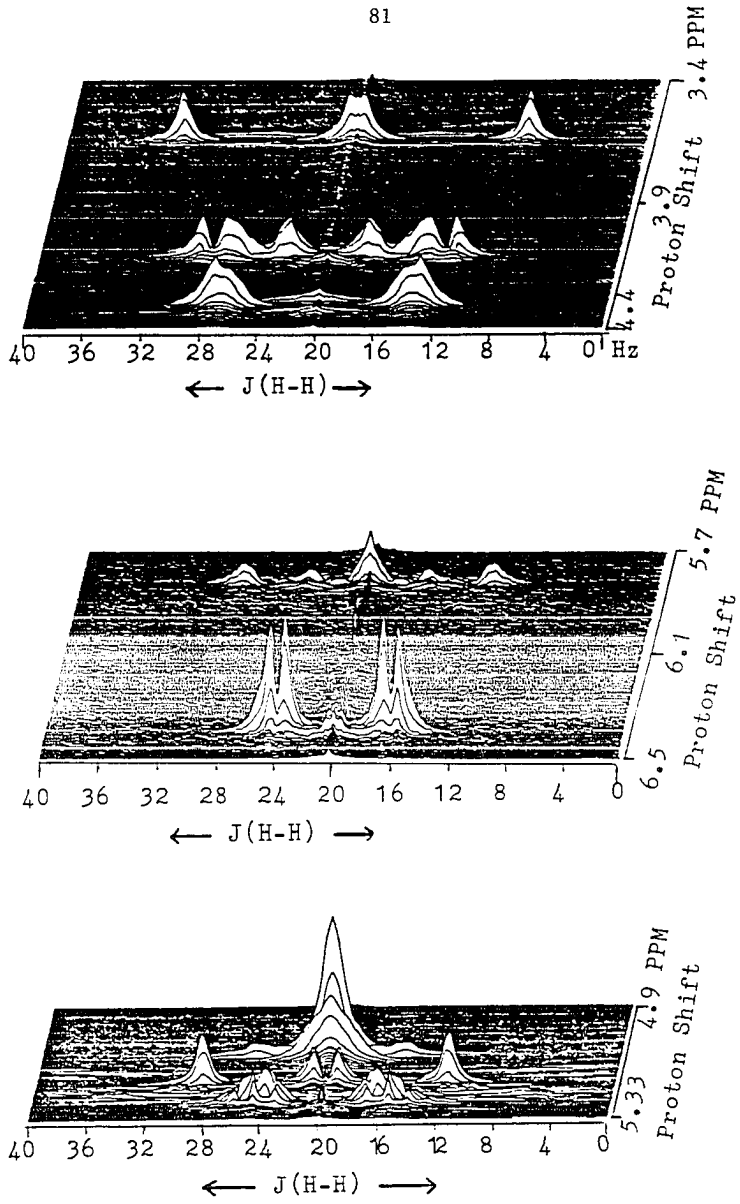


Figure 30. Stacked plots of the homonulear 2DJ spectrum of 52 in $CDCl_3$. ^1H chemical shifts are plotted along the vertical direction, and J (H-H) is plotted in the horizontal dimension.

18-hydroxyasteroxeniolide-A 9-acetate (52)

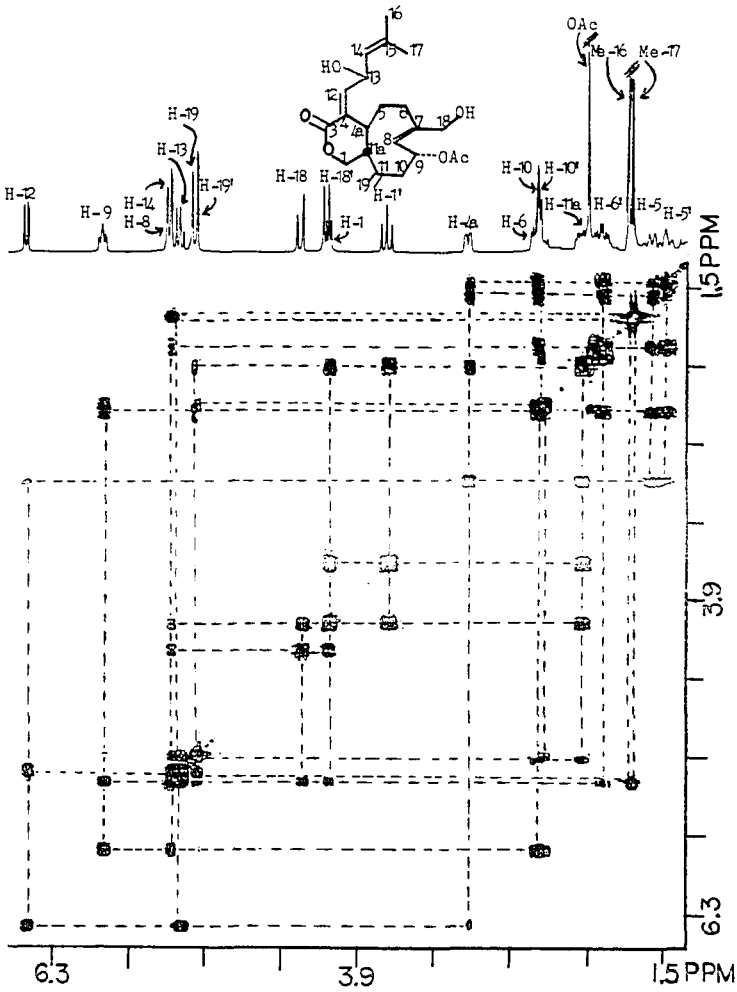


Figure 31a. Contour plot of the homocorrelated two-dimensional proton NMR spectrum (symmetrized) of 52 in $CDCl_3$ at 300 MHz. The final $s(F_2,F_1)$ matrix plot consisted of 512 X 512 data points. Off-diagonal contours establish direct proton spin-coupling connectivities.

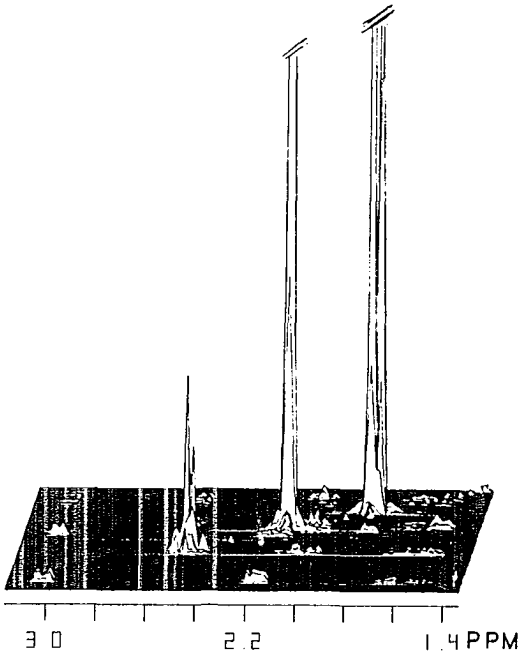


Figure 31c. Stacked plot of the high field region of homocorrelated 2D proton spectrum of 52 in $CDCl_3$ at 300 MHz.

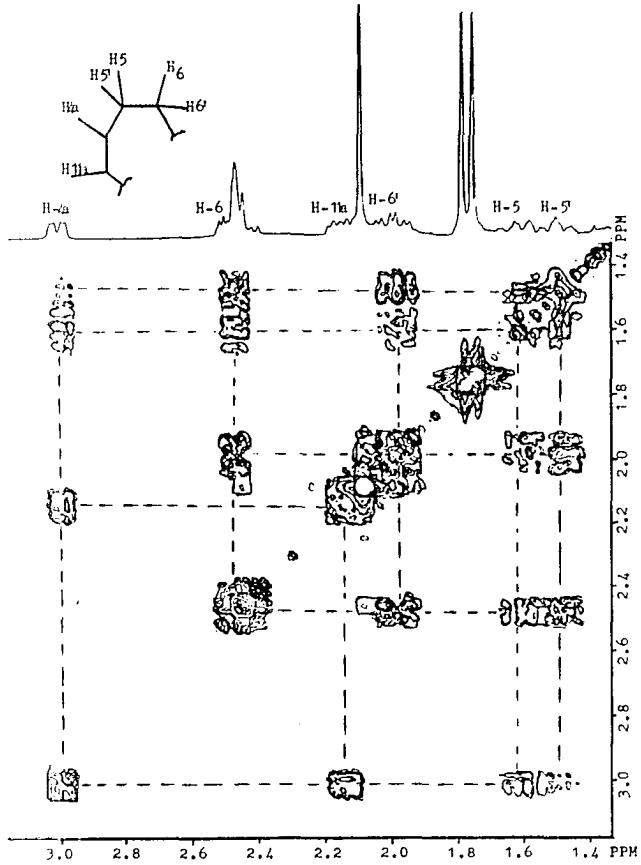


Figure 31b. Contour plot of the high-field region of homocorrelated 2D proton spectrum of 52 in $CDCl_3$ at 300 MHz. Off-diagonal contours establish direct proton spin-coupling connectivities.

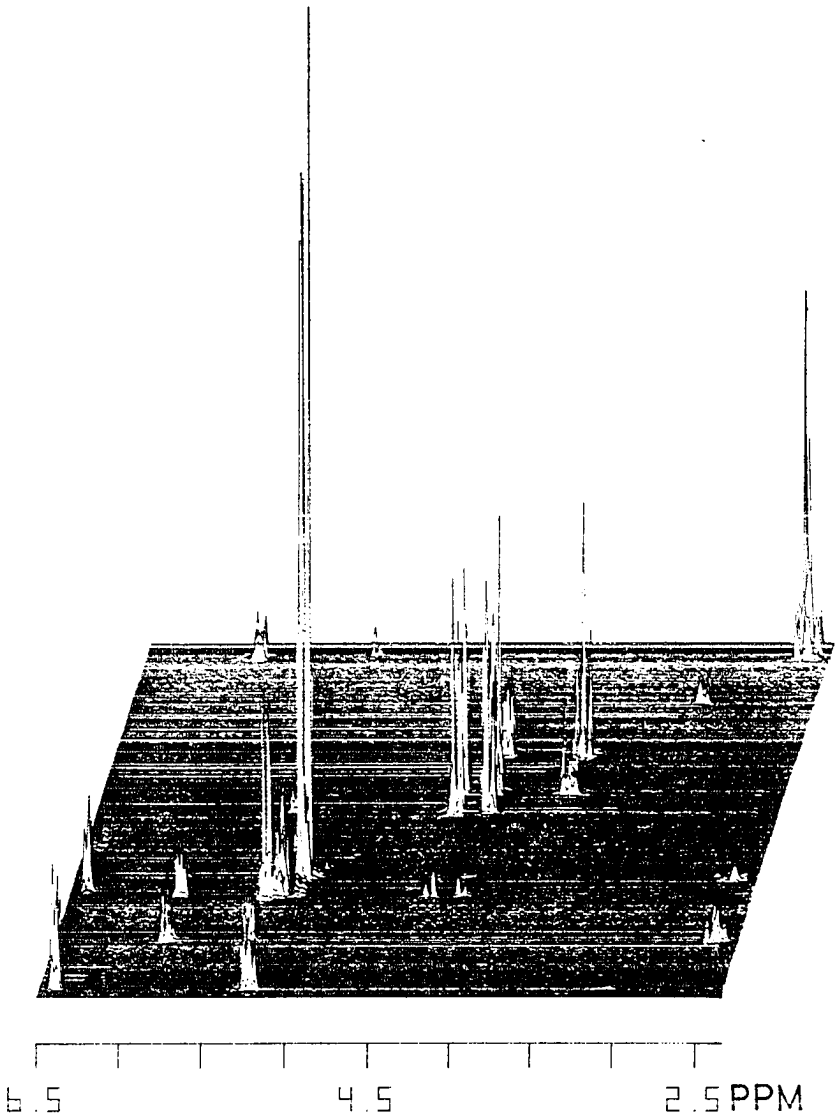


Figure 31d . Stacked plot of the down-field region of homo-correlated 2-D proton spectrum of 52 in $CDCl_3$ at 300 MHz.

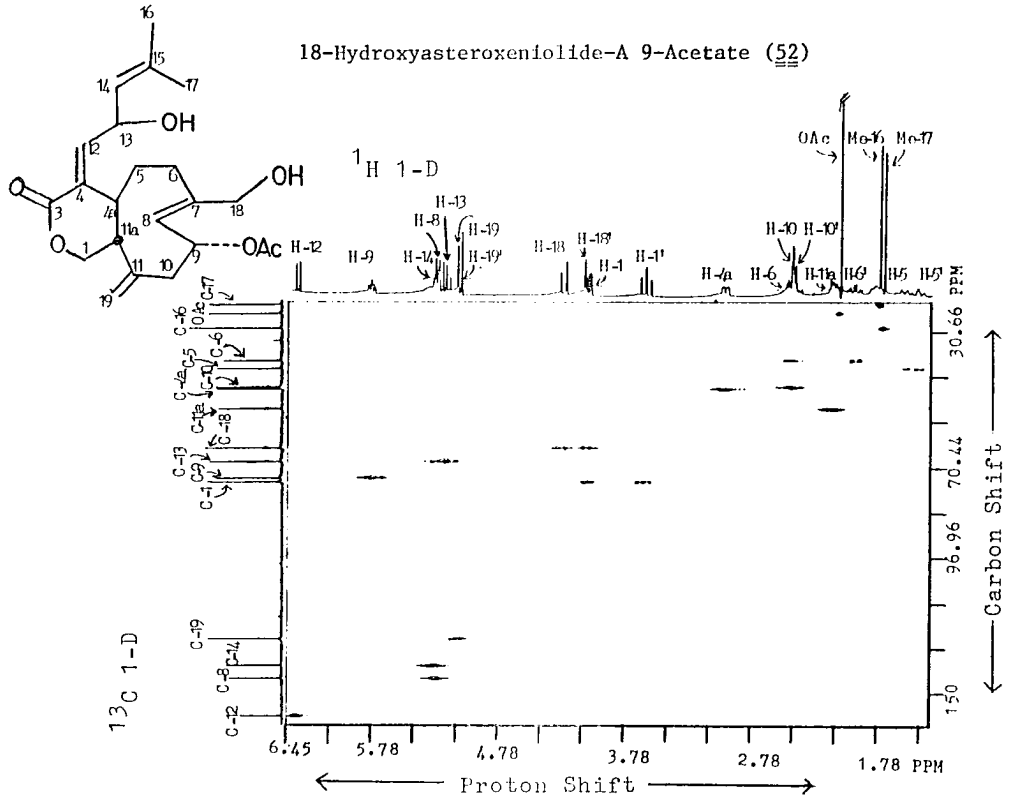


Figure 32. Contour plot of the heteronuclear correlated 2-D spectrum of 18-Hydroxy-asteroxeniolide-A 9-Acetate (52) in $CDCl_3$ at 75.4 MHz NMR. Carbon-13 chemical shift is plotted along the vertical direction, and proton chemical shift is plotted in the horizontal dimension. This 2-D spectrum establishes direct connectivitities between bonded nuclei.

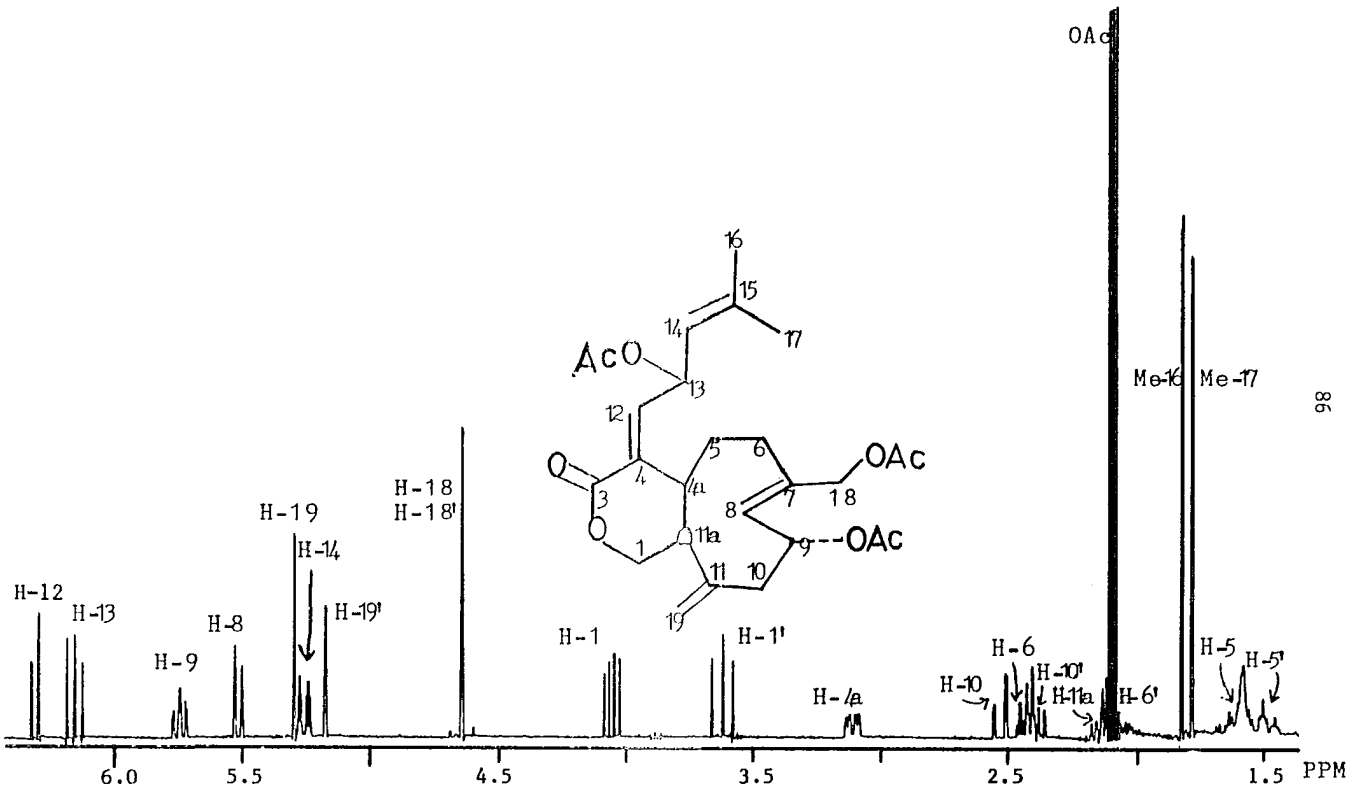


Figure 33. 300 MHz ^{1}H NMR spectrum of 53 in $CDCl_3$

Table 14. Results of NOEDS experiment with 52 in $CDCl_3$ at 300 MHz.

Proton irradiated chem shift, ppm	Proton (s) enhanced	% Enhancement
H-12, 6.39	H-14, 5.30	9
	H-13, 5.20	5
H-9, 5.82	H-18, 4.32	9
	H-18', 4.08	5
	H-10, 2.48	2
	H-10', 2.46	2
	H-11a, 2.14	2
H-4a, 3.02	H-19', 5.07	3
	H-13, 5.20	7.3
	H-8, 5.31	13
	H-6', 2.01	2
H-11a, 2.14	H-9, 5.82	2
	H-18, 4.32	5
	H-18', 4.08	6
	H-1, 4.09	4
H-6', 2.01	H-6, 2.49	12
	H-4a, 3.02	3
	H-13, 5.20	4
	H-8, 5.31	7
H-13, 5.31	H-12, 6.39	5
	H-6', 2.01	3
	H-4a, 3.02	10
H-1', 3.64	H-1, 4.09	17
	H-19', 5.07	4
	H-11a, 2.14	6
H-18, 4.32	H-9, 5.82	7
	H-18', 4.08	17

Table 15. Results of NOEDS experiment with 53 in $CDCl_3$ solution at 300 MHz.

Proton irradiated chem shift, ppm	Proton (s) enhanced	% Enhancement
H-12, 6.29	H-14, 5.25	8
H-14, 5.25	H-12, 6.29	8
	H-13, 6.15	4
H-4a, 3.10	H-8, 5.50	8
	H-13, 6.15	17
	H-6', 2.07	4
	H-19, 5.29	4

^{1}H NMR NOEDS experiment was also performed on the acetate derivative 53 of compound 52 where the chemical shifts of H-8, H-13, and H-24 are very different (see Table 11 and Figure 33). The results of these experiments (Table 15) supported the previous assignment. Thus, the structure of 52 is completely elucidated.

Structure elucidation of Asteroxeniolide-A (54):

Silica gel chromatography of fraction F' from Scheme 3 afforded 25 fractions. One of the fractions eluted with hexane-acetone (1:1) fraction 21, was resolved by HPLC with silica gel and hexane-acetone (75:25) as eluent to yield four fractions. Repeated chromatography of the fourth fraction by HPLC afforded 3 mg of asteroxeniolide-A (54) as a colorless oil; $[\alpha]_D^{25}$ + 26° (c. 0.29, $CHCL_3$). The molecular ion of 54 was not observed in the high resolution mass spectrum. However, an ion correlating to M^+-18 was detected at 314.1918 which is in agreement with the formula $C_{20}H_{26}O_3$ (calculated 314.1882). Consideration of this data with the observation of a peak at m/z 332 in the low resolution mass spectrum confirmed the formula of 54 to be $C_{20}H_{28}O_4$ (7 unsaturations). The ^{13}C NMR spectra (Figure 33) of 54 indicated the presence of three methyl groups, 4 sp^3 methylenes and 1 exocyclic terminal methylene, 7 methines, and 5 quaternary carbons. The IR spectrum of 54 showed a broad hydroxyl band at 3420 cm^{-1} and a band at 1712 cm^{-1} which was assigned to an α,β-unsaturated δ lactone. The UV spectrum of 54 exhibited a band at 217 nm which supported the latter moiety.

Analysis of the ^{1}H and ^{13}C NMR data (Tables 11 and 12 and Figures 33, 34, and 35) of 54 indicated the existence of four double bonds

(>C=CH_2, —CH=C<, —CH=C(Me)—, —CH=C(Me)(Me)), a conjugated carbonyl group, an allylic secondary hydroxyl [C-9 67.03 ppm (d)], and a doubly allylic secondary hydroxyl [C-13 64.92 ppm (d)]. The presence of two hydroxyl groups in 54 was suggested by the loss of two molecules of water in the mass spectrum [m/z 314 (M^+-H_2O) and 296 (M^+-$2H_2O$)]. Comparison of the ^1H and ^{13}C NMR data of 54 with data of previously isolated xeniolide-A[6] (7) indicated that compound 54 possesses the same bicyclic skeleton as xeniolide-A (7). From the analysis of the ^1H and ^{13}C NMR data (see Tables 11 and 12) and UV data, it was clear that compound 54 differs from xeniolide-A (7) in the structure of the side chain (see the structures of 54 and xeniolide-A), but 54 clearly has a side chain identical to 52. The structural features of 54 (see Tables 11 and 12) are very similar to those of compound 52 except that compound 54 possesses a methyl group at C-7 (rather than a primary alcohol group) and it lacks the acetyl group at C-9.

The proposed structure of 54 was confirmed by ^1H difference decoupling (DDS) and homocorrelated 2-D (COSY) (see Figure 35) spectroscopy experiments; all expected proton couplings were observed.

The stereochemical features of 54 are similar to those proposed for compound 52 according to the ^1H and ^{13}C NMR data of 52 and 54 (see Tables 11 and 12). The assignment of the trans configuration of the ring fusion in 54 was based on the similarities of the coupling constants ($J_{11a,4a} \approx 3.7$ Hz) observed for 54 and 52. The E configuration was assigned to C-7,8 on the basis of the ^{13}C NMR chemical shift observed for Me-18 at 17.32 ppm (rather than at 22-25 for the Z configuration).[24] The α configuration of the hydroxyl group at C-9 was based on

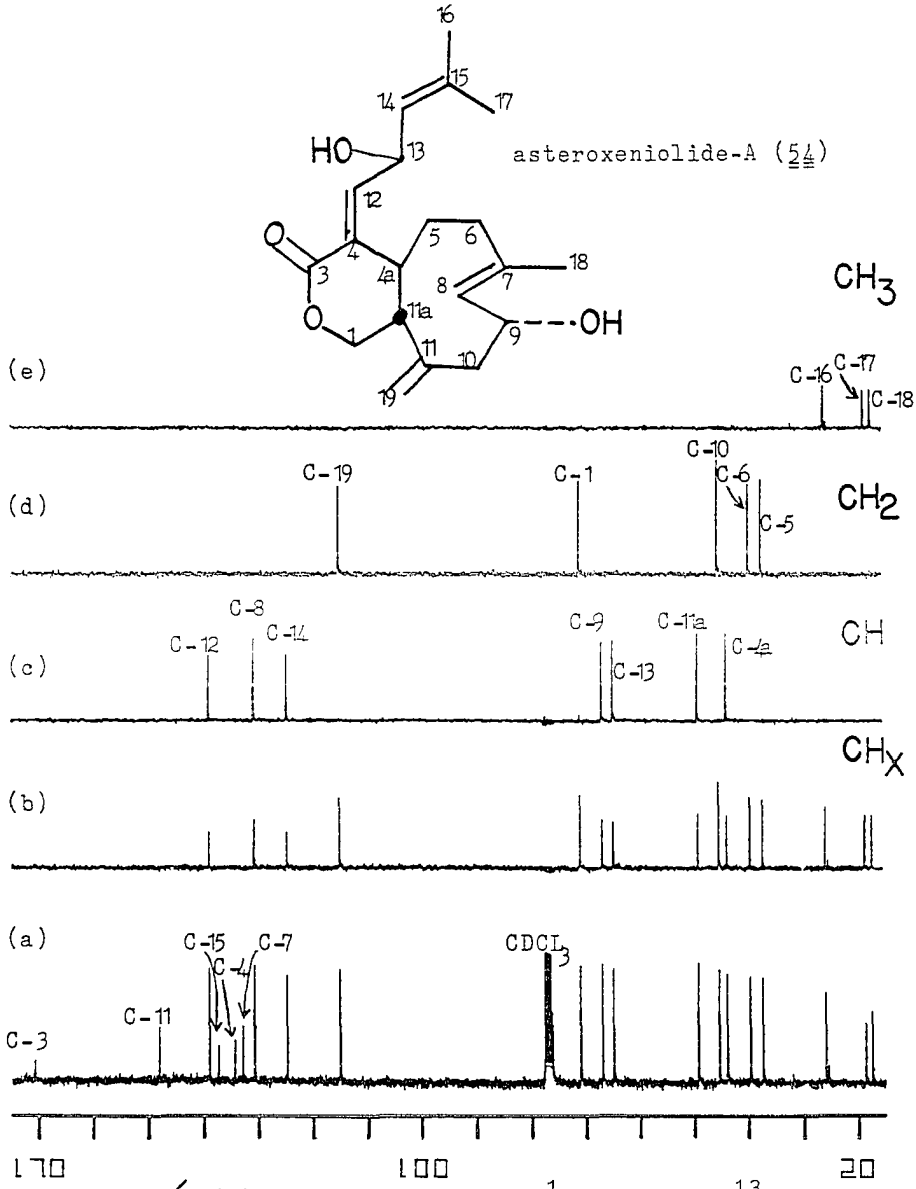


Figure 33. (a) 75.4 MHz broadband ^{1}H decoupled ^{13}C NMR spectrum of 54 in $CDCl_3$. (b) all protonated carbons. (c) methine carbons. (d) methylene carbons. (e) methyl carbons. Spectra b-e were recorded at 75.4 MHz in $CDCl_3$ and resulted from a DEPT experiment.

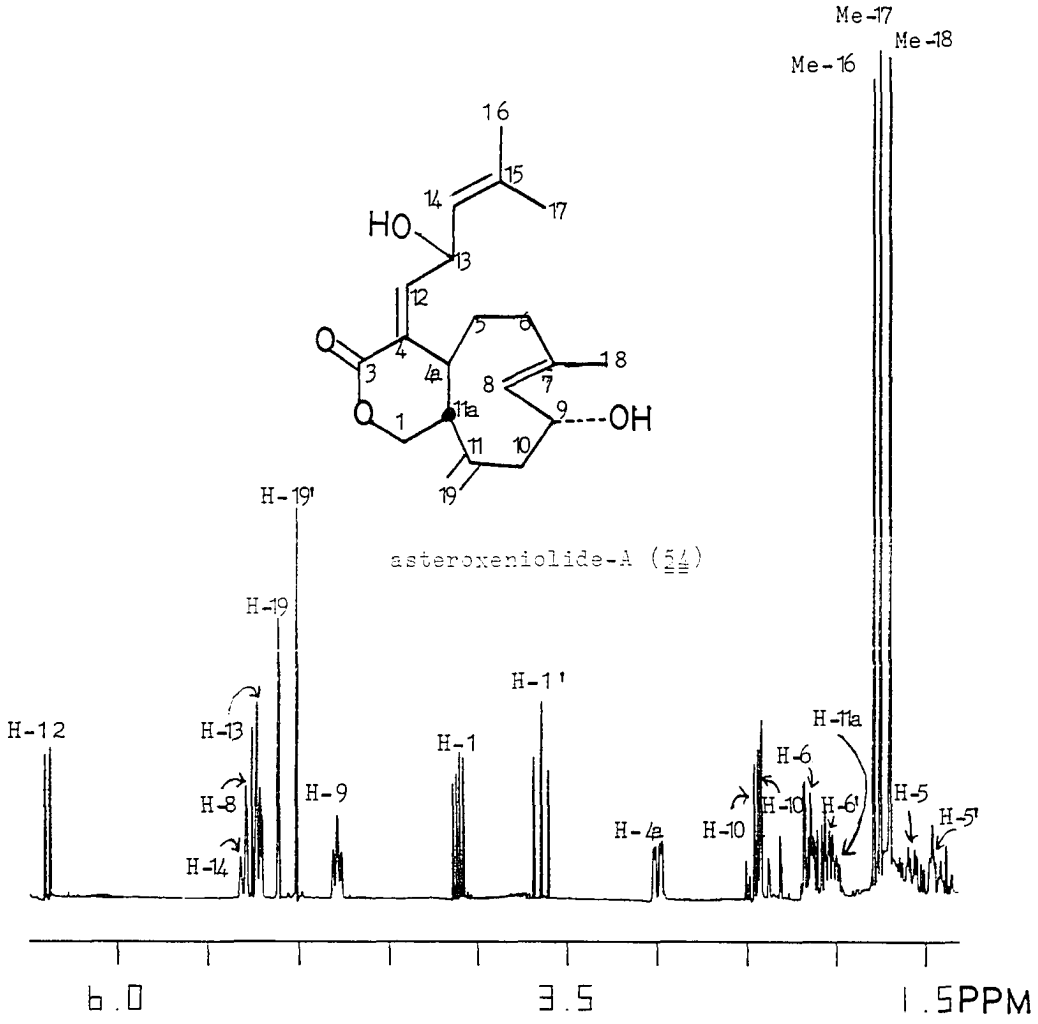


Figure 34. 300 MHz proton NMR spectrum of 54 in $CDCl_3$.

asteroxeniolide-A (54)

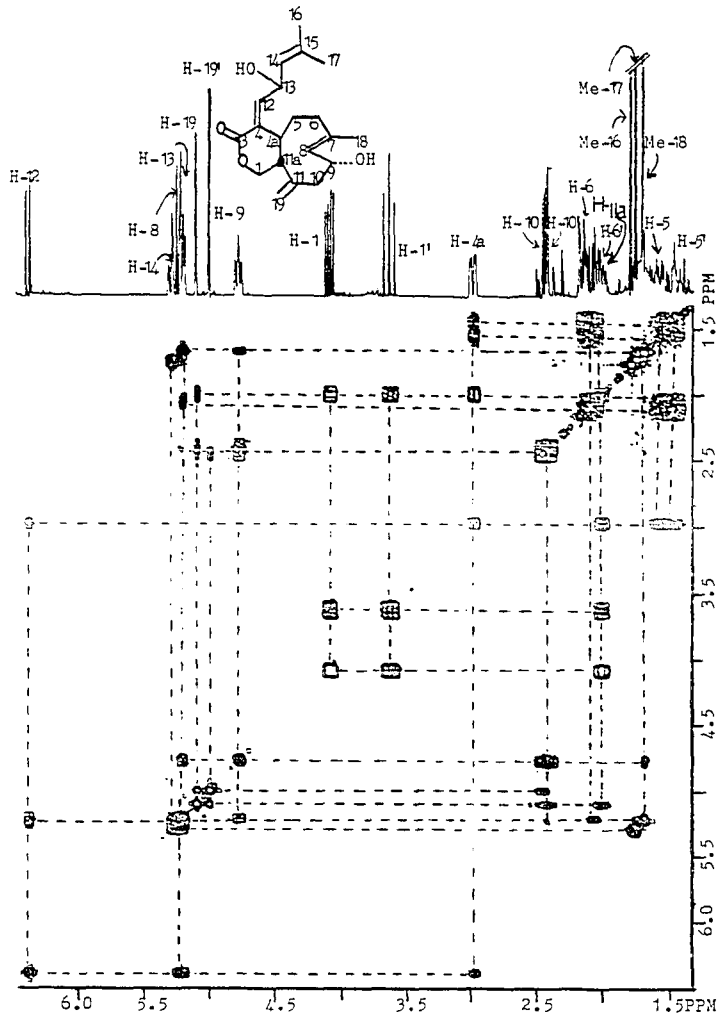


Figure 35. Contour plot of the homocorrelated 2-D proton NMR spectrum of 54 in $CDCl_3$ at 300 MHz. Off-diagonal peaks establish direct proton spin-coupling connectivities.

the similarities of the corresponding ^{1}H and ^{13}C NMR chemical shifts for 52 and xeniolide-A (7) (see Tables 11 and 12). Similarly, C-4,12 and C-13 in 52 were assigned the same stereochemistry as in 54 on the basis of the correlation of ^{1}H and ^{13}C NMR data associated with their side chains (see Tables 11 and 12). Thus, the structure of 54 is completed.

Structure elucidation of Asteroxeniolide-A 9-Acetate (55):

The $CHCL_3$ extract, fraction F' of Scheme 3, was chromatographed on a column of silica gel. Elution with 7:3 hexane-acetone yielded a fraction (fraction 14) which was further chromatographed by HPLC to give nine fractions. Repeated chromatography of fraction 6 led to the isolation, in trace quantities, of an oil, asteroxeniolide-A 9-acetate (55), 3 mg, $[\alpha]_D^{25}$ -1 (c. 0.3, $CHCL_3$). The molecular ion of 55 was not observed in the high resolution mass spectrum. However, a peak relating to a loss of one molecule of water was detected at 356.19796 which is consistent with the formula $C_{22}H_{28}O_4$ (cald 356.1988). IR (OH group), ^{1}H and ^{13}C NMR (22 carbons and 29 non-exchangeable protons) and high resolution mass spectral data taken together confirmed the formula of 55 to be $C_{22}H_{30}O_5$ (8 unsaturations). The ^{13}C NMR spectra of 55 (Figure 36) revealed the presence of 4 methyls, 4 methylenes, and one exocyclic terminal methylene, 7 methines, and 6 quaternary carbons. The IR spectrum of 55 exhibited a broad hydroxyl band at 3450 cm^{-1}, and bands appropriate for an acetate (1730 cm^{-1}) and an α,β-unsaturated δ lactone (1712 cm^{-1}). The latter moiety was consistent with the UV band at 217 nm.

The ^{1}H NMR spectrum of 55 (see Figure 37) is almost identical to that of asteroxeniolide-A (54) except for the existence of an additional acetate methyl group signal at 2.1 ppm and a downfield shift of the H-9 signal to 5.67 ppm from 4.78. Additionally, the ^{13}C NMR spectrum of 55 is almost identical to that of 54 except that in 55 the chemical shifts of C-7, C-8, C-9, and C-10 were changed relative to their counterparts in 54 (see Table 12) and an acetate carbonyl carbon signal appeared at 170.58 ppm in the spectrum of 55. Consequently, compound 55 was determined to be asteroxeniolide-A 9-acetate (55). The proposed structure of 55 was confirmed by DDS and homocorrelated 2-D (COSY) spectroscopy (Figure 38 represents the contour plot of the COSY spectrum which contains off-diagonal peaks that establish direct proton spin-coupling connectivities).

The stereochemical features of 55 are assumed to be identical to those proposed for asteroxeniolide-A (54), since the ^{1}H NMR chemical shifts and J values, and the ^{13}C NMR data are virtually identical for the two compounds (54 and 55) except as noted.

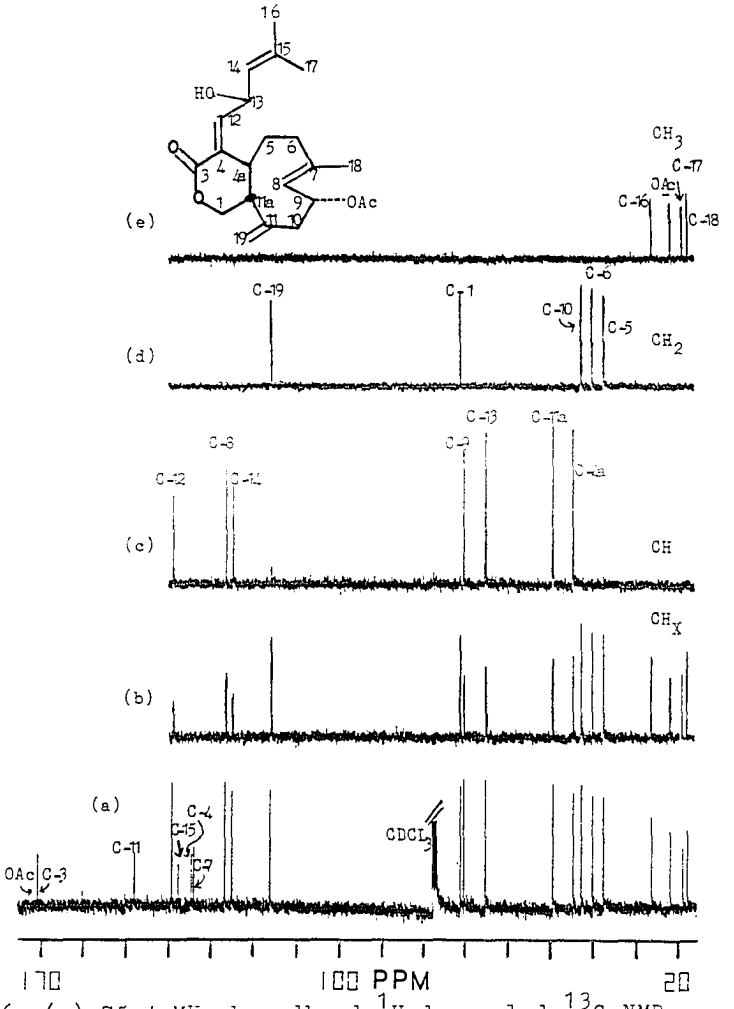


Figure 36. (a) 75.4 MHz broadband ^{1}H decoupled ^{13}C NMR spectrum of 55 in $CDCl_3$. (b) all protonated carbons. (c) methine carbons. (d) methylene carbons. (e) methyl carbons. Spectra b-e were recorded at 75.4 MHz in $CDCl_3$ and resulted from a DEPT experiment.

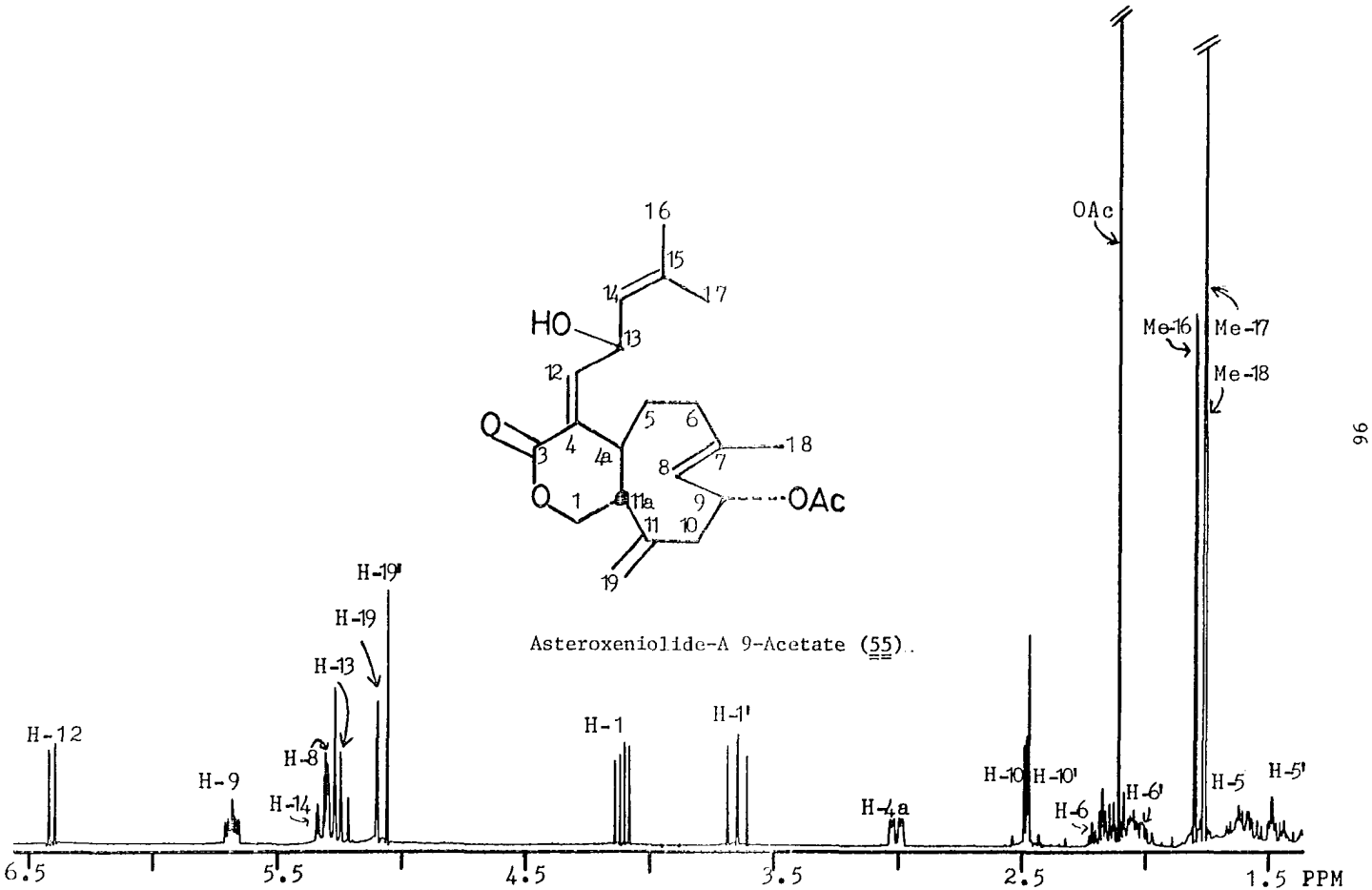


Figure 37. 300 MHz proton NMR spectrum of 55 in $CDCl_3$

Asteroxeniolide-A 9-Acetate (55)

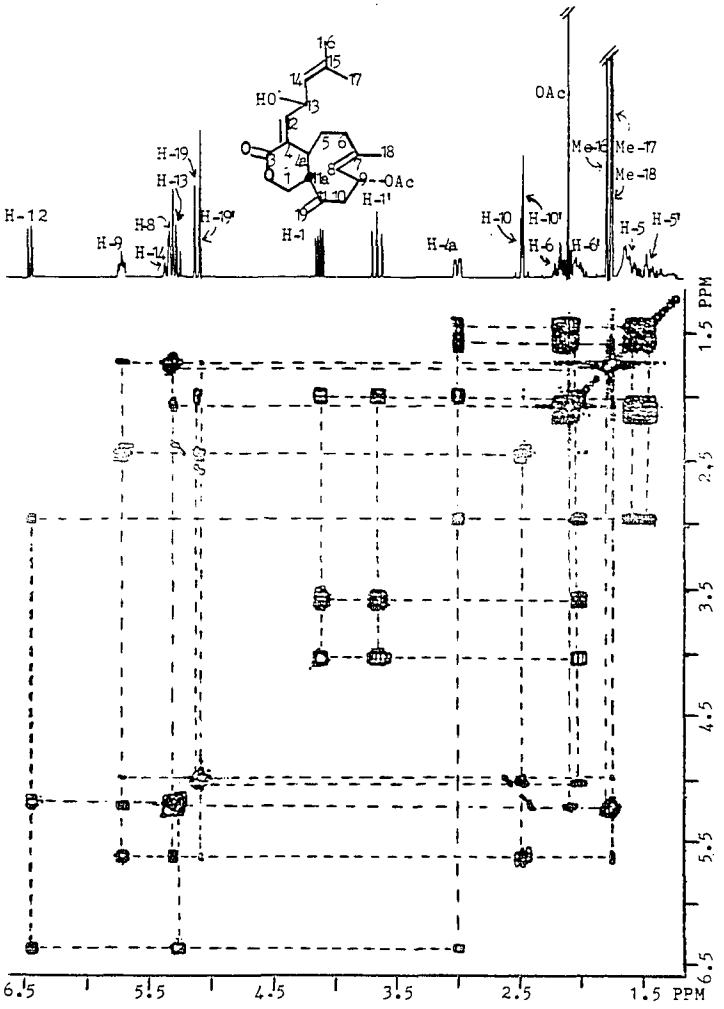


Figure 38. Contour plot of the homocorrelated 2-D proton NMR spectrum of 55 in $CDCl_3$ at 300 MHz. Off-diagonal peaks establish direct proton spin-coupling connectivities.

Identification of Xeniolide-B(13):

The twenty-third fraction of Scheme 4 was chromatographed by HPLC using hexane-acetone (80:20) as eluent to give 4 fractions. The second fraction contained 23 mg of a light yellow oil which was identified as xeniolide-B (13) on the basis of identity of the IR, UV, ^{1}H and ^{13}C NMR data observed in this work and values reported[6] for xeniolide-B (13).

Identification of Xeniolide-B 9-Acetate (14):

The fifteenth fraction of Scheme 4 was chromatographed by HPLC using a column of silica gel and hexane-acetone (83:17) as eluent to give pure xeniolide-B 9-acetate (14), 65 mg, as a yellow oil. The molecular formula $C_{22}H_{30}O_5$ (8 unsaturations) was deduced from the following data: (a) the low resolution mass spectrum displayed the molecular ion at m/z 374; (b) the ^{1}H NMR spectrum (see Figure 40) showed signals for 29 non-exchangable protons; (c) IR absorptions occurred at 3440 cm^{-1} (OH) and 1730 (acetate); (d) the ^{13}C NMR spectra (see Figure 41) exhibited signals for 22 carbons including 2 carbonyl ester carbons. The ^{1}H NMR spectrum (see Figure 40) of 14 was very similar to that of xeniolide-B (13) except for the presence of an extra acetate methyl group signal at 2.06 ppm and a downfield shift of the H-9 signal from 4.0 to 5.55 ppm. Furthermore, the ^{13}C NMR spectrum of 14 contained two additional carbon signals at 21.25 (q) and 170.54 ppm (s) which corresponded to an acetate group. Hence, it was concluded that 14 was the xeniolide-B 9-acetate (14), a known compound isolated by Kashman and Growiess.[6] However, Kashman reported that the chemical shift of the H-8, H-9, and H-13 signals in xeniolide-B 9-acetate (14) were at 4.45 (m),

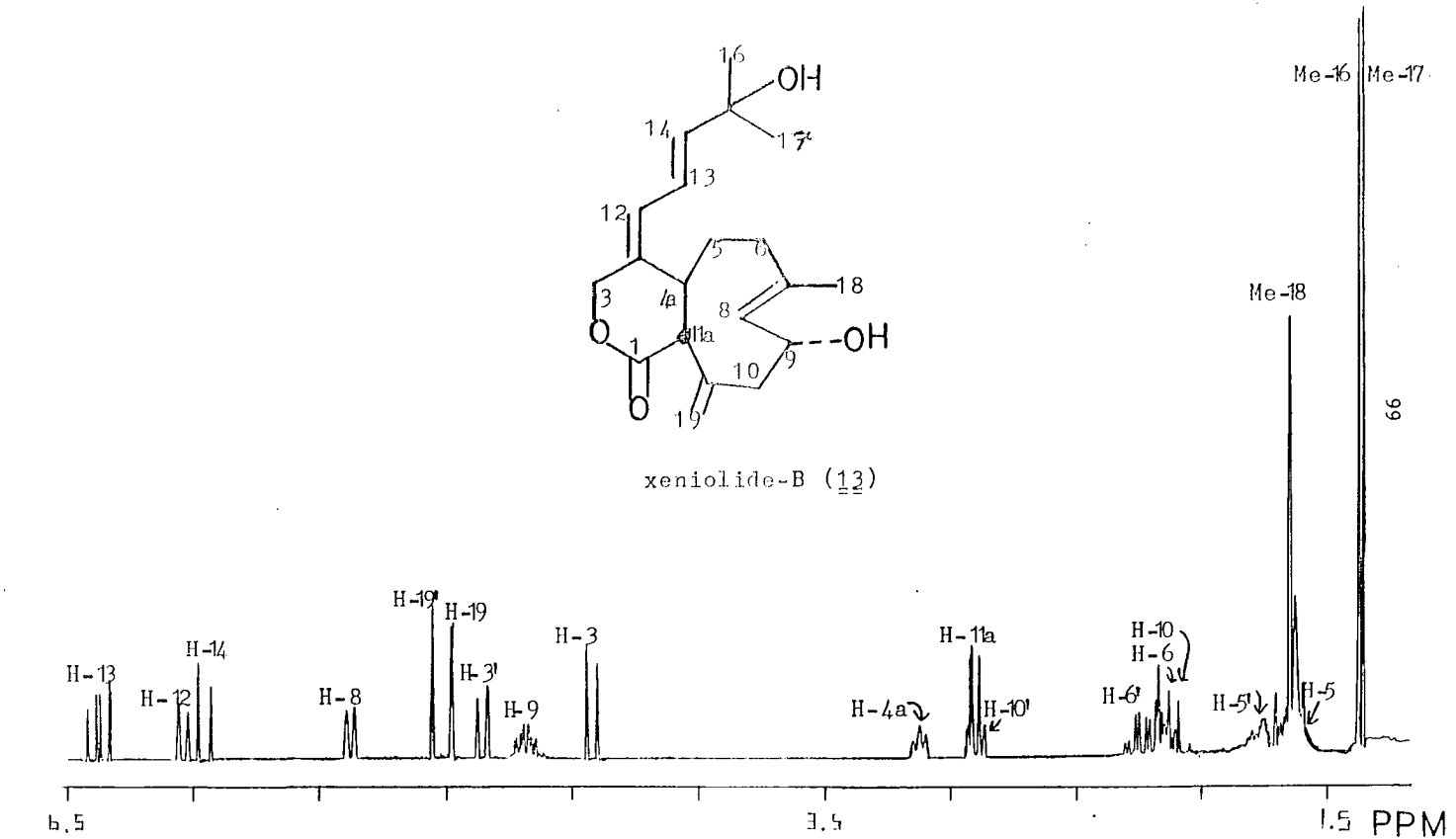


Figure 39. 300 MHz proton NMR spectrum of xeniolide-B (13) in $CDCl_3$

Table 16. ^{1}H NMR Data of Xeniolide-B and its Derivatives*

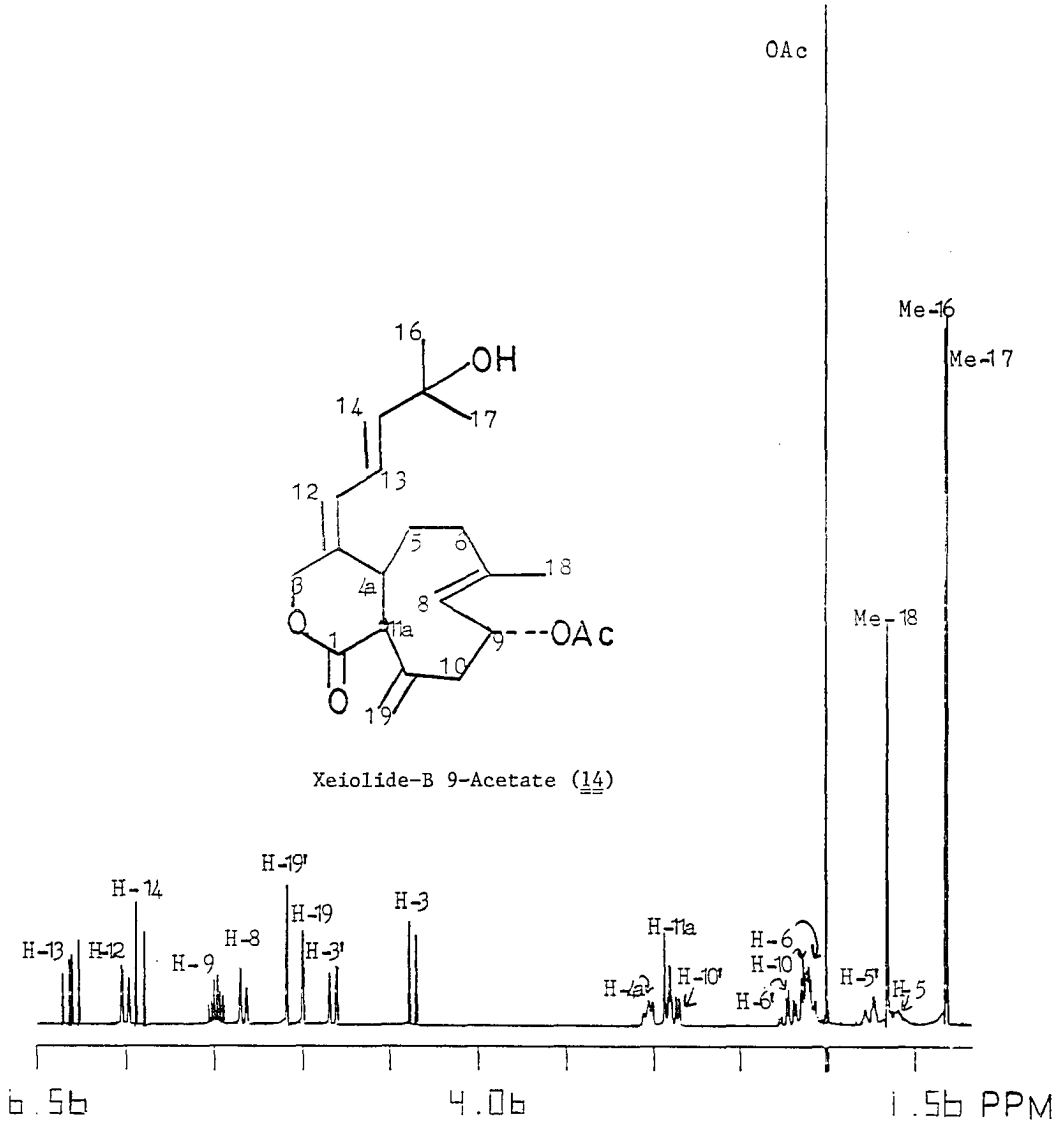
	13	14	15
H-3	4.42 br d 12	4.42 br d 12.1	4.42 br d 12.1
H-3'	4.85 br d 12	4.88 br d 12.1	4.89 br d 12.1
H-4a	3.12 br dd 9.5;6	3.09 br dd 9.1;6	3.11 br dd 10.4;4.8
H-5	1.65 m	1.65 m	1.96 br d 15
H-5'	1.81 dq 14;3.8	1.82 dq 14.2;3.8	1.75 tdd 15;4.8;2.5
H-6	2.17 m	2.17 m	1.44 ddd 15;3.3;3.0
H-6'	2.27 td 11.8;3.8	2.27 td 11.8;3.8	2.12 ddd 13.3;4.5;2.5
H-8	5.37 br d 10	5.39 br d 10.2	3.0 d 9.5
H-9	4.69 ddd 10;9;5.4	5.45 ddd 10.2;9.2;5.4	3.70 ddd 10;9.5;5.1
H-10	2.16 br dd 13.5;9	2.16 br dd 13.6;9.2	2.21 ddd 13.4;10;2
H-10'	2.89 br dd 13.5;5.4	2.94 br dd 13.6;5.4	3.01 br dd 13.4;5.1
H-11a	2.89 br d 9.5	2.98 br d 9.1	3.17 d 9.8
H-12	6.04 br d 11	6.06 br d 11.1	6.06 br d 11.3
H-13	6.37 dd 15;11	6.37 dd 15.1;11.1	6.23 dd 15;11.3
H-14	5.95 d 15	5.97 d 15.1	5.95 d 15
Me-16	1.38 s	1.38 s	1.32 s
Me-17	1.39 s	1.39 s	1.34 s
Me-18	1.65 br s	1.72 br s	1.24 s
H-19	4.98 br s	5.07 br s	5.15 br s
H-19'	5.05 br s	5.15 br s	5.22 br s
OAc		2.06 s	

*Spectra were recorded in $CDCl_3$ at 300 MHz with TMS as internal standard. The values are given in δ units. Assignments were established by spin-decoupling experiments.

Table 17. ^{13}C NMR Data of Xeniolide-B Derivatives[a]

C#	14	15
C-1	172.9 s	172.25 s
C-3	70.65 t	70.58 t
C-4	136.95 s	136.86 s
C-4a	36.91 d	36.05 d
C-5	37.33 t	36.05 t
C-6	39.55 t	37.89 t
C-7	136.01 s	59.63 s
C-8	126.0 d	67.23 d
C-9	72.25 d	71.26 d
C-10	39.62 t	38.84 t
C-11	141.2 s	138.92 s
C-11a	57.0 d	57.25 d
C-12	127.66 d	128.39 d
C-13	121.08 d	121.69 d
C-14	145.20 d	145.66 d
C-15	70.65 s	70.78 s
C-16	29.71 q	29.81 q
C-17	29.82 q	29.89 q
C-18	19.23 q	19.61 q
C-19	120.68 t	120.82 t
OAc	170.54 s 21.25 q	

[a]Spectra were recorded in $CDCl_3$ at 75.4 MHz. Multiplicities were obtained by DEPT and assignments were made by comparison to other compounds in this series.



Xeiolide-B 9-Acetate (14)

Figure 40. 300 MHz proton NMR spectrum of Xeniolide-B 9-Acetate (14) in $CDCl_3$.

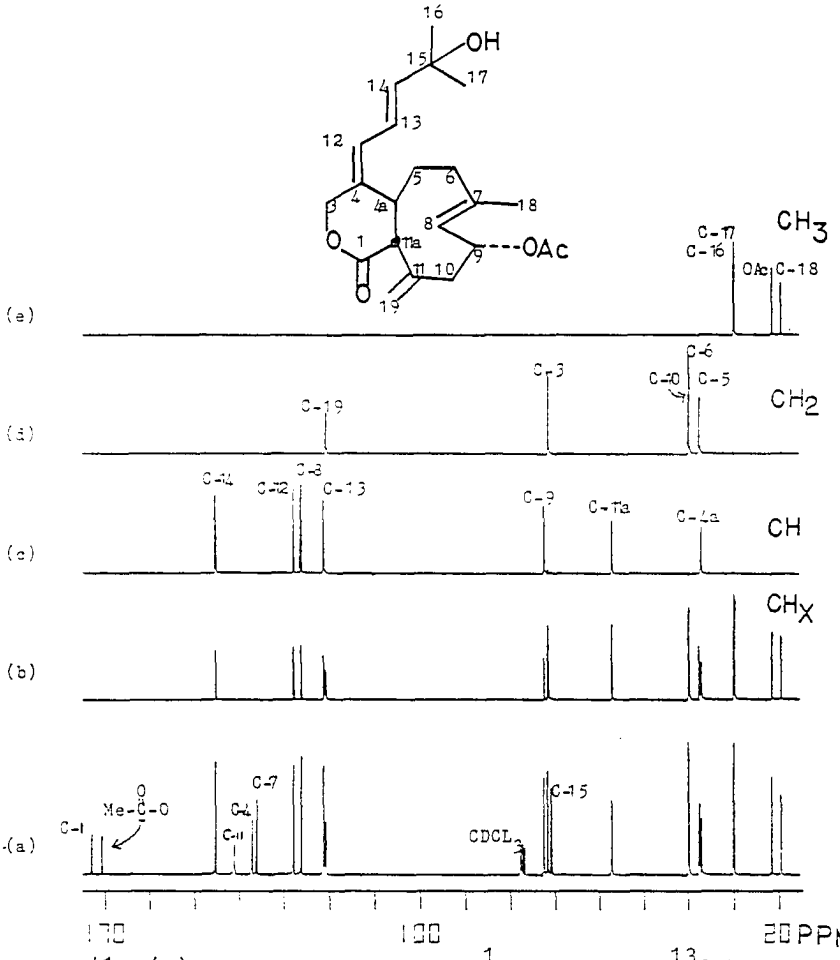


Figure 41. (a) 75.4 MHz broadband ^{1}H decoupled ^{13}C NMR spectrum of Xeniolide-B 9-Acetate (14) in $CDCl_3$. (b) all protonated carbons. (c) methine carbons. (d) methylene carbons. (e) methyl carbons. Spectra b-e were obtained in $CDCl_3$ at 75.4 MHz and resulted from a DEPT experiment.

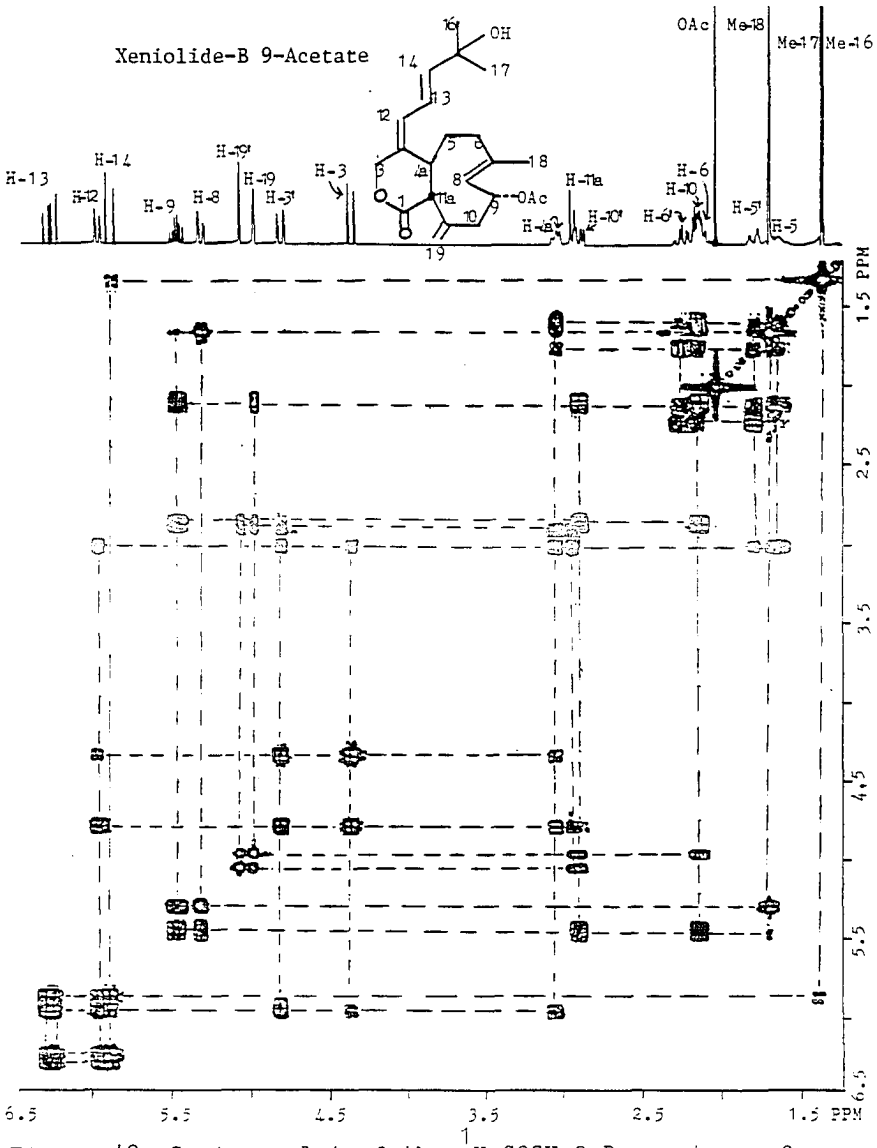
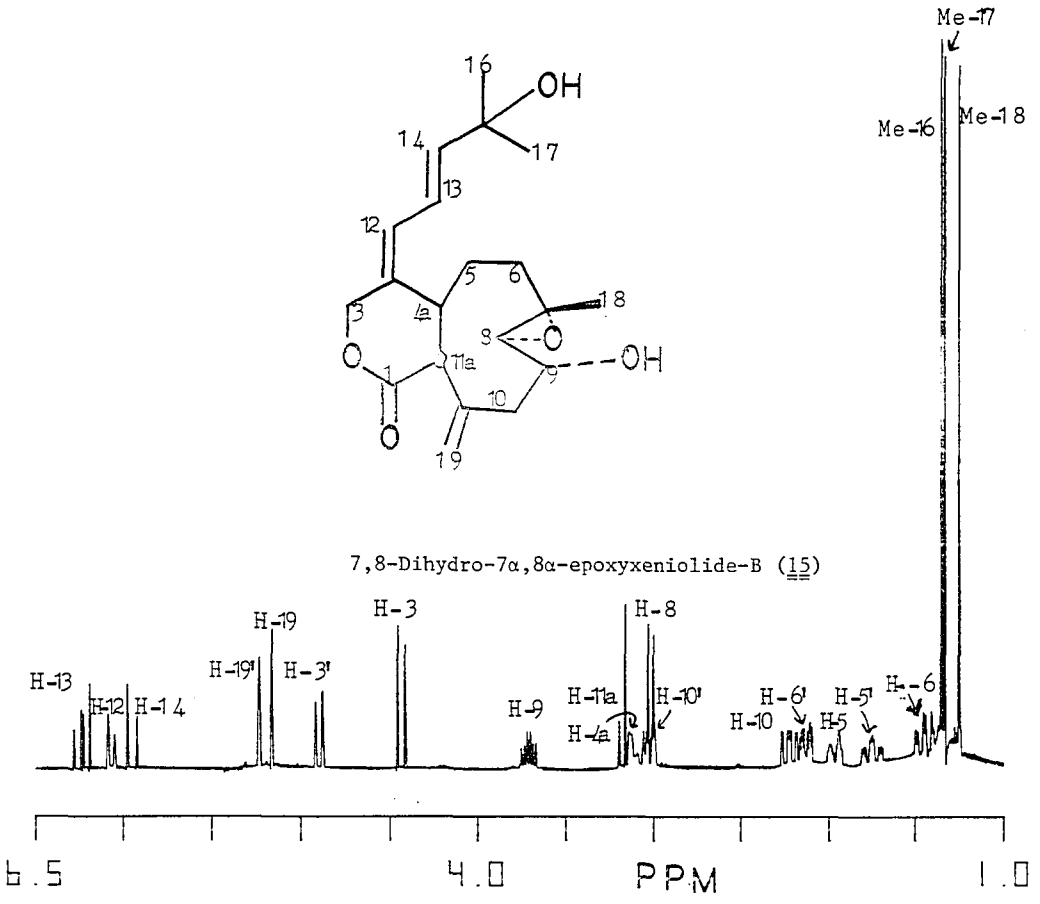


Figure 42. Contour plot of the ^{1}H COSY 2-D spectrum of Xeniolide-B 9-Acetate (14) in $CDCl_3$ at 300 MHz. Off-diagonal contours confirm direct proton spin-coupling connectivities.

4.45 (m), and 6.43 ppm (dd), respectively. We performed ^{1}H difference decoupling and COSY (see Figure 42) NMR experiments to establish the chemical shifts and multiplicities of all the proton signals in 14. Our results indicated that the chemical shifts of H-8, H-9, and H-13 signals were at 5.39 (br d), 5.55 (ddd), and 6.37 (dd), respectively. Our observed ^{13}C NMR data (see Table 17) is identical to that reported[6] by Kashman for xeniolide-B 9-acetate (14).

Identification of 7,8-Dihydro-7α,8α-epoxyxeniolide-B (15):

Silica gel chromatography of the $CHCl_3$ extract of Scheme 1 gave 9 fractions. Fractions 8 and 9 were combined and rechromatographed over silica gel to yield 7 fractions. The sixth of these fractions was subjected to HPLC to afford pure 7,8-dihydro-7α,8α-epoxyxeniolide-B (15), 1 mg, as a white powder. The molecular formula $C_{20}H_{28}O_5$ (7 unsaturations) was obtained for 15 from the following data: (a) the low resolution mass spectrum exhibited the molecular ion at 348; (b) the ^{1}H NMR spectrum (see Figure 43) showed signals for 26 non-exhangeable protons; (c) the IR spectrum showed OH (3500) cm^{-1} and carbonyl ester (1730) absorptions; (d) the ^{13}C NMR spectra indicated the presence of 20 carbons including two carbons each deshielded by a single oxygen, [70.59 ppm (C-9, d) and 70.78 (C-15, s)], 2 other carbon signals due to an epoxide group, [59.63 ppm (C-7, s) and 67.23 (C-8, d)], and a lactone carbonyl carbon, [172.25 (C-1, s)]. The ^{1}H NMR data (see Figure 43) of 15 were very close to those of 7,8-dihydro-7α,8α-epoxyxeniolide-B isolated by Kashman[6] and Growiess and hence identity between these two compounds was assumed. However, Kashman reported that the chemical



7,8-Dihydro-7α,8α-epoxyxeniolide-B (15)

Figure 43. 300 MHz proton NMR spectrum of 7,8-dihydro-7α,8α-epoxy-xeniolide-B (15) in $CDCl_3$

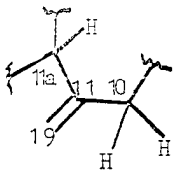
shift of H-13 signal in 7,8-dihydro-7α,8α-epoxyxeniolide-B was at 6.35 ppm. Our 300 MHz ^{1}H NMR analysis of the spectrum of 15 indicated that H-13 signal is at 6.23 ppm. Also, Kashman did not report the ^{13}C NMR chemical shifts of 7,8,-dihydro-7α,8α-epoxyxeniolide-B. We would like to report the ^{13}C NMR data of 7,8-dihydro-7α,8α-epoxyxeniolide-B (15), as well as the complete assignments of the ^{1}H NMR spectrum of 15.

Structure elucidation of Asterospicin (56):

Silica gel chromatography of the $CHCl_3$ extract of Scheme 1 afforded 9 fractions. Fractions 3-7 were combined and rechromatographed on a column of silica gel to give 6 fractions. The fourth of these was subjected to HPLC to yield 3 fractions, the first of which contained 8 mg of oily material. This oily material was resolved by HPLC using a reverse-phase column with MeOH-H_2O (60:40) as eluent to give 3 fractions. Evaporation of the second fraction left 2.2 mg of asterospicin (56) as a colorless oil. The molecular formula $C_{20}H_{28}O_4$ was established for 56 by the analysis of its high resolution mass spectrum. The molecular ion was also observed in the low resolution mass spectrum (M^+ = 332). The ^{13}C NMR spectra (Figure 44) of 56 indicated the presence of 3 methyls, 4 sp^3 methylenes and one exocyclic terminal methylene, 5 sp^3 methines and 3 sp^2 methines, and 4 quaternary carbons. The IR spectrum exhibited a broad hydroxyl band at 3440 cm^{-1}. The presence of (OH) group was unequivocally confirmed by acetylation of 56 to give the expected 9-acetate 57 in which H-9 signal experienced a downfield shfit to 4.74 from 3.74 ppm. A study of the ^{1}H and ^{13}C NMR data suggested the

existence of three double bonds ($CH_2=C<$, $-CH=CH-CH=C<^{Me}_{Me}$), an epoxide group ($-CH\overset{O}{-}C<^{Me}$), and an acetal group, 105.64 ppm (C-1, d).

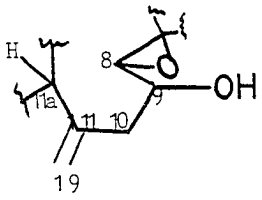
In the ^{13}C NMR spectrum (Figure 44d) of 56, the signal at 115.60 ppm (C-19, t)*, together with the proton signals at 5.14 (H-19') and 5.33 ppm (H-19), suggested an exocyclic terminal methylene group. Irradiation of H-19' signal sharpened the broad singlet at 5.33 ppm (H-19) and caused removal of small allylic couplings from the proton signals at 2.25 ppm (H-11a), 2.40 (H-10), and 2.58 (H-10'). This suggested that H-10, H-10', and H-11a were allylic to the terminal methylene protons. These coupling results led to the establishment of partial structure A of 56.



A

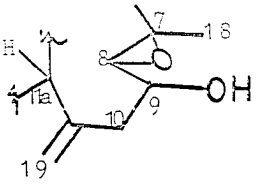
A ABM pattern was established between the proton signals at 2.40 ppm (H-10), 2.58 (H-10'), and 3.74 (H-9). The AB part which arises from the H-10 and H-10' proton signals of this ABM system was found to couple allylically to the terminal methylene proton signals (H-19 and H-19'). The M part (H-9) of this ABM pattern was also coupled vicinally to the methine proton at 2.75 ppm (H-8) which was assigned to the epoxide group. Therefore, partial structure A could be extended into B.

*assignment was made by analogy to other compounds in this series.



B

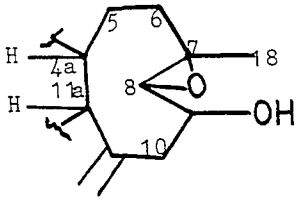
A small W-coupling (J = 0.9 Hz) was confirmed (by homonuclear 2-DJ and COSY experiments) between the epoxide proton signal at 2.75 ppm (H-8) and the methyl singlet at 1.38 ppm (Me-18), see Figures 45k, 45i, and 46. Thus, C-8 was connected to C-7 and this enlarged partial structure B into C.



C

In the high-field region (1-2.3 ppm) of the ^{1}H NMR spectrum (Figure 47) of 56, an ABMX system was confirmed between the proton signals at 1.83 ppm (H-5), 1.53 (H-5'), 1.05 (H-6'), and 2.24 (H-6). The carbon connectivity in this ABMX spin system was established by homocorrelated 2-D experiment (see Figures 46 and 47) in which all the proton-spin coupling connectivities were shown very clearly. C-7 was determined to be bonded to C-6 because a small W-coupling was observed (see Figures 45h, 45k, and 46) between H-6' and Me-18. The signal assigned to H-5' (1.53

ppm of this ABMX system was coupled vicinally (J = 10.5 Hz) to the one-proton signal at 1.76 ppm (H-4a), see Figure 47. Therefore, C-5 was connected to C-4a. Moreover, H-4a was also coupled (J = 4.5 Hz) vicinally to H-11a, see Figure 3b. This proved the connection between C-4a and C-11a and completed the carbocyclic partial structure D.

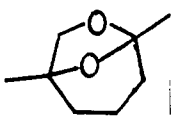


D

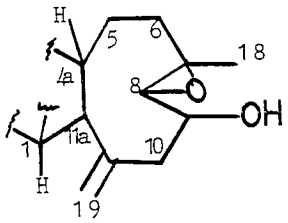
In the ^{1}H NMR spectrum of 56, a proton singlet at 5.32 ppm (H-1) was resolved by homonuclear 2-DJ experiment, Figure 45b, into a quintet (J = 0.8 Hz). In the COSY spectrum (Figure 46) of 56, the H-1 signal was coupled (J = 0.8 Hz) to H-11a signal. This small coupling between H-11a and H-1 was attributed to a dihedral angle near 90° between H-11a and H-1. Consequently, C-11a was fixed to C-1, see partial structure E. This linkage was supported further by a small W-coupling (J = 0.8 Hz) observed between H-1 and H-4a signals, see Figure 46.

An AB pattern was established between the proton signals at 3.35 ppm (H-3) and 3.96 (H-3'), see Figure 46, J = 6.7 Hz. This small geminal coupling was also found in frontalin[27] in which the carbon bearing the two protons due to this AB system is adjacent to an oxygen atom and is a part of bicyclic acetal system, see structure of frontalin. Based on the frontalin analogy, C-3 was connected to C-1 through

an ether oxygen. Indeed, H-3 was found to be long-range coupled to H-1 (see Figure 46).

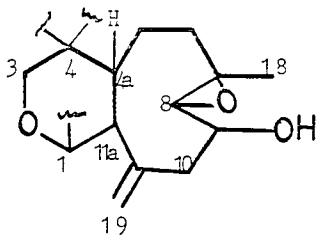


FRONTALIN



E

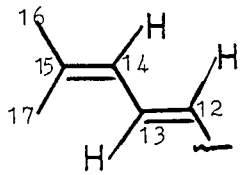
Furthermore, a 1.9 Hz W-coupling was established between H-3 and H-4a. The lack of any vicinal coupling between either H-3 or H-3' signals and other proton signal(s) in the COSY spectrum of 56 suggested that C-3 could be attached to a quaternary carbon. The C-3, C-4, and G-4a chain was fixed on the basis of the W-coupling observed between H-4a and H-3 (see Figure 46). Therefore, partial structure D was extended to F.



F

In the downfield region of the ^{1}H NMR spectrum of 56, an ABM spin system was confirmed for the olefinic proton signals at 5.73 ppm (H-12), 6.47 (H-13), and 5.86 (H-14). It was clear from the coupling constant (J = 15.7 Hz) that H-12 and H-13 were trans and H-13 signal was also coupled vicinally to H-14 signal (J = 10.3 Hz). Irradiation of H-14 signal changed the H-13 signal from a double doublet into a doublet and

sharpened both the vinylic methyl signlets at 1.80 and 1.78 ppm. Conversely, irradiation of either of the vinylic methyl signals sharpened the broad proton signal at 5.86 ppm (H-14). This indicated that these methyls (Me-16 and Me-17) were vinylic and also geminal. Therefore, a diene chain was constructed as a partial structure G.



G

A close examination of the ^{1}H COSY spectrum (see Figure 46) of 56 indicated that H-12 signal was found to couple with long-range couplings to H-3, H-3', H-4a, and H-14 signals. Furthermore, the lack of any vicinal coupling for H-12 signal other than H-13 coupling suggested that C-12 must be connected to a quaternary carbon. The chemical shift of the carbon signal at 88.26 ppm (C-4, s) suggested that it must be deshieleded by an oxygen substituent and adjacent to a double bond. Consideration of the long-range couplings observed between H-12 and H-3, H-3', and H-4a signals and the fact that C-12 must be connected to a quaternary carbon led to the conclusion that C-4 must be linked to C-12. The chemical shift of the carbon signal at 105.64 ppm (C-1, d) suggested an acetal carbon which bears two oxygens, one of which was connected to C-3 as mentioned above and the second oxygen could be connected to only one position which was C-4. Thus, the skeleton of 56 was established.

The stereochemical features of 56 were established by NOE experiments (see Table 20). The E configuration of the epoxide group at C-

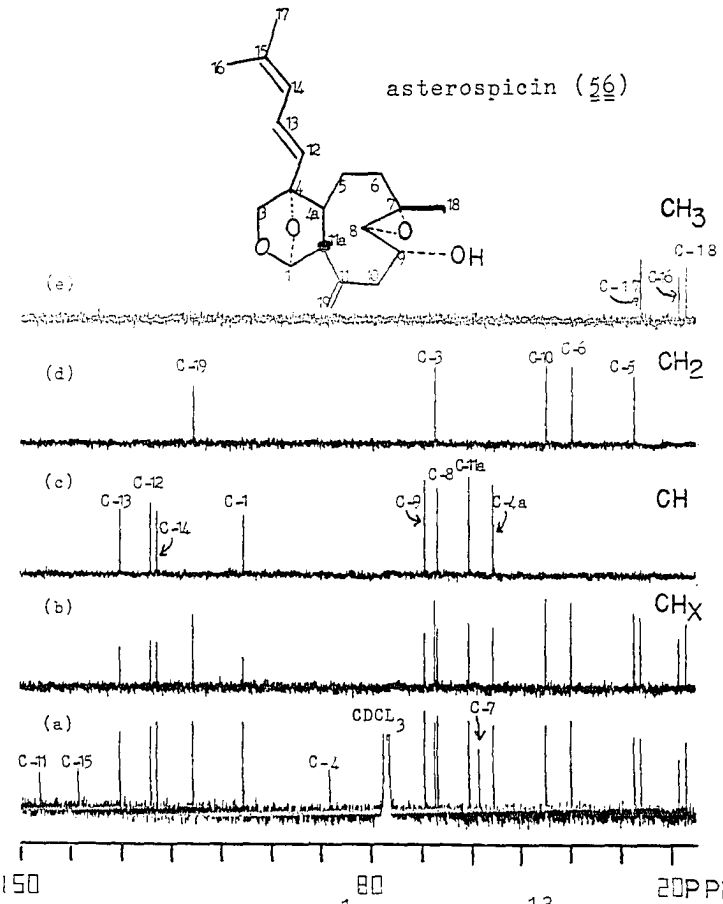


Figure 44. (a) 75.4 MHz broadband ^{1}H decoupled ^{13}C NMR spectrum of 56 in $CDCl_3$. (b) all protonated carbons. (c) methine carbons. (d) methylene carbons. (e) methyl carbons. Spectra b-e were recoreded at 75.4 MHz NMR in $CDCl_3$ and resulted from a DEPT experiment.

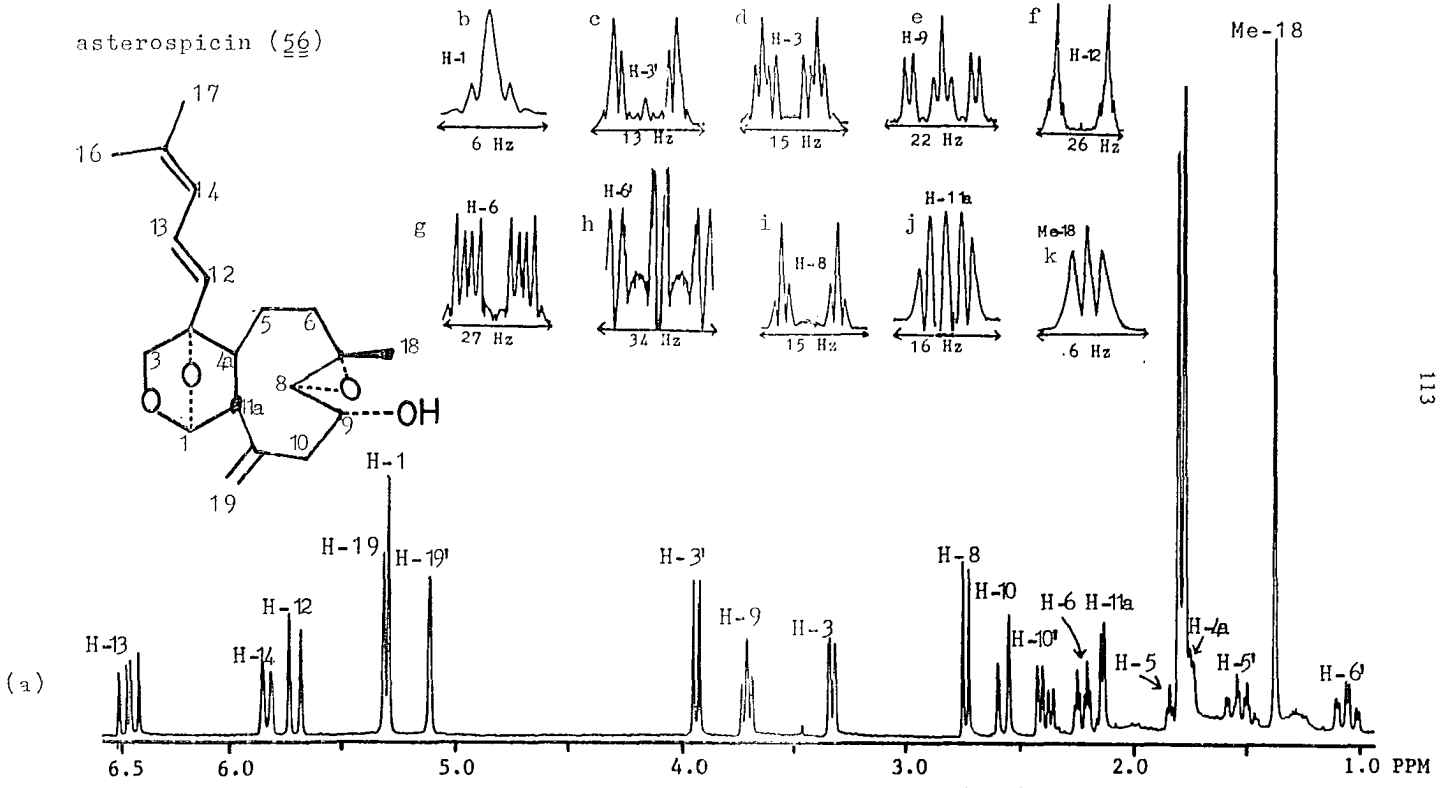


Figure 45. (a) 300 MHz proton NMR spectrum of 56 in $CDCl_3$. (b-k) are slices from homonuclear two-dimensional J-resolved (2DJ) spectrum in $CDCl_3$ at 300 MHz.

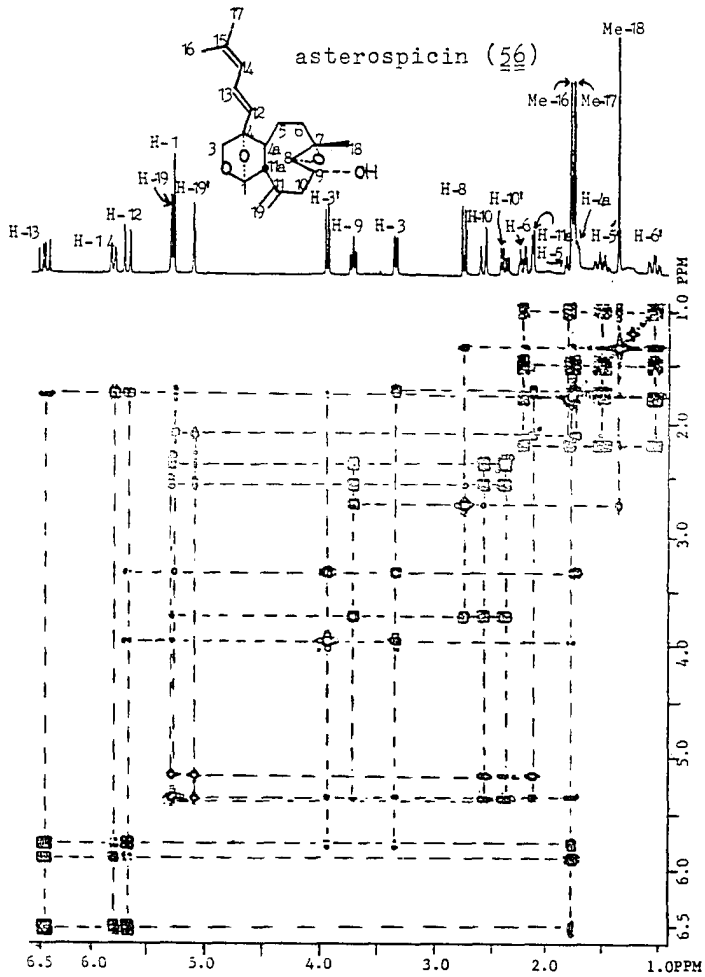


Figure 46. Contour plot of the homocorrelated (COSY) two-dimensional proton NMR spectrum of 56 in $CDCl_3$ at 300 MHz. Off-diagonal responses establishing proton spin-coupling connectivities.

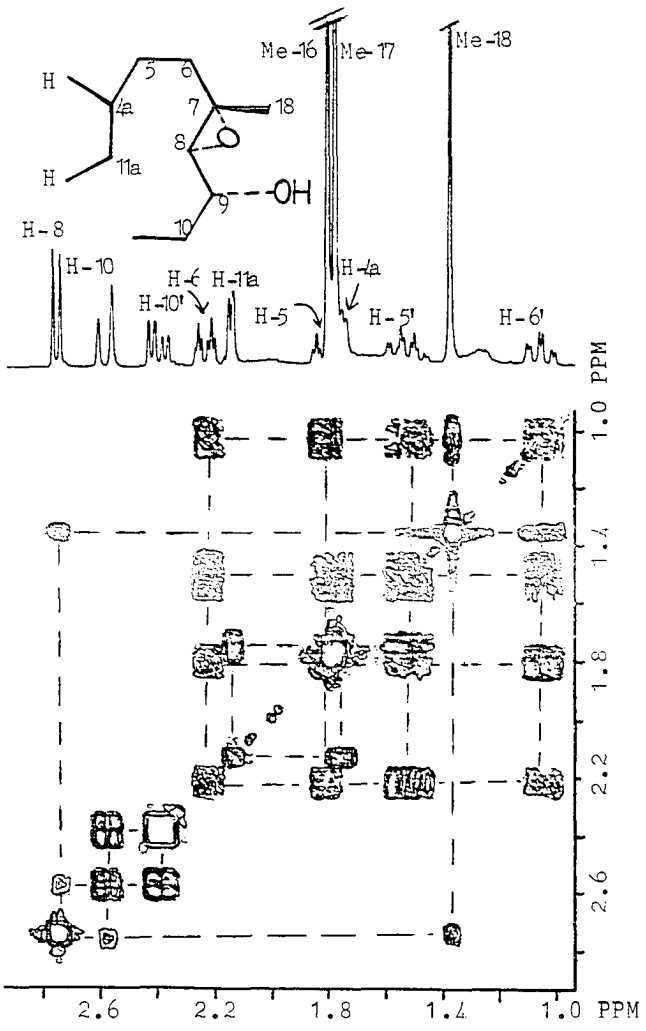


Figure 47. Contour plot of the up-field portion of COSY spectrum of asterospicin (56) in $CDCl_3$ at 300 MHz. Off-diagonal peaks denote spin coupling connectivities.

Table 18. Proton NMR Data of the Asterospicins*

	56[a]	57	58	59
H-1	5.32 quintet 0.8	5.30 s	5.30 s	5.30 s
H-3	3.35 ddd 6.8;1.9;0.8	3.34 dd 6.7;2	3.32 dd 7.7;2	3.34 dd 7.7;2
H-3'	3.96 br dd 6.8;0.8	3.94 d 6.7	3.96 d 7.7	3.96 d 7.7
H-4a	1.76 br ddd 10.5;4.7;1.9	1.74 br ddd 10.5;4.5;2	1.74 br ddd 10;4.3;2	1.74 br ddd 10;4.3;2
H-5	1.83 dt 14.5;3.5	1.82 dt 14.5;3.5	1.78 dt 14.5;3.5	1.78 dt 14.5;3.5
H-5'	1.53 dddd 14.5;13.5;10.5;2.2	1.53 dddd 14.5;13;10.5;3.5	1.53 dddd 14.5;13.5;10;3.5	1.53 dddd 14.5;13.5;10;3.5
H-6	2.24 ddd 13.5;3.5;2.2	2.25 dt 13.4;3.5	2.24 dt 13.9;3.5	2.24 dt 13.9;3.5
H-6'	1.05 tdd 13.5;3.5;0.9	1.04 ddd 13.4;13;3.5	1.05 ddd 13.9;13.5;3.5	1.05 ddd 13.9;13.5;3.5
H-8	2.75 dt 7.8;0.9	2.91 d 7.8	2.73 d 7.7	2.73 d 7.7
H-9	3.74 ddd 7.8;5.9;1.9	4.74 ddd 7.8;5.2;1.5	3.74 br dd 7.7;6.5	3.74 br dd 7.7;6.5
H-10	2.40 ddd 14.2;5.9;1.8	2.38 br dd 14.8;5.2	2.39 br dd 13.9;6.5	2.39 br dd 13.9;6.5
H-10'	2.58 ddd 14.2;1.9;1	2.61 br d 14.8	2.58 br d 13.9	2.58 br d 13.9
H-11a	2.15 br dt 4.7;1.8	2.13 br dd 4.5;1.5	2.15 br d 4.3	2.15 br d 4.3
H-12	5.73 d quintet 15.6;0.8	5.72 d 15.7	6.12 br d 15.4	6.12 br d 15.4
H-13	6.47 dd 15.6;10.3	6.46 dd 15.7;10.4	5.73 dd 15.4;6.5	5.71 dd 15.4;6.5
H-14	5.86 br d 10.3	5.85 br d 10.4	3.26 br d 6.5	3.27 br d 6.5
Me-16	1.80 br s	1.79 br s	1.37 s	1.37 s
Me-17	1.78 br s	1.77 br s	1.26 s	1.26 s
Me-18	1.38 t 0.8	1.40 s	1.36 s	1.36 s
H-19	5.33 br s	5.35 br s	5.31 br s	5.31 br s
H-19'	5.14 br s	5.04 br s	5.14 br s	5.14 br s
OAc		2.1 s		

[a]Multiplicity and coupling constants were obtained from a homonclear 2-DJ experiment.
*Spectra were recorded in $CDCl_3$ at 300 MHz with Me_4Si as internal standard except for compounds 60 and 61 in which their spectra were obtained in 5% CD_3OD in $CDCl_3$. The values are given in δ units.

(Continued Table 18)

	60	61	62	63	64
H-1	5.29 t 0.9	5.29 t 0.9	5.28 s	5.39 s	6.10 d 4.7
H-3	3.28 dd 6.8;2	3.29 dd 6.8;2	5.04 d 12.1	6.10 s	9.61 d 2
H-3'	3.94 d 6.8	3.94 d 6.8	2.56 d (OH) 12.1		
H-4a	1.73 br ddd 10.5;4.6;2	1.73 br ddd 10.5;4.6;2	1.74 br dd 10.5;3.7	1.78 br dd 10;4.2	2.25 br t 9.6
H-5	1.78 dt 14.2;3.6	1.78 dt 14.2;3.6	1.86 dt 13.3;3.7	1.93 dt 13.6;3.4	2.19 br dd 12.8;5.5
H-5'	1.50 dddd 14.2;13.5;10.5;2.3	1.50 dddd 14.2;13.5;10.5;2.3	1.41 tdd 13.4;10.5;3.7	1.55 m	1.55 m
H-6	2.20 ddd 13.5;3.6;2.3	2.20 ddd 13.5;3.6;2.3	2.22 dt 12.3;3.7	2.26 dt 12.2;3.4	1.88 br dd 12.5;3.8
H-6'	1.0 tdd 13.5;3.6;0.9	1.0 tdd 13.5;3.6;0.9	1.99 ddd 13.4;12.3;3.7	2.02 td 12.2;3.4	1.82 br dd 12.5;5.5
H-8	2.73 dt 7.8;0.9	2.73 dt 7.8;0.9	5.08 br d 6.7	5.16 br d 7.8	5.20 br d 9.3
H-9	3.66 ddd 7.8;5.9;1.9	3.66 ddd 7.8;5.9;1.9	4.69 br s	5.61 br dd 7.8;5.9	5.51 ddd 9.3;8.2;5.4
H-10	2.35 ddd 14.2;5.9;1.5	2.35 ddd 14.2;5.9;1.5	2.42 br d 13.7	2.43 br dd 13.6;5.9	2.10 br dd 13;8.2
H-10'	2.55 ddd 14.2;1.9;1	2.55 ddd 14.2;1.9;1	2.46 br dd 13.7;5.4	2.50 br d 13.6	2.65 br dd 13;5.4
H-11a	2.11 br ddd 4.6;2;0.9	2.11 br ddd 4.6;2;0.9	1.76 br dd 3.7;1.5	1.76 br d 4.2	2.44 br dd 9.6;4.7
H-12	6.04 ddt 15.6;1.7;0.9	6.04 ddt 15.6;1.7;0.9	5.76 d 15.7	5.70 d 15.6	5.57 d 15.2
H-13	5.81 dd 15.6;5.9	5.82 dd 15.6;5.9	6.72 dd 15.6;11	6.68 dd 15.6;11	6.60 dd 15.2;10.8
H-14	3.91 dd 5.9;1.7	3.92 dd 5.9;1.7	5.90 br d 11	5.88 br d 11	5.85 br d 10.8
Me-16	1.18 d 0.6	1.18 d 0.6	1.81 br s	1.80 br s	1.79 br s
Me-17	1.09 dd 0.6;.03	1.09 dd 0.6;0.3	1.81 br s	1.81 br s	1.81 br s
Me-18	1.34 t 0.9	1.34 t 0.9	1.65 br s	1.71 br s	1.65
H-19	5.27 br s	5.27 br s	5.24 br s	5.26 br s	5.09 br s
H-19'	5.12 br s	5.12 br s	4.94 br s	4.92 br s	5.05 br s
OAc				2.06 s, 2.07 s	2.06 2s

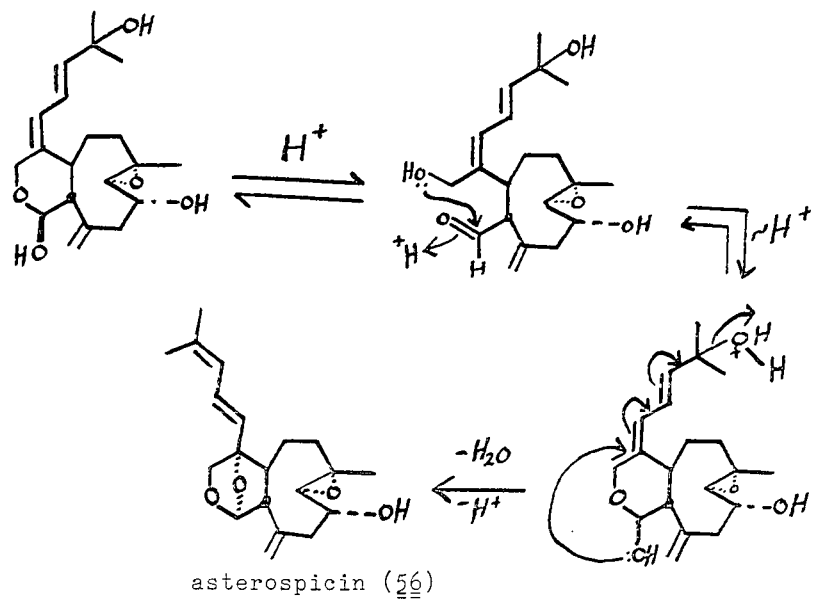
Table 19. ^{13}C NMR Chemical Shifts (ppm) of the Asterospicins*

C#	56	57	58	59	60	61	62
C-1	105.64 d	105.53 d	105.71 d	105.71 d	105.63 d	105.67 d	104.40 d
C-3	67.37 t	67.34 t	67.25 t	67.19 t	67.19 t	67.21 t	93.51 d
C-4	88.26 s	88.25 s	87.37 s	87.39 s	87.78 s	87.76 s	90.10 s
C-4a	55.62 d	55.86 d	55.59 d	55.61 d	55.44 d	55.44 d	55.10 d
C-5	27.49 t	27.36 t	27.37 t	27.37 t	27.23 t	27.31 t	28.75 t
C-6	39.82 t	39.82 t	39.72 t	39.72 t	39.58 t	39.62 t	39.82 t
C-7	58.58 s	58.23 s	58.47 s	58.47 s	58.79 s	58.77 s	132.26 s
C-8	66.82 d	63.38 d	66.74 d	66.74 d	66.91 d	66.87 d	130.30 d
C-9	69.36 d	72.34 d	69.34 d	69.34 d	68.71 d	68.88 d	67.10 d
C-10	44.88 t	42.49 t	44.86 t	44.86 t	44.75 t	44.75 t	46.86 t
C-11	146.11 s	145.62 s	145.92 s	145.90 s	145.86 s	145.90 s	147.80 s
C-11a	60.55 d	60.48 d	60.46 d	60.46 d	60.31 d	60.37 d	60.26 d
C-12	122.86 d	122.79 d	130.01 d	130.00 d	133.46 d	133.30 d	121.06 d
C-13	130.22 d	130.20 d	128.99 d	129.00 d	125.80 d	126.29 d	131.00 d
C-14	124.14 d	124.10 d	63.18 d	63.22 d	78.29 d	78.76 d	124.35 d
C-15	138.43 s	138.47 s	57.45 s	57.45 s	72.40 s	72.43 s	138.12 s
C-16	18.52 q	18.51 q	24.56 q	24.56 q	25.92 q	26.26 q	18.56 q
C-17	26.09 q	26.08 q	18.70 q	22.77 q	23.56 q	23.82 q	26.07 q
C-18	17.02 q	17.05 q	17.01 q	17.01 q	16.86 q	16.93 q	16.95 q
C-19	115.60 t	116.17 t	115.68 t	115.68 t	115.54 t	115.63 t	114.17 t
OAc		171.00 s					
		21.28 q					

*Spectra were recorded in $CDCl_3$ at 75.4 MHz with Me_4Si as internal standard. The values are given in δ units. Assignments of multiplets were made by DEPT experiments. Spectra of 60 and 61 were obtained in 5% CD_3OD in $CDCl_3$.

Table 20. Results of NOEDS Experiment with Asterospicin (56) in $CDCl_3$ solution at 300 MHz.

Proton(s) irradiated	Proton(s) enhanced	%Enhancement
H-1	H-11a	7
H-11a	Me-18	3
	H-1	9
H-3'	H-3	25
	H-5	6
	H-11a	4
H-8	H-4a	10
H-4a	H-8	12
	H-19	2
	H-12	4
H-5'	H-3'	4
	H-11a	3
Me-18	H-11a	4
	H-9	9



asterospicin (56)

SCHEME 5

7,8 was assigned on the basis of te Overhauser enhancement observed between Me-18 and H-9 and the lack of any NOE between Me-18 and H-8. The trans configuration of the ring fusion was established by the following: (a) the observation of an NOE effect between H-8 and H-4a; (b) the Overhauser enhancements observed between Me-18, H-9, and H-11a; (c) the similarities of the coupling constants ($J_{4a,11a}$ = 4.5 Hz) observed for 56 and 7,8-dihydro-7α,8α-epoxyxeniolide-A (50) (see Table 18); (d) the absence of any NOE between H-11a and H-4a. The α configuration of the C-1 to C-4 oxygen bridge was assigned on the basis of the W-coupling (J = 1.9 Hz) observed between H-3 and H-4a and the Overhauser enhancement between H-3', H-5', and H-11a. In 4β,1β-configuration, none of these Overhauser effects or the W-coupling between H-3 and H-4a would be expected.

The structure of 56 was confirmed further when it was found that treatment of 7,8-dihydro-7α,8α-epoxyxenialactol (44) with acetic acid, produced compound 56. The mechanism of the latter conversion is shown in Scheme 5

Structure elucidation of 14,15-Dihyro-14ξ,15ξ-epoxyasterospicin-1 (58) and 14,-15-Dihydro-14ξ,15ξ-epoxyasterospicin-2 (59):

The $CHCL_3$ extract of Scheme 1 was chromatographed over silica gel. Repeated chromatography on silica gel of fractions 3-7 (Scheme 2) led to an 8 mg fraction that when subjected to HPLC gave 3 fractions, one of which was resolved by HPLC using a reverse-phase C_{18} column and MeOH-H_2O (6:4) to yield two fractions, 0.3 mg each, of pure 14,15-dihydro-14ξ,15ξ-epoxyasterospicin-1 (58) and 14,15-dihydro-14α,15α-epoxyastero-

spicin-2 (59) as colorless oils. The molecular formula $C_{20}H_{28}O_5$ (7 unsaturations) was obtained for 58 from the following spectral data: (a) the highest mass peak in the low resolution mass spectrum was observed at 348 corresponding to M^+; (b) the ^1H NMR spectrum (Figure 48) contains 27 non-exchangeable and one exchangeable protons; (c) the IR spectrum showed a broad hydroxyl band at 3500 cm^{-1}; (d) the ^{13}C NMR spectra of 58 indicated the presence of 20 carbons including a carbon deshielded by a secondary hydroxyl group,* 69.34 ppm (C-9, d), 4 other carbons deshielded by oxygens (corresponding to two epoxide groups), 58.47 ppm (C-7, s), 66.74 (C-8, d), 63.18 (C-14, d), 57.45 (C-15, s), and an acetal carbon. The ^{13}C NMR spectra of 58 revealed the existence of 3 methyls, 4 sp^3 methylenes and one exocyclic terminal methylene, 6 sp^3 methines and 2 sp^2 methines, and 4 quaternary carbons. Analysis of the ^1H decoupling experiments suggested that 58 possesses the same carbon framework as asterospicin except that 58 contains an epoxide group at C-14,15 rather than a double bond in asterospicin. The ^1H and ^{13}C NMR data of 58 and asterospicin are similar except for some differences due to the presence of an epoxide group at C-14,15 in 58 (see Table 22).

Three methyl singlets were found in the ^1H NMR spectrum of 58, one of which was assigned to the epoxide methyl group at C-7 on the basis of the observation of a w-coupling between it (Me-18; 1.36 ppm) and H-6' (1.05) and a W-coupling between Me-18 and H-8. The other two methyl singlets were assigned to the geminal dimethyl groups at C-15 since irradiation of H-14 (3.26) ppm in 58 sharpened the two methyls at 1.26

*assignment was made by analogy to other compounds in this series.

Table 21. ^{1}H and ^{13}C NMR chemical shifts of some signals in 58 and asterospicin (spectra of both compounds were taken under the same experimental conditions).

H#	58	56
12	6.12	5.73 ppm
13	5.73	6.47
14	3.26	5.86
16	1.36	1.80
17	1.26	1.78

C#	58	56
12	130.01 ppm	122.86
13	128.99	130.22
14	63.18	124.14
15	57.45	138.43
16	24.26	18.52
17	18.70	26.09

Table 22. ^{1}H NMR chemical shifts for some signals in 58 and 59 in $CDCl_3$ (spectra of 58 and 59 were obtained under the same experimental conditions).

H#	58	59
13	5.73 ppm	5.71
14	3.26	3.27
16	1.26	1.28
3	3.32	3.34

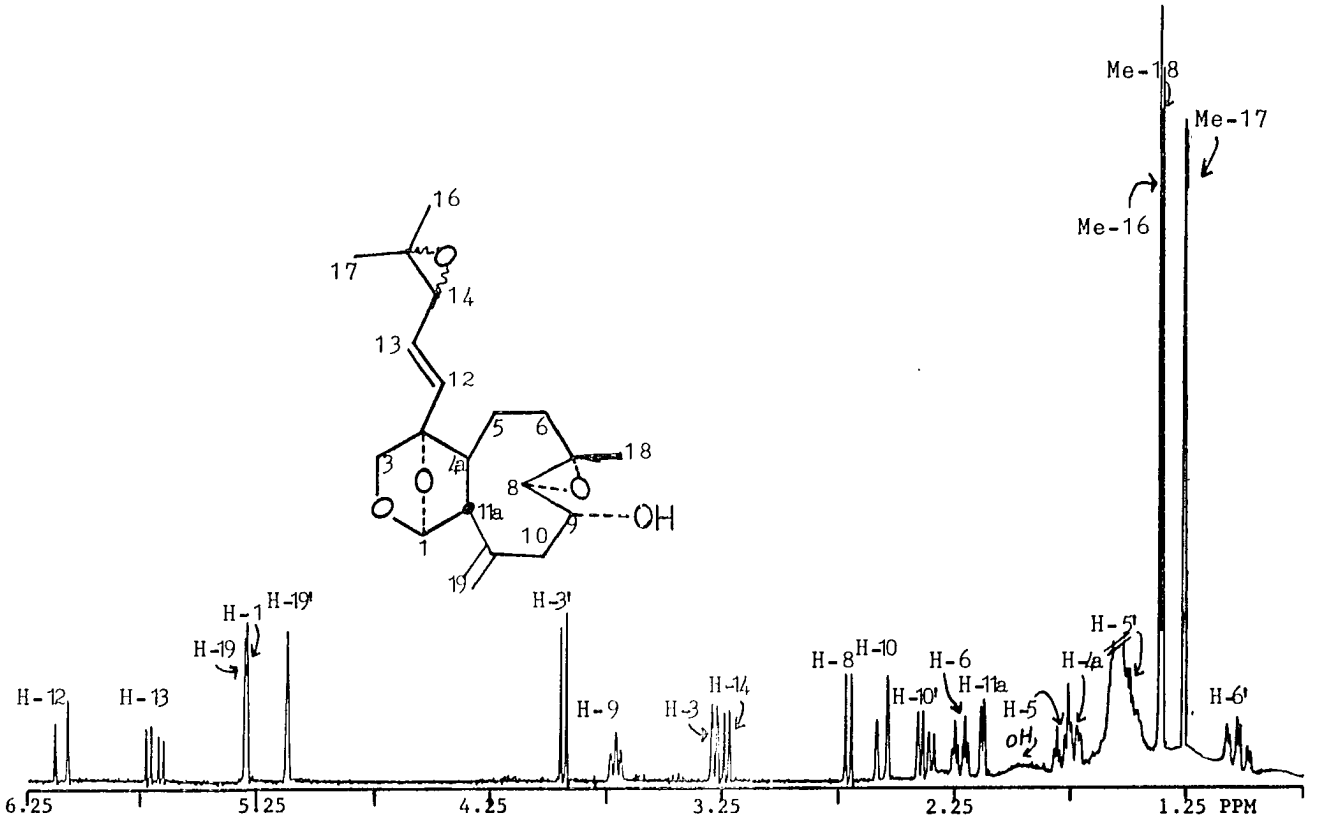


Figure 48. 300 MHz proton NMR spectrum of 58 in $CDCl_3$.

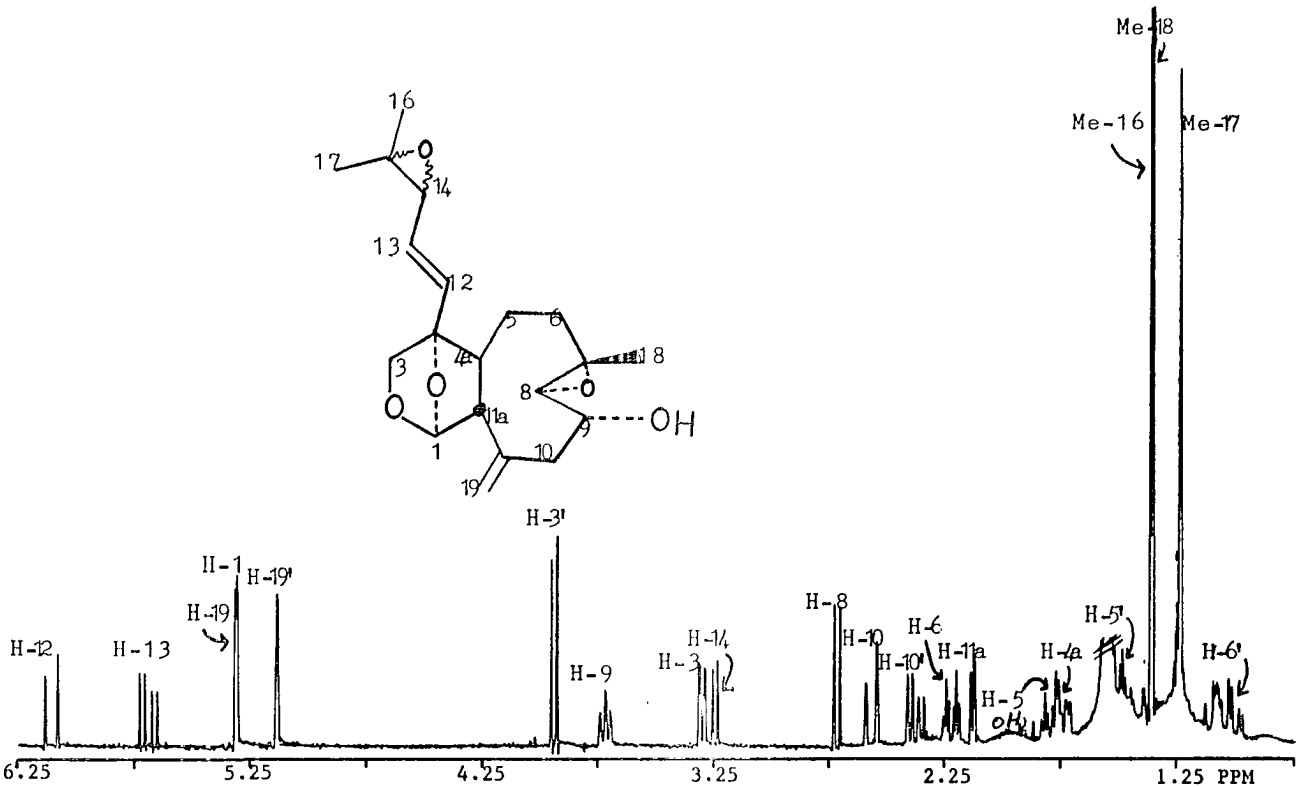


Figure 49. 300 MHz proton NMR spectrum of 59 in $CDCl_3$.

and 1.37 ppm and caused a removal of a vicinal coupling from H-13 signal and also removed an allylic coupling from H-12 signal. Hence, a structure like asterospicin, but with a modified side chain, was established for 58.

The mass spectrum of 59 was identical to that of 58. This suggested that both 58 and 59 possessed the same molecular formula. Moreover, the ^{13}C NMR spectra of 58 and 59 are nearly identical (see Table 19) except that the Me-17 signal in 58 was observed at 18.7 ppm while in 59 at 22.77. The ^{1}H NMR spectra of 58 and 59 (see Figures 48 and 49) are virtually identical except for some very minor differences in respect to the ^{1}H NMR chemical shifts of four proton signals, H-3, H-13, H-14, and H-17 (see Table 22).

Based on the above evidence we concluded that compounds 58 and 59 are epimers in regard to their C-14 configuration. The trans configuration of the double bond at C-12,13, in 58 and 59 was assigned on the basis of the magnitude of the coupling constant ($J_{H_{12,13}}$ = 15.4 Hz). The remaining sterochemical features of 58 and 59 are assumed to be identical to those proposed for asterospicin (56), since the ^{1}H NMR chemical shifts and J values and ^{13}C NMR data (see Tables 18 and 19) are virtually identical for these three compounds (56, 58, and 59) except as noted. The configuration at C-14 was not determined for 58 or 59.

Structure elucidation of 14,15-dihydro-14ξ,15-dihydroxyasterospicin-1 (60) and 61:

Silica gel chromatography of the $CHCL_3$ extract of Scheme 1 yielded 9 fractions of which 8 and 9 were combined and rechromatographed on a

column of silica gel to give 7 fractions. The most polar fraction was subjected to HPLC using a silica gel column and hexane-acetone (1:1) to give a 3.5 mg of colorless oil* which was resolved further into 2 pure compounds 60 and 61 by HPLC with a reverse-phase C_{18} column and MeOH-H_2O (25:75) as eluent. The molecular formula $C_{20}H_{30}O_6$ (6 unsaturations) was deduced for 60 from the following spectral data: (a) the molecular ion was observed in the field-desorption spectrum at m/z 366 (low resolution); (b) the high resolution mass spectrum contained a peak corresponding to M^+-18 at m/z 348.1909 which was consistent with the formula $C_{20}H_{28}O_5$ (calcd, 348.1937); (c) the ^1H NMR spectrum (Figure 50) showed signals for 27 non-exchangeable and 3 exchangeable protons; (d) IR absorption was observed at 3500 cm^{-1} (OH) group(s); (e) the ^{13}C NMR data revealed 20 carbons including an acetal carbon, 105.63 ppm (C-1, d), epoxide carbons, 58.79 (C-7, s), 66.91 ppm (C-8, d), and 3 carbons each deshielded by a single oxygen, 68.71 ppm (C-9, d), 78.29 (C-14, d), and 72.40 (C-15, s). The ^{13}C NMR spectra (Figure 51) indicated the presence of 3 methyls, 4 sp^3 methylenes and one exocyclic terminal ethylene, 6 sp^3 methines and 2 sp^2 methines, and 4 quaternary carbons. The ^1H and ^{13}C NMR data of 60 and 61 are very similar (see Figures 50a, 51 and 52) except for very minor differences (see Table 23) in the chemical shifts due to the configuration of the hydroxyl group at C-14. Comparison of the ^1H and ^{13}C NMR data of 60 and asterospicin (56) (see Tables 18 and

*This oily mixture of 60 and 61 was soluble in $CHCL_3$ solution but after resolving this oily mixture into 60 and 61 by HPLC, pure 60 and 61 were slightly soluble in $CHCL_3$. For this reason, all ^1H and ^{13}C NMR spectra were obtained in a mixture of 5% CD_3OD in $CDCL_3$. However, 60 and 61 are soluble in excess of $CDCL_3$ (see Experimental for ^1H NMR data of 60 and

Table 23. (a) ^{1}H NMR Chemical Shifts for some Signals of 60 and 61 in 5% CD_3OD in $CDCl_3$. (b) ^{13}C NMR Chemical Shifts for some Signals of 60 and 61.

(a) H#	60	61	(b) C#	60	61
3	3.28 ppm	3.29 ppm	12	133.46 ppm	133.30
13	5.81	5.82	13	125.80	126.29
14	3.91	3.92	14	78.29	78.76
			16	25.92	26.26
			17	23.56	23.82

Table 24. (a) ^{1}H NMR Chemical Shifts for some Signals of 56 and 60 in $CDCl_3$. (b) ^{13}C NMR Chemical Shifts for some Signals of 56 and 60.

(a) H#	56	60	(b) C#	56	60
12	5.73ppm	6.08 ppm	4	88.26 ppm	87.78 ppm
13	6.47	5.86	12	122.86	133.46
14	5.86	4.0	13	130.22	125.80
16	1.80	1.15	14	124.14	78.29
17	1.78	1.25	15	138.43	72.40
			16	18.52	25.92
			17	26.09	23.56

19) suggested that both 60 and asterospicin (56) possess the same carbon skeleton and they differ only in the structure of the side chain at C-4. The ^{1}H and ^{13}C NMR chemical shift differences between 60 and asterospicin (56) are shown in Table 24. The structure of 60 was also confirmed by DDS, COSY, and homonuclear 2-DJ spectroscopy (see Figures 50-54). The assignment of the geminal dimethyl groups at C-15 in 60 was established by a COSY experiment (see Figure 53b) in which off-diagonal peaks indicated a 4-bond spin coupling between the two methyl signals at 1.09 and 1.18 ppm.

The stereochemistries at C-4a, C-7,8, C-9, and C-11a were established by NOE experiments. The results of these experiments were similar to the NOE results obtained for asterospicin (56). Thus, 60 has the same stereochemistry as asterospicin (56). It was not possible to determine the configuration of the hydroxyl group at C-14 in 60 or 61 by NMR spectroscopy.

The proposed structures of 60 and 61 were further confirmed when xenialactol (8) was treated with m-chloroperbenzoic acid and 4 products were obtained, two of which were identified as 60 and 61, see Scheme 6. Compound 45 is 7,8-dihydro-7α,8α-epoxyxenialactol and contains a peroxide group at C-15. Compound 44 is the 7,8-dihydro-7α,8α-epoxyxenialactol (44), a natural product which is also isolated from this organism and discussed above. The formation of 60 and 61 from xenialactol (8) can be explained by the mechanism shown in Scheme 6.

The ^{1}H NMR spectrum (see Figure 55) of 45 was nearly identical to that of 44 except for some signals which appeared at slightly different chemical shifts, (H-12) 5.89 ppm, (H-13) 6.26, (H-14) 5.69, (Me-16 and

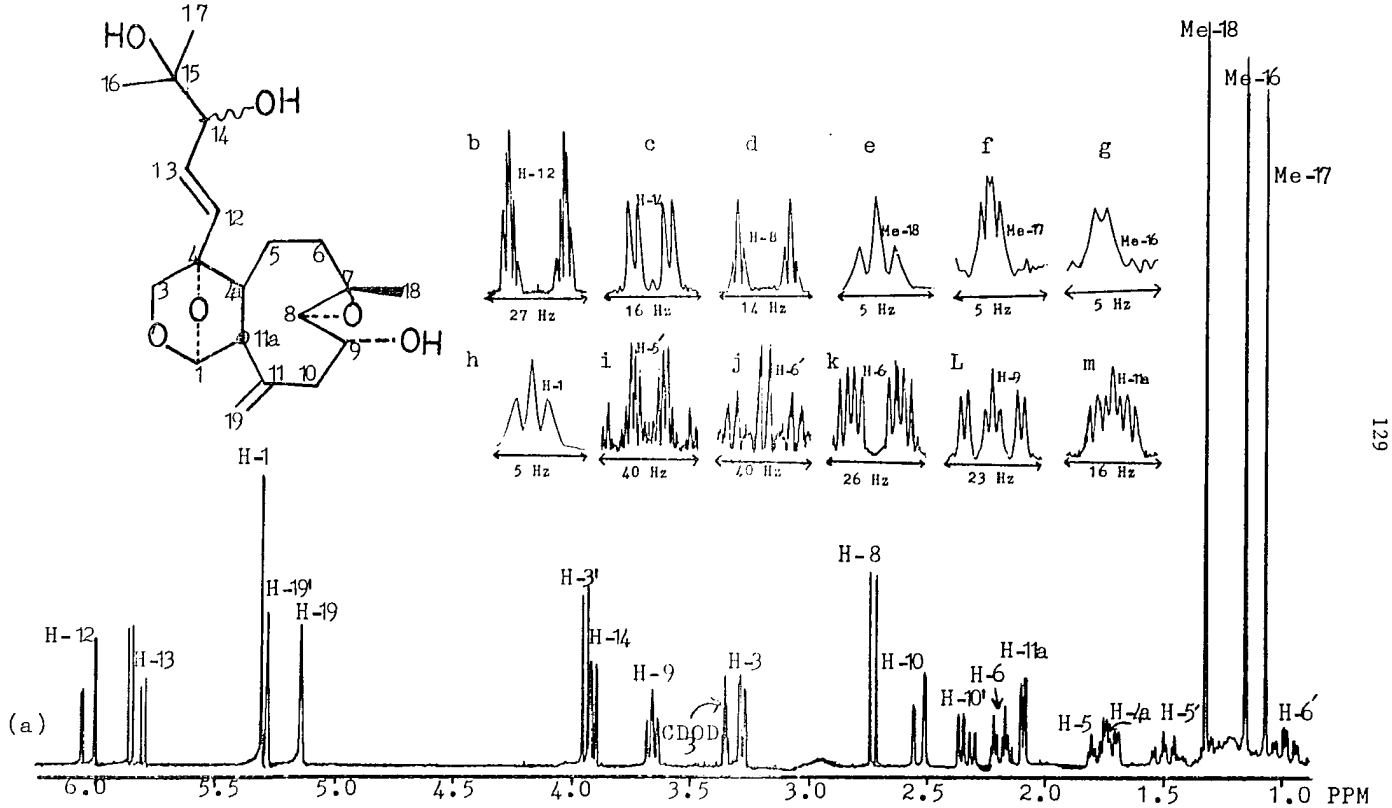


Figure 50. (a) 300 MHz ^{1}H NMR spectrum of 60 in $CDCl_3$ +5% CD_3OD. (b-m) Slices from homonuclear two-dimensional J-resolved (2DJ) spectrum in $CDCl_3$+5% CD_3OD.

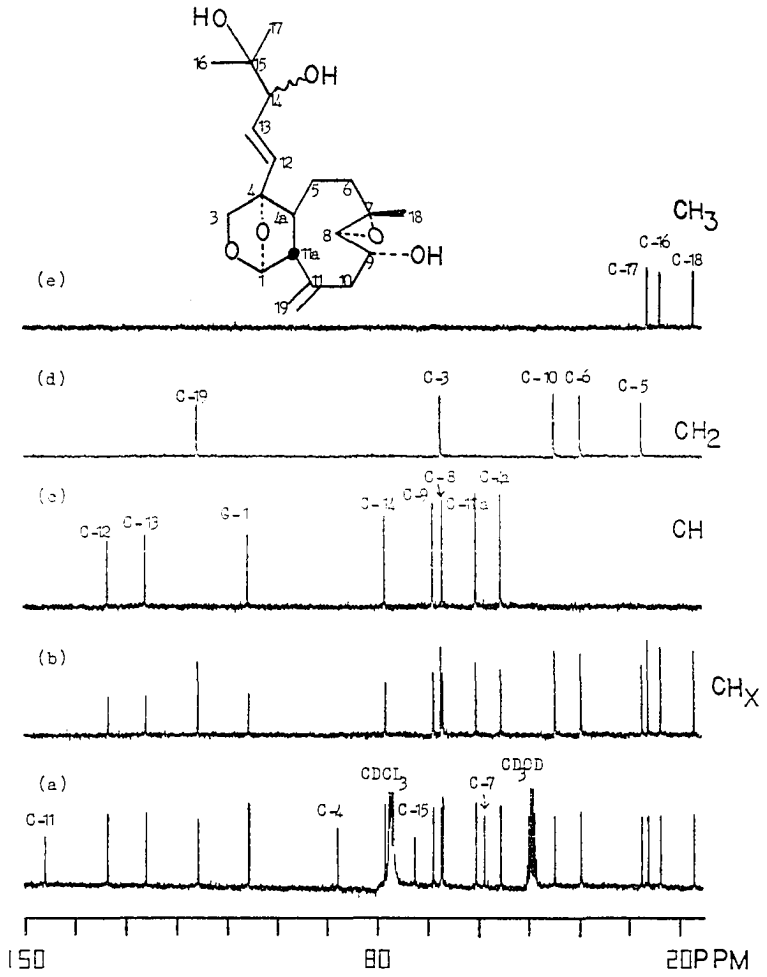


Figure 51. (a) 75.4 MHz broadband ^{1}H decoupled ^{13}C NMR spectrum of 60 in $CDCl_3$ + CD_3OD 5%. (b) all protonated carbons. (c) methine carbons. (d) methylene carbons. (e) methyl carbons. Spectra b-e were recorded at 75.4 MHz in $CDCl_3$ + CD_3OD 5% and resulted from a DEPT experiment.

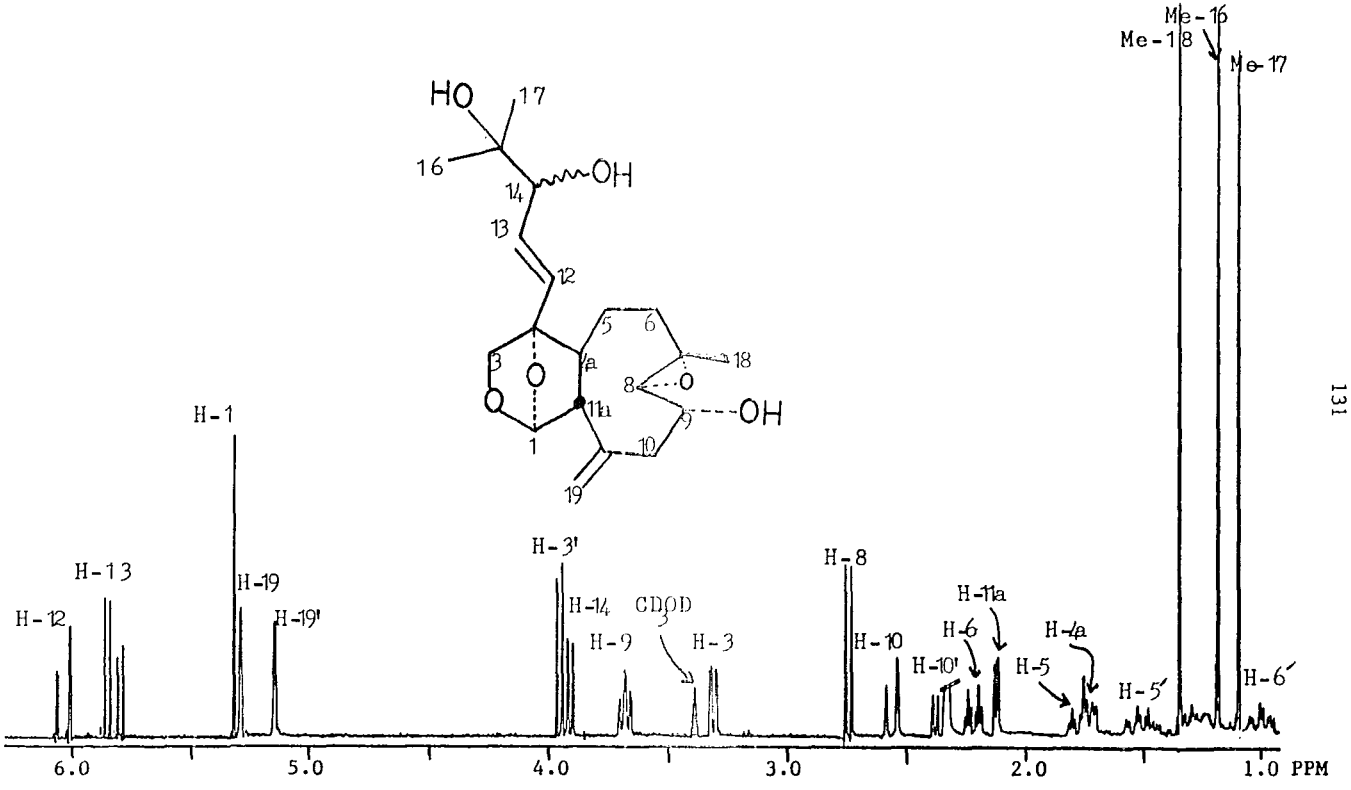


Figure 52. 300 MHz proton NMR spectrum of 61 in $CDCl_3$+5% CD_3OD.

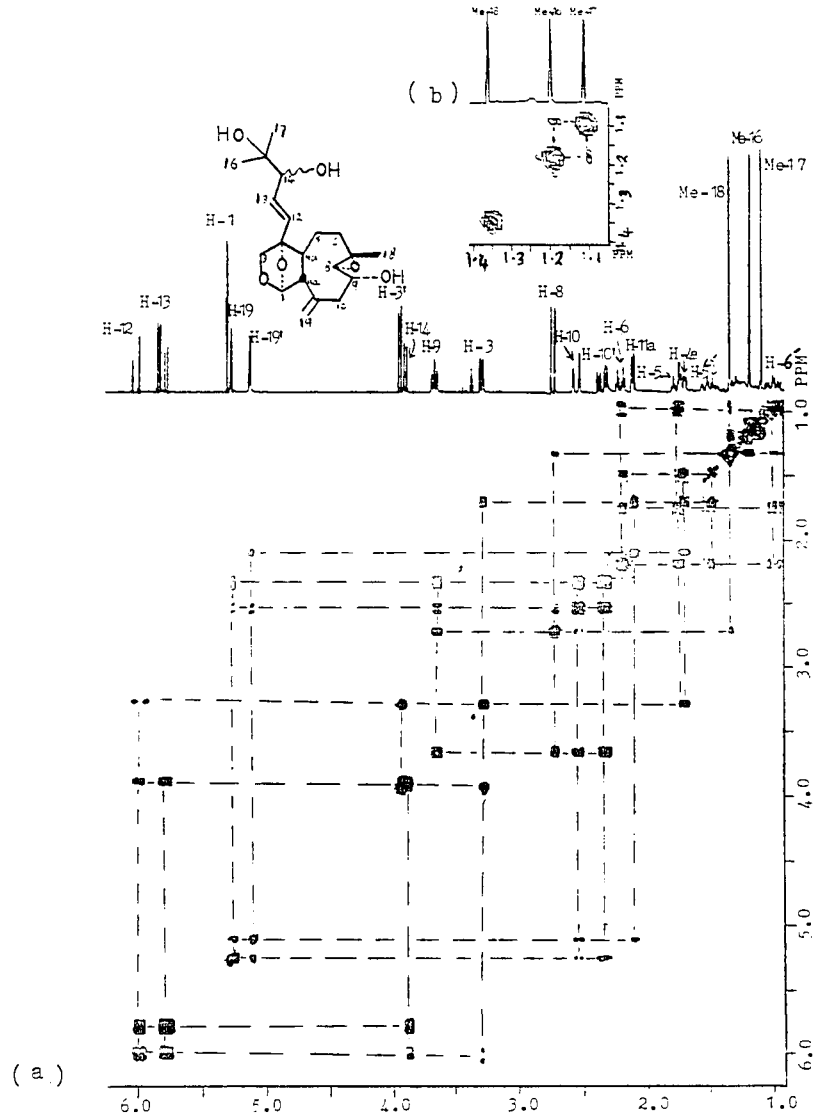


Figure 53. (a) Contour plot of the ^{1}H COSY spectrum of 60 in $CDCl_3$ + CD_3OD 5% at 300 MHz. Off-diagonal contours confirm direct proton spin-coupling connectivities. (b) Higher contour slices of the high-field region of 60. Off-diagonal cross peaks arise from the ^{4}J coupling between the gem-dimethyls of 60.

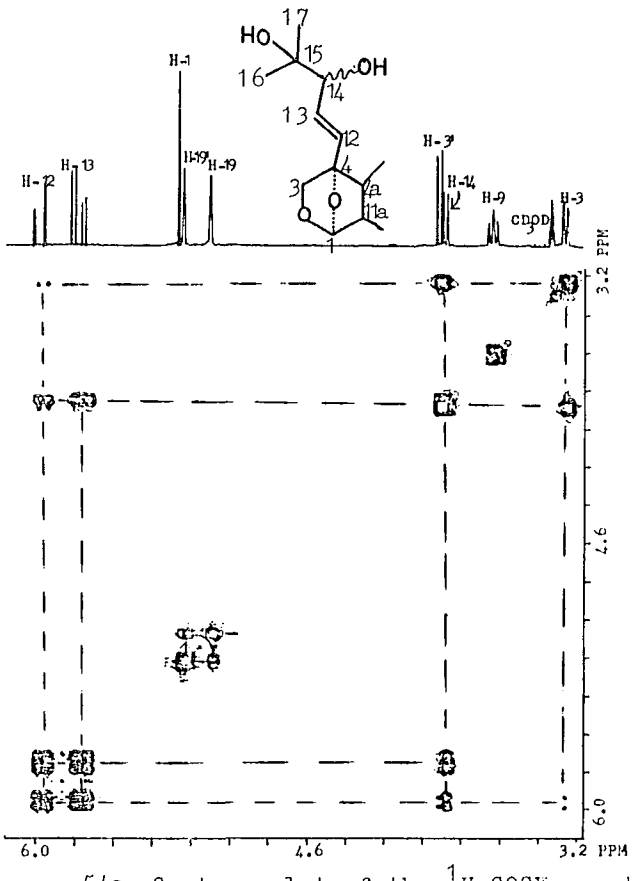


Figure 54a. Contour plot of the ^{1}H COSY spectrum (downfield region) of 60 in $CDCl_3$ + CD_3OD 5% at 300 MHz. Off-diagonal peaks establish direct proton spin-coupling connectivities.

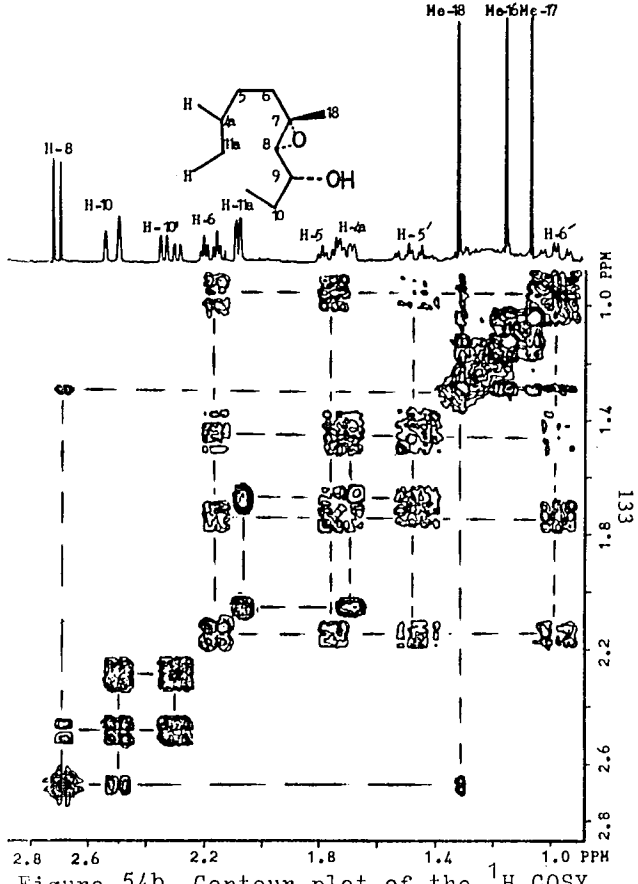


Figure 54b. Contour plot of the ^{1}H COSY spectrum (highfield region) of 60 in $CDCl_3$ + CD_3OD 5% at 300 MHz. Off-diagonal peaks establish direct proton spin-coupling connectivities.

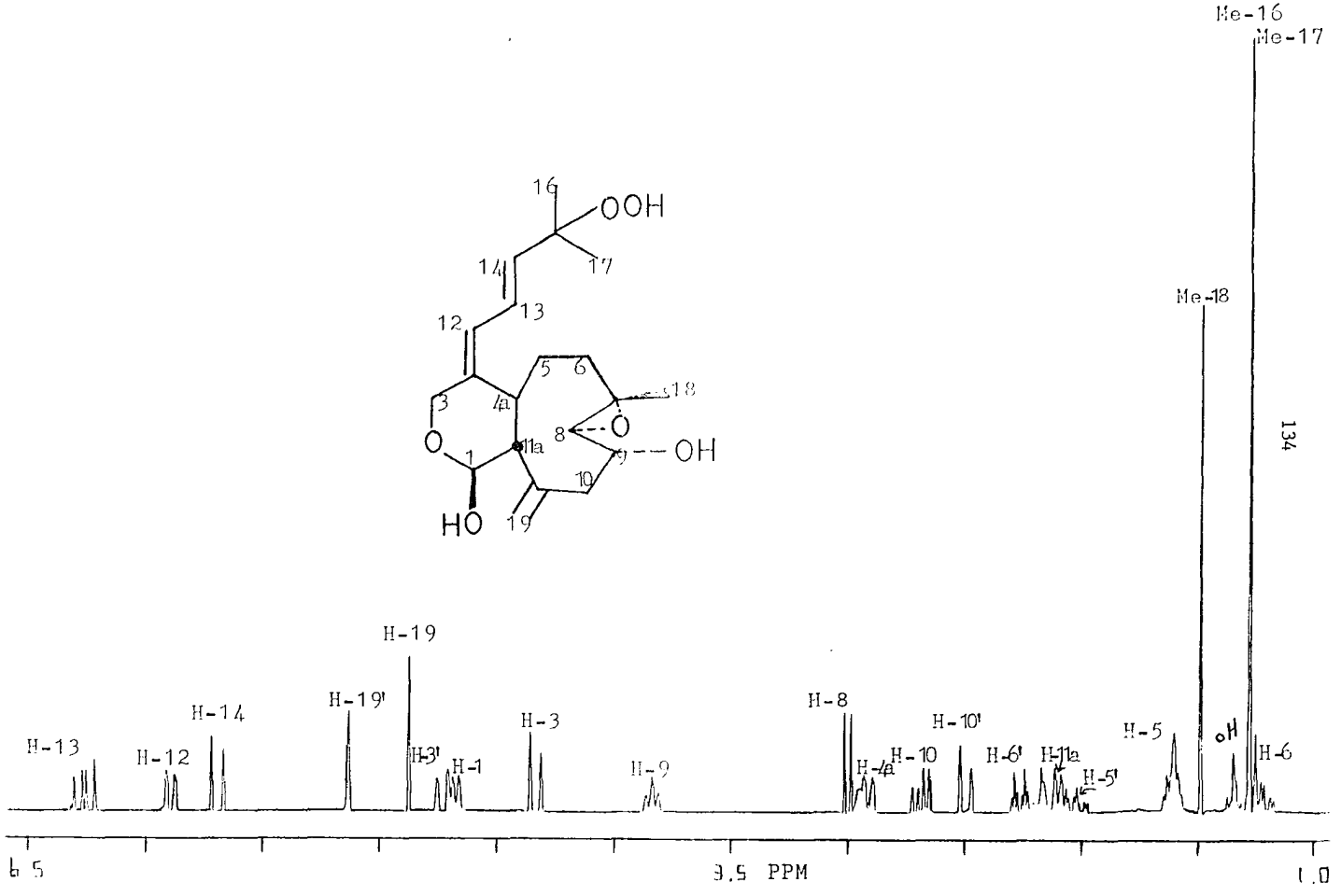


Figure 55. 300 MHz proton NMR spectrum of 45 in $CDCl_3$.

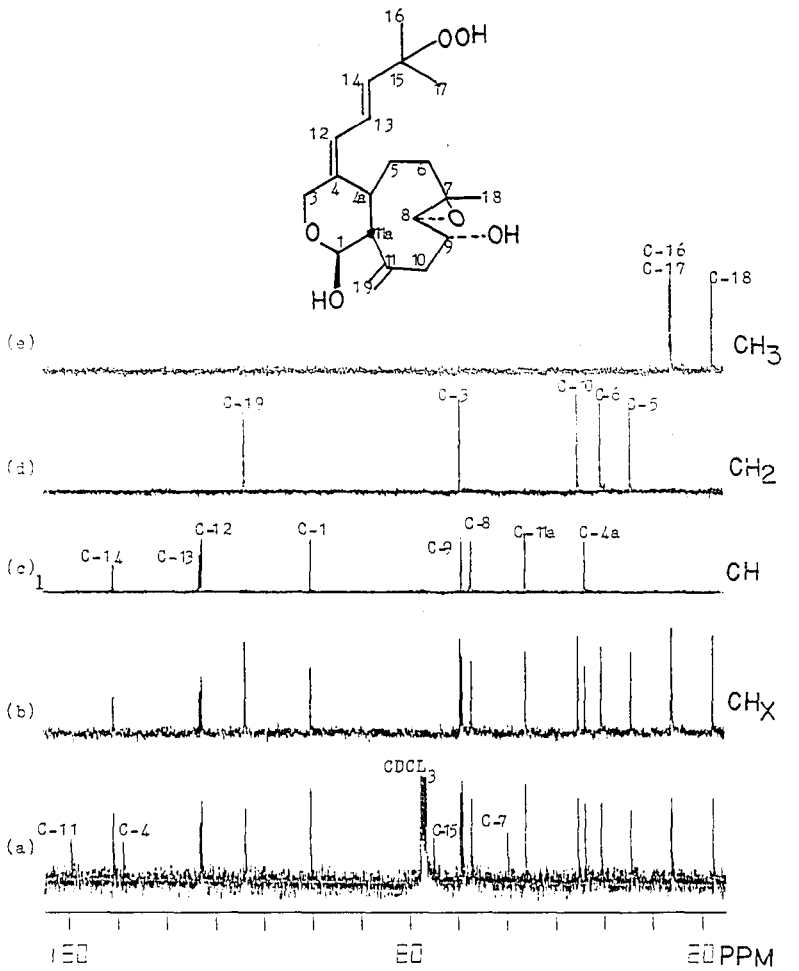
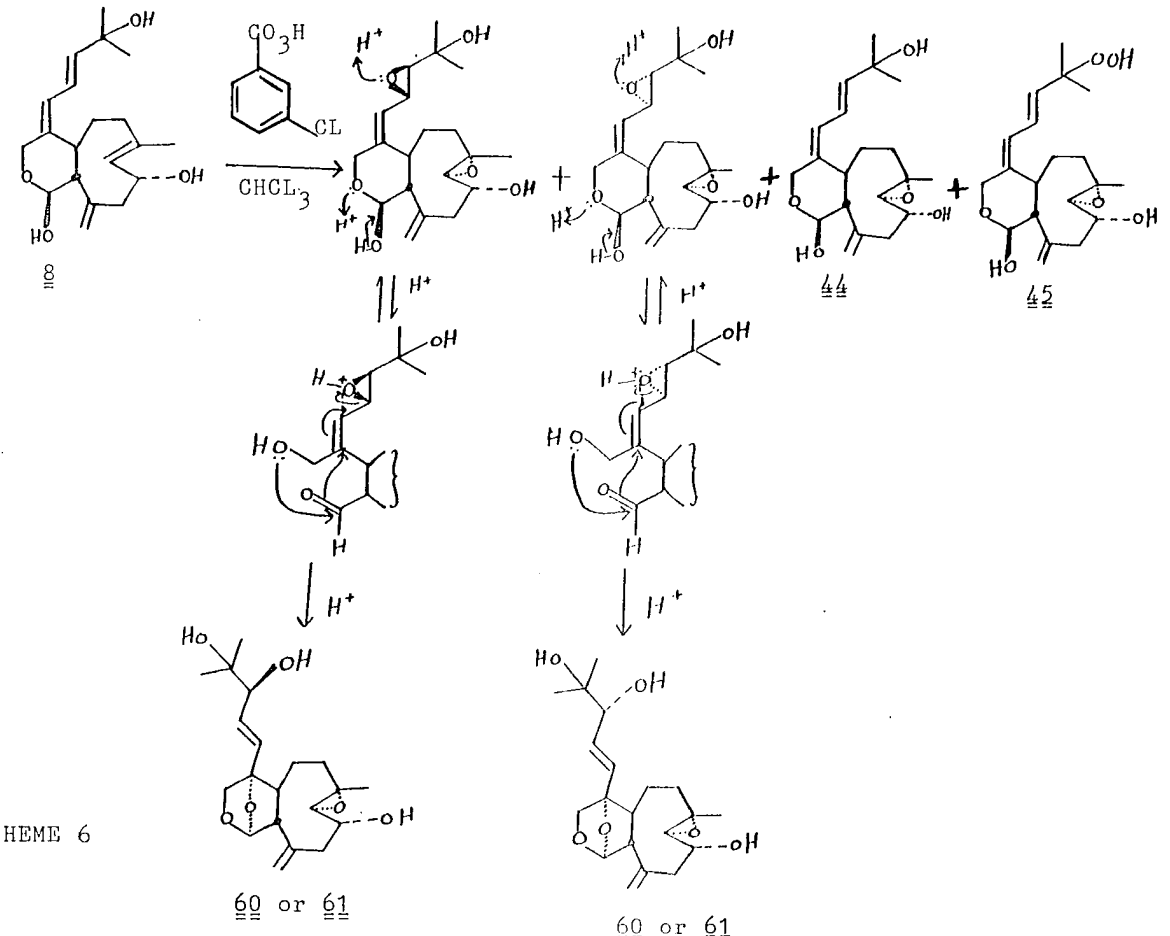


Figure 56. (a) 75.4 MHz broadband ^{1}H decoupled ^{13}C NMR spectrum of 45 in $CDCl_3$. (b) all protonated carbons. (c) methine carbons. (d) methylene carbons. (e) methyl carbons. Spectra b-e were recorded in $CDCl_3$ at 75.4 MHz and resulted from a DEPT experiment.



8

44

45

60 or 61

60 or 61

SCHEME 6

Me-17) 1.28. Hence, a structure like 44 could be postulated with a minor difference in the side chain. The ^{13}C NMR spectrum (see Figure 56) of 45 was also similar to that of 44 except for a shift in the C-14, C-15, C-16, and C-17 signals to 140.60, 74.83, 25.87, and 25.96 ppm, respectively. These shifts are consistent with the β-effects from having a peroxide group at C-15. Thus, the presence of peroxide group at C-15 in 45 was confirmed (see discussion of 49). Reaction of 45 with zinc dust in acetic acid in an attempt to reduce the peroxide group to an alcohol gave asterospicin (56). This can be explained, since in acidic solution 45 can undergo dehydration after reduction to give asterospicin (56).

Structure elucidation of 3-α-Hydroxy-7,8-deepoxy-7,8-dehydro-asterospicin (62):

The $CHCL_3$ extract, fraction F' of Scheme 3 was chromatographed over silica gel. One of the fractions eluted with hexane-acetone (7:3), fraction 14, was subjected to HPLC to give 2.2 mg of 3-α-hydroxy-7,8-deepoxy-7,8-dehydro-asterospicin (62) as a colorless oil; UV (EtOH) λ_{max} 235 nm (ε = 15119). The molecular formula $C_{20}H_{28}O_4$ (7 unsaturations) was established for 62 from the following data: (a) a peak corresponding to the molecular ion was detected in the field-desorption mass spectrum at m/z 332; (b) the high resolution mass spectrum exhibited an ion corresponding to M^+-30, m/z 302.18377 which is in agreement with the formula $C_{19}H_{26}O_3$ (calculated, 302.18820); (c) the ^{13}C NMR spectrum (Figure 57) exhibited signals for 20 carbons including a carbon deshielded by a secondary hydroxyl group,* 67.1 ppm (C-9, d) and a

hemiacetal carbon, 93.51 ppm (C-3, d) and an acetal carbon, 105.4 ppm (C-1, d); (d) the ^{1}H NMR spectrum (see Figure 58) showed signals for 26 non-exchangeable and 2 exchangeable protons; (e) IR absorption was observed at 3450 cm^{-1} (OH) group(s). The ^{13}C NMR spectra (Figure 57) revealed the existence of 3 methyls, 3 sp^3 methylenes and one exocyclic terminal methylene, 5 sp^3 methines and 4 sp^2 methines, and 4 quaternary carbons. The presence of hydroxyl groups were confirmed by the acetylation of 62 to give the expected 3,9-diacetate 63 in which two proton signals were shifted, H-9 signal from 4.69 to 5.61 ppm and H-3 signal from 5.04 to 6.10 ppm. In addition, the 1,9-diacetate 64 was obtained.

Analysis of the results of the ^{1}H difference decoupling spectroscopy (DDS) experiments (see Table 25) indicated that compound 62 possesses the same carbon skeleton as asterospicin (56). The ^{1}H and ^{13}C NMR data of 62 and asterospicin (see Tables 18 and 19) are similar except for some differences (see Table 26) which reveal that 62 possesses a hydroxyl group at C-3 and a double bond at C-7,8.

When 62 was acetylated using a mixture of acetic anhydride-pyridine, two diacetate derivatives were obtained (see Figures 59 and 60). The first diacetate 63 was the expected derivative but the second diacetate was identified as a rearranged product 64 (see Scheme 7). The structure of compound 64 was established by ^{1}H DDS experiments and by analysis of the low resolution mass spectrum data (see Scheme 8). The presence of an aldehyde group in 64 was confirmed because when the proton signal at 9.63 ppm was irradiated, the H-4a signal was sharpened and vice versa. The proton signal at 6.10 ppm was assigned to the

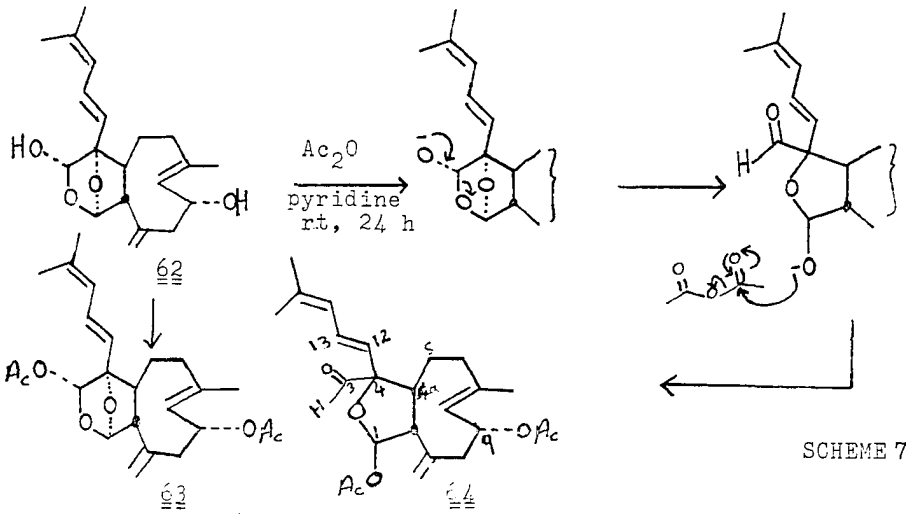
Table 25. Results of ^{1}H Difference Decoupling (DDS) Experiments* with (62)

Irradiated proton, chemical shifts, ppm, multiplicity (J, Hz)	effect of ^{1}H decoupling
H-13, 6.72 dd(15.6, 11)	H-14, 5.90 br d(11) ⟶ br s
	H-12, 5.76 d(15.6) ⟶ s
H-14, 5.90 br d(11)	H-13, 6.72 dd(15.6, 11) ⟶ d(15.6)
	Me-16, Me-17, 1.81 br s ⟶ sharpened
H-12, 5.76 d(15.6)	H-13, 6.72 dd(15.6, 11) ⟶ d(11)
H-1, 5.28 br s	H-11a, 1.76 br dd(3.7, 3) ⟶ sharpened
	H-4a, 1.74 br dd(10.5, 3.7) ⟶ sharpened
H-19', 5.24 d(1.3)	H-19, 4.94 br s ⟶ sharpened
	H-11a, 1.76 br dd(3.7, 3) ⟶ sharpened
H-8, 5.08 br d(6.7)	H-9, 4.69 br m ⟶ br t
H-3, 5.04 br d(12.1)	Me-18, 1.65 br s ⟶ sharpened
	H-6', 1.99 ddd(13.4, 12.3, 3.7) ⟶ sharpened
	OH, 2.56 br d(12.1) ⟶ br s
H-19, 4.94 br s	H-19', 5.24 d(1.3) ⟶ s
	H-10', 2.46 br dd(13.7, 5.4) ⟶ sharpened
	H-10, 2.42 br d(13.7) ⟶ sharpened
	H-11a, 1.76 br dd(3.7, 3) ⟶ sharpened
H-9, 4.69 br m	H-10, 2.42 br d(13.7) ⟶ sharpened
	H-10', 2.46 br dd(13.7, 5.4) ⟶ br d(13.7)
	H-8, 5.08 br d(6.7) ⟶ br s
OH, 2.56 br d(12.1), exchangable	H-3, 5.04 br d(12.1) ⟶ s
H-10', 2.46 br dd(13.7, 5.4)	H-9, 4.69 br m ⟶ br d(6.7)
	H-19, 4.94 br s ⟶ sharpened
	H-19', 5.24 d(1.3) ⟶ sharpened
H-6, 2.22 dt(12.3, 3.7)	H-6', 1.99 ddd(13.4, 12.3, 3.7) ⟶ dd(13.4, 3.7)
	H-5, 1.86 dt(13.4, 3.7) ⟶ dd(13.4, 3.7)
	H-5', 1.41 tdd(13.4, 10.5, 3.7) ⟶ td(13.4, 10.5)
H-6', 1.99 ddd(13.4, 12.3, 3.7)	H-6, 2.22 dt(12.3, 3.7) ⟶ t(3.7)
	H-5, 1.86 dt(13.4, 3.7) ⟶ dd(13.4, 3.7)
	H-5', 1.41 tdd(13.4, 10.5, 3.7) ⟶ ddd(13.3, 10.5, 3.7)
	H-8, 5.08 br d(6.7) ⟶ sharpened
H-5, 1.86 dt(13.4, 3.7)	H-5', 1.41 tdd(13.4, 10.5, 3.7) ⟶ ddd(13.4, 10.5, 3.7)
	H-6, 2.22 dt(12.3, 3.7) ⟶ dd(13.4, 3.7)
	H-6', 1.99 ddd(13.4, 12.3, 3.7) ⟶ dd(13.4, 12.3)
Me-16, Me-17, 1.81 br s	H-14, 5.90 br d(11) ⟶ sharpened
Me-18, 1.65 br s	H-8, 5.08 br d(6.7) ⟶ sharpened

*Spectra were recorded in $CDCl_3$ at 300 MHz with Me_4Si as internal standard. The values are given in ppm downfield from TMS.

Table 26. (a) ^{1}H NMR Chemical Shifts Differences between 62 and 56. (b) Carbon-13 NMR Chemical Shifts Differences between 62 and 56

(a) H#	62	56	(b) C#	62	56
3'	5.04	3.96	1	104.4	105.64
3	2.56 (OH)	3.35	3	93.51	67.37
5'	1.41	1.53	4	90.1	88.26
6'	1.99	1.05	7	132.26	58.58
8	5.08	2.75	8	131.0	66.82
9	4.69	3.74	9	67.1	69.36
10'	2.46	2.58			
11a	1.76	2.15			
13	6.72	6.47			
18	1.65	1.38			
19	4.94	5.14			
19'	5.24	5.33			
6'	1.99	1.05			



62 63 64

SCHEME 7

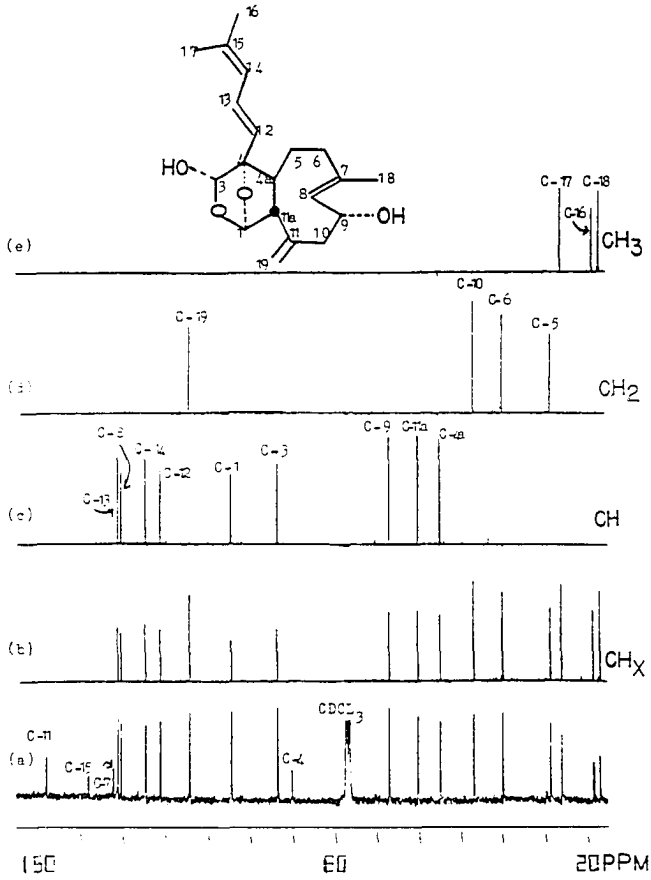


Figure 57. (a) 75.4 MHz broadband ^{1}H decoupled ^{13}C NMR spectrum of 62 in $CDCl_3$. (b) all protonated carbons. (c) methine carbons. (d) methylene carbons. (e) methyl carbons. Spectra b-e were recorded at 75.4 MHz in $CDCl_3$ and resulted from a DEPT experiment.

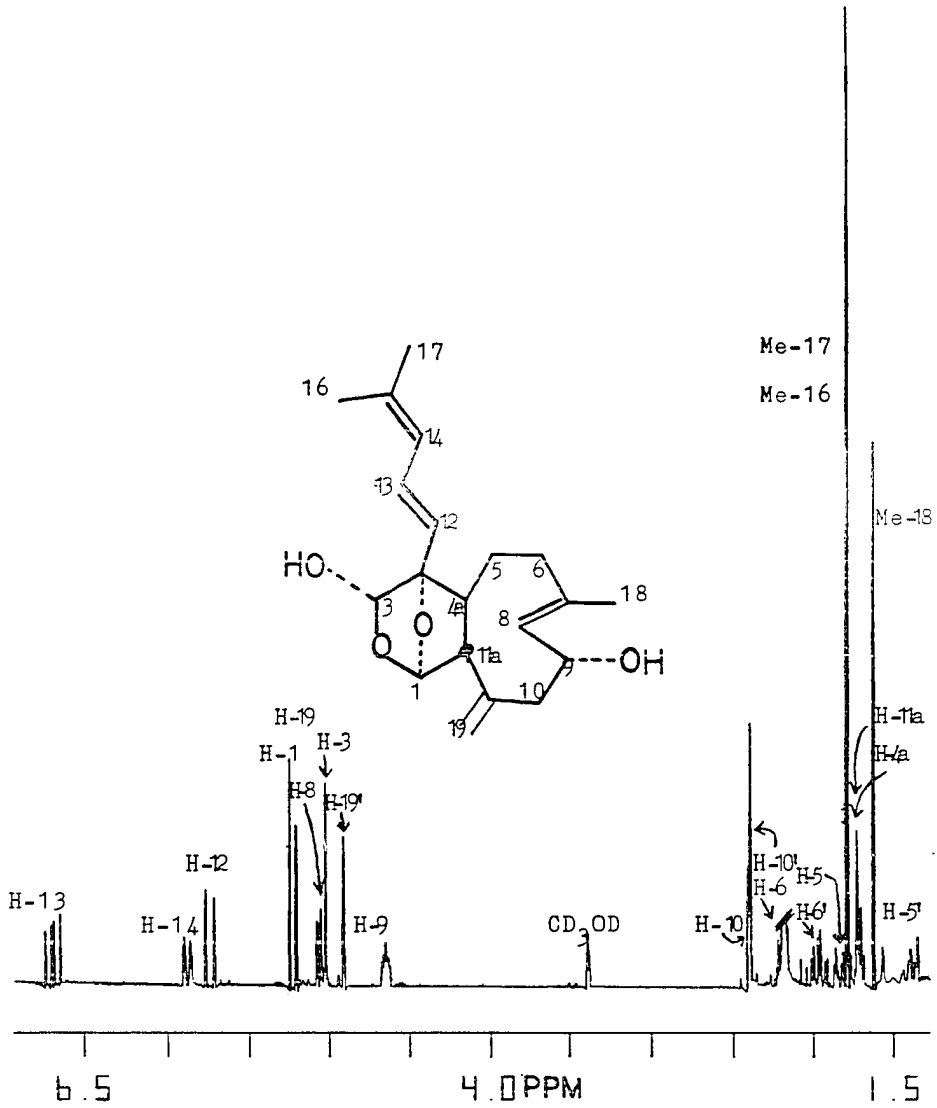


Figure 58. 300 MHz proton NMR spectrum of 62 in $CDCl_3$+ %5 CD_3OD .

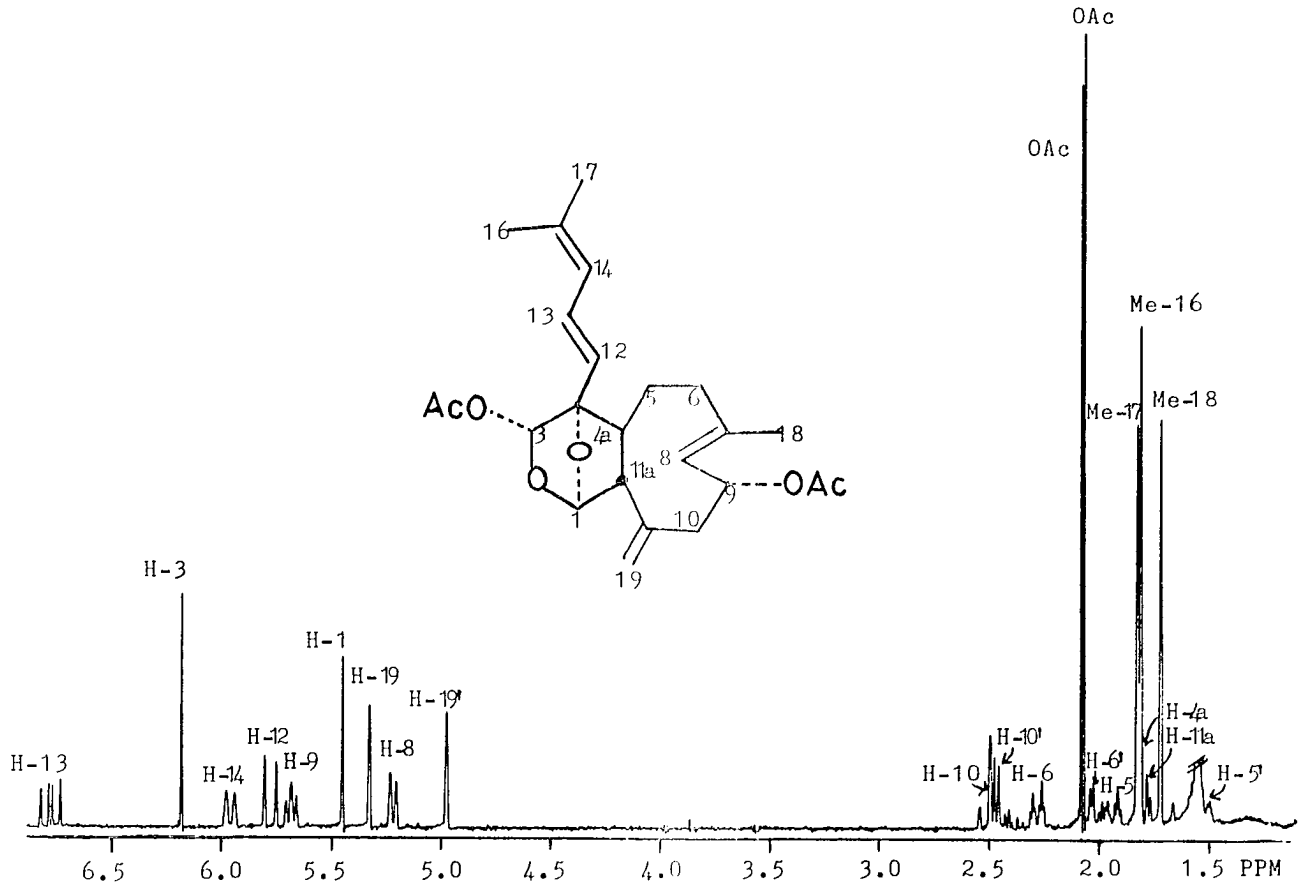


Figure 59. 300 MHz proton NMR spectrum of 63 in $CDCl_3$.

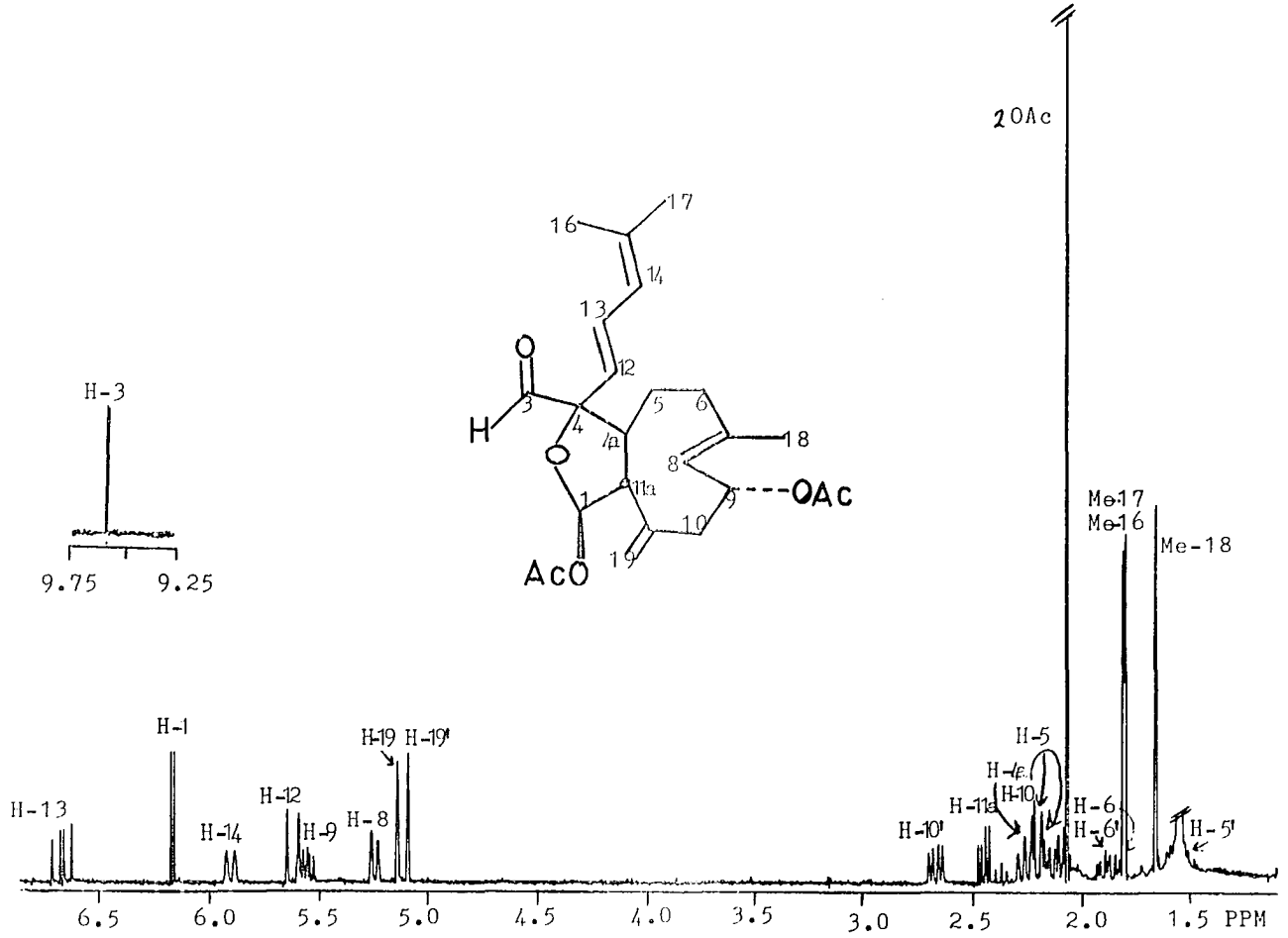
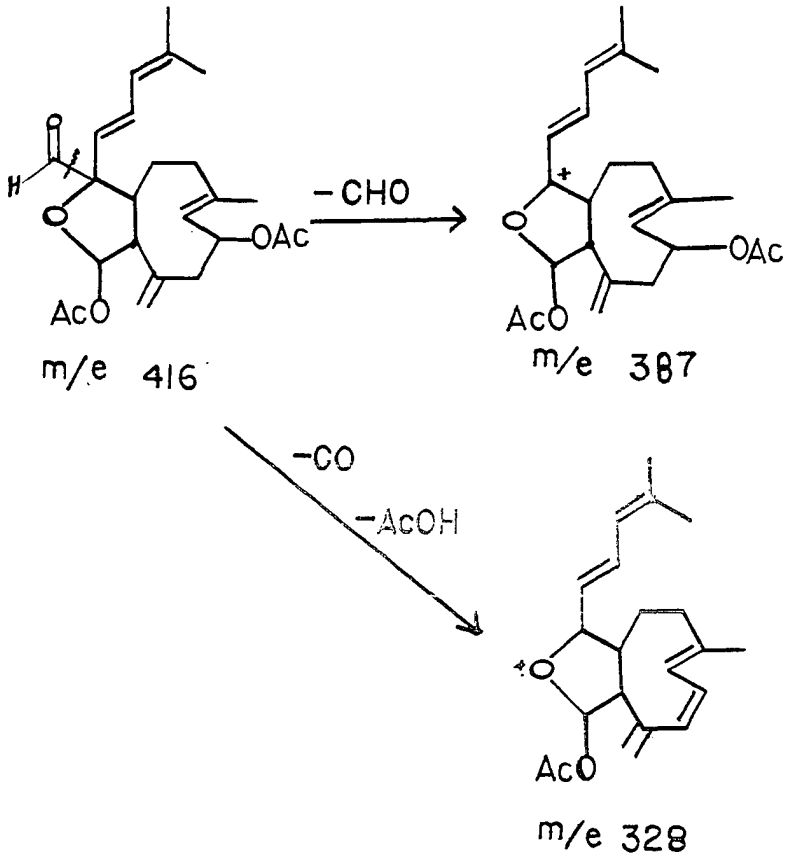


Figure 60. 300 MHz proton NMR spectrum of 64 in $CDCl_3$.

acetylated hemiacetal group (H-1) on the basis of the chemical shift observed for the H-1 signal between 62 and 64.

The sterochemical features of 62 were established by NOE experiments. The E configuration of the C-7,8 double bond was confirmed by the observation of an NOE effect between Me-18 and H-9 and the lack of any NOE between Me-18 and H-8. The trans ring fusion (H-11a, H-4a) was assigned because of the similarities of the J values observed for the coupling constants ($J_{11a,4a} \simeq 4$ Hz) and of the ^{13}C NMR chemical shifts of C-4a and C-11a (see Tables 18 and 19) for 62 and asterospicin. The α configuration of the hydroxyl group at C-3 was established on the basis of the observation of an NOE effect between H-3 and H-5' and the absence of any W-coupling between H-3 and H-4a. The α configuration of the bridge-head oxygen was also assigned by the Overhauser enhancement observed between H-3 and H-5'. Thus, the structure of 62 is fully elucidated.

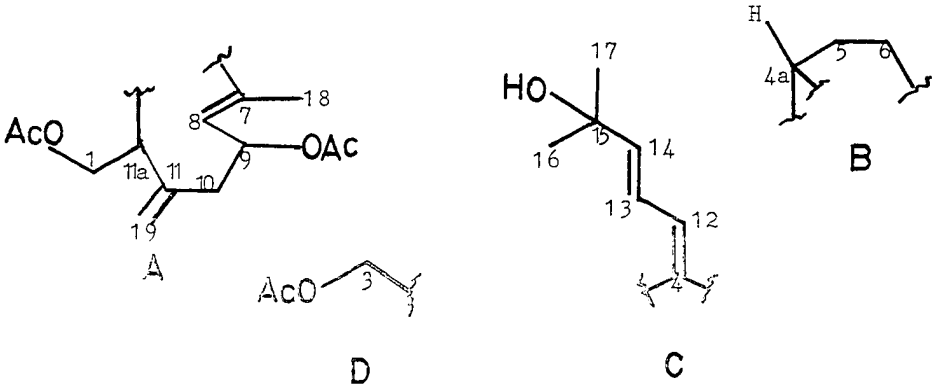


Scheme 8. Possible Mass Spectral Fragmentation Pathways for 64.

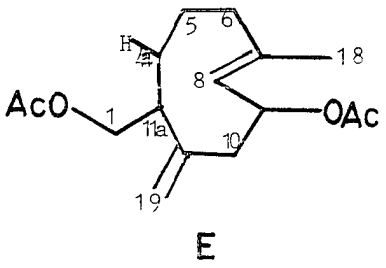
Structure elucidation of Asterospiculin (65):

The fourteenth fraction (250 mg) of Scheme 4 was chromatographed by HPLC using silica gel and hexane-acetone (82:18) to give 9 fractions. The fourth of these fractions was subjected to HPLC using a reverse-phase C_{18} column and MeOH-H_2O (7:3) as eluent to yield two fractions. The first fraction contained a known compound [13-epi-9-0-deacetyl-xenicin (3)]. The second fraction contained 90 mg of pure asterospiculin (65) as a viscous oil: $[\alpha]_D^{25}$-163.37° (c. 0.51, $CHCL_3$); UV (EtOH) λ_{max} 241 nm (ε = 15900). The molecular formula $C_{26}H_{38}O_7$ (8 unsaturations) was established for 65 from the analysis of the field-desorption mass spectrum (M^+ at 462, $C_{26}H_{38}O_7$) and high resolution mass spectrum (M^+-18, obs, 444.2518, calcd, 444.2512, $C_{26}H_{36}O_6$). The ^{13}C NMR spectra (Figure 61) revealed the presence of 6 methyls, 5 sp^3 methylenes and one exocyclic terminal methylene, 3 sp^3 methines and 4 sp^2 methines, and 4 quaternary carbons, in addition to 3 carbonyl carbons. The IR spectrum exhibited a broad hydroxyl band at 3450 and acetate(s) band at 1730 cm^{-1}. The ^1H, ^{13}C, and cross-heterocorrelated 2-D NMR spectra (Figures 61-64) of 65 indicated the existence of three acetate groups. The analysis of ^{13}C and cross-heterocorrelated 2-D NMR spectra (Figures 61 and 64) suggested the presence of 4 carbons each deshielded by a single oxygen, 72.44 ppm (C-9, d), 70.81 (C-15, s), 64.95 (C-1, t), and 60.95 (C-3, t). The three acetate groups were assigned to C-1, C-3, and C-9 on the basis of the ^1H NMR chemical shifts observed for (H-1 and H-1'), (H-3 and H-3'), and (H-9), respectively and by analogy to other compounds in this series. The hydroxyl group in 65 was connected to C-15 on the basis of the similarities of the ^{13}C NMR chemical shifts

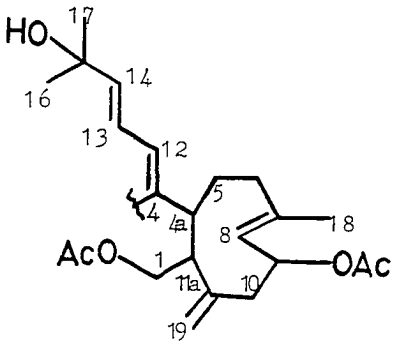
observed for C-15 in 65 and in other compounds of this series. (see Tables 28, 8, and 5). Analysis of the results of the ^{1}H difference decoupling (DDS) and ^{1}H COSY experiments (see Figure 63) suggested that 65 contained 4 double bonds (—CH=CH-CH=C<, —CH=C<(Me), CH_2=C<) incorporated in partial structures A and C while the remaining carbons were associated with partial structures B and D.



H-8 in partial structure A was found to be coupled allylically to H-6 (see Figure 63) in partial structure B. Therefore, C-7 was connected to C-6. The proton signal at 2.27 ppm (H-4a) was coupled to H-11a signal ($J_{11a,4a}$ = 10.7 Hz). This suggested that C-11a could be joined to C-4a (see Figure 63). Thus, a 9-membered-ring carbocyclic partial structure E was constructed.



In the COSY spectrum (Figure 63) of 65, off-diagonal contours clearly indicate a small allylic coupling between H-4a and H-12. This proved C-4a, C-4 linkage and extended partial structure E into F.



F

The proton signals at 4.75 ppm (H-3) and 4.69 (H-3') which comprise an AB spin system (partial structure D) were found to have allylic couplings with H-12 signal as shown in COSY spectrum (Figure 63). Therefore, C-4, C-3 connection was fixed and the structure of 65 is established.

The stereochemical features of 65 were established by NOE experiments (see Table 29). The E configuration of C-7,8 double bond was confirmed by the observation of an NOE effect between Me-18 and H-9 signals and the absence of any enhancement between Me-18 and H-8 signals. Overhauser enhancements were also observed between H-9, H-11a, and Me-18. This suggested that H-9, H-11a, and Me-18 are on the same face (β face). Irradiation of H-4a signal enhanced both H-8 and H-12 signals. This observation led to the conclusion that H-4a and H-8 are on the same face (α-face). The trans configuration of the ring fusion

Table 27. Proton NMR Data of Asterospiculins*

	65	66	67	68	69	70
H-1	4.02 dd 11.4;4.9	4.09 dd 11.2;4.8	4.05 dd 11.2;4.8	4.05 dd 11.2;4.8	4.19 dd 11.5;4.8	4.23 dd 11.5;4.7
H-1'	3.79 dd 10.7;11.4	3.82 t 11.2	3.80 t 11.2	3.83 t 11.2	3.76 t 11.5	3.83 t 11.5
H-3	4.69 d 12.6	4.70 d 12.7	4.68 d 13	4.71 d 13	3.86 br d 12.6	4.39 d 12.6
H-3'	4.75 d 12.6	4.77 d 12.7	4.75 d 13	4.80 d 13	3.98 br d 12.6	4.49 d 12.6
H-4a	2.27 br ddd 10.7;4.2;1.8	2.26 br dt 11.2;3.5	2.26 br dt 11.2;3.5	2.26 br dt 11.2;3.5	1.72 ddd 11.5;4.3;2.2	1.70 br dd 11.5;5
H-5	1.44 br t 13.5	1.73 br ddt 14.2;5.1;3.5	1.75 br ddt 14.2;5.1;3.5	1.75 br ddt 14.2;5.1;3.5	2.05 m	2.18 br ddd 14;5;2.5
H-5'	1.61 br d 13.5	1.63 br ddd 14.2;11.8;3.5	1.63 br ddd 14.2;11.8;3.5	1.63 br ddd 14.2;11.8;3.5	1.38 m	1.51 m
H-6	1.88 dt 3.5;13.3	1.11 br ddd 13;11.8;3.5	1.10 br ddd 13;11.8;3.5	1.11 br ddd 13;11.8;3.5	1.06 br dd 13.3;10.8	1.09 br t 12.5
H-6'	1.99 m	2.0 br dd 13;5.1	2.0 br dd 13;5.1	2.0 br dd 13;5.1	2.04 m	2.05 m
H-8	5.25 br d 10.3	3.05 d 10	3.0 d 10	3.01 d 10	2.89 d 10	2.90 d 10
H-9	5.50 dt 5.3;10.3	4.78 dt 5;10	4.78 dt 5;10	4.78 dt 5;10	4.70 ddd 11;10;5	4.72 ddd 11;10;5
H-10	1.95 br dd 12.7;10.3	2.06 br dd 13.5;10	2.06 br dd 13.5;10	2.06 br dd 13.5;10	1.86 ddd 13.5;11;2.1	1.87 ddd 13.6;11;2
H-10'	2.56 br dd 12.7;5.3	2.74 br dd 13.5;5	2.74 br dd 13.5;5	2.74 br dd 13.5;5	2.68 ddt 13.5;5;1.8	2.70 br dd 13.6;5
H-11a	2.45 br dt 4.9;10.7	2.64 br dt 4.8;11.2	2.60 br dt 4.8;11.2	2.60 br dt 4.8;11.2	2.48 br dt 4.8;11.5	2.54 ddd 12.2;11.5;4.7
H-12	6.07 br d 11	6.02 br d 11	5.18 br d 9	5.21 br d 9	3.76 br d 5.7	3.62 br d 5.6
H-13	6.42 dd 15.2;11	6.44 dd 15.2;11	3.68 dd 9;2.5	3.68 dd 9;2.5	5.76 dd 15.3;5.7	5.73 dd 15.5;5.6
H-14	5.86 d 15.2	5.88 d 15.2	2.84 d 2.5	2.85 d 2.5	5.99 br d 15.3	6.02 br d 15.5
Me-16	1.34 s	1.35 s	1.32 s	1.32 s	1.41 s	1.43 s
Me-17	1.34 s	1.35 s	1.32 s	1.32 s	1.37 s	1.40 s
Me-18	1.68 br s	1.28 s	1.27 s	1.27 s	1.23 s	1.26 s
H-19	4.99 br s	5.17 br s	5.17 br s	5.17 br s	5.12 br s	5.15 br s
H-19'	5.0 br s	5.23 br s	5.22 br s	5.22 br s	5.17 br s	5.20 br s
OAc	1.93 s;2.05 s;2.08 s	1.97 s;2.10 2s	1.97 s;2.09 2 s	1.97 s;2.09 2s	1.96 s; 2.05 s	1.98 s;2.07 s;2.09 s

*Spectra were recorded in $CDCl_3$ at 300 MHz with Me_4Si as internal standard. The values are given in δ units. Assignments were established by ^1H difference decoupling experiments (DDS).

Table 28. ^{13}C NMR Data of Asterospiculins*

C#	65[a]	66	69
C-1	64.95 t	64.66 t	63.27 t
C-3	60.95 t	60.4 t	59.31 t
C-4	137.87 s	137.6 s	67.9 s
C-4a	46.92 d	46.96 d	37.89 d
C-5	33.29 t	31.47 t	27.16 t
C-6	39.75 t	38.19 t	39.0 t
C-7	138.01 s	59.85 s	59.60 s
C-8	124.03 d	63.54 d	63.41 d
C-9	72.44 d	72.34 d	72.33 d
C-10	36.58 t	32.57 t	32.19 t
C-11	142.16 s	143.4 s	139.5 s
C-11a	52.37 d	52.29 d	51.21 d
C-12	130.99 d	131.21 d	60.79 d
C-13	121.52 d	121.31 d	120.66 d
C-14	143.68 d	144.18 d	144.52 d
C-15	70.81 s	70.87 s	70.64 s
C-16	29.73 q	29.75 q	30.03 q
C-17	29.73 q	29.75 q	29.99 q
C-18	19.49 q	19.77 q	19.63 q
C-19	120.12 t	123.62 t	122.0 t
OAc	170.94 s, 170.61 s, 170.57 s, 21.3 q, 20.97 q, 20.89 q	170.93 s, 170.57 s, 170.49 s, 21.25 q, 20.98 q, 20.88 q	170.52 s, 170.85 s, 20.8 q, 21.2 q

*Spectra were recorded in $CDCl_3$ at 75.4 MHz. [a]Assignments were made by a heterocorrelated 2-D experiment. Multiplicities were made by DEPT experiments.

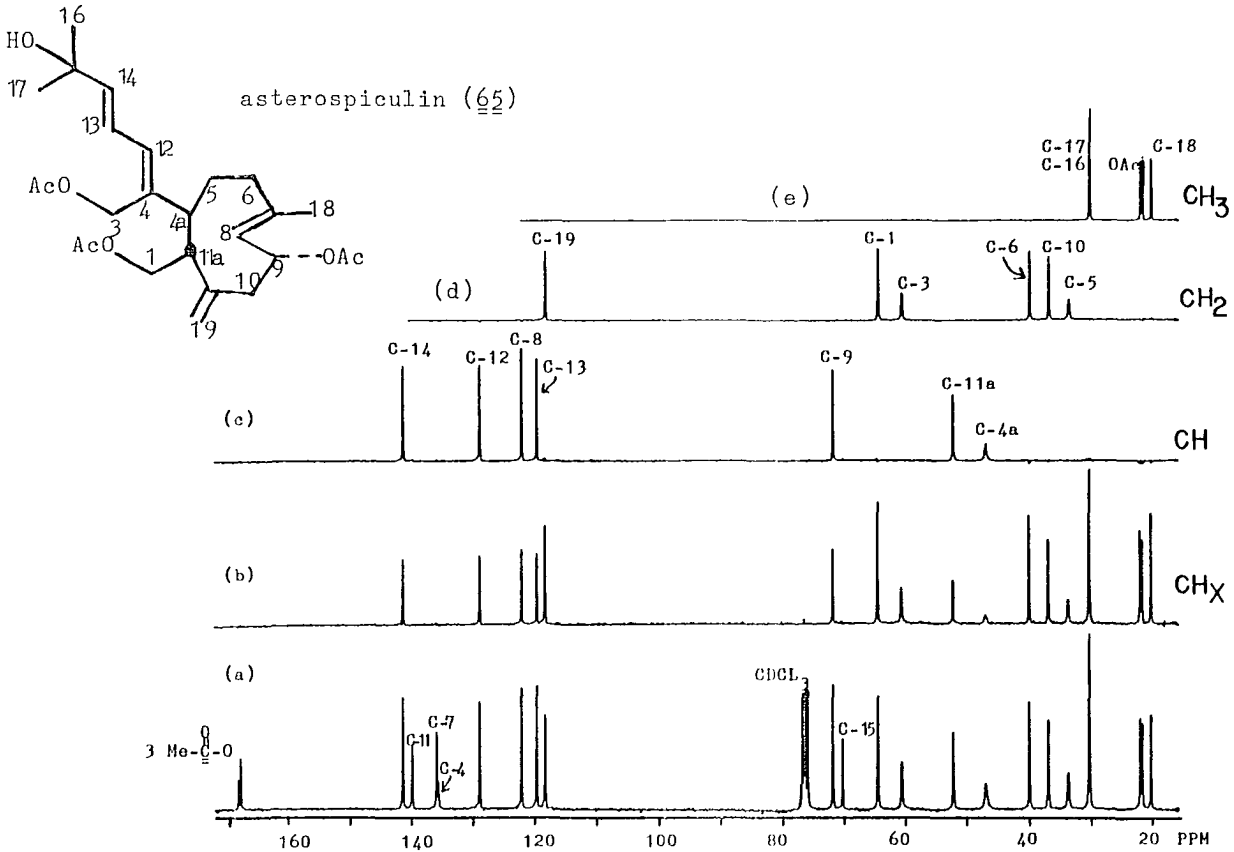


Figure 61. (a) 75.4 MHz broadband ^{1}H decoupled ^{13}C NMR spectrum of 65 in $CDCl_3$. (b) all protonated carbons. (c) methine carbons. (d) methylene carbons. (e) methyl carbons. Spectra b-e were recorded at 75.4 MHz in $CDCl_3$ and resulted from a DEPT experiment.

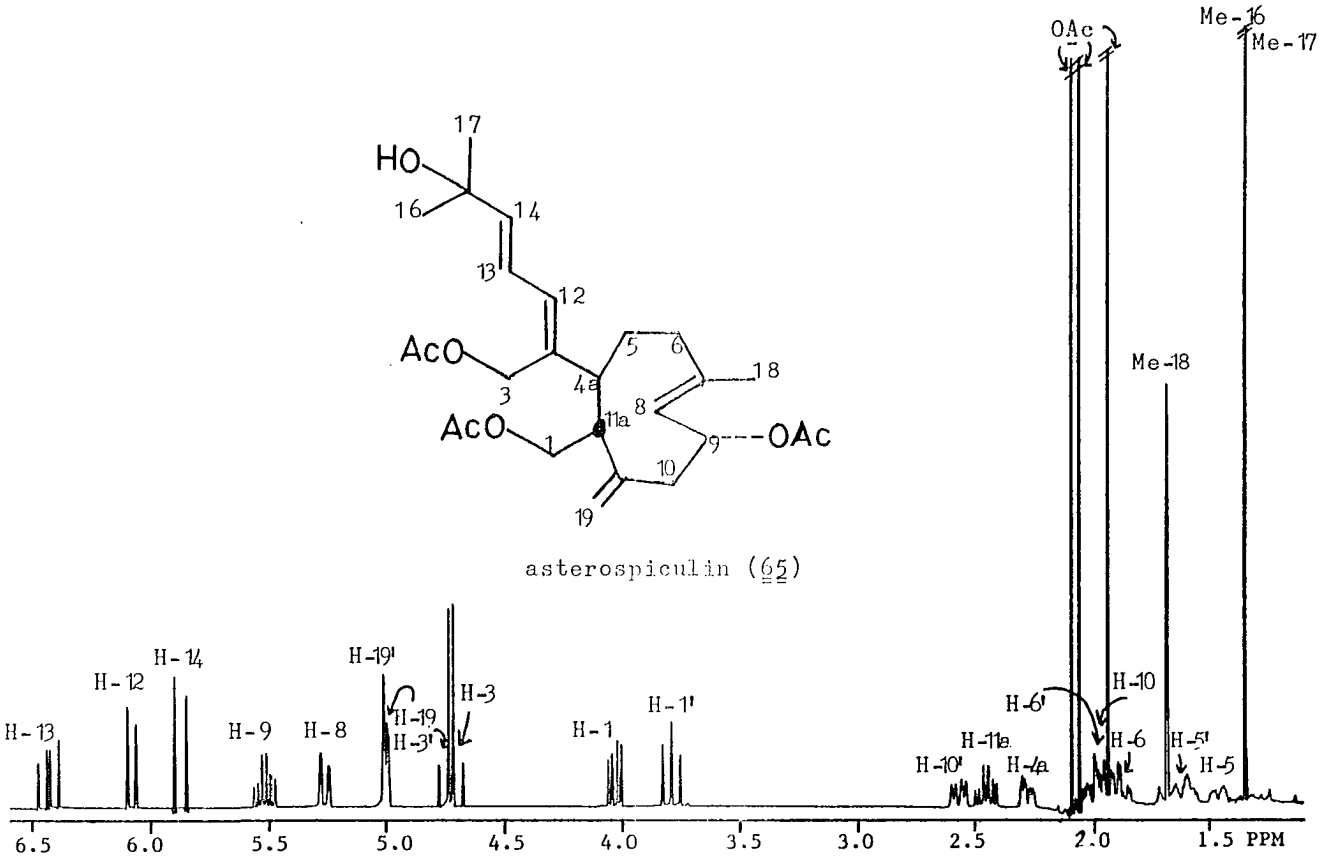


Figure 62. 300 MHz proton NMR spectrum of 65 in $CDCl_3$.

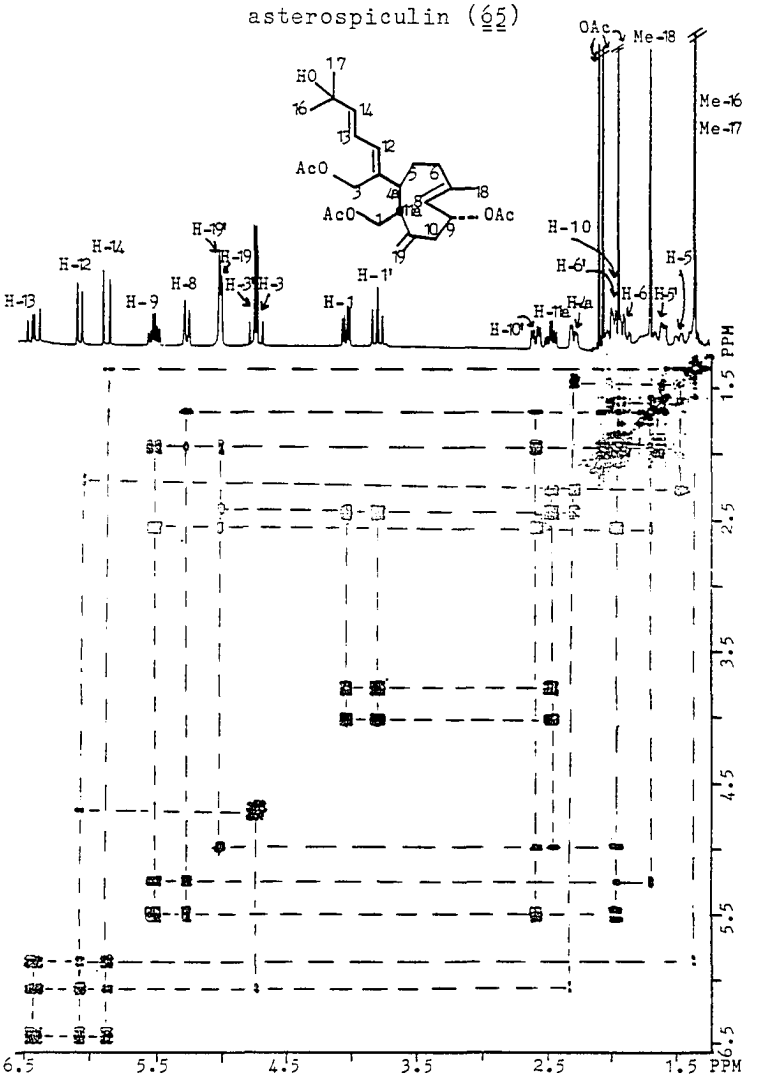


Figure 63. Contour plot of COSY spectrum of 65 in $CDCl_3$ at 300 MHz. Off-diagonal peaks confirm direct proton spin-coupling connectivities.

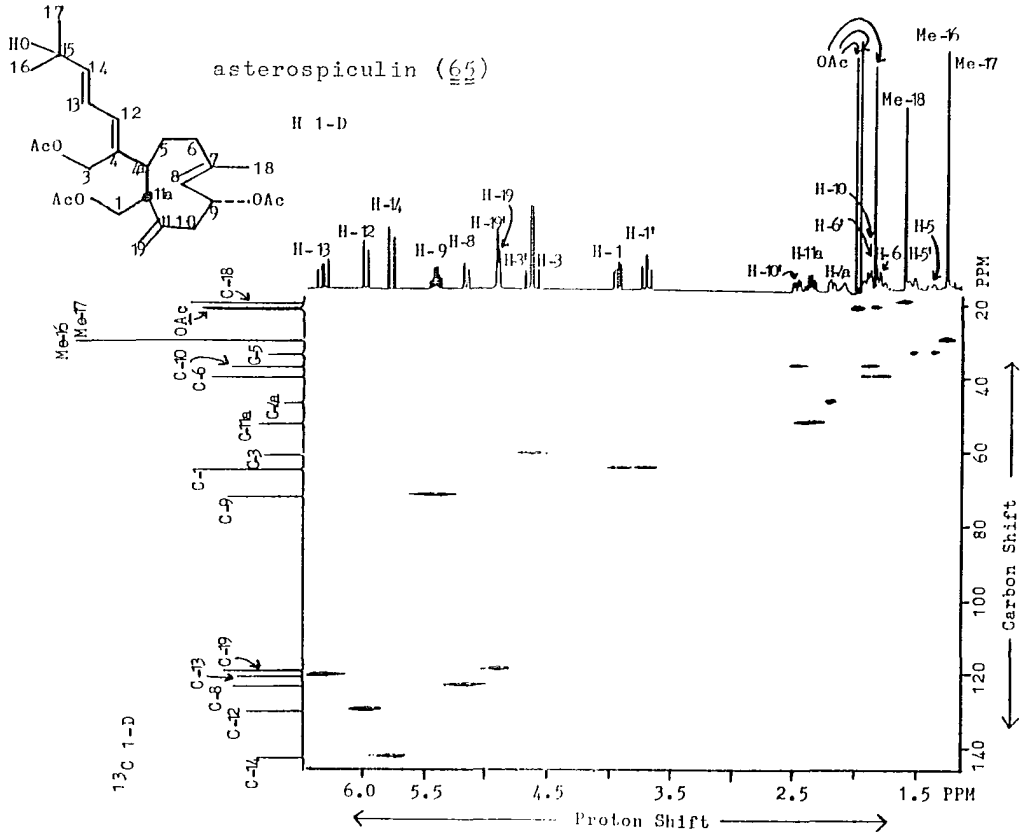


Figure 64. Contour plot of the heteronuclear correlated 2-D spectrum of 65 in $CDCl_3$ at 75.4 MHz NMR. Carbon-13 chemical shift is plotted along the vertical direction, and proton chemical shift is plotted in the horizontal dimension. This 2-D spectrum establishes direct connectivities between bonded nuclei.

was established on the basis of the observation of NOE effects between H-4a/H-8 and H-11a/H-18. The stereochemistry of the diene at C-4, 12, 13, and 14 was confirmed as Z,E on the basis of the magnitude of the coupling constant $J_{13,14}$ = 15 Hz and of the observation of Overhauser enhancements between H-13, H-3, and H-3' and also between H-12 and H-4a (see Table 29). Thus, the structure of 65 is completely elucidated.

Table 29. Results of NOEDS experiment with asterospiculin (65) in $CDCl_3$ at 300 MHz.

Proton(s) irradiated	Proton(s) enhanced	% Enhancement
Me-16 and Me-17	H-14	12
	H-13	8
Me-18	H-9	13
	H-11a	4
H-4a	H-8	12
	H-19	5
	H-12	11
H-13	H-3	12
	H-3'	12
H-8	H-4a	11
H-12	H-14	11
	H-4a	10

Structure elucidation of 7,8,-Dihydro-7α,8α-epoxyasterospiculin (66):

The fourteenth fraction (250 mg) (see Scheme 4) was chromatographed by HPLC with silica gel and hexane-acetone (82:18) as eluent to give 9 fractions. The ninth of these fractions was resolved by HPLC using a reverse-phase C_{18} column and MeOH-H_2O as a mobile phase to yield two fractions. The second fraction contained 3.7 mg of 40 which was discussed above. The first fraction contained 1.9 mg of 7,8-dihydro-7α,8α-epoxyasterospiculin (66) as a colorless oil; UV (EtOH) λ_{max} 241 nm (ε = 15900). The molecular formula $C_{26}H_{38}O_8$ (8 unsaturations) was deduced for 66 from the following spectral data: (a) the low resolution mass spectrum showed a ion peak at 460 (M^+-H_2O); (b) IR (OH) band at 3440 cm^{-1} and acetate(s) band at 1730 cm^{-1}; (c) the ^1H NMR spectrum exhibited signals that accounted for 37 non-exchangeable protons; (d) the ^{13}C NMR spectra of 66 contained signals for 26 carbons including 6 carbons representing 3 acetate groups,* 2 carbon signals due to an epoxide group, [59.85 ppm (C-7, s) and 63.54 (C-8, d)], 4 carbons each deshielded by a single oxygen, [64.66 ppm (C-1, t), 60.40 (C-3, t), 72.34 (C-9, d), and 70.87 (C-15, s)].* The ^{13}C NMR spectra revealed the presence of 6 methyls, 5 sp^3 methylenes and one exocyclic terminal methylene, 4 sp^3 methines and 3 sp^2 methines, and 4 quaternary carbons, in addition to the three acetate carbonyl carbons.

Analysis of the ^1H and ^{13}C NMR data of 66 suggested the presence of three double bonds (CH_2=C<, >C=CH-CH=CH-), an epoxide group, three acetate groups, and a tertiary hydroxyl group.*

*assignments were made by analogy to asterospiculin (65).

Comparison of the ^{1}H and ^{13}C NMR data of 66 with the data of asterospiculin (65) (see Tables 27 and 28) indicated the carbon skeleton of 66 to be identical with that of asterospiculin (65) except that 66 possesses an epoxide group at C-7,8. The ^{1}H NMR spectrum of 66 (see Figure 65) is similar to that of asterospiculin (65) except that the H-5, H-6, H-8, H-9, and H-18 signals in 66 appeared at 1.73 ppm, 1.11, 3.05, 4.78, and 1.28, respectively.

The proposed structure of 66 was established by ^{1}H difference decoupling (DDS) and ^{1}H COSY NMR experiments (see Figure 66).

The geometry of the epoxide group at C-7,8 was assigned as trans, on the basis of the NOE results (irradiation of Me-18 enhanced H-9) (see Table 30). Since the ^{1}H and ^{13}C NMR data of 66 were very close to that of asterospiculin (65) (see Tables 27 and 28), we proposed that 66 has the same stereochemistry as asterospiculin (65) at C-11a, C-4a, C-4, 12, 13, 14, and C-9. Moreover, the results of NOE experiment (see Table 30) supported these assignments.

The structure of 66 was further confirmed when 65 was treated with m-chloroperbenzoic acid and 3 products were obtained (85:8:7) (see Scheme 9). The major product was found to be 7,8-dihydro-7α,8α-epoxy-asterospiculin (66), identical in all aspects (NMR, TLC, IR) with the natural material. The minor products 67 and 68 were deduced to be dieponides having the same overall structures, 7α,8α-13ξ,14ξ-diepoxy-7, 8:13,14-tetrahydroasterospiculin, and differing only in the configuration of the epoxide group at C-13,14. The structures proposed for 67 and 68 (see Table 27), were established by DDS experiments and compari-

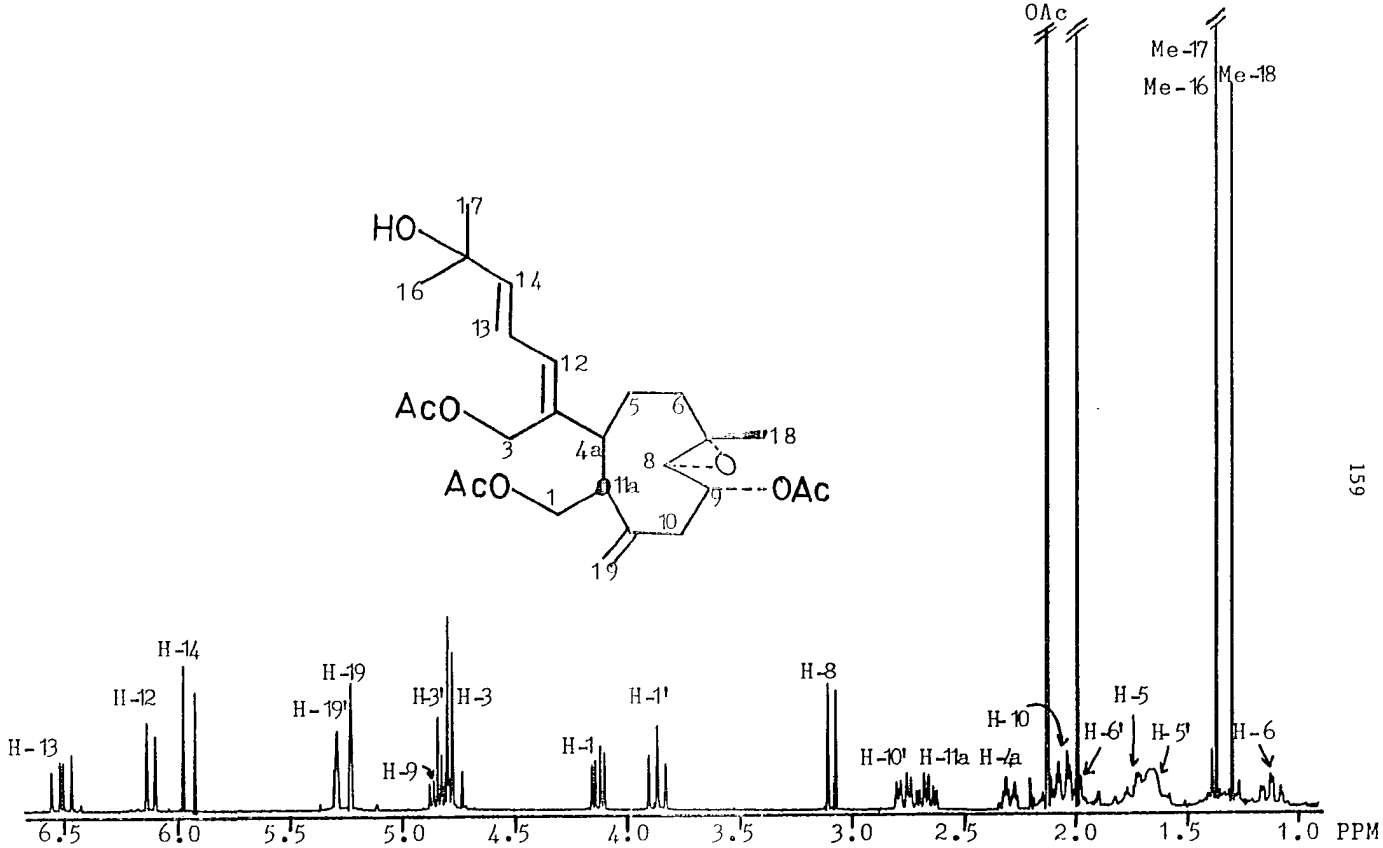


Figure 65. 300 MHz proton NMR spectrum of 66 in $CDCl_3$.

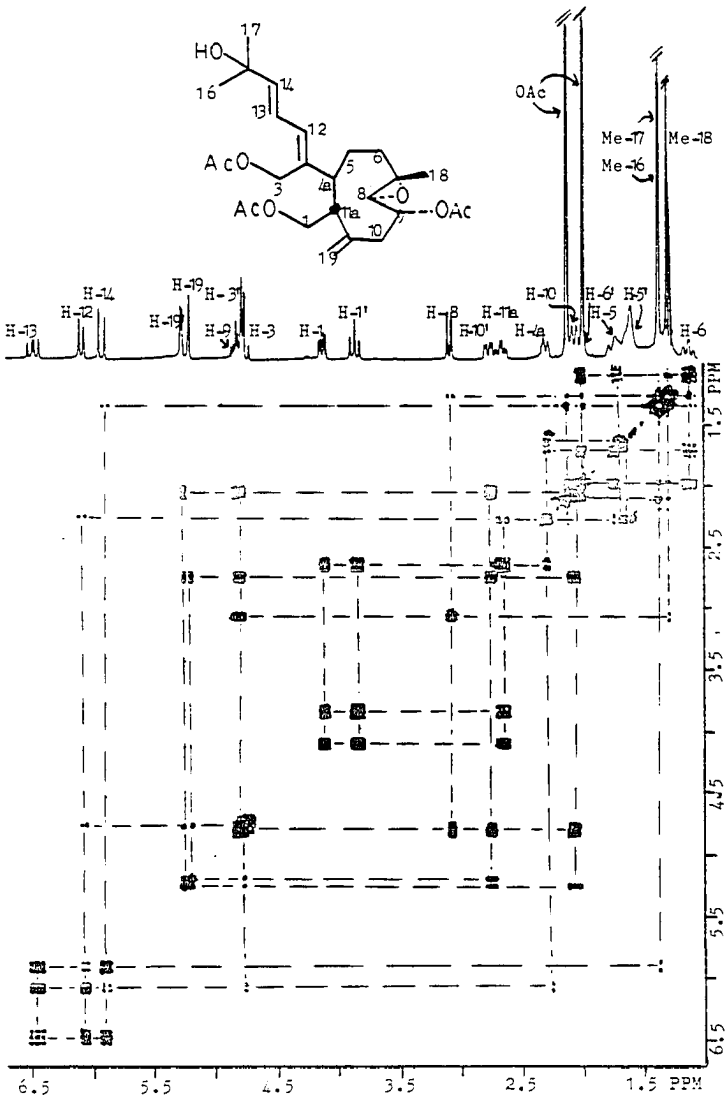
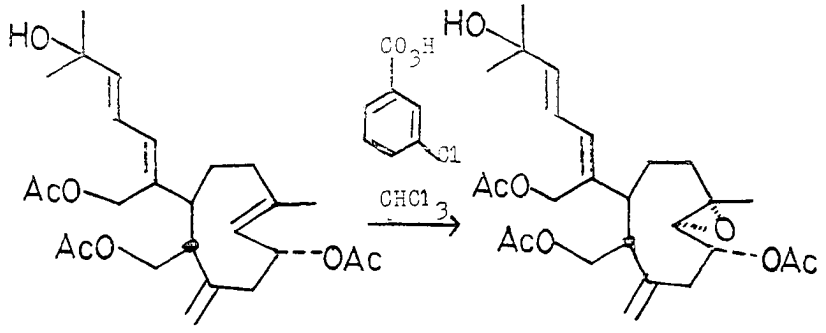


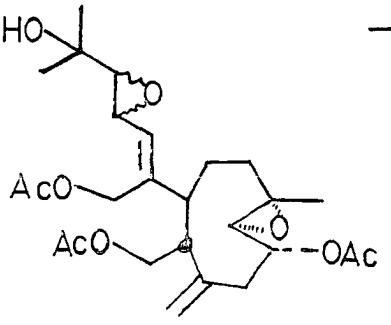
Figure 66. Contour plot of ^{1}H COSY spectrum of 66 in $CDCl_3$ at 300 MHz. Off-diagonal contours establish direct proton spin-coupling connectivities.

Table 30. Results of NOEDS experiment with 66 in $CDCl_3$ at 300 MHz.

Proton(s) irradiated	Proton(s) enhanced	%Enhancement
Me-18	H-11a	3
	H-9	9
H-8	H-4a	11
H-4a	H-8	11
	H-12	12
H-12	H-4a	12
	H-14	11
H-13	H-3	9
	H-3'	9



65 66



SCHEME 9

67, 68

sons of their ^{1}H NMR data with that of 65. The configuration of the epoxide group at C-13,14 in both 67 and 68 was not determined.

Structure elucidation of 4ξ,12ξ:7α,8α-Diepoxy-4,12:7,8-tetrahydro-3-O-deacetylasterospiculin (69):

The CCL_4 extract of Scheme 1 was chromatographed on a column of silica gel (15 X 6.5 cm). Elution was started with hexane-EtOAc (8:2) and the amount of EtOAc was increased stepwise and collecting 25 fractions. The most polar fraction was subjected to HPLC using a silica gel column and hexane-acetone (7:3) as eluent to give 3 fractions. The third of these fractions contained 2.7 mg of 4ξ,12ξ:7α,8α-diepoxy-4,12:7,8-tetrahydro-3-O-deacetylasterospiculin (69) as a white powder; $[\alpha]_D^{25}$ 0.0. The molecular formula $C_{24}H_{36}O_8$ (7 unsaturations) was obtained for 69 from the following spectral data: (a) the molecular ion was detected in the field-desorption spectrum at m/z 452 (low resolution); (b) the high resolution mass spectrum showed a peak corresponding to M^+-78 at m/z 374.2125 which was in agreement with the formula $C_{22}H_{30}O_5$ (calcd, 374.2093); (c) the ^{1}H NMR spectrum (Figure 67) exhibited signals for 34 non-exchangeable protons; (d) IR absorption occurred at 3500 cm^{-1} (OH) group(s); (e) the ^{13}C NMR data indicated the presence of 24 carbons including 4 carbons each deshielded by a single oxygen, [63.27 ppm (C-1, t), 59.31 (C-3, t), 72.33 (C-9, d), 70.64 (C-15, s)], 4 other carbons deshielded by oxygens, correlating to two epoxide groups [59.60 ppm (C-7, s), 63.41 (C-8, d), 67.90 (C-4, s), 66.79 (C-12, d), and two carbonyl acetate carbons, 170.52 ppm (s), 170.85 (s). The ^{13}C NMR spectra (Figure 68) of 69 indicated the

existence of 5 methyls, 5 sp^3 methylenes and one exocyclic terminal methylene, 5 sp^3 methines and 2 sp^2 methines, and 4 quaternary carbons, in addition to the two carbonyl acetate carbons.

Analysis of ^1H difference decoupling, ^{13}C, and ^1H COSY NMR experiments (see Figures 68-69) suggested the presence of the following moieties: two double bonds (CH_2=C<, -CH=CH-), two epoxide groups, two acetate groups,* a tertiary hydroxyl group,* and a primary alcohol group. The presence of the latter moiety was confirmed by acetylation of 69 to give 70 (see Table 27) in which two proton signals were shifted, H-3 signal from 3.86 ppm to 4.39 and H-3' signal from 3.98 to 4.49.

Comparison of the ^1H and ^{13}C NMR data (see Tables 27 and 28) of 69 with the data of 66 suggested that 69 has the same carbon framework as compound 69. However, 69 possesses an additional epoxide group at C-4,12 and lacks the acetyl group at the C-3 oxygen. The ^1H NMR spectrum of 69 (see Figure 67) is similar to that of 66 except that H-3, H-3', H-4a, H-11a, H-12, and H-13 signals appeared at 3.86 ppm, 3.98, 1.72, 2.48, 3.76, and 5.76, respectively (see Table 27).

The carbon connectivity in 69 was established by DDS and confirmed by COSY NMR experiments (Figure 69 exhibits the contour plot of the COSY spectrum which confirms direct proton spin-coupling connectivities).

The trans configuration at C-13,14 was assigned on the basis of the magnitude of the coupling constant observed $J_{H_{13,14}}$ = 15 Hz. The configuration of the epoxide group at C-4,12 was not determined. The E

*assignments were made by analogy to other compounds in this series.

configuration of the epoxide group at C-7,8 was established by NOE, irradiation of Me-18 signal enhanced the H-9 signal. Because ^{1}H and ^{13}C NMR data for 69 were very close to those of compound 66 (see Tables 27 and 28), we proposed that 69 possesses the same stereochemistry at C-11a, C-4a, and C-9. Furthermore, the results of the NOE experiment supported these assignments.

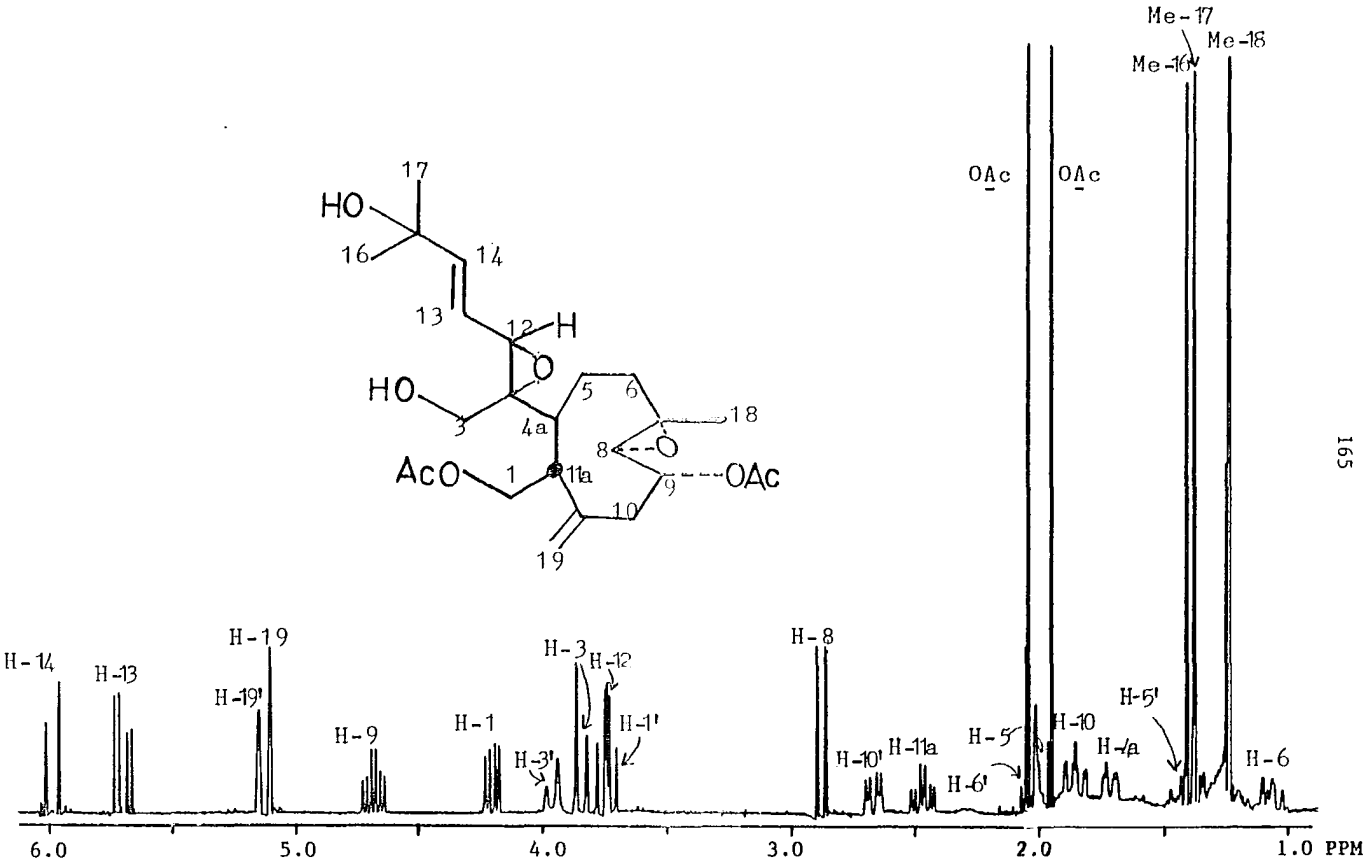


Figure 67. 300 MHz proton NMR spectrum of 69 in $CDCl_3$.

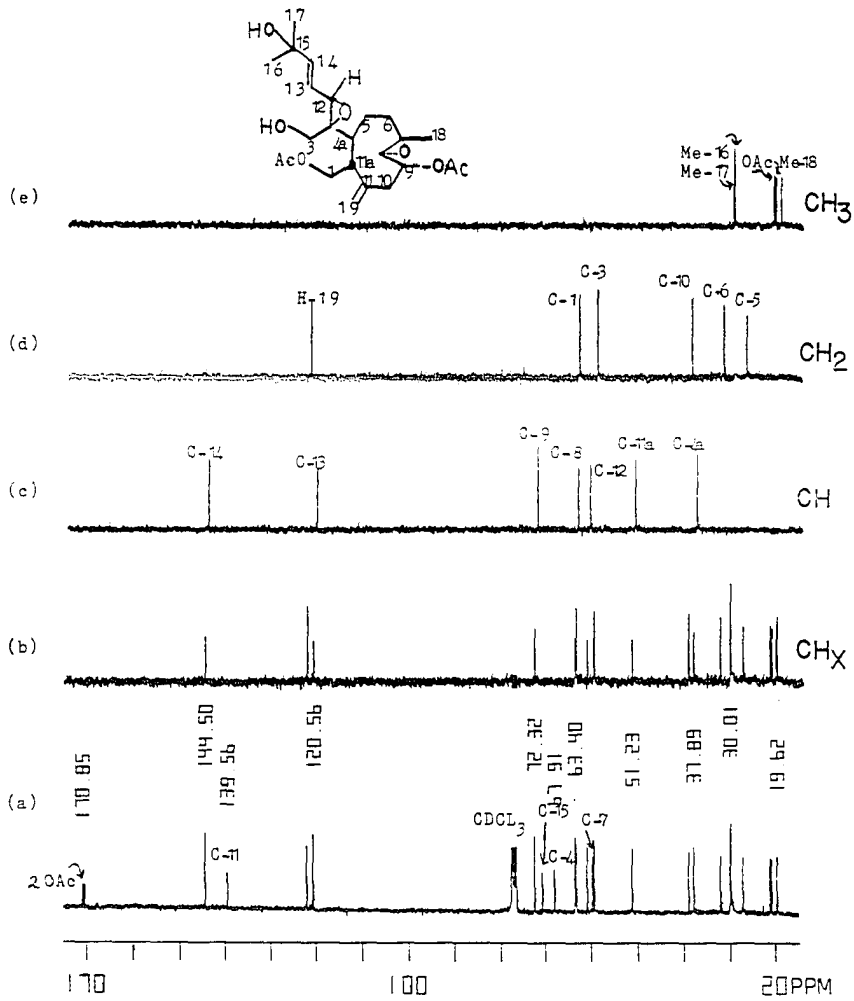


Figure 68. (a) 75.4 MHz broadband ^{1}H decoupled ^{13}C NMR spectrum of 69 in $CDCl_3$. (b) all protonated carbons. (c) methine carbons. (d) methylene carbons. (e) methyl carbons. Spectra b-e were recorded in $CDCl_3$ at 75.4 MHz and resulted from a DEPT experiment.

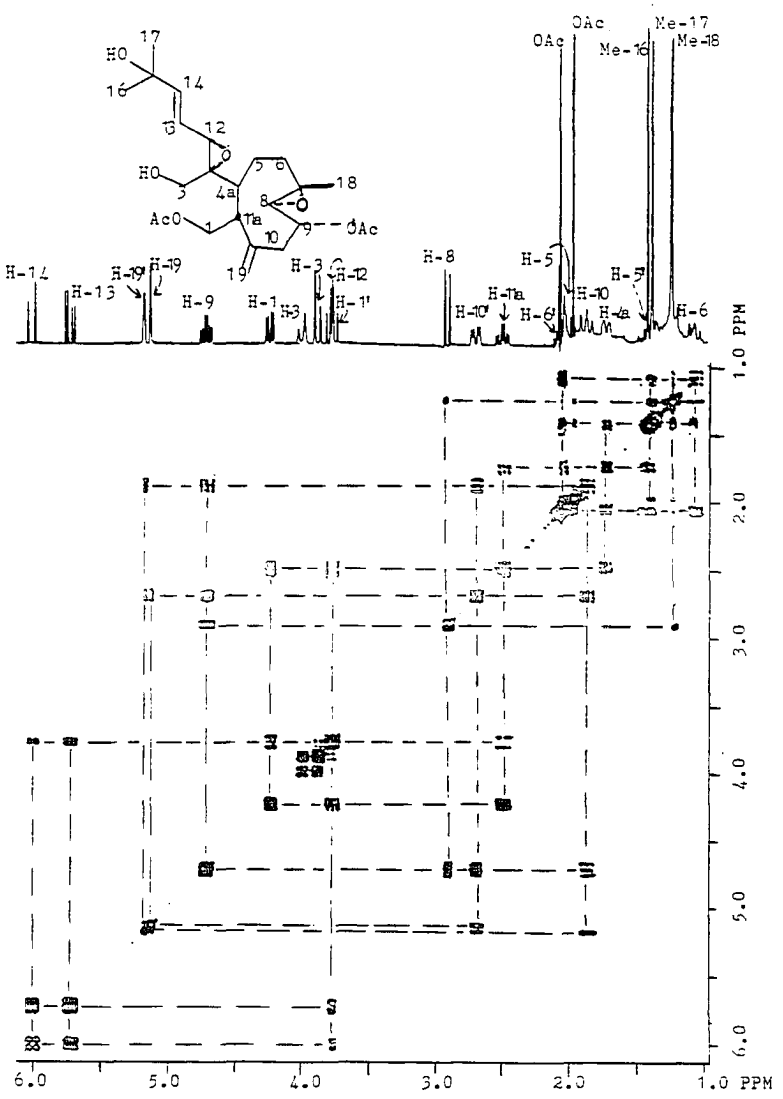


Figure 69. Contour plot of the homonuclear correlated 2-D of 69 in $CDCl_3$ at 300 MHz. Off-diagonal peaks establish direct proton spin-coupling connectivities.

Identification of Peridinin (71):

The nineteenth fraction of Scheme 4 was chromatographed by HPLC with a silica gel column and hexane-acetone (75:25) as eluent to yield 6 fractions. The fourth of these fractions was purified by HPLC using a reverse-phase C_{18} column and MeOH-H_2O (95:5) as a mobile phase to afford 7.5 mg of a pure orange-red pigment. Preliminary analysis of the ^1H and ^{13}C NMR data of 71 suggested that compound 71 is a member of the carotenoid family (see Figures 70 and 71). In depth analysis revealed that the ^1H, ^{13}C NMR and mass spectral properties of the orange pigment matched those reported[28] for peridinin and hence identification was confirmed.

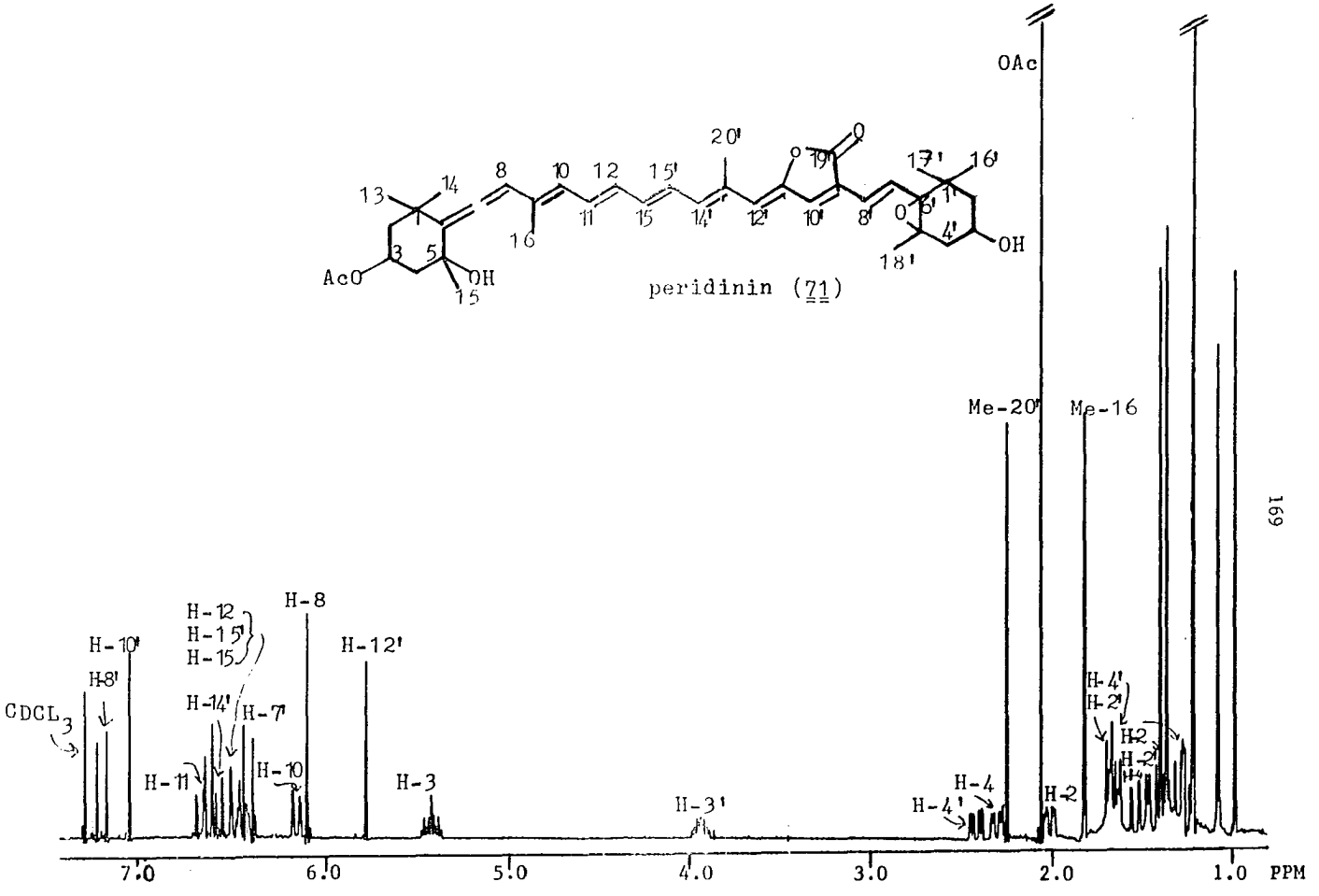


Figure 70. 300 MHz proton NMR spectrum of Peridinin (71) in $CDCl_3$.

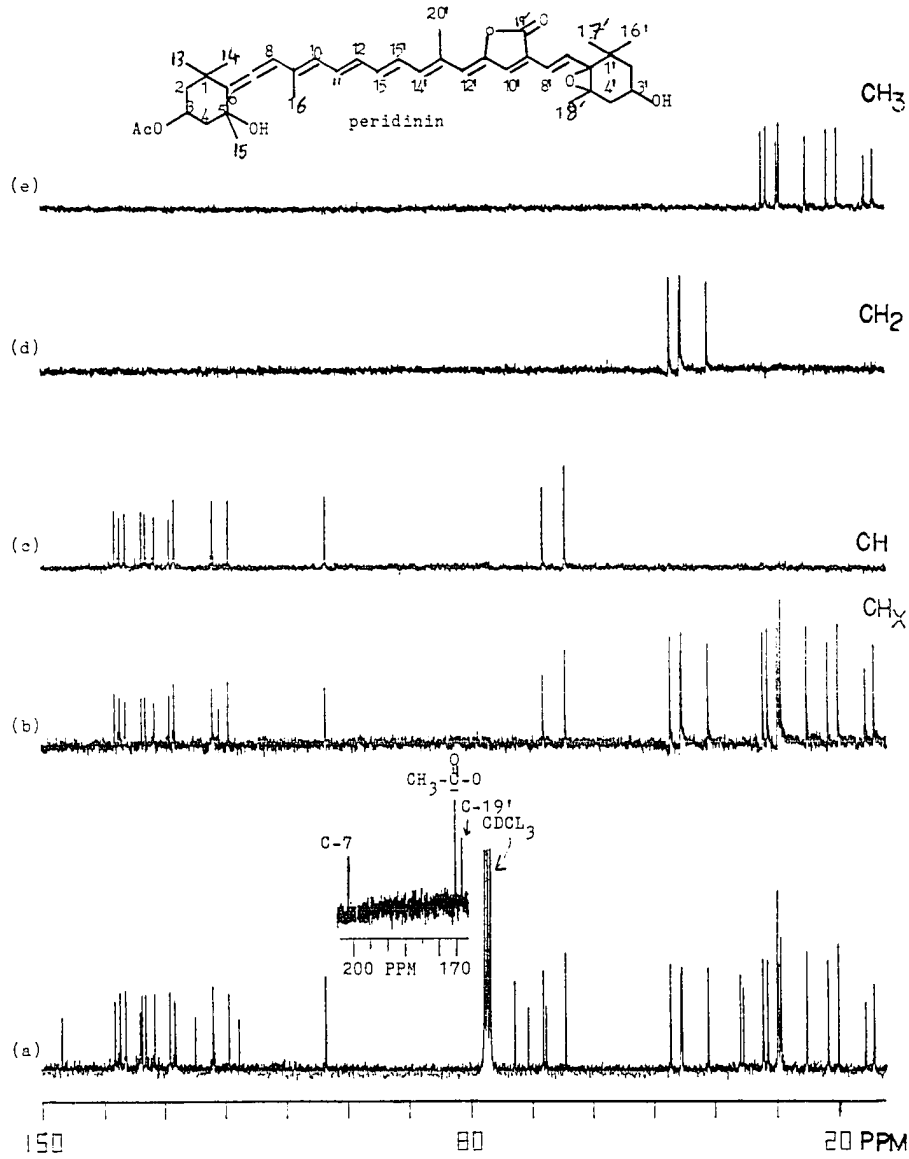


Figure 71. (a) 75.4 MHz broadband ^{13}C NMR spectrum of peridinin (71) in $CDCl_3$. (b) all protonated carbons. (c) methine carbons. (d) methylene carbons. (e) methyl carbons. Spectra b-e were recorded in $CDCl_3$ at 75.4 MHz and resulted from a DEPT experiment.

Structure elucidation of (22R)-24ξ-Methyl-5α-cholestane-3β,5,6β,22,24-pentaol 6-Acetate (72):

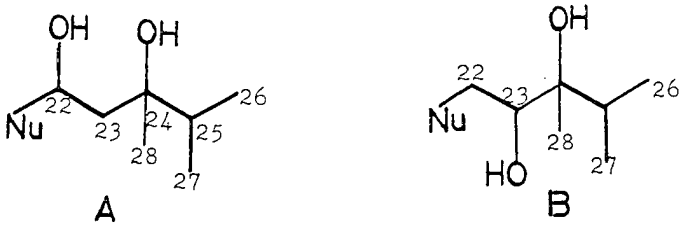
The twenty-first fraction (0.31 g) of Scheme 4 was chromatographed by HPLC with silica gel and hexane-acetone as eluent to give 4 fractions. The most polar fraction (fraction 4) was chromatographed by HPLC using a reverse-phase C_{18} column and MeOH-H_2O (8:2) as a mobile phase to afford two fractions. The first fraction contained 3 mg of asteroxeniolide-A (54), a new compound which was discussed above in this section. The second fraction contained 180 mg of 72 as a colorless oil: $[\alpha]_D^{25}$ -29.2 (c. 0.31, $CHCL_3$). The molecular formula $C_{30}H_{52}O_6$ (5 unsaturations) of 72 was deduced from the following considerations: (a) the molecular ion was detected in the low resolution field desorption mass spectrum at m/z 508; (b) the highest mass peak in the high-resolution mass spectrum was observed at m/z 369.27794 which was in agreement with the formula $C_{25}H_{37}O_2$, (calcd. 369.27936); (c) the ^{13}C NMR spectra (Figure 72) exhibited signals for 30 carbons; (d) IR absorptions occurred at 3440 cm^{-1} (OH) and 1728 (acetate); (e) the ^1H NMR spectrum in pyridine d_5 showed four exchangeable proton signals. The ^{13}C spectrum showed signals for 9 methylenes, 9 methines, and 4 quaternary carbons in addition to the carbonyl acetate carbon, (this accounts for 48 non-exchangeable protons). Two of the four quaternary carbons were each deshielded by a single oxygen, [77.15 ppm (C-5, s)] and [78.13 (C-24, S)], and two other carbons were each deshielded by a secondary hydroxyl group,* [69.54 ppm (C-3, d)] and [72.54 (C-22, d)]. The presence of two

*assignments were established by heteronuclear 2-D experiment.

secondary alcohol groups in Z2 was established by acetylation (see Scheme 11) of Z2 to give Z5 in which two proton signals were shifted from 4.07 ppm (3α-H) to 5.14 and 4.07 (H-22) to 5.19 (see Figures 73a and 74). The ^{1}H NMR spectrum (Figure 73a) of Z2 in $CDCl_3$ indicated the presence of one acetate [2.05 ppm (s)]. Furthermore, the ^{13}C NMR spectrum (Figure 72) of Z2 also confirmed the acetate group, [23.11 ppm (OAc, q)] and [169.7 ppm (CH_3-$\overset{O}{\overset{\|}{C}}$-O, s)]. The ^{1}H NMR spectrum (Figure 73a) displayed signals for methyl groups typical of a sterol with an additional methyl group in the side chain: 0.70 (s, C-18), 0.85 (3H, d, J = 7.5 Hz, C-27), 0.95 (3H, d, J = 7.5 Hz, C-21), 0.98 (3H, d, J = 7.5 Hz, C-26), 1.07 (s, C-28), and 1.14 (s, C-19). The broad signal at 4.06 ppm in Z2 suggested a 3α proton with a hydroxyl group being at 3β position. The lower chemical shift observed for 3α-H signal was attributed to the deshielding effect from having an axial 6α-hydroxyl group.[29] The methine proton signal which was observed at 4.71 ppm (6α-H) was deshielded by an acetate group (hydrolysis of Z2 gave Z3 in which H-6 signal was shifted to 3.52 ppm). H-6 in Z2 was established to be in an equatorial position on the basis of the coupling constants (J = 3 Hz) observed between H-6 and both 7α-H and 7β-H signals. The assigned location of the acetylated hydroxyl group in Z2 was confirmed by a single frequency on-resonance decoupling experiment in which H-6 signal in the ^{1}H NMR spectrum was selectively irradiated while observing ^{13}C fully coupled spectrum. The result showed a sharpening in C-5 signal which confirmed the C-5,6 connection and established the assignment of C-5.

The high resolution mass spectrum of Z2 displayed a fragment ion at m/z 123 which was consistent with the formula C_9H_{15}. This ion corre-

sponded to the loss of rings A→D plus $2H_2O$ (M^+-2H_2O-rings A→D) which led to the conclusion that the side chain in 72 consisted of 9 carbons. Moreover, a peak was observed at m/z 131 for $C_7H_{15}O_2$ which corresponded to a cleavage between C-20 and C-22 (see Figure 76). This suggested that two of the four hydroxyl groups in 72 were present in the side chain from C-22 onward. The latter formula ($C_7H_{15}O_2$) presumably contained 2 hydroxyls and an isopropyl group connected to a blocked position (see Figure 76). The presence or an isopropyl group in 72 was established by ^1H difference decoupling experiments in which the latter moiety was also confirmed to be next to a blocked position [irradiation at 2.03 ppm (H-25) collapsed only the two doublets at 0.98 ppm (Me-26) and 0.85 (Me-27) to two singlets]. Based on the above spectral data, two possible partial structures (A and B) for the fragment $C_7H_{15}O_2$ were postulated.



To determine which of these structures, A or B, was present in 72, an oxidation reaction was performed (see Scheme 10) on 72 using Jones reagent which produced a diketone 74 whose ^1H NMR spectrum lacked the two methine proton signals due to H-3 and the side chain -CHOH-, but contained two AB patterns which were consistent with the expectations for the two isolated methylene groups at C-4 and C-23 of the oxidized form of a sterol with side chain A above. This was consistent with the

presence of two acetylatable hydroxyl groups in Z2 at C-3 and C-22. Therefore, partial structure A is the correct one. The low resolution mass spectrum of Z4 was also consistent with this structure (see Figure 77). Therefore, the location of the secondary hydroxyl groups in Z2 were fixed to C-3 and C-22. The high resolution mass spectrum of Z2 was fully consistent with the proposed structure (see Figure 76).

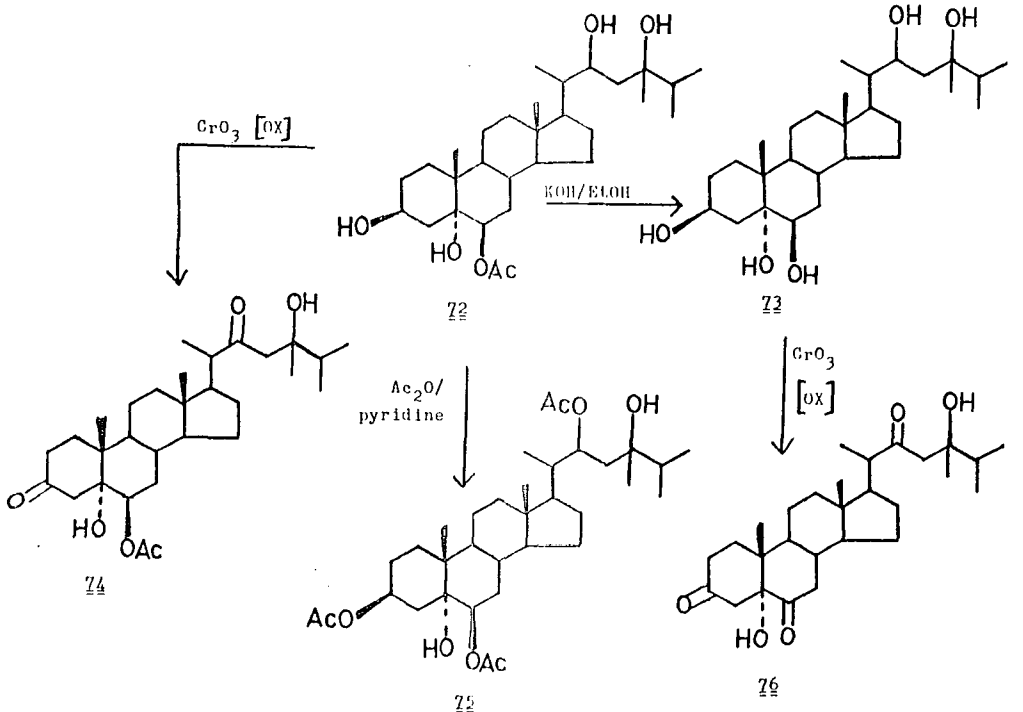
Hydrolysis of Z2 with KOH in aqueous ethanol gave the pentaol Z3 (see Scheme 10), the 6-hydroxy derivative of Z2. The ^{1}H NMR spectrum of Z3 in $CDCL_3$ was similar to that of Z2 except that Z3 lacked the acetate group methyl signal at 2.05 ppm and the H-6 signal was shifted to 3.52 ppm. This further confirmed that C-6 bore the acetate group in Z2. The ^{1}H NMR spectrum of Z3 in pyridine-d_5 revealed the presence of 5 hydroxyl groups (exchangeable signals at 5.29 (s), 5.92 (s), 6.16 (d, J = 4.5 Hz, coupled to H-6), 6.07 (d, J = 3 Hz, coupled to H-3), and 6.03 (d, J = 4.5 Hz, coupled to H-22). The ^{13}C NMR spectra (see Figure 78) and the low resolution mass spectrum of Z3 supported the structure of Z3 (see Figure 79).

Oxidation of Z3 using Jones reagent (see Scheme 10) gave 3,6,22-triketone Z6 whose ^{1}H NMR spectrum lacked the three methine proton signals due to H-3α, H-6α, and H-22 and contained signals assignable to H-4α, and H-4β, AB system at 2.28 ppm and 2.92; to H-23 and H-23', AB pattern at 2.50 ppm and 2.66; and to H-7α (2.73 ppm) and H-7β (2.18 ppm), part of an ABM system (see Figure 80 and Experimental). The low

resolution mass spectrum of 76 was in full agreement (see Figure 81) with the structure 76.

The configurations of the hydroxyl group in 72 at C-24 was not determined. However, the configuration at C-22 could be assigned as 22R on the basis of the coincidence of the ^{13}C chemical shift of C-20 (42.5 ppm) in 72 with that reported for C-20 (40.3)* in (22S)-22-hydroxycholesterol (cf. 42.6 for C-22 in (22R)-22-hydroxycholesterol).*

*Letourneux, L.; Khuong-Huu, Q.; Gut, M.; Lukacs, G. *J. Org. Chem. 1975, 40,* 1674.


CrO3 [OX]
KOH/ETOH
Ac2O/
pyridine
CrO3
[OX]
OH
OH
HO
HO
OAc
O
OH
AcO
72
73
74
75
76

SCHEME 10

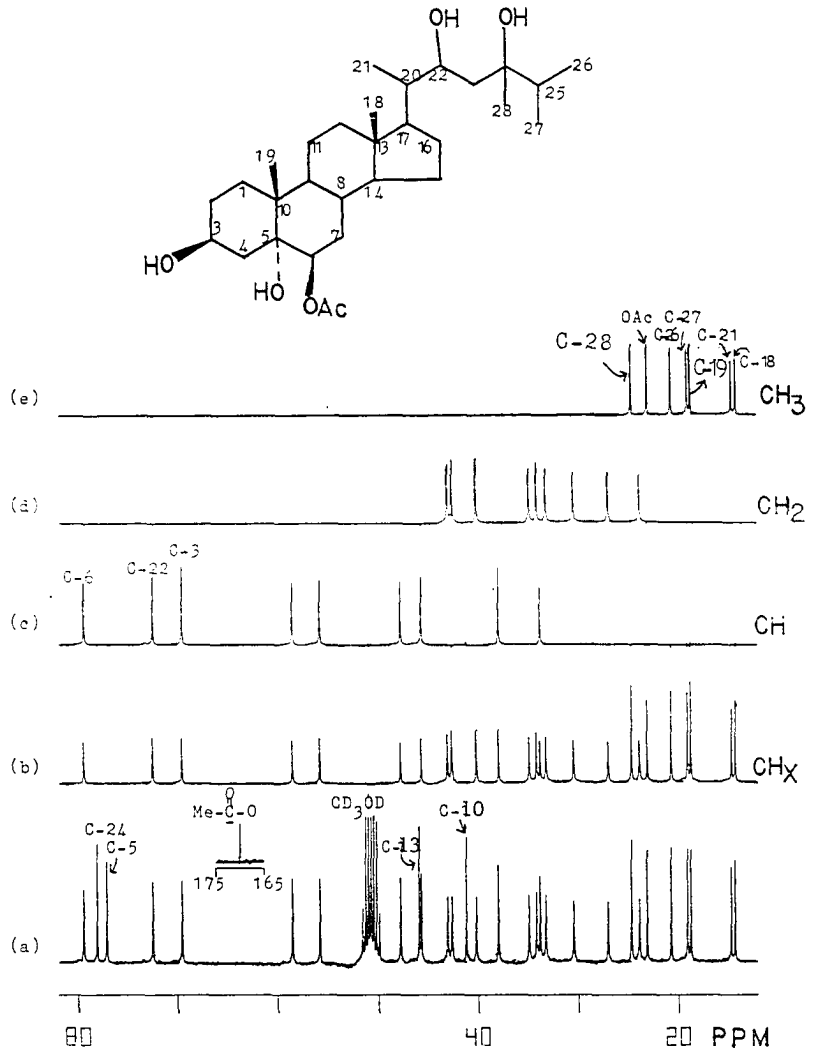


Figure 72. (a) 75.4 MHz broadband ^{13}C NMR spectrum of 72 in CD_3OD. (b) all protonated carbons. (c) methine carbons. (d) methylene carbons. (e) methyl carbons. Spectra b-e were recorded in CD_3OD at 75.4 MHz and resulted from a DEPT experiment.

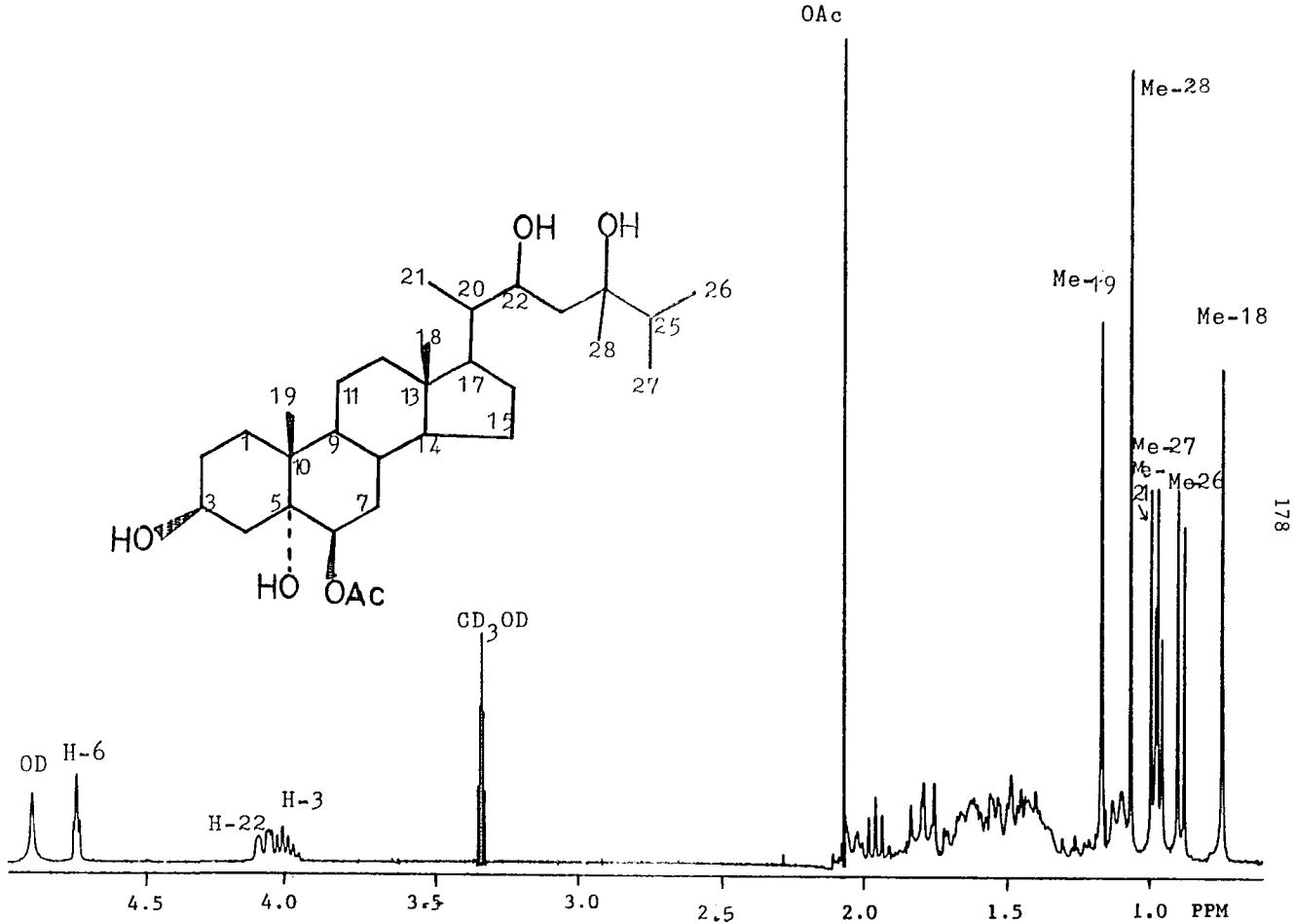


Figure 73. 300 MHz proton NMR spectrum of 72 in CD_3OD.

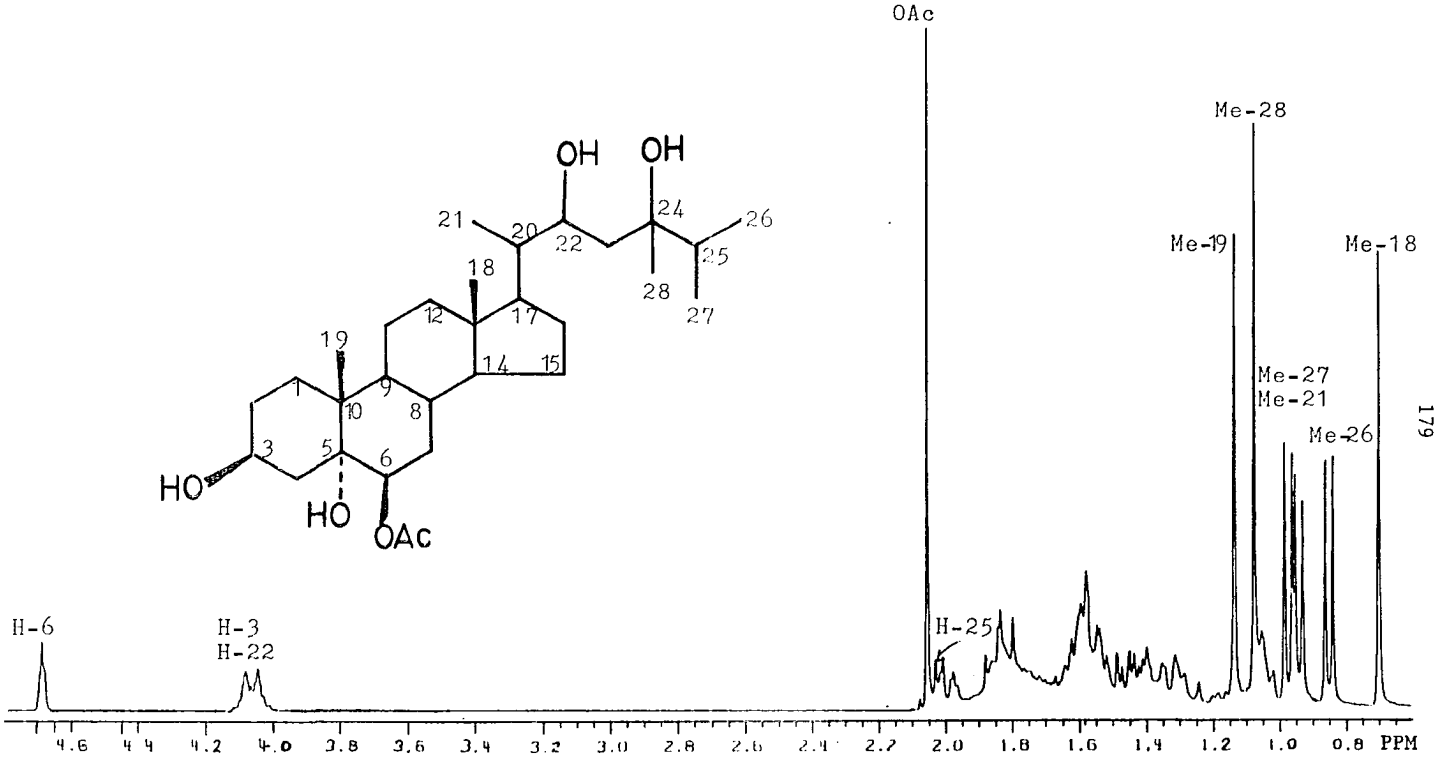


Figure 73a . 300 MHz proton NMR spectrum of 72 in $CDCl_3$

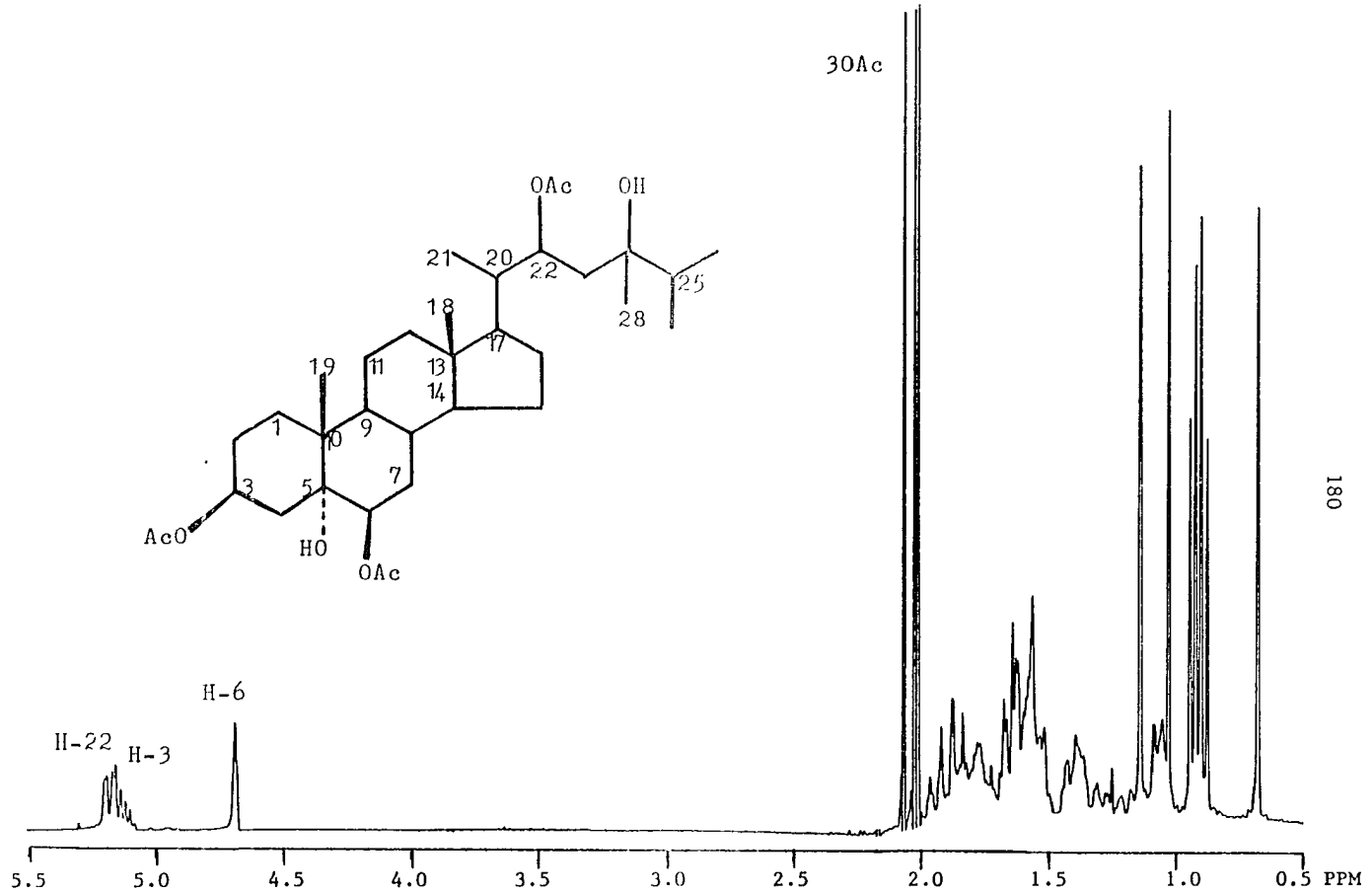


Figure 74. 300 MHz proton NMR spectrum of 75 in $CDCl_3$.

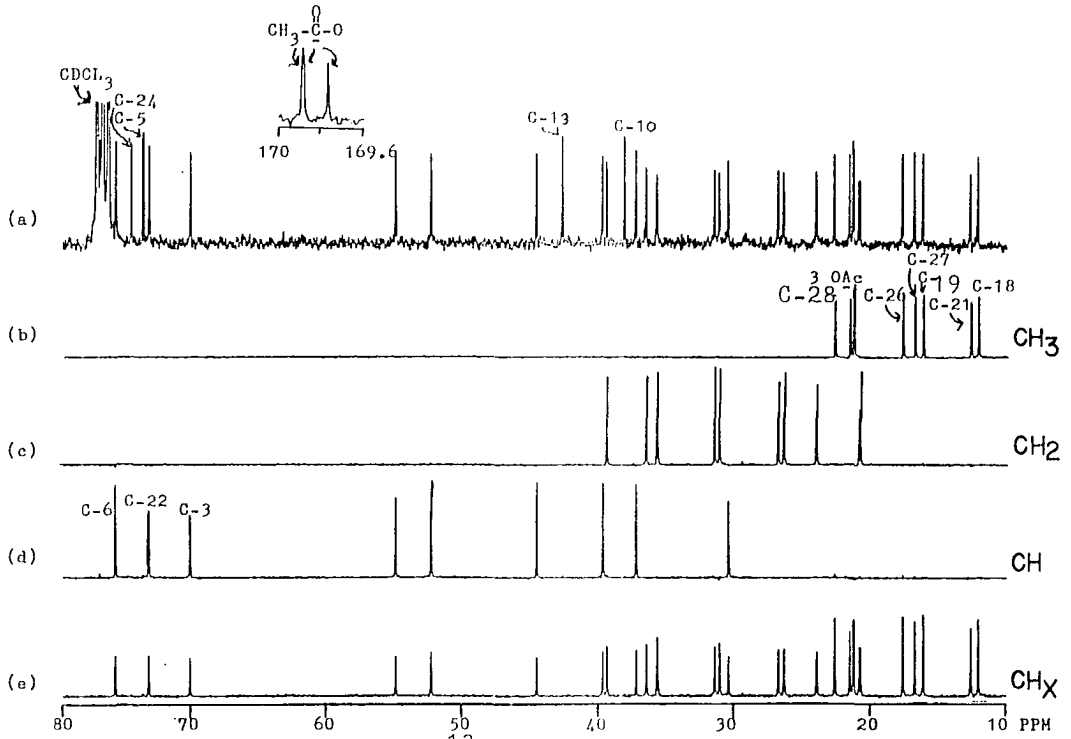


Figure 75. (a) 75.4 MHz broadband ^{13}C NMR spectrum of 75 in $CDCl_3$. (e) all protonated carbons. (d) methine carbons. (c) methylene carbons. (b) methyl carbons. Spectra b-e were recorded in $CDCl_3$ at 75.4 MHz and resulted from a DEPT experiment.

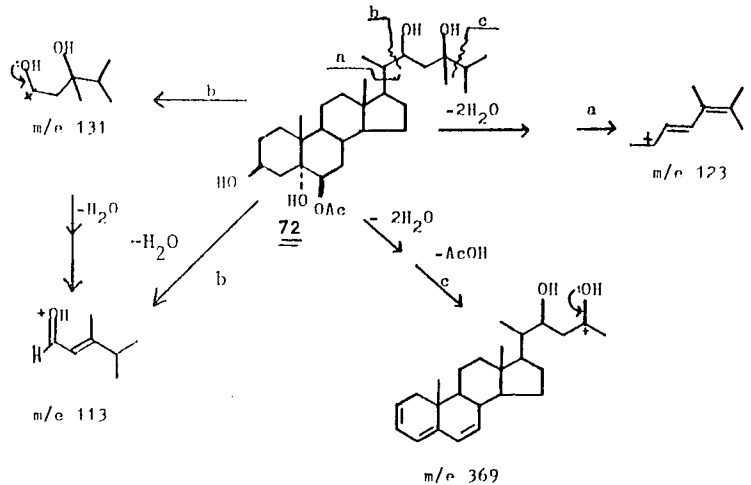


Figure 76. Possible Mass Spectral Fragmentation Pathways for 72.

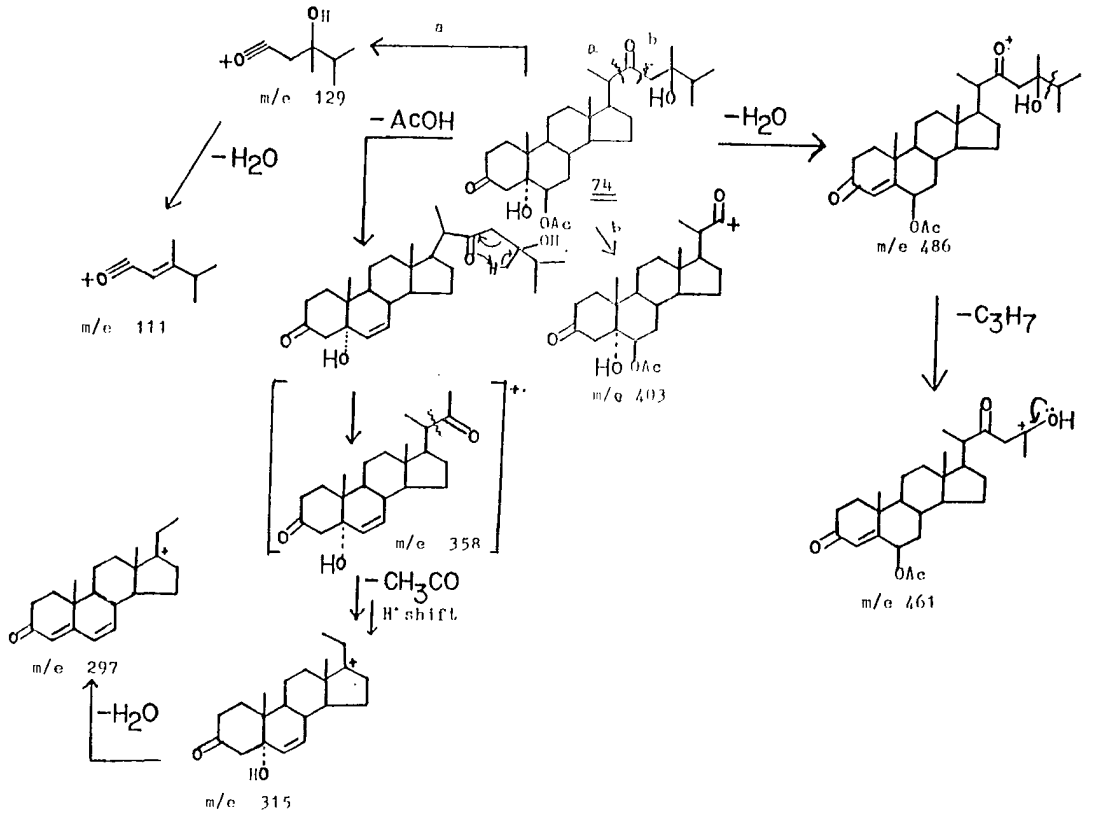


Figure 77. Possible Mass Spectral Fragmentation Pathways for 74.

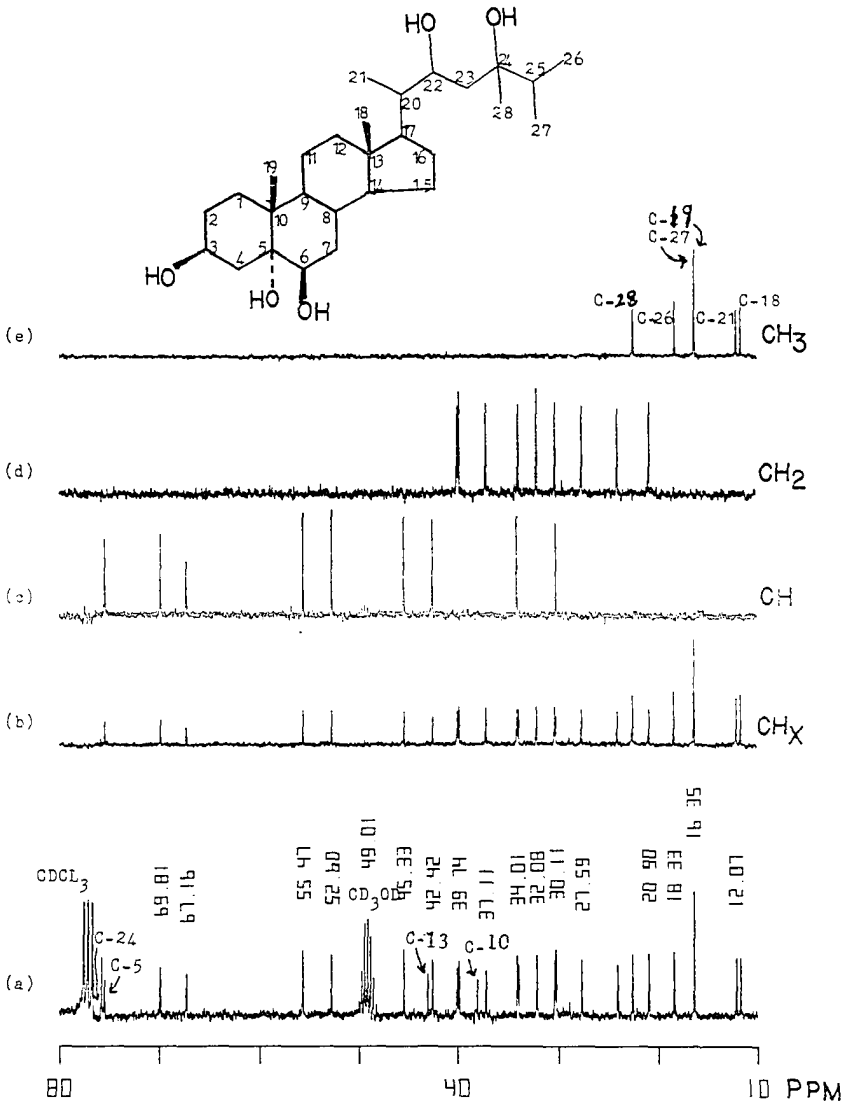


Figure 78. (a) 75.4 MHz broadband ^{13}C NMR spectrum of 73 in 5% CD_3OD in $CDCl_3$. (b) all protonated carbons. (c) methine carbons. (d) methylene carbons. (e) methyl carbons. Spectra b-e were recorded in 5% CD_3OD in $CDCl_3$ at 75.4 MHz and resulted from a DEPT experiment.

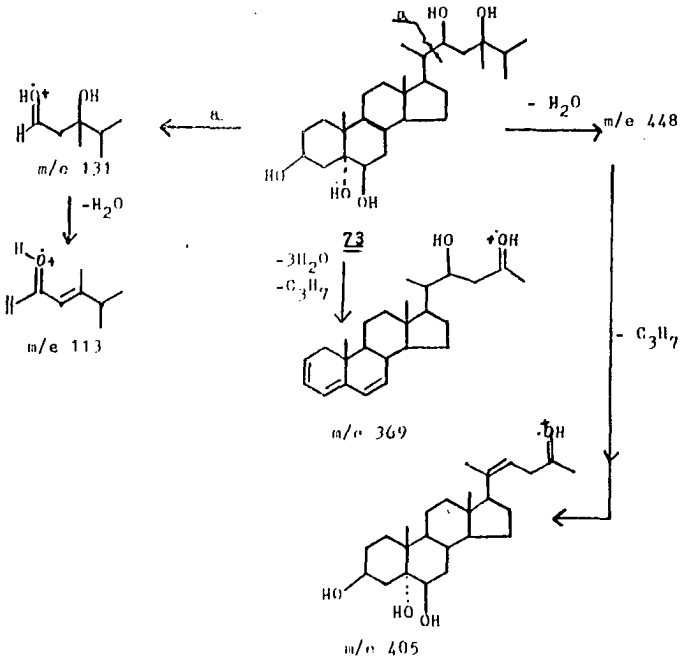


Figure 79. Possible Mass Spectral Fragmentation Pathways for 73.

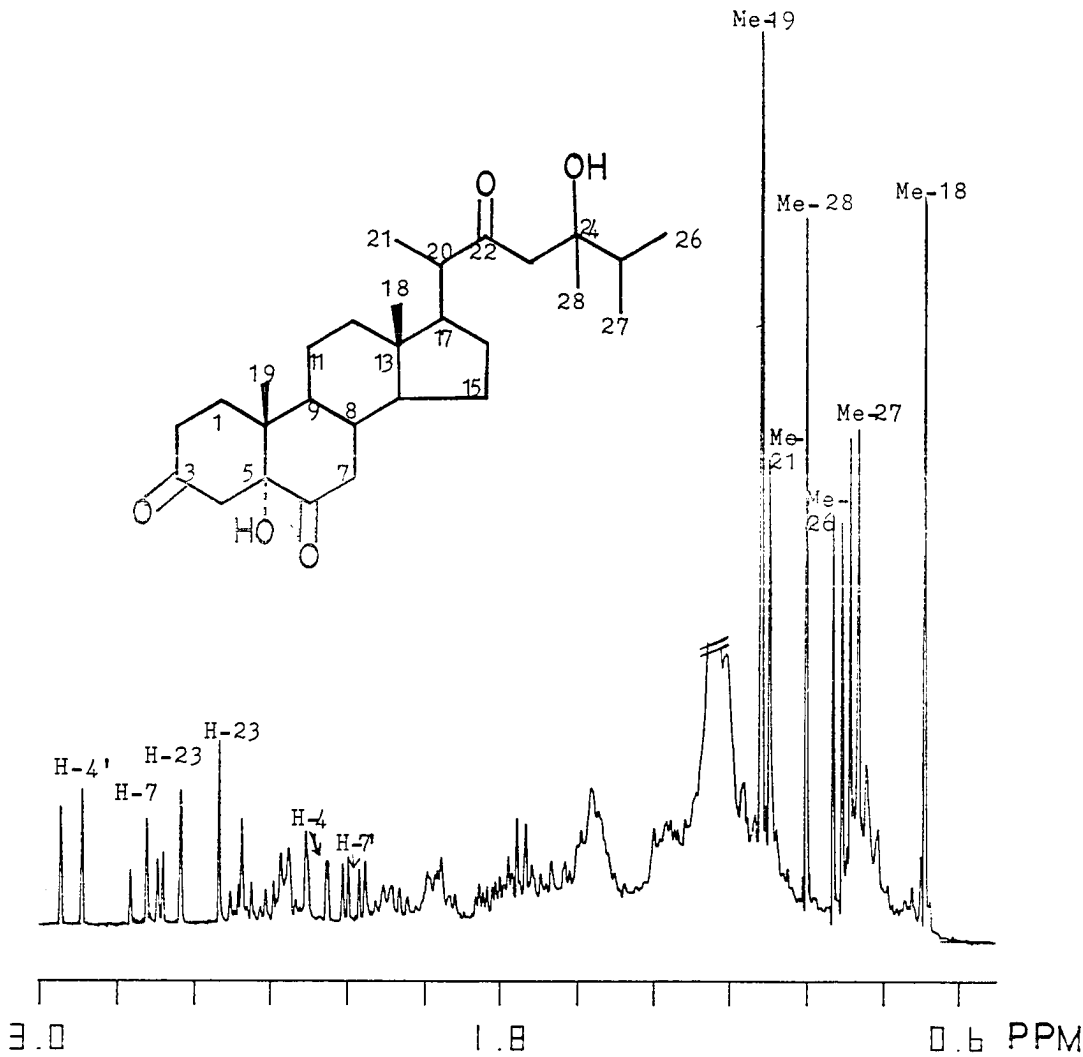


Figure 80. 300 MHz proton NMR spectrum of 76 in $CDCl_3$.

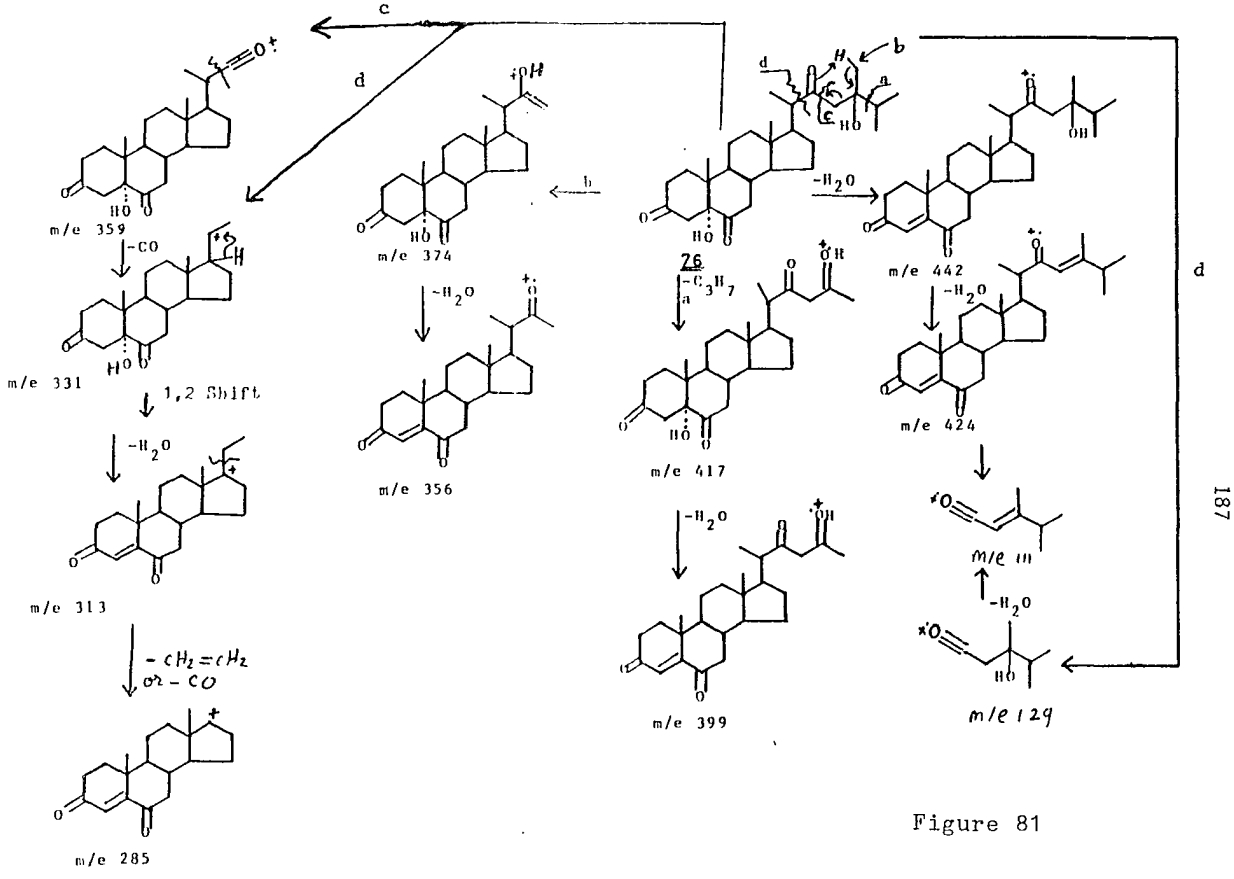


Figure 81

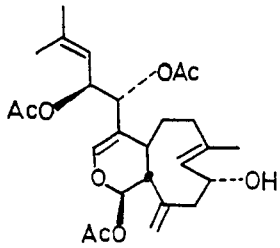
Possible Mass Spectral Fragmentation Pathways for 76

SUMMARY

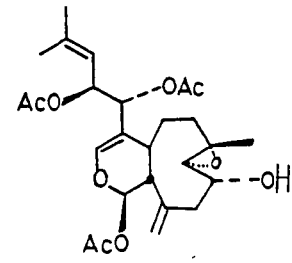
Seven known compounds and twenty-one new compounds have been isolated from *Asterospicularia randalli*. Six of the seven known natural products, 13-epi-9-O-deacetylxenicin (3), xenialactol (8), xeniolide-A (7), xeniolide-B (13), xeniolide-B 9-acetate (14), and 7,8-dihydro-7α,8α-epoxyxeniolide-B (15), have been isolated previously from *Xenia macrospiculata* and *Xenia obscuronata*. The seventh known compound, peridinin (71), has been isolated from various marine and fresh-water dinoflagellates (Chart 2).

One of the twenty-one new compounds described is the novel steroid 72. Compound 40 is the 7α,8α-epoxide of the known 13-epi-9-O-deacetylxenicin (3). Isoxenialactol (42) is the geometric isomer at C-12 of the known xenialactol (8). Compound 43 is the 9-Acetate and compound 44 the 7α,8α-epoxide of the known xenialactol (8). Compounds 47, 48, 49, and 50 are the 9-deoxy, 9-deoxy-7α,8α-epoxy, 15-dehydroxy-15-acetoxydioxy, and 7α,8α-epoxy analogs of the known xeniolide-A (7), respectively. Compound 52 possesses the same carbon skeleton as xeniolide-A (7), but it contains a hydroxyl group at C-13 rather than at C-15, there is a hydroxyl group at C-18, and the hydroxyl group at C-9 is acetylated. Compound 54 is the 18-deoxy-9-O-deacetyl analog of compound 52. Compound 55 is the 9-acetate of asteroxeniolide-A (54). Asterospicin (56) has a novel tricyclic skeleton which possesses a 9-membered-ring as in any other compound in this series. Compounds 58 and 59 are epimeric 14,15-epoxides of the asterospicin (56). Compounds 60 and 61 appear to arise from ring opening of the 14,15-epoxides 58 and 59, respectively and are epimeric at C-14. Compound 62 is

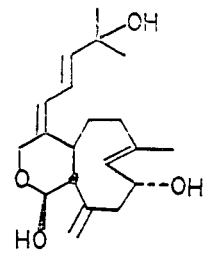
similar to asterospicin, but lacks the epoxide group at C-7,8 and contains an additional hydroxyl group at C-3. Asterospiculin (65) is a novel 9-membered ring carbocyclic diterpenoid compound. Compound 66 is the 7α,8α-epoxide of asterospiculin (65). Compound 69 is similar to 7α,8α-epoxyasterospiculin (65), but has a free alcohol group at C-3 and contains an additional epoxide group at C-4,12 (see Chart 2).



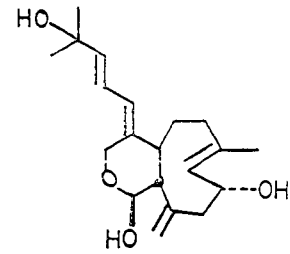
3



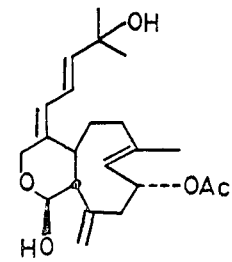
40



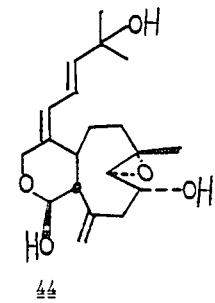
8



42

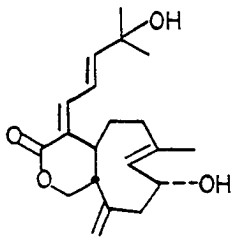


43

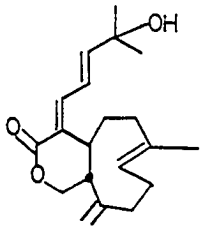


44

Chart 2

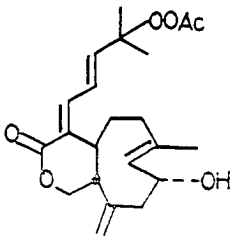


7

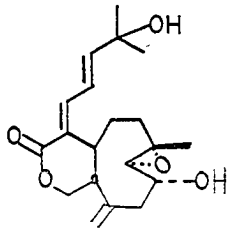


47

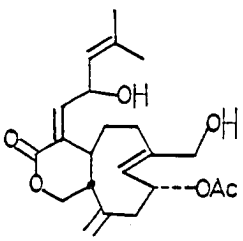
48 7,8-epoxide of 47



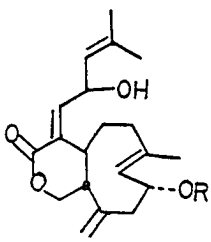
49



50



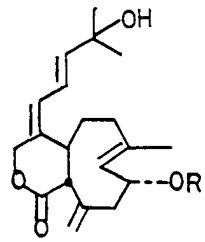
52



54 R = H

55 R = Ac

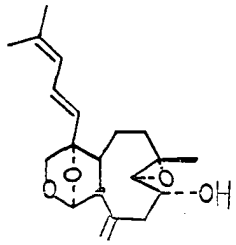
Chart 2 (Continued)



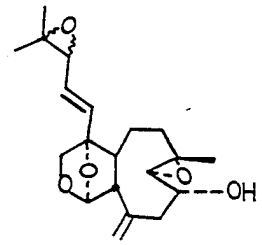
13 R = H

14 R = Ac

15 7,8-epoxide of 13

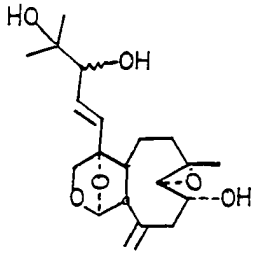


16



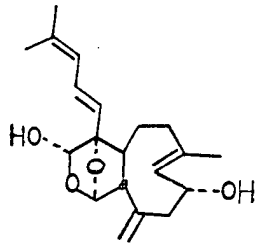
58

59 epimer of 58



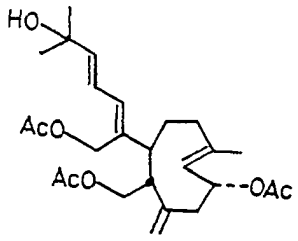
60

61 epimer of 60



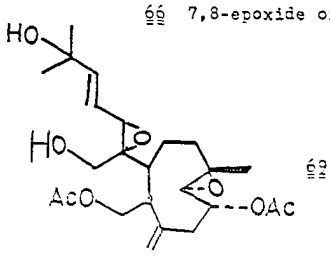
62

Chart 2 (Continued)

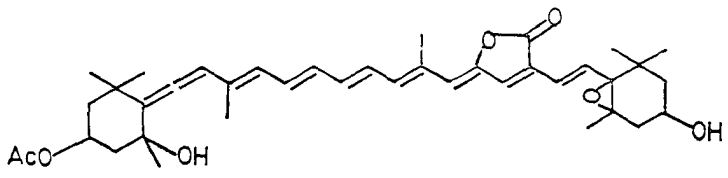


65

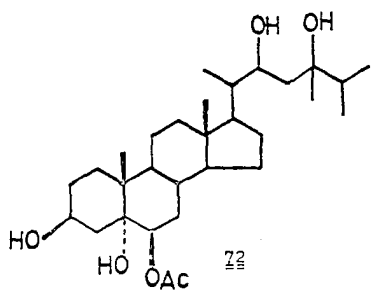
66 7,8-epoxide of 65



69



71



72

Chart 2 (Continued)

Experimental

Melting points were taken on an A.H. Thomas Unimelt apparatus and are uncorrected. Infrared spectra were taken on a Perkin-Elmer 2 98 spectrophotometer. Ultraviolet spectra were taken in ethanol on a Perkin-Elmer Lambda 3 UV/VIS spectrophotometer. Optical rotations were recorded on a Perkin-Elmer 141 polarimeter. Low resolution mass spectra were taken on a Hewlett-Packard 5985B GC/MS system. High resolution mass spectra were obtained on CEC-110 (Dupont, Monrovia, CA) spectrometer. NMR spectra were acquired on a Varin XL-300 spectrometer in the solvents specified; signals are reported in parts per million (δ) downfield from internal tetramethylsilane. The chromatographic adsorbent used was Brinkmann silica gel 60 (230-400 mesh). Thin layer chromatograms were run on precoated Macherey-Nagel polygram sil G/UV_{254} (0.25 mm) plates. A sulfuric acid-vanillin spray was used to visualize TLC plates. Some chromatographies were run on a Hitachi CLC-5 centrifugal preparative liquid chromatograph. Altex 5-μ particle 9.6 mm X 25 cm preparative silica gel (Li Chrosorb 60) and Adsorbosphere 5-μ particle 9.6 X 25 cm reverse phase C_{18} columns were used for HPLC separations.

Extraction and partition procedure of the first collection: The freshly thawed soft coral, *Asterospicularia randalli*, collected and shipped frozen from Guam Is. in May of 1979 was allowed to soak in ethanol (4 liters) for 24 h, and then in a mixture of MeOH-$CHCL_3$ (1:1) (8

liters) for 24 h. Ethanol and $CHCL_3$–MeOH extracts were combined, 63.4g, after the solvents were evaporated. One liter of water was added to the combined crude extracts and then the aqueous suspension was partitioned against CH_2CL_2 (3 X 1500 mL) to give an aqueous layer designated fraction B, and a CH_2CL_2 solution. The CH_2CL_2 solution was evaporated to yield 45.2 g of extract designated as fraction A. Fraction A was dissolved in 0.5 liter of 10% aqueous methanol and partitioned against hexane (3 X 1500 mL). The hexane solution was evaporated to give 25 g of extract (fraction C). The 10% aqueous methanol solution was diluted with more water (200 mL) and then it was partitioned against CCL_4 (3 X 1000 mL). The CCL_4 solution was evaporated to give 9.6 g of extract (fraction D). The aqueous methanol solution was further diluted with water (100 mL) and partitioned with $CHCL_3$ (2 X 1000 mL) to give 3.5 g of chloroform solubles, fraction F. The remaining aqueous methanol solution was labeled fraction E. Fractions E and B have not been studied any further (see Scheme 1).

Extraction and partition procedure of the second collection: The freshly thawed soft coral, *Asterospicularia randalli*, collected and shipped frozen from Guam Is. in April of 1983 was allowed to soak in ethanol (15 liter for 24 h, and then in a mixture of MeOH–$CHCL_3$ (1:1) (15 liter) for three days. Ethanol and $CHCL_3$–MeOH extracts were combined (71.2 g) after the solvents were evaporated using a cyclone evaporator.[30] Two liters of water were added to the combined crude extracts and then the aqueous suspension was partitioned against CH_2CL_2 (3 X 2500 mL) to give an aqueous layer designated fraction B', and a CH_2CL_2 solution. The CH_2CL_2 solution was evaporated to yield 50.1 g of extract

designated as fraction A'. Fraction A' was dissolved in 1.3 liter of 10% aqueous methanol and partitioned against hexane (3 X 2500 mL). The hexane solution was evaporated to give 30 g of extract (fraction C'). The 10% aqueous methanol solution was diluted with more water (250 mL) and then it was partitioned against chloroform (3 X 2500 mL). The $CHCL_3$ solution was evaporated to give 15 g of extract (fraction F'). The aqueous methanol solution was labeled fraction E'. Fractions E' and B' have not been studied any further (see Scheme 3).

Isolation of 13-Epi-9-O-deacetylxenicin (3): The fourteenth fraction (see Scheme 4) (250 mg) was chromatographed by HPLC on a column of silica gel with hexane-acetone (82:18) as eluent to give 9 fractions. The fourth of these fractions was resolved by HPLC using a reverse-phase C_{18} column and MeOH-H_2O (7:3) to yield 2 fractions. Evaporation of the first fraction left 42 mg of pure 13-epi-9-O-deacetylxenicin (3) as a colorless oil. Compound 3 has the following spectral data: IR (neat) 3450, 2970, 2920, 2850, 1735, 1660, 1440, 1380, 1240, 1155, 1030, 980, 940, 900, 870 cm^{-1}; for ^1H and ^{13}C NMR data see Tables 1 and 2; low resolution mass spectrum (70 eV) m/z (relative intensity) 476.3 [M^+, (2)], 416.1 [M^+-AcOH, (3)], 365.2 (15), 356.2 [M^+-2AcOH, (18)], 349.2 (45), 307.3 (40), 247.2 (41), 229.1 (42), 201.1 (26), 159.1 (34), 157.1 (28), 131.0 (41), 105.1 (46), 85.1 (100).

Isolation of 13-Epi-9-O-deacetyl-7,8,-dihydro-7α,8α-epoxyxenicin (40): A portion (15 g) of fraction F' (see Scheme 4) was chromatographed over silica gel eluting with 9:1 hexane-acetone and increasing the amount of acetone while collecting 450 mL fractions. Fraction 14 (eluted with 7:3 hexane-acetone) (250 mg) yielded a yellow oil. This

oil was passed through a silica gel Sep-Pak column (elution with 7:3 hexane-acetone) to yield 220 mg of light yellow oil which was further resolved by HPLC using silica gel with 8 3:17 hexane-acetone as eluent to afford 9 fractions. Fraction 9 (8 mg) was purified by HPLC using a reverse-phase C_{18} column with a mobile phase of MeOH-H_2O (55:45) to produce two fractions. The first fraction contained a pure compound (2 mg) which will be discussed later in this thesis. The second fraction contained 3.7 mg of 13-epi-9-O-deacetyl-7,8,-dihydro-7α,8α-epoxyxenicin (40) as a colorless oil; [α] + 25.68 (c 0.37, $CHCL_3$); IR ($CHCL_3$) 3500, 3050, 2940, 2850, 1735, 1660, 1440, 1380, 1250, 1180, 1040, 975, and 910 cm^{-1}; for ^1H and ^{13}C NMR data (see Tables 1 and 2) low resolution mass spectrum (70 eV) m/z (relative intensity) 433.1 (1), 373.3 (2), 372.2 (1), 366.2 (19), 365.2 (90), 323.1 (52), 305.1 (27), 263.1 (58), 245.1 (57), 147.0 (39), and 85.1 (100).

Acetylation of 13-Epi-9-O-deacetyl-7,8-dihydro-7α,8α-epoxyxenicin (40): Acetic anhydride (0.02 mL) was added dropwise to a solution of 40 (1.5 mg) in pyridine (0.02 mL) and the reaction mixture was allowed to react at room temperature for 24 h. Excess reagents were then evaporated under nitrogen, and the residue was purified by HPLC using a silica gel column. Elution with 8:2 hexane-acetone gave the corresponding tetraacetate (41) (1 mg) as a white powder; for ^1H and ^{13}C NMR data see Tables 1 and 2 low resolution mass spectrum (12 eV) m/z (relative intensity) 415.1 [M^+-(59 + 60); 1], 408.2 (26), 407.1 (79), 355.3 (5), 347.2 (19), 305.2 (60), 287.1 (51), 254.1 (76), 189.0 (31), and 146.9 (51).

Isolation of Xenialactol (8): A 15 g sample of $CHCL_3$ extract (fraction F' of Scheme 4) was chromatographed on a Büchner funnel column

of silica gel (24 X 9 cm) using a water aspirator as a source of vacuum.[29] The material was eluted using a step gradient beginning with hexane-acetone (9:1) and then increasing the amount of acetone and collecting 45 mL fractions. One of the fractions eluted with hexane-acetone (1:1) was a brown oily material (300 mg). Addition of 15 mL of a mixture of hexane-acetone (7:3) to the brown oily material caused a yellow solid (110 mg) to precipitate. The light yellow solid (all attempts to crystalize this solid failed) was identified as xenialactol (8); UV (EtOH) λ_{max} 242 nm (ε = 15180); IR (neat) 3400, 3010, 2975, 2940, 2850, 1640, 1450, 1380, 1350, 1250, 1130, 1080, 970, 905, 876 cm^{-1}; for ^{1}H and ^{13}C NMR data see Tables 4 and 5; low resolution mass spectrum (70 eV) m/z (relative intensity) 316.3 [M^{+}-H_2O (50)], 298.3 [M^{+}-$2H_2O$, (17)], 283.3 [M^{+}-$2H_2O$-Me, (17)], 270.2 [M^{+}-$2H_2O$-CO, (24)], 253.3 (17), 201.1 (42), 199.1 (30), 185.2 (48), 145.1 (57), 109.2 (43), 95.1 (100).

Isolation of Isoxenialactol (42): A portion (15 g) of fraction F" (see Scheme 4) was chromatographed over silica gel eluting with hexane-acetone first and then hexane-acetone mixtures with increasing amounts of acetone and collecting 450 mL fractions. Fraction 25 was eluted with 1:1 hexane-acetone (100 mg) and yielded a brown oily residue. This oily fraction was passed through a silica gel Sep-Pak column (elution with 6:4 hexane-acetone) to give 80 mg of yellow oil which was further chromatographed by HPLC using silica gel with 75:25 hexane-acetone as eluent to yield 8 fractions. Fraction 4 (0.9 mg) contained isoxenialactol (42), which was obtained as a white powder. Compound 42 has the following spectral properties: IR (neat) 3450, 2980, 2950, 2850, 1640,

1450, 1382, 1533, 1250, 1130, 1070, 975, 905, 875 cm^{-} for ^{1}H and ^{13}C NMR data see Tables 4 and 5; low resolution mass spectrum (12 eV) m/z (relative intensity) 334.2 [M^{+}, (9)], 316.2 [$M^{+}-H_2O$, (53)], 298.1 [$M^{+}-2H_2O$, (65)], 280.2 [$M^{+}-3H_2O$, (14)], 270.1 (60), 225.2 (56), 214.1 (63), 199.2 (78), 173.1 (70), 171.1 (83), 159.1 (73), 157.1 (76), 145.0 (96), 143.1 (65), 95.2 (100).

Isolation of Xenialactol 9-Acetate (43): Silica gel chromatography of the $CHCL_3$ extract of Scheme 4 yielded 25 fractions. Elution of hexane-acetone (6:4) gave a red oily fraction. A 15 mg portion of this oily material was resolved by HPLC with a reverse-phase C_{18} column and MeOH-H_2O (7:3) as a mobile phase to afford 2 fractions. The first fraction contained a colorless oil, 5 mg, of xenialactol 9-acetate (43). Compound 43 possesses the following spectral data: UV (EtOH) λ_{max} 241 nm (ε = 14000); IR (neat) 3440, 2970, 2930, 2850, 1730, 1640, 1440, 1370, 1250, 1120, 1110, 1050, 1030, 990, 960, 900 cm^{-1}; for ^{13}C and ^{1}H NMR data see Tables 4 and 5; low resolution mass spectrum (12 eV) m/z (relative intensity) 376.4 [M^{+}, (3)], 358.4 [$M^{+}-H_2O$, (12)], 340.1 [$M^{+}-2H_2O$, (4)], 316.2 [M^{+}-AcOH, (15)], 298.2 [M^{+}-AcOH-H_2), (36)], 283.1 (38), 270.2 (37), 269.1 (34), 255.2 (59), 252.2 (41), 237.2 (73), 227.2 (54), 213.2 (58), 212.2 (96), 211.2 (81), 209.1 (88), 199.1 (64), 170.0 (89), 183.1 (100), 171.1 (91), 157.0 (71), 145.0 (88), 120.2 (42).

Isolation of 7,8-Dihydro-7α,8α-epoxyxenialactol (44): A 15 g sample of $CHCL_3$ extract, fraction F', (see Scheme 4) was chromatographed on a silica gel column (24 X 9 cm). The material was eluted starting with a hexane-acetone (9:1) mixture and increasing the amount of acetone. Elution with hexane-acetone (1:1) gave a fraction (fraction 2 5,

100 mg) which was passed through a silica gel Sep-Pak column to obtain 80 mg of yellow oil. This oily material was chromatographed by HPLC using silica gel with 75:25 hexane-acetone and collecting eight fractions. Fraction 4 (3.5 mg) was rechromatographed by HPLC using the same solvent mixture to afford 2.9 mg of triol 44 as a white powder in ca. 95% purity. Compound 44 exhibits the following spectral properties: UV (EtOH) λ_{max} 241 nm (ϵ = 15320); IR (neat) 3400, 3010, 2980, 2930, 2860, 1640, 1450, 1380, 1350, 1250, 1130, 1080, 970, 900, 880 cm^{-1}; for ^{1}H and ^{13}C NMR data see Tables 5 and 4; low resolution mass spectrum (12 eV) m/z (relative intensity) 350.2 (11), 332.1 (46), 314.1 (11), 286.2 (20), 271.1 (17), 207.2 (17), 199.1 (36), 187.2 (60), 173.1 (59), 161.0 (88), 145.0 (100), 130.0 (27); high-resolution mass spectrum, observed m/z (composition, interpretation, calculated millimass) 332.19594 ($C_{20}H_{28}O_4$, $M^+ - H_2O$, 332.19876), 314.18948 ($C_{20}H_{26}O_3$, $M^+ - 2H_2O$, 314.1882), 286.19325 ($C_{19}H_{26}O_2$, $M^+ - H_2O - CH_2O_2$, 286.19328).

Acetylation of 7,8-Dihydro-7α,8α-epoxyxenialactol (44): Acetic anhydride (15 µL) was added to a solution of 44 (1 mg) in pyridine (25µ L) and the reaction mixture was allowed to react at room temperature for 24 h. The excess of reagents was evaporated under nitrogen and the residue was chromatographed by HPLC using a silica gel column with 8:2 hexane-acetone as eluent to yield the 1,9-diacetate 46; for ^{1}H and ^{13}C NMR data see Tables 5 and 4.

Isolation of Xeniolide-A (7): The $CHCL_3$ extract, fraction F' of Scheme 4, was chromatographed (see isolation of xenialactol) over silica gel. Repeated chromatography of fraction 22, Scheme 4, by HPLC using a silica gel column and hexane-acetone (8:2) led to the isolation of

xeniolide-A (7) as an oil which decomposed after 4 weeks even in low temperature. Compound 7 possesses the following spectral data: UV (EtOH) λ_{max} 265 nm (ε = 15000); IR (neat) 3450, 2970, 2930, 2875, 1712, 1638, 1605, 1440, 1380, 1240, 1160, 1100, 1035, 970, 900, 850 cm^{-1}; for ^{1}H and ^{13}C NMR data see Tables 7 and 8; low resolution mass spectrum (20 eV) m/z (relative intensity) 314.2 [$M^{+}-H_2O$, (9)], 299.3 [$M^{+}-H_2O$-Me, (7)], 296.1 [$M^{+}-2H_2O$, (7)], 280.9 (7), 271.2 (17), 253.3 (22), 218.3 (31), 199.1 (22), 185.1 (30), 157.1 (48), 144.8 (63), 132.9 (58), 131.1 (100), 105.0 (56), 91.1 (51), 81.0 (32).

Isolation of 9-Deoxyxeniolide-A (47): A 15 g sample of fraction F' (see Scheme 4) was chromatographed over a column of silica gel. The material was eluted first with hexane-acetone (9:1), fraction 10, and then increasing amounts of acetone. One of the fractions eluted with hexane-acetone (7:3) fraction 10, was a yellowish oil. This oily fraction was resolved by HPLC with silica gel and hexane-acetone (88:12) to give 5 fractions. Fraction 5 was rechromatographed by HPLC using a reverse-phase C_{18} column and MeOH-H_2O (7:3) as eluent to yield, in trace amounts, 1.2 mg of ca. 88% pure 9-deoxyxeniolide-A (47) as a colorless oil. Compound 47 possesses the following spectral data: IR (neat) 3440, 2945, 2830, 2840, 1712, 1635, 1455, 1440, 1380, 1245, 1200, 1160, 1020, 995, 885 cm^{-1}; for ^{1}H and ^{13}C NMR data see Tables 7 and 8.

Isolation of 9-Deoxy-7,8-dihydro-7α,8α-epoxyxeniolide-A (48): A 15 g sample of the $CHCL_3$ extract of Scheme 3 was chromatographed on a column of silica gel (24 X 9 cm). Elution started with hexane-acetone (9:1) and then the amount of acetone was increased. One of the fractions eluted with hexane-actone (7:3), fraction 14, was rechromatographed by HPLC using a silica gel column and hexane-acetone (82:18) as

eluent to give 9 fractions, of which fraction 6 was further purified by HPLC using a reverse-phase C_{18} column and MeOH-H_2O (6:4) as eluent to yield 1 mg of 9-deoxy-7,8-dihydro-7α,8α-epoxyxeniolide-A (48) as a colorless oil. Compound 48 possesses the following spectral properties: $[\alpha]_D^{25}$ +3° (c. 0.1 $CHCL_3$); UV (EtOH) λ_{max} 267 nm (ε = 15300); IR (neat) 3430, 2950, 2820, 2835, 1714, 1635, 1460, 1450, 1380, 1240, 1200, 1170, 1025, 990, 900, 885 cm^{-1}; for ^1H and ^{13}C NMR data see Tables 7 and 8; low resolution mass spectrum (12 eV) m/z (relative intensity) 332.2 [M^+, (21)], 314.2 [M^+-H_2O (11)], 289.1 (100), 271.1 (34), 253.0 (34), 215.1 (34), 197.1 (25), 159.0 (49).

Isolation of 15-Dehydroxy-15-acetyldioxyxeniolide-A (49): The $CHCL_3$ extract, fraction F' of Scheme 3 was chromatographed on a column of silica gel. Elution was started with hexane-acetone (9:1) and the amount of acetone was increased stepwise and 450 mL fractions were collected. One of the fractions eluted with hexane-acetone (7:3), fraction 14 of Scheme 4, was rechromatographed by HPLC using a silica gel column with hexane-acetone (82:18) to give 9 fractions. Fraction 7 therefore was resolved by HPLC with a reverse-phase C_{18} column and MeOH-H_2O (7:3) to afford 2 fractions. Evaporation of the second fraction left 1.7 mg of 15-dehydroxy-15-acetyldioxyxeniolide-A (49) as a colorless oil; compound 49 has the following spectral properties: $[\alpha]_D^{25}$ -12.9° (c. 0.17 $CHCL_3$); UV (EtOH) λ_{max} 265 nm (ε = 14500); IR (neat) 3440, 2980, 2930, 2845, 1730, 1712, 1640, 1600, 1440, 1385, 1370, 1320, 1250, 1190, 1120, 1030, 990, 900 cm^{-1}; for proton and carbon-13 NMR data ($CDCL_3$) see Tables 7 and 8; ^1H NMR (C_6D_6) (number of protons, multiplicity, J in Hz, assignment) δ 1.24 (3, br s, Me-18), 1.36 (1, m, H-5), 1.40 (6, s, Me-16

and Me-17), 1.43 (1, m, H-5'), 1.68 (3, s, oAc), 1.76 (1, br dt, 12.2, 5.5, H-11a), 1.80 (1, dt, 12.2, 3.5, H-6), 1.93 (1, br dd, 13.5, 5.4, H-10), 1.96 (1, br d, 13.5, H-10'), 1.99 (1, ddd, 13, 12.2, 5.4, H-6'), 3.0 (1. br dd, 10.5, 5.5, H-4a), 3.33 (1, dd, 12.2, 11.6, H-1), 3.72 (1, dd, 12.2, 5.5, H-1'), 4.31 (1, ddd, 8.4, 5.4, 1.8, H-9), 4.66 (1, br s, H-19), 4.84 (1, br s, H-19'), 5.18 (1, br d, 8.4, H-8), 5.98 (1, d, 15.4, H-14), 6.48 (1, dd, 15.4, 11.4, H-13), 7.08 (1, br d, 11.4, H-12); low resolution mass spectrum (70 eV) m/z (relative intensity) 315.3 [M^+-AcOO, (2)], 314.3 [M^+-AcOOH, (7)], 296.2 [M^+-AcOOH-H_2O, (1)], 273.1 (15), 199.1 (12), 185.2 (11), 171.1 (20), 159.1 (25), 145.1 (34), 131.1 (53), 117.2 (43), 105.1 (67), 91.1 (100), 83.1 (61), 79.2 (76), 77.1 (64).

Isolation of 7,8-Dihydro-7α,8α-epoxyxeniolide-A (50): A 3 g sample of $CHCL_3$ extract, Scheme 1, was chromatographed on a column of silica gel (40 X 2.5 cm). The material was eluted first with chloroform-acetone (9:1) and then increasing the amount of acetone and collecting 9 fractions. Elution with chloroform-acetone (75:25) gave fractions 8 and 9 which were combined and rechromatographed over silica gel using chloroform-acetone (85:15) as eluent and increasing the amount of acetone and collecting 7 fractions. Fraction 5 was resolved by HPLC with silica gel and hexane-acetone (6:4) as a mobile phase to give 4 fractions. Fraction 3 contained pure 7,8-dihydro-7α,8α-epoxyxeniolide-A (50) as a white powder, 7 mg, $[\alpha]_D^{25}$ +22.1° (c. 0.19 $CHCL_3$); UV (EtOH) λ_{max} 268 nm (ε = 15270); IR ($CHCL_3$) 3600, 3440, 2970, 2930, 2870, 1712, 1638, 1610, 1455, 1385, 1365, 1320, 1180, 1105, 1035, 970, 910 cm^{-1}; for ^1H and ^{13}C NMR data see Tables 7 and 8; HRMS, observed m/z (composition, interpre-

tation, calculated millimass) 348.19259 ($C_{20}H_{28}O_5$, M^+, 348.19367), 330.18089 ($C_{20}H_{26}O_4$, M^+-H_2O, 330.18311), 305.17305 ($C_{18}H_{25}O_4$, M^+-Ac, 305.17528), 135.08053 ($C_9H_{11}O$, $M^+-C_{11}H_{17}O_4$, 135.08099), 91.05346 (C_7H_7, $M^+-C_{13}H_{21}O_5$, 91.05477); low resolution mass spectrum (70 eV) m/z (relative intensity) 348.3 (7), 330.2 (2), 305.2 (26), 289.2 (27), 161.1 (31), 145.1 (53), 135.1 (47), 121.1 (56), 119.2 (53), 109.2 (51), 91.1 (100), 81.1 (63), 77.1 (60).

Acetylation of 7,8-Dihydro-7α,8α-epoxyxeniolide-A (50): Compound 50 (2 mg) was allowed to react with 0.01 mL of acetic anhydride-pyridine (1:3) at room temperature overnight. Cold water (1.3 mL) was added and the resulting mixture was dried under nitrogen. The residue was purified by HPLC using a silica gel column and hexane-acetone (8:2) as eluent to give compound 51; for ^1H and ^{13}C NMR data see Tables 7 and 8.

Isolation of 18-Hydroxyasteroxeniolide-A 9-Acetate(52): A 15 g sample of the $CHCL_3$ extract (fraction F') of Scheme 3 was chromatographed on a Büchner funnel column of silica gel (24 X 9 cm) and using a water aspirator as a source of vacuum.[29] The material was eluted using a step gradient beginning with hexane-acetone (9:1) and then increasing the amount of acetone and collecting 450 mL fractions. Two of the fractions eluted with hexane-acetone (1:1) was a brown oily material (300 mg). Addition of 15 mL of a mixture of hexane-acetone (7:3) to the brown oily material caused a yellow solid (110 mg) to precipitate. This yellow solid was discussed above (see isolatin of 8). After removal of the yellow solid by filtration, the remaining solution was chromatographed by HPLC using a silica gel column and hexane-acetone (8:2) as

eluent to yield four fractions. The first fraction contained pure 52, yellow oil, 45.2 mg, $[\alpha]_D^{25}$ 0.00 ($CHCL_3$); IR ($CHCL_3$) 3430, 3030, 2940, 2860, 1725, 1715, 1640, 1450, 1380, 1340, 1260, 1180, 1130, 1020, 960, 840 cm^{-1}; UV (EtOH) λ_{max} 217 nm (ε = 11764); for ^1H and ^{13}C NMR data see Tables 11 and 12; mass spectrum (12 eV, low resolution), m/z (relative intensity) 390.2 [M^+ $C_{22}H_{30}O_6$ (4)], 372.2 (11), 362.4 (20), 361.3 (82), 331.1 (14), 330.3 (19), 313.1 (14), 312.1 (34), 302.3 (28), 301.2 (93), 299.1 (13), 297.2 (33), 284.1 (39), 283.1 (37), 271.2 (27), 270.2 (31), 269.2 (100), 267.2 (25), 244.2 (26), 243.2 (71), 241.2 (47), 229.2 (37), 228.2 (28), 227.2 (32), 225.2 (32), 199.2 (91), 197.1 (44), 183.2 (48), 181.1 (37), 173.2 (44), 171.2 (58), 159.1 (40), 157.1 (51), 145.0 (44), 83.1 (45), and 81.0 (14).

Acetylation of 18-Hydroxyasteroxeniolide-A 9-Acetate(52): Compound 52 (1.5 mg) was allowed to react with 0.075 mL of acetic anhydride-pyridine (1:3) at room temperature overnight. Cold water (1 mL) was added and the resulting mixture was dried under nitrogen. The residue was purified by HPLC using a silica gel column and hexane-acetone (8:2) as eluent to give compound 53; for ^1H and ^{13}C NMR data see Tables 11 and 12; low resolution mass spectrum (70 eV), m/z (relative intensity) 474.1 [M^+ $C_{26}H_{34}O_8$ (4)], 432.1 (26), 415.2 (30), 414.1 (33), 373.1 (100), 372.1 (18), 330.2 (13), 313.1 (14), 312.2 (23), 297.1 (16), 269.0 (14), 253.1 (14), 225.0 (13), 199.0 (13), 195.0 (14), 143.0 (25), 131.0 (25), 117.1 (20), and 105.1 (23).

Isolation of Asteroxeniolide-A (54): The 21st fraction (0.19 g) obtained upon elution with hexane-acetone (1:1) from a silica gel column of the $CHCL_3$ extract (fraction F') (see isolation of compound 52) was

chromatographed by HPLC using a silica gel column and hexane-acetone (75:25) as eluent to afford four fractions. The fourth fraction (60 mg) was rechromatographed by HPLC using a reverse-phase column and MeOH-H_2O (8:2) as eluent to afford two fractions. The first fraction (4.5 mg) was purified by HPLC using a reverse-phase column and MeOH-H_2O (1:1) as eluent to give pure 54, light yellow oil, 3 mg; $[\alpha]_D^{25}$ +26° (c. 0.29, $CHCL_3$); UV (EtOH) λ_{max} 217 nm (ε = 11547); IR (neat) 3420, 3080, 2980, 2930, 2850, 1710, 1640, 1442, 1382, 1218, 1190, 1025, 990, 910, 850, and 760 cm^{-1}; for ^1H and ^{13}C NMR data see Tables 11 and 12, respectively; mass spectrum (12 eV, low resolution), m/z (relative intensity) 332.1 [M^+ $C_{20}H_{28}O_4$ (0.8)], 314.1 (12), 300.1 (12), 299.1 (50), 296.2 (13), 286.1 (21), 285.1 (24), 281.1 (22), 272.1 (19), 271.1 (100), 231.1 (39), 230.1 (30), 218.1 (71), 173.0 (58), 171.1 (59), 159.1 (55), 157.0 (38), 145.0 (57), 133.0 (47), 131.1 (50), 105.1 (35), 95.1 (56), and 83.1 (41).

Isolation of Asteroxeniolide-A 9-Acetate (55): The 14th fraction (0.25 mg) obtained upon elution with hexane-acetone (7:3) from a silica gel column of the $CHCL_3$ extract (fraction F', Scheme 3) was chromatographed by HPLC using a silica gel column with 82:18 hexane-acetone as eluent to give nine fractions. The sixth faction was chromatographed further by HPLC using a C_{18} reverse-phase column (6:4 MeOH-H_2O) to obtain three fractions. The second fraction contained pure 55, light yellow oil, 4 mg; $[\alpha]_D^{25}$ -1° (c. 0.3, $CHCL_3$); UV (EtOH) λ_{max} 217 nm (ε = 11429); IR (neat) 3450, 3080, 2940, 2850, 1725, 1730, 1640, 1450, 1380, 1370, 1370, 1260, 1180, 1030, 990, 900, 840 cm^{-1}; for ^1H and ^{13}C NMR

data see Tables 11 and 12, respectively; mass spectrum (12 eV, low resolution), m/z (relative intensity) 356 [M^+-H_2O $C_{22}H_{28}O_4$ (27)], 345.1 (100), 341.3 (66), 296.2 (64), 286.1 (37), 281.1 (60), 271.1 (79), 253.3 (48) 241.1 (41), 230.1 (70), 218.1 (73), 190.1 (34), 171.2 (37), 157.0 (67), 143.0 (62), 107.2 (60), 95.1 (75), and 83.1 (64).

Isolation of Xeniolide-B (13): The twenty-third and twenty-fourth fraction (155 mg) of Scheme 4 was chromatographed by HPLC using a silica gel column and hexane-acetone (8:2) as eluent to give 4 fractions. The second of these fractions contained 23 mg of pure xeniolide-B (13) as a light yellow oil. Compound 13 has the following spectral data: UV (EtOH) λ_{max} 241 nm (ε = 15000); IR (neat) 3440, 2970, 2930, 2845, 1730, 1660, 1640, 1455, 1380, 1360, 1248, 1160, 1020, 970, 910, 870 cm^{-1}; for ^1H NMR data see Table 16; low resolution mass spectrum (70 eV) m/z (relative intensity) 314.3 [M^+-H_2O, (2)], 256.2 (5), 230.1 (13), 207.1 (28), 187.2 (22), 173.1 (33), 159.1 (41), 147.0 (42), 133.1 (42), 129.0 (60), 115.1 (40), 105.1 (67), 91.1 (100), 79.1 (61), 69.1 (50).

Isolation of Xeniolide-B 9-Acetate (14): A 0.1 g portion of the fifteenth fraction of Scheme 4 was chromatographed by HPLC using silica gel and hexane-acetone (83:17) to yield a pure Xeniolide-B 9-acetate (14), 65 mg, as a yellow oil. Compound 14 has the following data: UV (EtOH) λ_{max} 241 nm (ε = 15000); IR (neat) 3440, 2970, 2930, 2850, 1730, 1660, 1640, 1450, 1380, 1365, 1250, 1164, 1020, 970, 910, 870 cm^{-1}; for ^1H and ^{13}C NMR data see Tables 16 and 17; low resolution mass spectrum (12 eV) m/z (relative intensity) 374.5 [M^+, (9)], 314.2 [M^+-AcOH, (30)], 299.3 [M^+-AcOH-Me, (12)], 296.1 [M^+-AcOH-H_2O, (34)], 280.9 (27), 256.1

(100), 225.3 (57), 212.0 (46), 185.0 (85), 169.1 (44), 144.8 (38), 131.0 (51), 109.2 (22).

Isolation of 7,8-Dihydro-7α,8α-epoxyxeniolide-B (15): The $CHCL_3$ extract (3 g) of Scheme 1 was chromatographed on a column of silica gel. The material was eluted initially with chloroform-acetone (9:1) and then gradually the amount of acetone was increased while 9 fractions were collected. Fractions 8 and 9 were combined and rechromatographed over silica gel using chloroform-acetone (85:15) as eluent initially and then increasing the amount of acetone and collecting 7 fractions. The sixth of these fractions was subjected to HPLC with a silica gel column and hexane-acetone (7:3) as a mobile phase to give pure 7,8-dihydro-7α,8α-epoxyxeniolide-B (15), 1 mg, as a white powder. Compound 15 has the following spectral data: IR (neat) 3500, 2975, 2930, 2850, 1730, 1640, 1450, 1380, 1360, 1250, 1160, 1025, 975, 910, 875 cm^{-1}; for ^1H and ^{13}C NMR data see Tables 16 and 17; low resolution mass spectrum (70 eV) m/z (relative intensity) 348.1 [M^+, (16)], 330.2 [M^+-H_2O, (7)], 227.1 (7), 207.0 (15), 161.0 (21), 148.9 (30), 147.0 (80), 133.0 (32), 119.1 (36), 107.1 (43), 95.1 (59), 75.1 (100).

Isolation of Asterospicin (56): A 3 g portion of the $CHCL_3$ extract of Scheme 1, was chromatographed on a column of silica gel (40 X 2.5 cm). The sample was eluted first with chloroform-acetone (9:1) and the amount of acetone was increased stepwise and 9 fractions were collected. Fractions 3 to 7 were combined and rechromatographed on a short column of silica gel to give 6 fractions. The fourth of these fractions was subjected to HPLC using a reverse-phase C_{18} column with MeOH-H_2O (75:25) as eluent to yield 3 fractions. The first fraction, 8 mg, was resolved

by HPLC using a reverse-phase C_{18} column with MeOH-H_2O (6:4) as a mobile phase to afford 3 fractions. Evaporation of the second fraction left 2.2 mg of pure asterospicin (56) as a colorless oil. 56 has the following spectral data: UV (EtOH) λ_{max} 235 nm (ε = 16100); IR (neat) 3440, 2960, 2925, 2850, 1660, 1640, 1440, 1385, 1220, 1080, 1040, 990, 910, 880 cm^{-1}; for ^1H and ^{13}C NMR data see Tables 18 and 19; low resolution mass spectrum (70 eV) m/z (relative intensity) 332.3 (4), 302.3 (12), 286.3 (5), 185.1 (12), 171.2 (24), 159.0 (17), 157.0 (26), 145.0 (34), 143.1 (35), 131.1 (32), 129.1 (31), 119.2 (24), 117.1 (25), 109.1 (100), 105.1 (45), 96.1 (10), 95.1 (38), 93.1 (24), 91.1 (50), 81.1 (35), 79.1 (38), 77.1 (27); high-resolution mass spectrum, observed m/z (composition, interpretation, calculated millimass) 332.20101 ($C_{20}H_{28}O_4$, M^+, 332.19876), 314.18902 ($C_{20}H_{26}O_3$, M^+-H_2O, 314.1882), 303.19493 ($C_{19}H_{27}O_3$, M^+-CHO, 303.19602), 302.18691 ($C_{19}H_{26}O_3$, M^+-CH_2O, 302.1882), 286.19516 ($C_{19}H_{26}O_2$), 244.14824 ($C_{16}H_{20}O_2$), 199.11159 ($C_{14}H_{15}O$), 185.09594 ($C_{13}H_{13}O$), 109.06562 (C_7H_9O), 81.07028 (C_6H_9).

Acetylation of Asterospicin (56): Compound 56 (1 mg) was allowed to react with 0.008 mL of acetic anhydride-pyridine (1:3) at room temperature overnight. The excess reagents were dried under nitrogen and the residue was chromatographed by HPLC using a silica gel column with hexane-acetone (8:2) to give compound 57; for ^1H and ^{13}C NMR data see Tables 18 and 19: low resolution mass spectrum (12 eV) m/z (relative intensity) 345.0 [M^+-CHO, (5)], 344.1 [M^+-CH_2O, (27)], 314.2 [M^+-AcOH, (10)], 225.2 (14), 197.1 (31), 172.1 (13), 164.2 (32), 147.1 (23), 135.1 (24), 133.2 (23), 124.2 (20), 109.1 (100), 95.0 (24).

Dehydration of 7,8-Dihydro-7α,8α-epoxyxanialactol (44): To a solution of compound 44 (2 mg) in $CHCL_3$ (2 mL) at 4 C° was added 0.25 mL of glacial acetic acid with stirring for 4 h. The excess of the reagents were dried under nitrogen and the residue was chromatographed by HPLC with a silica gel column and hexane-acetone (7:3) as a mobile phase to give asterospicin (56) (1.2 mg), identical in all aspects (NMR, IR, TLC) with the natural material.

Isolation of 14,15-Dihydro-14ξ,15ξ-epoxyasterospicin-1 (58) and 14,15-Dihydro-14ξ,15ξ-epoxyasterospicin-2 (59): A 3 g sample of the $CHCL_3$ extract, see Scheme 1, was chromatographed on a column of silica gel. Elution started with chloroform-acetone (9:1) and the amount of acetone was increased stepwise and 9 fractions were collected. Fractions 3-7 were combined (0.6 g) and rechromatographed on a short column of silica gel using chloroform-acetone (8:2) initially and increasing the amount of acetone and collecting 6 fractions. Fraction 4, 8 mg, was then subjected to HPLC with a reverse-phase C_{18} column and MeOH:H_2O (75:25) to give 3 fractions. The first fraction was resolved further by HPLC with MeOH-H_2O (6:4) to yield two fractions containing compounds 58 and 59 as colorless oils, 0.3 mg each. Compound 58 has the following spectral data: IR (neat) 3500, 2960, 2920, 2840, 1610, 1450, 1380, 1225, 1090, 1024, 990, 910, 885 cm^{-1}; for ^1H and ^{13}C NMR data of 58 and 59 see Tables 18 and 19; low resolution mass spectrum (70 eV) m/z (relative intensity) 348.3 [M^+, (2)], 330.4 [M^+-H_2O, (2)], 279.2 (8), 167.0 (21), 149.0 (82), 129.0 (34), 113.1 (24), 109.1 (43), 105.1 (29), 97.1 (95), 95.2 (53), 86.0 (62), 85.1 (77), 84.1 (100), 83.1 (81), 75.1 (63), 71.1 (87).

Isolation of 14,15-Dihydro-14ξ,15-dihydroxyasterospicin-1 (60) and 14,15-Dihydro-14ξ,15-dihydroxyasterospicin-2 (61): A 3 g portion of the $CHCL_3$ extract of Scheme 1 was chromatographed on a column of silica gel (40 X 2.5 cm). The materials were eluted first with chloroform-acetone (9:1) and then increasing amounts of acetone and collecting 9 fractions. Elution with chloroform-acetone (75:25) gave fractions 8 and 9 which were combined and rechromatographed over silica gel using chloroform-acetone (85:15) as eluent and increasing the amount of acetone and collecting 7 fractions. The last of these fractions (fraction 7) was purified by HPLC with silica gel and hexane-acetone (1:1) as a mobile phase to give a mixture of 1.5 mg of 60 and 61 as white powder. This mixture was resolved by HPLC with a reverse-phase C_{18} column and MeOH-H_2O (25:75) as eluent to give pure 60 (0.5 mg) and 61 (0.4 mg) as colorless oils. Compound 60 has the following spectral data: IR (neat) 3500, 2980, 2930, 2860, 1630, 1440, 1385, 1260, 1220, 1160, 1080, 1040, 990 900, 885 cm^{-1}; 300-MHz ^1H NMR ($CDCL_3$) (assignment, multiplicity, J in Hz) δ 6.08 (H-12, br d, 16), 5.86 (H-13, dd, 16, 6), 5.33 (H-1, s), 5.32 (H-19, br s), 5.15 (H-19', br s), 4.00 (H-14, br d, 6), 3.97 (H-3', d, 6.8), 3.74 (H-9, br dd, 7.6, 6.5), 3.31 (H-3, dd, 6.8, 2), 2.74 (H-8, d, 7.6), 2.59 (H-10', br d, 14.5), 2.40 (H-10, br dd, 14.5, 6.5), 2.15 (H-11a, br dd, 4.16, 1.2), 1.82 (H-5, br dt, 14.2, 3.6), 1.75 (H-4a, br dd, 10.5, 4.6), 1.54 (H-5', dddd, 14.2, 13.5, 10.5, 3.6), 1.38 (Me-18, s), 1.25 (Me-16, s), 1.15 (Me-17, s), 1.05 (H-6', br td, 13.5, 3.6); low resolution mass spectrum (12 eV) m/z (relative intensity) 348.4 [M^+-H_2O, (4)], 284.3 (37)], 279.1 (19), 241.2 (10), 227.2 (14), 213.1 (31), 199.2 (20), 193.1 (20), 185.2 (45), 171.1 (30), 167.1 (60), 149.0 (93), 129.0

(100), 115.2 (31), 97.1 (92), 83.1 (53). IR data of 61 are identical to those of 60; ^{1}H NMR ($CDCL_3$) data of 61 are identical to those of 60 except for the following protons in respect to the chemical shift; δ 5.84 (H-13, dd, 16, 6), 3.98 (H-14, br d, 6), 3.32 (H-3, dd, 6.8, 2); low resolution mass spectrum of 61 was identical to that of 60; high-resolution mass spectrum of 60, observed m/z (composition, interpretation, calculated millimass) 348.19087 ($C_{20}H_{28}O_5$, M^+-H_2O, 348.19368), 336.19306 ($C_{19}H_{28}O_5$, M^+-CH_2O, 336.19368), 333.170 ($C_{19}H_{25}O_5$, M^+-MeO, 333.17020), 330.18382 ($C_{20}H_{26}O_4$, M^+-2H_2O, 330,18311), 308.16148 ($C_{17}H_{24}O_5$, $M^+-C_3H_6O$, 308.16238), 290.15286 ($C_{17}H_{22}O_4$, $M^+-H_2O-C_3H_6O$, 290.15181), 272.14137 ($C_{17}H_{20}O_3$, $M^+-2H_2O-C_3H_6O$, 272.14125), 262.15491 ($C_{16}H_{22}O_3$), 244.14803 ($C_{16}H_{20}O_2$), 201.12669 ($C_{14}H_{17}O$), 125.06129 ($C_7H_9O_2$), 97.06499 (C_6H_9O).

Expoxidation of Xenialactol (8): To a solution of xenialactol (8) (35 mg) in $CHCL_3$ (10 mL) at 2 C° was added a solution of m-chloroperbenzoic acid (20 mg) in $CHCL_3$ (3 mL) dropwise with stirring over a period of 1.5 h. Stirring was continued for another 2 h. The reaction mixture was extracted with 20% Na_2CO_3 solution, and the solution was washed with water, and evaporated to give 30 mg of an oily material. The oily mixture was chromatographed on a preparative TLC plate using hexane-acetone (1:1) as eluent to yield two fractions. The first fraction (10 mg) (Rf = 0.6) was purified by HPLC with a reverse-phase column and MeOH-H_2O (1:1) to yield 8 mg of compound 45 as a colorless oil. The second fraction (12 mg) (Rf = 0.47) was subjected to HPLC using a reverse-phase C_{18} column and MeOH-H_2O (1:1) to give two fractions. The first fraction contained a mixture of two compounds. This mixture was resolved by HPLC using reverse-phase and MeOH-H_2O (25:75) to afford two pure compounds

which were found to be 60 (2.5 mg) and 61 (1.9 mg), identical in all respects with the natural material. The second fraction (6 mg) was found to be 7,8-dihydro-7α,8α-epoxyxenialactol (44), identical in all respects (TLC, NMR, IR) with the natural material. Compound 45 has the following spectral data: UV (EtOH) λ_{max} 240 nm (ε = 15700); IR (neat) 3450, 3010, 2980, 2940, 2840, 1640, 1445, 1380, 1355, 1250, 1130, 1080, 980, 905, 885 cm^{-1}; for ^{1}H and ^{13}C NMR data of 45 see Tables 4 and 5.

Reduction of compound 45: Compound 45 (3 mg) in acetic acid (1 mL) was treated with zinc dust (22 mg) and stirred for 4 h at room temperature. The reaction mixture was diluted with $CHCl_3$ and extracted with 15% Na_2CO_3 solution. The organic solution was washed with water and evaporated to give 1.6 mg of oily material which was chromatographed by HPLC using a silica gel column and hexane-acetone (65:35) as eluent to give 1 mg of asterospicin (56), identical in all respects (TLC, NMR, IR) with the natural material.

Isolation of 3α-Hydroxy-7,8-deepoxy-7,8-dehydroasterospicin (62): Silica gel chromatography of the $CHCL_3$ extract, fraction F', of Scheme 3 afforded 25 fractions. Fraction 14 (250 mg) was subjected to HPLC with a silica gel column and hexane-acetone (82:18) as eluent to give 9 fractions. The fifth of these fractions (12 mg) was resolved by HPLC using a reverse-phase C_{18} column and MeOH-H_2O as a mobile phase to yield 3 fractions. Evaporation of the first fraction left 2.2 mg of 3α-hydroxy-7,8-deepoxy-7,8-dehydroasterospicin (62) as a colorless oil; UV (EtOH) λ_{max} 235 nm (ε = 15119); IR (neat) 3450, 2930, 2850, 1655, 1640, 1440, 1380, 1090, 1040, 918, 885 cm^{-1}; for ^{1}H and ^{13}C NMR data of 62 see Tables 18 and 19; low resolution mass spectrum 70 eV) (m/z, relative

intensity) 305.1 (2), 304.1 (6), 303.2 (24), 287.2 (6), 286.1 (34), 161.1 (11), 159.1 (19), 145.1 (23), 143.0 (16), 131.0 (25), 129.0 (20), 121.1 (20), 119.1 (26), 111.1 (11), 109.1 (100), 107.1 (28), 105.1 (37), 95.2 (28), 93.2 (28), 91.1 (59), 83.1 (26), 81.1 (64), 79.1 (49), and 77.0 (37); high resolution mass spectrum, observed m/z (compsosition, interpretation, calculated millimass) 317.17090 ($C_{19}H_{25}O_4$, ,M^+-Me, 317.17529), 302.18377 ($C_{19}H_{26}O_3$, M^+-CH_2O, 302.18820), 286.18912 ($C_{19}H_{26}O_2$), M^+-CO-H_2O, 286.19328), 109.06606 (C_7H_9O, M^+-CH_2O-$C_{12}H_{17}O_2$, 109.06534), 91.05465 (C_7H_7, M^+-CH_2O-H_2O-$C_{12}H_{17}O_2$, 91.05478), 81.07130 (C_6H_9, M^+-CH_2O-$C_{12}H_{17}O_2$-CO, 81.07042), 79.05597 (C_6H_7, M^+-$2CH_2O$-$C_{12}H_{17}O_2$, 79.05478); FAB mass spectrum, m/z 332.

Acetylation of 3-α-hydroxy-7,8-deepoxy-7,8-dehydroasterospicin (62): A 1 mg portion of 62 was allowed to react with acetic anhydride-pyridine (1:3) (0.08 mL) at room temperature overnight. The excess reagents were dried under nitrogen and the resultant residue was chromatographed by HPLC using a silica gel column and hexane-acetone (9:1) as eluent to give 63 (0.3 mg) and 64 (0.2 mg) as white powders; for ^1H NMR data of 63 and 64 see Table 18; low resolution mass spectrum of 63 (12 eV): m/z (relative intensity) 388.1 [M^+-CO, (4)], 387.1 [M^+-CHO, (8)], 263.1 (5), 221.0 (12), 207.0 (12), 189.1 (7), 167.1 (10), 155.1 (11), 149.0 (46), 125.1 (21), 109.0 (43), 99.0 (59), 97.1 (55), 85.1 (73), 75.1 (100); low resolution mass spectrum of 64: (12 eV) m/z relative intensity) 388.1 [M^+CO, (1)], 387.1 [M^+-CHO, (6)], 357.1 [M^+-AcO, (1)], 356.2 [M^+-AcOH, (1)], 239.1 [M^+-CO-AcO, (22)], 328.2 [M^+-CO-AcOH, (100)], 328.2 [M^+-CO-2AcOH, (9)], 250.2 (8), 235.1 (13), 207.0 (9), 175.0 (7), 147.0 (18), 109.1 (81), 79.1 (13), 75.1 (30).

Isolation of Asterospiculin (65): The fourteenth fraction (250 mg) of Scheme 4 was chromatographed by HPLC with a silica gel column and hexane-acetone as eluent to give 9 fractions. The fourth fraction (127 mg) was resolved by HPLC using a reverse-phase C_{18} column and MeOH-H_2O (7:3) to yield 2 reactions. The first fraction contained a known compound, 13-epi-9-O-deacetylxenicin (3). The second fraction contained 90 mg of pure asterospiculin (65) as a viscous oil; $[\alpha]_D^{25}$ -163.37° (c. 051, $CHCL_3$); UV (EtOH) λ_{max} 241 nm (ε = 15900); IR (neat) 3540, 3020, 2970, 2925, 2850, 1730, 1640, 1440, 1375, 1365, 1240, 1150, 1020, 980, 900 cm^{-1}; for ^1H and ^{13}C NMR data see Tables 27 and 28: low resolution mass spectrum (12 eV) m/z (relative intensity) 462.3 [M^+, (1)], 444.2 [M^+-H_2O, (7)], 402.2 [M^+-AcOH, (28)], 384.1 [M^+-AcOH-H_2O (8)], 342.2 [M^+2AcOH, (14)], 324.2 [M^+-2AcOH-H_2O, (20)], 282.2 [M^+-3AcOH, (28)], 264.2 [M^+-3AcOH-H_2O, (26)], 249.0 (29), 209.1 (62), 183.1 (44), 155.0 (39), 145.1 (85), 131.0 (82), 107.1 (100), 91.1 (81); high-resolution mass spectrum, observed m/z (composition, interpretation, calculated millimass) 444.25183 ($C_{26}H_{36}O_6$, M^+-H_2O, 444.25119), 348.22931 ($C_{24}H_{32}O_4$, M^+-AcOH, 348.23006), 324.20845 ($C_{22}H_{28}O_2$, M^+-2AcOH-H_2O, 324.20893), 282.19514 ($C_{22}H_{26}O$, M^+-3AcOH, 282.1984), 264.18813 ($C_{20}H_{24}$, M^+-3AcOH-H_2O, 264.18780), 249.16353 ($C_{19}H_{21}$, M^+-3AcOH-H_2O-Me, 249.16433), 107.08612 (C_8H_{11}), 105.07103 (C_8H_9), 93.07041 (C_7H_9); FD spectrum, m/z, 462.

Isolation of 7,8-Dihydro-7α,8α-epoxyasterospiculin (66): The fourteenth fraction (250 mg) (see Scheme 4) was chromatographed by HPLC with a silica gel column and hexane-acetone (82:18) as eluent to give 9 fractions. The ninth fraction (8 mg) was resolved by HPLC using a

reverse-phase C_{18} column and MeOH-H_2O as a mobile phase to give 2 fractions. The first fraction contained 3.7 mg of 40 which was discussed above. The second fraction contained 1.9 mg of 7,8-dihydro-7α,8α-epoxyasterospiculin (66) as a colorless oil; UV (EtOH) λ_{max} 241 nm (ε = 15900); IR (neat) 3440, 2980, 2930, 2850, 1730, 1640, 1440, 1375, 1240 1080, 1030, 970, 910, 875 cm^{-1}; for ^1H and ^{13}C NMR data see Tables 27 and 28; low resolution mass spectrum (12 eV): m/z (relative intensity): 460.1 [M^+-AcOH, (3)], 418.2 [M^+-AcHO, (3)], 400.1 [M^+-H_2O-AcOH, (8)] 358.2 [M^+-2 AcOH, (4)], 340.2 [M^+-2 AcOH-H_2O, (9)], 298.2 [M^+-3 AcOH, (4)], 280.2 [M^+-3 AcOH-H_2O, (22)], 265.1 (12), 239.2 (20), 209.2 (27), 197.1 (30), 185.1 (31), 161.0 (36), 149.0 (67), 147.1 (64), 145.0 (74), 134.0 (90), 60.1 (100).

Epoxidation of Asterospiculin (65): To a solution of asterospiculin (65) (18 mg) in $CHCL_3$ (7 mL) at 2° was added a solution of m-chloroperbenzoic acid (10 mg) in $CHCL_3$ (2 mL) dropwise with stirring over a period of 2.5 h. Stirring was continued for another 2 h. The reaction mixture was extracted with 15% Na_2Co_3 solution, and then water, and the solvent was evaporated to give 14 mg of an oily material. The oily material was chromatographed by HPLC with a silica gel column and hexane-acetone (75:25) as eluent to yield 2 fractions. The first fraction (8.5 mg) was found to contain 7,8-dihydro-7α,8α-epoxyasterospiculin (66), identical in all respects (TLC, NMR, IR) with the natural material. The second fraction contained a mixture of 2 epimeric compounds 67 and 68 (0.8 mg each); for ^1H NMR data see Table 27.

Isolation of 4ξ,12ξ:7α,8α-Diepoxy-4,12:7,8-tetrahydro-3-O-deacetylasterospiculin (69): A 4 g portion of CCL_4 extract, see Scheme 1, was

chromatographed on a column of silica gel (15 X 6.5 cm). Elution started with hexane-EtOAc (8:2) then the amount of EtOAc was gradually increased while 25 fractions were collected. The most polar fraction (fraction 25) (10 mg) was subjected to HPLC using a silica gel column and hexane-acetone (7:3) as eluent to yield 3 fractions. The third fraction contained 2.7 mg of compound 69 as a white powder; $[\alpha]_D^{25}$ 0.0 (c.0.27, $CHCL_3$); IR (neat) 3500, 3025, 2930, 2850, 1730, 1445, 1380, 1240, 1040, 970, 950, 905, 875 cm^{-1}; for ^1H and ^{13}C NMR data see Tables 27 and 28; low resolution mass spectrum (70 eV) m/z (relative intensity) 337.2 [M^+-$C_6H_{11}O_2$ (2)], 295.2 (10), 235.2 (2), 217.1 (4), 199.1 (3), 159.2 (15), 105.1 (23), 97.2 (100); high-resolution mass spectrum, observed m/z (composition, interpretation, calculated millimass) 374.2125 ($C_{22}H_{30}O_5$, M^+-AcOH-H_2O, 374.20933), 337.16476 ($C_{18}H_{25}O_6$), 295.15744 ($C_{16}H_{23}O_5$, M^+-AcOH-H_2O-C_6H_7, 295.15455), 235.13560 ($C_{14}H_{19}O_3$, M^+-2 AcOH-H_2O-C_6H_7, 235.13342), 217.12444 ($C_{14}H_{17}O_2$, M^+-2 AcOH-H_2O-C_6H_7, 217.12286), 199.11378 ($C_{14}H_{15}O$, M^+-2 AcOH-3 H_2O-C_6H_7, 199.11229), 187.11362 ($C_{13}H_{15}O$), 175.11184 ($C_{12}H_{15}O$), 159.11823 ($C_{12}H_{15}$), 119.08641 (C_9H_{11}), 109.0666 (C_7H_9O), 98.07267 ($C_6H_{10}O$), 97.0657 (C_6H_9O); FD mass spectrum m/z, 452.

Acetylation of compound 69: Acetic anhydride (0.02 mL) was added to a solution of 69 (0.5 mg) in pyridine (0.02 mL) and the mixture was stirred at room temperature for 24 h. The excess of reagents were then removed with a stream of nitrogen and the residue was chromatographed by HPLC using a silica gel column and hexane-acetone (8:2) as eluent to give pure 70: ^1H NMR data see Table 27; low resolution mass spectrum

(12 eV) m/z (relative intensity) 338.1 [M^+-2 AcOH-2 H_2O, (12)], 337.2 (70), 217.0 (19), 199.1 (13), 159.0 (11), 97.1 (100).

Isolation of Peridinin (71): The nineteenth fraction (40 mg) of Scheme 4 was chromatographed by HPLC with a silica gel column and hexane-acetone (75:25) as eluent to give 6 fractions. The fourth of these fractions was purified by HPLC using a reverse-phase C_{18} column and MeOH-H_2O as a mobile phase to yield 7.5 mg of pure peridinin (71) as an orange-red pigment. Compound 71 has the following spectral data: IR ($CHCL_3$) 3400, 2970, 2940, 2870, 1940, 1750, 1735, 1600, 1525, 1450, 1370, 1255, 1165, 1135, 1070, 1030, 990, 900 cm^{-1}; 300-MHz ^1H NMR ($CDCL_3$) (number of protons, multiplicity, J in Hz, assignment) δ 0.97 (3, s), 1.07 (3, s), 1.20 (6, s), 1.27 (1, dd, 11.8, 10, H-2'), 1.35 (3, s), 1.38 (3, s), 1.40 (1, t, 12, H-2), 1.51 (1, dd, 13.2, 11.7, H-4), 1.64 (1, ddd, 12.5, 3.5, 1.7, H-2'), 1.64 (1, dd, 14.2, 8.9, H-4'), 1.80 (3, s, Me-16), 1.99 (1, ddd, 14.5, 4.1, 1.9, H-2), 2.04 (3, s, OAc), 2.23 (2, s, Me-20'), 2.29 (1, ddd, 13.6, 6.1, 1.9, H-4), 2.40 (1, ddd, 14.3, 5.1, 1.5, H-4'), 3.90 (1, m, H-3'), 5.37 (1, tt, 11.5, 4.2, H-3), 5.73 (1, s, H-12'), 6.05 (1, s, H-8), 6.10 (1, br d, 11.7, H-10), 6.37 (1, d, 14.6, H-7'), 6.35-6.46 (3, m, H-12, H-15, H-15'), 6.53 (1, br d, 10.6, H-14'), 6.60 (1, dd, 13.9, 11.4, H-11), 7.02 (1, s, H-10'), 7.16 (1, d, 15.4, H-8'). 75.4-MHz ^{13}C NMR ($CDCL_3$) δ 14.0 (q), 15.42 (q), 19.89 (q), 21.4 (q), 24.90 (q), 29.17 (q), 29.53 (q), 31.24 (q), 32.07 (q), 35.23 (s), 35.80 (s), 40.92 (t), 45.23 (t), 45.42 (t), 47.2 (t), 64.18 (d), 67.53 (s), 67.98 (d), 70.48 (s), 72.66 (s), 103.32 (d), 117.60 (s), 119.24 (d), 121.80 (d), 124.77 (s), 127.8 (d), 128.94 (d), 131.49 (d), 133.03 (d), 133.64 (d), 133.91 (s), 133.97 (s), 170.44 (s),

202.66 (s); low resolution mass spectrum (12 eV) m/z (relative intensity) 630.5 [M^+, (4)], 612.4 [M^+-H_2O, (21)], 552.3 [M^+-H_2O-AcOH, (15)], 275.1 (12), 234.1 (14), 223.1 (10), 221.2 (7), 207.0 (6), 197.0 (12), 181.1 (100), 149.2 (100).

Isolation of (22R)-24ξ-Methyl-5α cholestane-3β,5,6β ,22,24-pentaol 6-Acetate (72): The twenty-first fraction (0.31 g) of Scheme 4 was chromatographed by HPLC with a silica gel column and hexane-acetone (75:25) as eluent to give 4 fractions. The most polar fraction (fraction 4) was rechromatographed by HPLC using a reverse-phase C_{18} column and MeOH-H_2O (8:2) as a mobile phase to afford 2 fractions. The first fraction contained 3 mg of asteroxeniolide-A (54), a new compound which was discussed above in this section. The second fraction consisted of 180 mg of pure 72 as a colorless oil: $[\alpha]_D^{25}$ -29.2 (c. 0.31 $CHCL_3$); IR ($CHCL_3$) 3600, 3440, 2950, 2870, 1728, 1460, 1440, 1375, 1250, 1210, 1090, 1030 cm^{-1}; 300-MHz ^1H NMR ($CDCL_3$) (number of protons, multiplicity, J in Hz, assignment) δ 0.70 (3, s, Me-18), 0.85 (3, d, 7.5 Hz, Me-27 or Me-26), 0.95 (3, d, 7.5 Hz, Me-21), 0.98 (3, d, 7.5 Hz, Me-26 or Me-27), 1.07 (3, s, Me-28), 1.14 (3, s, Me-19), 2.03 (1, sept, 7.5 Hz, H-25), 2.05 (3, s, OAc), 4.07 (1, m, H-3), 4.07 (1, br d, 11 Hz, H-22), 4.70 (1, br t, 3.0 Hz, H-6); 300-MHz ^1H NMR (CD_3OD) δ 0.74 (3, s, Me-18), 0.89 (3, d, 7 Hz, Me-27 or Me-26), 0.96 (3, d, 7 Hz, Me-21), 0.98 (3, d, 7 Hz, Me-26 or Me-27), 1.06 (3, s, Me-28), 1.16 (3, s, Me-19), 2.05 (3, s, Me-C(=O)-O), 4.0 (1, tt, 11, 5.4 Hz, H-3), 4.06 (1, br d, 11 Hz, H-22), 4.71 (1, t, 3 Hz, H-6); 75.4-MHz ^{13}C NMR (CD_3OD) δ 14.29 (Me-18, q), 14.72 (Me-21, q), 18.77 (Me-19, q), 19.07 (Me-27 or Me-26, q), 20.76 (Me-26 or Me-27, q), 23.11 (CH_3-C(=O)-O, q), 23.91 (t), 24.72 (Me-

28, q), 27.03 (t), 30.49 (t), 33.21 (t), 33.78 (d), 34.11 (t), 34.87 (t), 37.96 (d), 40.21 (t), 41.20 (C-10, s), 42.63 (t), 43.05 (t), 45.7 (d), 45.92 (C-13, s), 47.75 (d), 55.85 (d), 58.50 (d), 69.54 (C-3, d), 72.54 (C-22, d), 77.15 (C-5, s), 78.13 (C-24, s), 79.49 (C-6, d), 169.70 ($CH_3\underset{O}{\underline{C}}$-O, s); low resolution field desorption mass spectrum, observed M^+ (508); high-resolution mass spectrum, observed m/z (composition, interpretation, calculated millimass) 369.27794 ($C_{25}H_{37}O_2$, M^+-2 H_2O-AcOH-C_3H_7, 369.27936), 360.26912 ($C_{23}H_{36}O_3$, M^+-OH-$C_7H_{15}O_2$, 360.26645), 300.24382 ($C_{21}H_{32}O$, M^+-AcOH-OH-$C_7H_{15}O_2$, 300.24532), 282.23263 ($C_{21}H_{30}$, M^+-AcOH-H_2O-OH-$C_7H_{15}O_2$, 282.23475), 131.10794 ($C_7H_{15}O_2$, M^+-$C_{23}H_{37}O_4$, 131.10721), 123.11900 (C_9H_{15}, M^+-$C_{21}H_{37}O_6$, 123.11738), 113.09858 ($C_7H_{13}O$, M^+-H_2O-$C_{23}H_{37}O_4$, 113.09664); low resolution mass spectrum (12 eV) m/z (relative intensity) 369.3 (4), 360.3 (8), 300.3 (53), 282.2 (6), 131.1 (19), 123.2 (9), 113.2 (100), 99.2 (16), 95.2 (15).

Acetylation of compound 72: Compound 72 (12 mg) was allowed to react with 3 mL of acetic anhydride-pyridine (1:3) at room temperature for 24 h. The excess of reagents were evaporated in vacuo, and the resultant oily residue was purified by HPLC using a silica gel column and hexane-acetone (8:2) as eluent to yield 8 mg of pure 75: 300-MHz ^1H NMR ($CDCL_3$) (number of protons, multiplicity, J in Hz, assignment) δ 0.69 (3, s, Me-18), 0.88 (3, d, 7.5 Hz, Me-27), 0.91 (3, d, 7.5 Hz, Me-26), 0.94 (3, d, 7 Hz, Me-21), 1.03 (3, s, Me-28), 1.14 (3, s, Me-19), 4.69 (1, t, 3 Hz, H-6), 5.14 (1, tt, 11, 5.4 Hz, H-3), 5.19 (1, br d, 11 Hz, H-22); 75.4-MHz ^{13}C NMR ($CDCL_3$) δ 12.24 (C-18, q), 12.81 (C-21, q), 16.34 (C-19, q), 16.95 (C-27or C-26, q), 17.83 (C-26 or C-27, q), 21.01 (t), 21.47 (O$\underline{A}$c, q), 21.50 (O$\underline{A}$c, q), 21.75 (O$\underline{A}$c, q), 22.86 (C-28, q),

24.19 (t), 26.62 (t), 27.05 (t), 30.73 (d), 31.38 (t), 31.76 (t), 36.03 (t), 36.84 (t), 37.61 (d), 38.47 (C-10, s), 39.79 (t), 40.07 (d), 43.06 (C-13, s), 45.0 (d), 52.76 (d), 55.39 (d), 70.57 (C-3, d), 73.56 (C-22, d), 74.03 (C-5, s), 74.90 (C-24, s), 76.05 (C-6, d) 170.13 (s), 170.55 (s), 170.61 (s); low resolution mass spectrum (12 eV) m/z (relative intensity) 549.5 [$M^+-C_3H_7$, (3)], 514.4 [M^+-AcOH-H_2O, (9)], 489.3 [M^+-AcOH-C_3H_7, (26)], 472.4 [M^+-2 AcOH, (10)], 545.4 [M^+-2 AcOH-H_2O, (15)], 429.3 [M^+-2 AcOH-C_3H_7, (11)], 412.3 [M^+-3 AcOH, (16)], 411.4 [M^+-2 AcOH-H_2O-C_3H_7 (42)], 394.4 [M^+-3 AcOH-H_2O, (12), 276.3 [M^+-3 AcOH-$2H_2O$, (23)], 369.3 (51), 368.3 (60), 351.4 (74), 326.3 (83), 308.3 (100), 253.3 (35), 211.2 (33), 155.1 (84), 124.2 (64), 113.1 (79).

Hydrolysis of compound 72: A 2.5 mg of 72 was heated with 15% KOH in 95% ethanol (7 mL) under reflux for 25 min. The reaction mixture was cooled to room temperature, acidified, diluted with water and then extracted with $CHCL_3$. The organic layer was evaporated under nitrogen to afford an oily residue. The oily residue was purified by HPLC with silica gel and hexane-acetone (6:4) to yield pure 73 (1.5 mg) as a white powder. Compound 73 has the following spectral data: 300-MHz ^1H NMR ($CDCL_3$) (number of protons, multiplicity, J in Hz, assignment) δ 0.70 (3, s, Me-18), 0.88 (3, d, 7 Hz, Me-27), 0.96 (3, d, 7 Hz, Me-26), 0.99 (3, d, 7 Hz, Me-21), 1.08 (3, s, Me-28), 1.17 (3, s, Me-19), 3.52 (1, br t, 3.5 Hz, H-6), 4.08 (1, m, H-3), 4.10 (1, br d, 11 Hz, H-22); 300-MHz ^1H NMR (pyridine-d_5) δ 0.75 (3, s, Me-18), 1.02 (3, d, 7 Hz, Me-27), 1.21 (3, d, 7 Hz, Me-26), 1.24 (3, d, 7 Hz, Me-21), 1.34 (3, s, Me-28), 1.67 (3, s, Me-19), 4.17 (1, q, 4.5 Hz, H-6), 4.46 (1, m, H-3), 4.88 (1, m, H-22), 5.29 (1, s, 24-OH), 5.92 (1, s, 5-OH), 6.03 (1, d, 4.5 Hz, 22-

OH), 6.07 (1, d, 3 Hz, 3-OH), 6.16 (1, d, 4.5 Hz, 6-OH); 75.4-MHz ^{13}C NMR ($CDCL_3$ + 5% CD_3OD) δ 11.65 (C-18, q), 12.07 (C-21, q), 16.34 (C-19, q), 16.34 (C-27 or C-26, q), 18.33 (C-26 or C-27, q), 20.90 (t), 22.55 (C-28, q), 24.08 (t), 27.59 (t), 30.11 (d), 30.23 (t), 32.08 (t), 33.89 (t), 34.01 (d), 37.11 (t), 37.94 (C-10, s), 39.74 (t), 39.90 (t), 42.42 (d), 42.91 (C-13, s), 45.33 (d), 52.6 (d), 55.47 (d), 67.16 (C-3, d), 68.81 (C-22, d), 75.37 (C-5, s), 75.65 (C-6, d), 75.67 (C-24, s); low resolution mass spectrum (12 eV) m/z (relative intensity) 448.4 [M^+-H_2O, (0.7)], 405.3 [$M^+-H_2O-C_3H_7$, (1)], 369.3 [$M^+-3\ H_2O-C_3H_7$, (3)], 318.3 (50), 300.2 (49), 282.2 (9), 131.1 (21), 113.1 (100).

Oxidation of compound 72: A 2.5 mg of compound 72 was dissolved in 0.5 mL of acetone and one drop of Jones reagent was added. The reaction mixture was left for one hour and then diluted with $CHCL_3$, filtered and evaporated under a stream of nitrogen to give an oily residue which was chromatographed by HPLC using a silica gel column and hexane-acetone (7:3) as eluent to yield 1.5 mg of white solid 74: IR (neat) 3400, 2950, 2870, 1727, 1712, 1455, 1435, 1375, 1250, 1214, 1095, 1015 cm^{-1}; 300-MHz ^1H NMR ($CDCL_3$) (number of protons, multiplicity, J in Hz, assignment) δ 0.71 (3, s, Me-18), 0.87 (3, d, 7.5 Hz, Me-27), 0.92 (3, d, 7.5 Hz, Me-26), 1.10 (3, d, 7 Hz, Me-21), 1.11 (3, s, Me-28), 1.31 (3, s, Me-19), 2.02 (1, br d, 15 Hz, 4α-H), 2.07 (3, s, OAc), 2.46 (1, dq, 10.5, 7 Hz, 20β-H), 2.51 (1, d, 17 Hz, H-23), 2.64 (1, d, 17 Hz, H-23'), 2.90 (1, d, 15 Hz, 4β-H), 4.64 (1, br t, 3 Hz, H-6); 75.4-MHz ^{13}C NMR δ 12.36 (q), 15.92 (q), 16.2 (q), 16.8 (q), 17.72 (q), 21.1 (q), 21.39 (t), 22.82 (q), 24.4 (t), 27.45 (t), 30.57 (d), 31.35 (t), 33.64 (d), 37.26 (t), 37.74 (d), 38.94 (s), 39.60 (t), 42.97 (s), 45.35 (d), 48.0

(t), 49.0 (t), 50.87 (t), 51.90 (d), 54.92 (d), 73.86 (s), 75.79 (d), 75.79 (s), 169.80 (s), 210.30 (s), 210.35 (s); low resolution mass spectrum (12 eV) m/z (relative intensity) 486.4 [M^+-H_2O, (8)], 461.5 [M^+-H_2O-C_3H_7, (10)], 403.2 (32), 358.2 (10), 357.2 (13), 315.3 (25), 298.2 (18), 297.2 (79), 227.1 (20), 211.2 (18), 206.9 (51), 191.2 (29), 167.0 (72), 149.0 (42), 129.0 (13), 125.1 (63), 111.1 (100), 96.1 (57).

Oxidation of 73: Compound 73 (1.5 mg) was dissolved in 0.2 mL of acetone and treated with 0.05 mL of Jones reagent and the reaction mixture was left at room temperature for one hour. The reaction mixture was diluted with $CHCL_3$, filtered and evaporated to give an oil which was purified by HPLC using a silica gel column and hexane-acetone (7:3) as eluent to give 0.8 mg of white solid 76: 300-MHz ^1H NMR ($CDCL_3$) (number of protons, multiplicity, J in Hz, assignment) δ 0.698 (3, s, Me-18), 0.87 (3, d, 7 Hz, Me-27), 0.92 (3, d, 7 Hz, Me-26), 1.00 (3, s, Me-28), 1.10 (3, d, 7 Hz, Me-21), 1.12 (3, s, Me-19), 2.18 (1, dd, 13, 4 Hz, 7β-H), 2.28 (1, dd, 16, 2 Hz, 4α-H), 2.47 (1, m, H-20), 2.50 (1, d, 16.5 Hz, H-23), 2.66 (1, d, 16.5 Hz, H-23'), 2.73 (1, t, 13 Hz, 7α-H), 2.92 (1, d, 16 Hz, 4β-H); low resolution mass spectrum (12 eV) m/z (relative intensity) 442.4 [M^+-H_2O, (7)], 424.3 [M^+-$2H_2O$, (3)], 417.3 (15), 399.3 (8), 374.3 (10), 359.3 (32), 356.3 (20), 331.2 (63), 313.3 (100), 285.2 (43), 276.2 (50), 129.1 (9), 111.1 (82), 86.1 (41).

Bibliography

1. Gawel, M. J. *Micronesica, 1976, 12(2)*, 303.

2. (a) Tursch, B.; "Marine Natural Products", Vol. 2, Scheuer, P. J. Ed.; Academic Press, New York, San Francisco, and London, 1979; (b) Faulkner, D. J. *Tetrahedron, 1977, 33*, 1421.

3. Tursch, B.; Braekman, J. C.; Daloze, D.; and Kaisin, M. "Terpenoids from Coelenterates" in "Marine Natural Products, Chemical and Biological Perspectives" (Ed., P. Scheuer) Vol. 2 (Academic Press: New York (1978).

4. Weinheimer, A. J.; Middlebrook, R. E.; Bledsoe, J. O.; Jr., Marsico, W. E.; Karns, K. B. *Chem. Commun. 1968*, 384.

5. Vanderah, D. J.; Steudler, P. A.; Ciereszko, L. S.; Schmitz, F. J.; Ekstrand, J. D.; and Van der Helm, D. *J. Am. Chem. Soc. 1977, 99*, 5780.

6. Groweiss, A.; and Kashman, Y. *J. Org. Chem. 1980, 45*, 3814.

7. Groweiss, A.; and Kashman, Y. *Tetrahedron, 1983, 39*, 3385.

8. Akord, A.; Bowden, B. F.; Coll, J. C.; Fourneron, J. D.; and Mitchell, S. J. *Aust. J. Chem. 1981, 34*, 2657.

9. Kobayashi, M.; Yosuzawa, T.; Kobayashi, Y.; Kyogoku, Y.; and Kitagawa, I. *Tetrahedron Letters. 1981*, 4445.

10. Schwartz, R. E.; Scheuer, P. J.; Zabel, V.; and Watson, W. H. *Tetrahedron, 1981, 37*, 2725.

11. Bowden, B. F.; Coll, J. C.; Ditzel, E.; Mitchell, S. J.; and Robinson, W. T. *Aust. J. Chem. 1982, 35*, 997.

12. Finer, J.; Clardy, J.; Fenical, W.; Minale, L.; Riccio, R.; Battaile, J.; Kirkup, M.; and Moore, R. E. *J. Org. Chem. 1979, 44*, 2044.

13. Ravi, B. N.; and Wells, R. J. *Aust. J. Chem. 1982, 35*, 121.

14. Burns, K. P.; Englert, G.; Kazlauskas, R.; Murphy, P. T.; Schonholzer, P.; and Wells, R. J. *Ibid. 1983, 36*, 171.

15. Braekman, J. C.; Daloze, D.; Tursch, B.; Declercq, J. P.; Germain, G.; and Van-Meerssche, M. *Bull. Soc. Chim. Bleg. 1979, 88*, 71.

16. Hall, L. D.; and Sanders, J. K. M. *J. Amer. Chem. Soc., 1980, 102*, 5703.

17. Kuo, M.; and Gibbons, W. A. *J. Biol. Chem. 19779, 254*, 6278.

18 Aue, W. P.; Bartholdi, E.; Ernst, R. R. *J. Chem. Phys. 1976, 64*, 2229-46.

19. Freeman, R.; Morris, G.; Bax, A. *J. Magn. Reson. 1981, 42*, 164-68.

20. Baumann, R.; Wilder, G.; Ernst, R. R.; Wüthrich, K. *J. Magn. Reson. 1981, 44*, 402-06.

21. Bax, A.; Morris, G. A. *J. Magn. Reson. 1981, 42*, 501.

22. Doddrell, D. M.; Pegg, D. T.; Bendall, M. R. *J. Magn. Reson. 1982, 48*, 323.

23. Hall, L. D.; Sanders, J. M. *J. Am. Chem. Soc. 1980, 102*, 5703.

24. Couperus, P. A.; Chague, A. D. H.; Van-Dongen, J. P. M. *Org. Magn. Reson. 1976, 8*, 426.

25. Assignment was made by analogy to the ^{13}C NMR spectra of tert-Butanol vs tert-Butylhydroperoxide which were published under Sadtler Standard Carbon-13 Spectra, Sadlter Research Laboratories, Philidelphia, PA, Vol. 9, #1602C and Vol. 7, #1271C, respectively.

26. Aue, W. P.; Karham, J.; Ernst, R. R. *J. Chem. Phys. 1976, 64*, 4226.

27. Jarosz, S.; Hicks, D. R.; Fraser-Reid, B. *J. Org. Chem. 1982, 47*, 935.

28. Strain, H. H.; Suec, W. A.; Aitzetmullr, K.; Kiosen, H.; Norgord, S.; Liaaen-Jensen, S.; Haxo, F. T.; Wegfahrt, P.; Rapoport, H. *J. Am. Chem. Soc. 1971, 93*, 1823.

29. Schmitz, F. J.; Campbell, D. C.; Kubo, I. *Steroids 1976, 28*, 211. Bortolotto, M.; Braekman, J. C.; Daloze, D.; Tursch, B. *Bull. Soc. Chim. Belg. 1976, 85*, 27. Moldowan, J. M.; Tan, W. L.; Djerassi, C. *Steroids 1975, 26*, 107. Moldowan, J. M.; Tursch, B.; Djerassi, C. *Ibid. 1974, 24*, 387.

30. Mitchell, D. T.; Shildneck, P.; Dustin, J. *Ind. Engr. Chem. Anal. Ed. 1944, 16*, 754.

31. Green, B.; Kilcoyne, J. P.; Targett, N. M. *J. Org. Chem. 1979, 44*, 4962.

CHAPTER TWO

Introduction

The nudibranch, *Tridachia crispata*,[1] is a marine mollusc in the family of Elysiidae. It is a common species found in shallow water (6 m in depth) and occurs on reef and algal covered areas. It has been observed on dead parts of the coral *Acropora cervicornis*. *Tridachia crispata* lacks the shield of an external shell and its dorsal surface, which reaches 10 cm in length, has a group of ruffled ridges on its back. The cells of the dorsal surface contain chloroplasts, acquired from food eaten by the nudibranch, which produce secondary metabolites by photosynthetic processes using dissolved inorganic carbonate. *Tridachia crispata* which possesses functional chloroplasts obtained from siphonous marine algae.[5]

The chemistry of opisthobranch molluscs has turned out to be very interesting.[2] The absence of shell protection for opisthobranchs has led these marine molluscs to use other types of defense, such as toxic or noxious chemicals which are acquired from their diet.[3,4]

Faulkner et al. have investigated the chemistry of two marine molluscs, *Tridachiella diomedea*[6] and *Tridachia crispata*,[7,8] obtained from the Gulf of California, and the Caribbean, respectively. Figure 1 exhibits the structures of 4 metabolites which they have isolated from *Tridachiella diomedea* and *Tridachia crispata*.

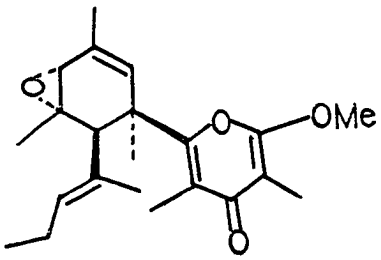
We have investigated *Tridachia crispata*, collected from Jamaica, Discovery Bay, and this work has resulted in the isolation of the known metabolites crispatone (3) and crispatene (4), and nine more new compounds (see Figure 2).

Results and Discussion

The nudibranch, *Tridachia crispata*, used in this work was collected from Jamaica, Discovery Bay, in June 1979, in shallow water (~ 1-2 m in depth). Eighty animals were shipped frozen to Oklahoma. The specimens were allowed to thaw for 24 h, and soaked in acetone (4000 mL) for one day. The acetone solution was concentrated and the concentrate diluted with H_2O and extracted with ether (2 X 3000 mL). The ether-soluble portion of the acetone extracts was chromatographed extensively according to Scheme 1 to yield 11 compounds which are described individually below.

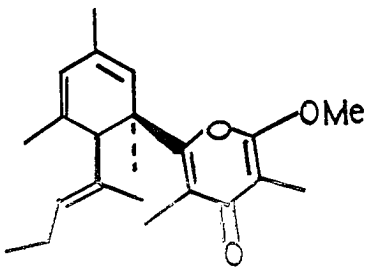
Figure 2 shows all the compounds which were isolated in this work.

Figure 1.



1 tridachione

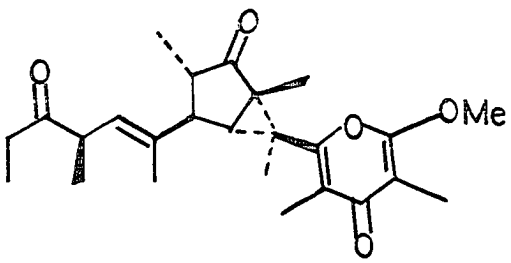
Tridachiella diomedea[6]



2. 9,10-deoxytridachione

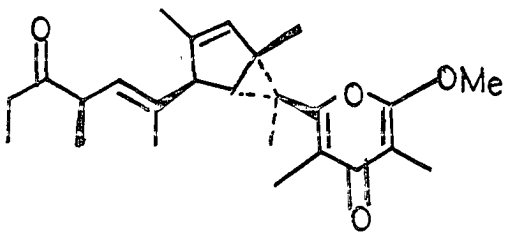
Tridachiella diomelea[6]

Placobranchus ocellatus[9]



3. crispatone

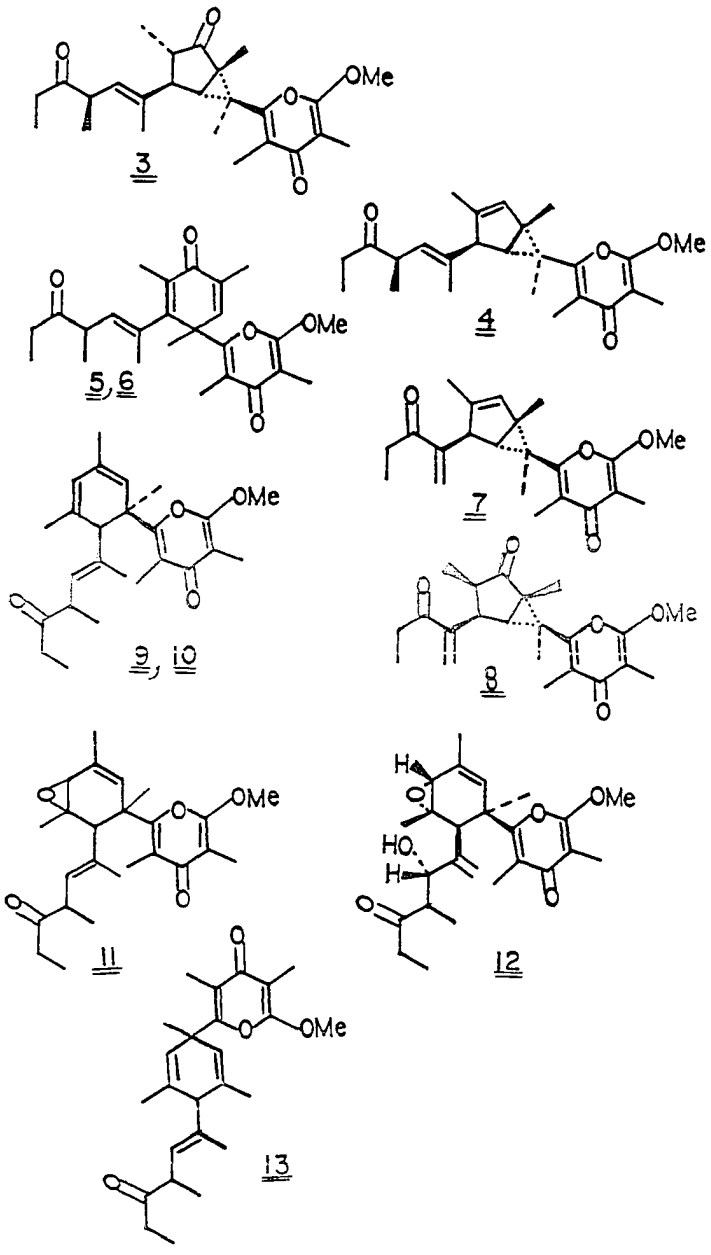
Tridachia crispata[7,8]

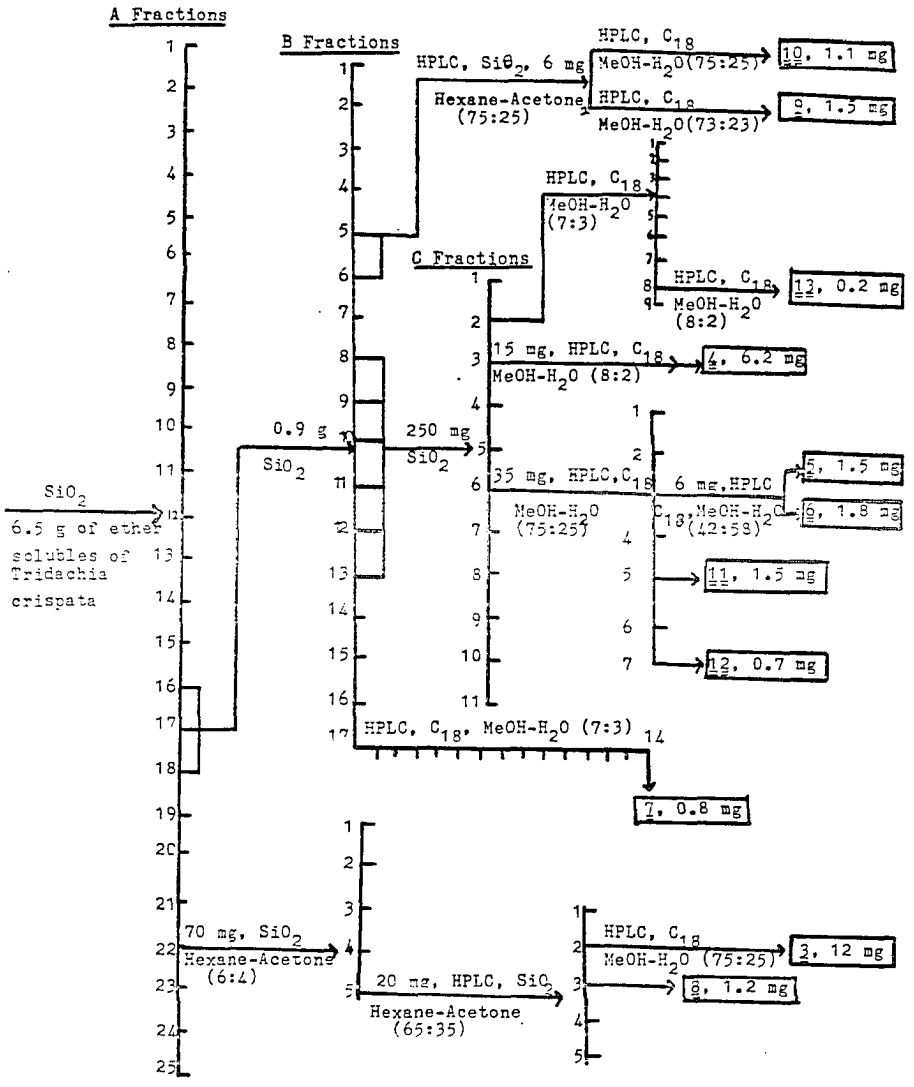


4. crispatene

Tridachia crispata[7,8]

Figure 2




A Fractions
B Fractions
C Fractions
SiO2
6.5 g of ether solubles of Tridachia crispata
0.9 g SiO2
250 mg SiO2
HPLC, SiO2, 6 mg
Hexane-Acetone (75:25)
HPLC, C18
MeOH-H2O(75:25)
10, 1.1 mg
HPLC, C18
MeOH-H2O(73:23)
9, 1.5 mg
HPLC, C18
MeOH-H2O (7:3)
HPLC, C18
MeOH-H2O (8:2)
13, 0.2 mg
15 mg, HPLC, C18
MeOH-H2O (8:2)
4, 6.2 mg
35 mg, HPLC, C18
MeOH-H2O (75:25)
6 mg, HPLC C18, MeOH-H2O (42:58)
5, 1.5 mg
6, 1.8 mg
11, 1.5 mg
12, 0.7 mg
HPLC, C18, MeOH-H2O (7:3)
7, 0.8 mg
70 mg, SiO2
Hexane-Acetone (6:4)
20 mg, HPLC, SiO2
Hexane-Acetone (65:35)
HPLC, C18
MeOH-H2O (75:25)
3, 12 mg
8, 1.2 mg

Scheme 1

Identification of Crispatone (3):

The ether-soluble fraction of acetone extracts of homogenized was chromatographed over silica gel to give 24 fractions. The twenty-second of these fractions was rechromatographed on a short column of silica gel to obtain 5 fractions. The most polar fraction (fraction 5, 20 mg) was subjected to HPLC with a silica gel column and hexane-acetone (65:35) as eluent to afford 5 fractions. The second of these fractions was further purified by reversed-phase HPLC to obtain 12 mg of pure crispatone (3) as a colorless oil. The molecular formula $C_{25}H_{34}O_5$ was deduced from the analysis of the high-resolution mass spectral data of 3. The ^{13}C NMR spectra (Figure 3) indicated the presence of 25 carbons including 9 methyls, one methylene, 5 methines, and 10 quaternary carbons. The ^1H NMR spectrum (Figure 4) displayed signals for 6 methyl singlets, two methyl doublets, and a methyl triplet. This isolate was identified as crispatone (3), isolated by Ireland and Faulkner[8] from , when it was found that the ^1H NMR and ^{13}C NMR data of the isolate and crispatone were identical.

Identification of Crispatene (4):

Fraction C3 (15 mg) of Scheme 1 was chromatographed by HPLC using a reverse-phase C_{18} column and MeOH-H_2O (8:2) as eluent to yield 10 fractions. The tenth of these fractions contained 6.2 mg of pure crispatene (4) as a colorless oil. The molecular formula $C_{25}H_{34}O_4$ was obtained for 4 from the following spectral data: (a) the low-resolution mass spectrum displayed the molecular ion at m/z 398; (b) the ^1H NMR spectrum showed signals which accounted for 34 non-exchangeable protons; (c) IR

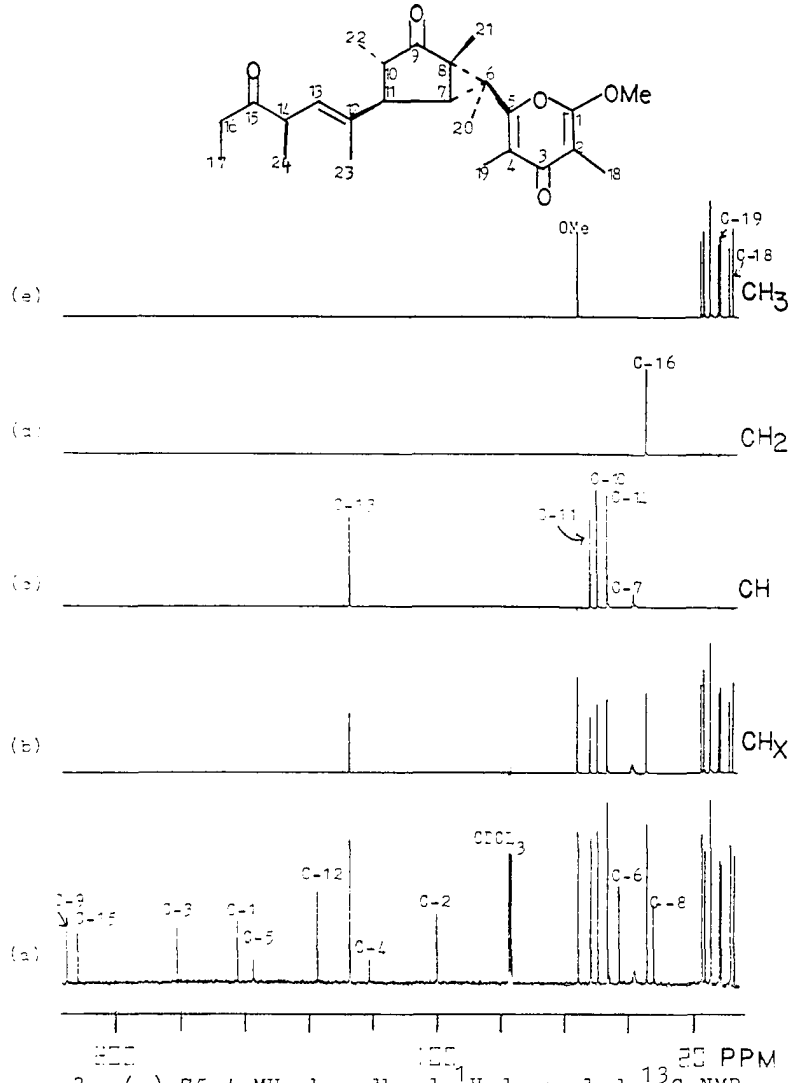


Figure 3. (a) 75.4 MHz broadband ^{1}H decoupled ^{13}C NMR spectrum of crispatone (3) in $CDCl_3$. (b) all protonated carbons. (c) methine carbons. (d) methylene carbons. (e) methyl carbons. Spectra b-e were recorded in $CDCl_3$ at 75.4 MHz and resulted from a DEPT experiment.

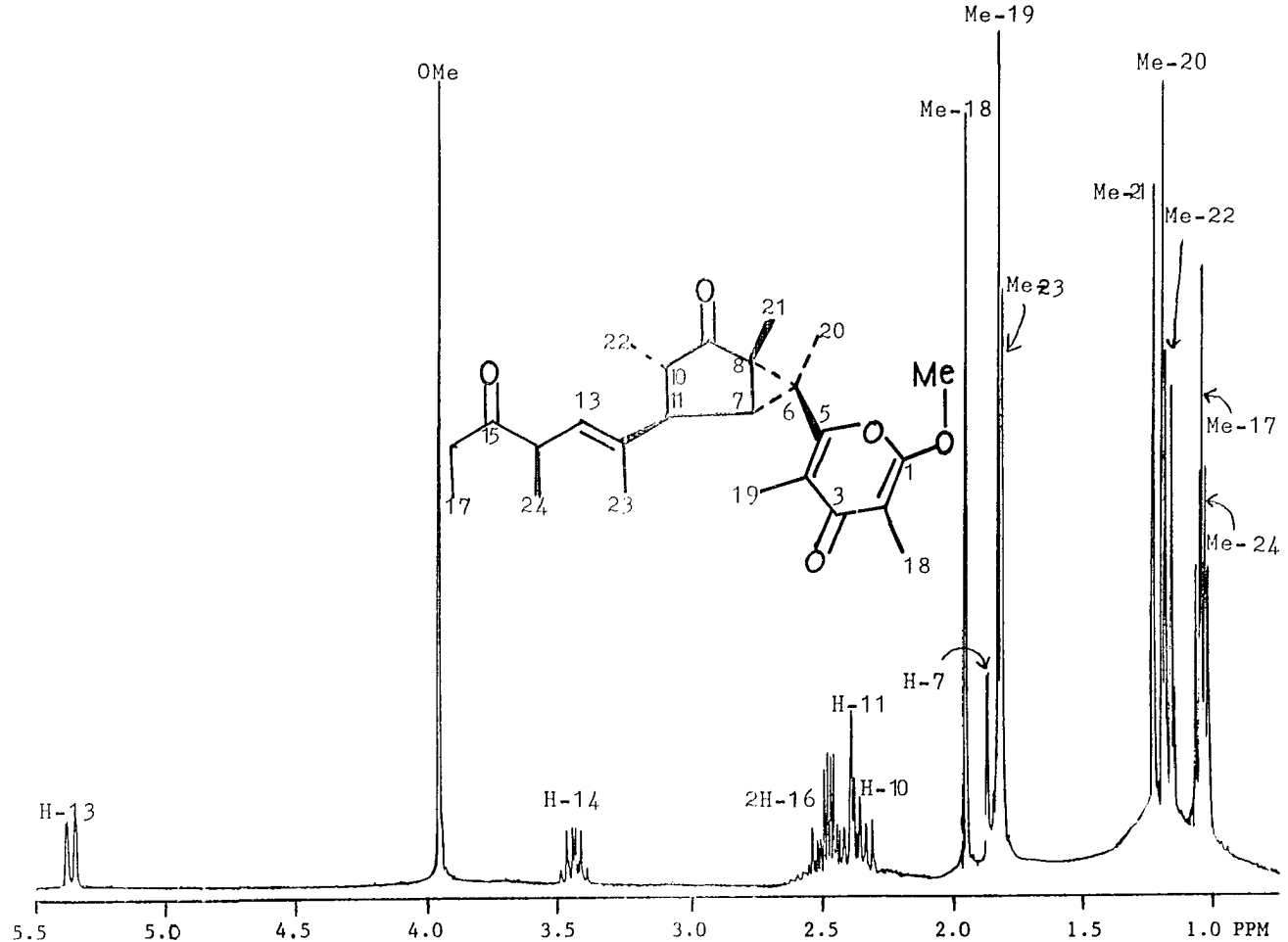


Figure 4. 300 MHz proton NMR spectrum of crispatone (2) in $CDCl_3$.

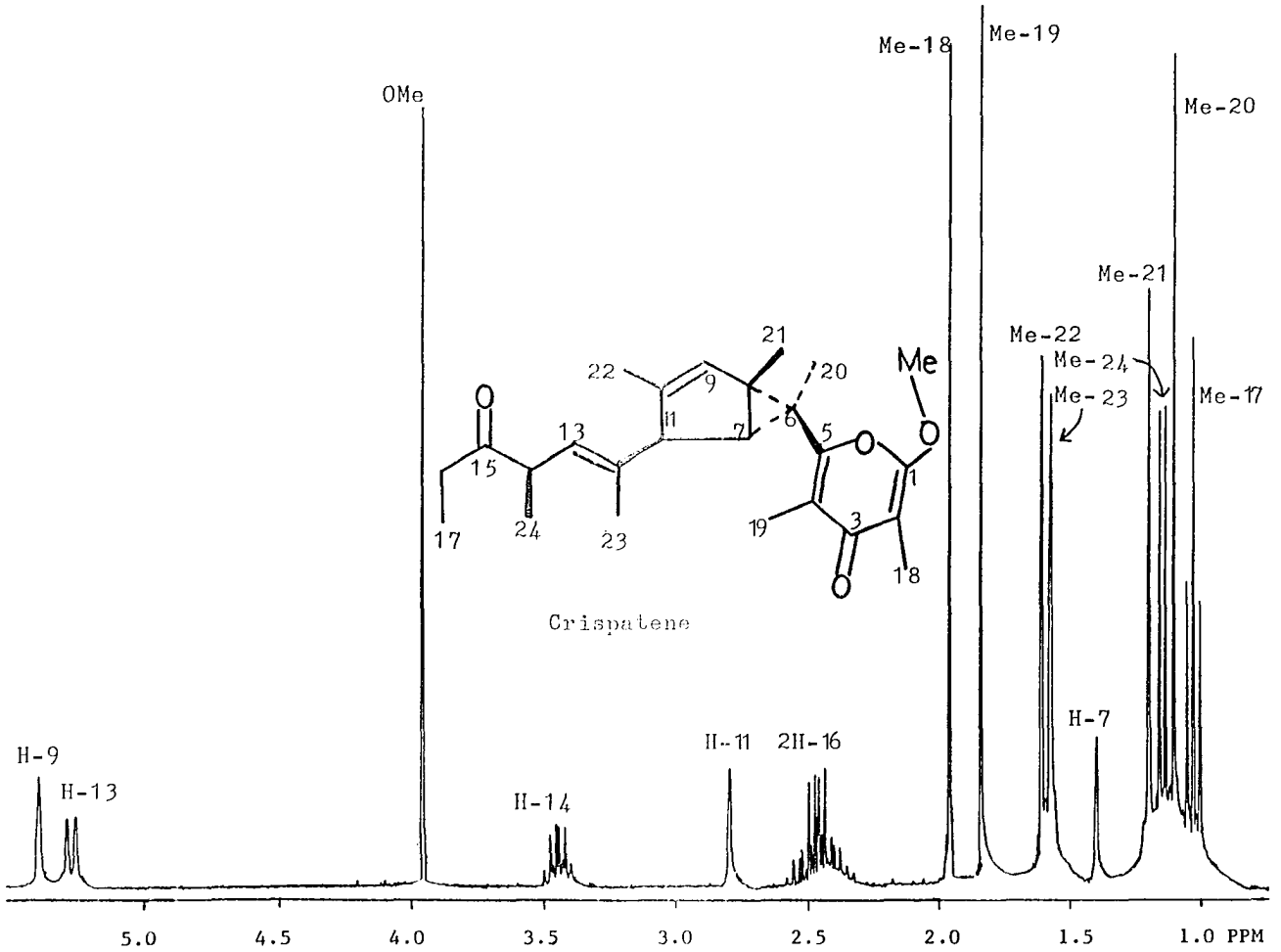


Figure 5 . 300 MHz proton NMR spectrum of crispatene (4) in $CDCl_3$.

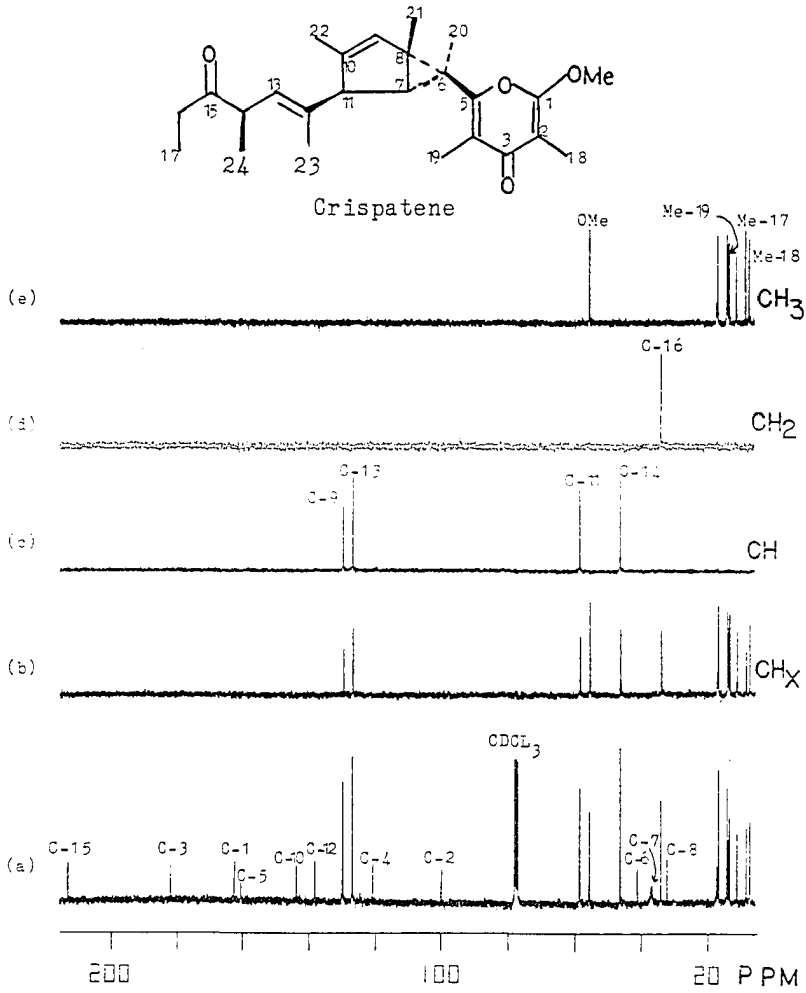
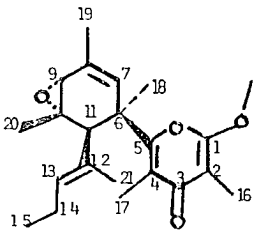


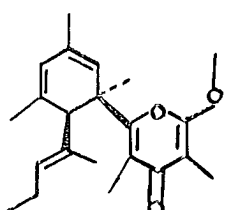
Figure 6. (a) 75.4 MHz broadband proton decoupled ^{13}C NMR spectrum of crispatene (4) in $CDCl_3$. (b) all protonated carbons. (c) methine carbons. (d) methylene carbons. (e) methyl carbons. Spectra b-e were recorded in $CDCl_3$ at 75.4 MHz and resulted from a DEPT experiment.

Table 1. ^{1}H NMR Data of Tridachione (1)*, 9,10-Deoxytridachione (2)*, 7[a], and 8[a]

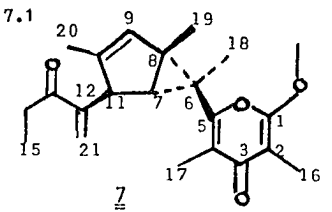


1

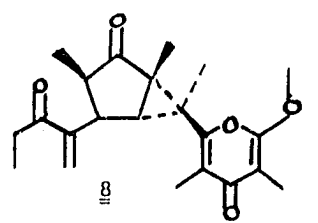
H#	1	2	7	8
H-7	6.10 s	5.56 s	1.41 br s	1.83 br s
H-9	3.11 s	5.65 s	5.45 br s	
H-10				2.50 dq 8.2, 7.1
H-11	2.91 s	2.71 s	3.52 br s	2.80 br d 8.2
H-13	5.18 t 7	5.09 t 7		
H-14	2.05 m		2.80 q 7	2.80 q 7.1
H-15	0.68 t 7	0.69 t 7	1.15 t 7	1.13 t 7.1
H-16	2.07 s	2.03 s	1.84 s	1.82 s
Me-17	2.0 s	1.81 s	1.98 s	1.95 s
Me-18	1.32 s	1.32 s	1.19 s	1.26 s
Me-19	1.80 s	1.69 s	1.28 s	1.25 s
Me-20	1.36 s	1.76	1.58 br s	1.05 d 7.1
Me-21	1.60 s	1.60 s	5.72 s 6.21 s	5.92 s 6.22 s
Me-22				
Me-23				
Me-24				
OMe	3.93 s	3.95 s	3.96 s	3.96 s



2



7



8

*Spectra[8] were recorded in $CDCl_3$ at 220 MHz with TMS as internal standard.

[a]Spectra were recorded in $CDCl_3$ at 300 MHz with TMS as internal standard, assignments were made by spin decoupling and NOE experiments and by analogy to compounds 3 and 4. All values are given in δ units.

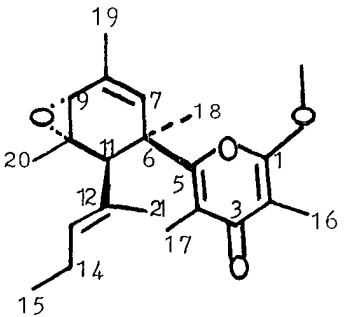
Table 1a. ^{1}H NMR Data* of crispatone (3), Crispatene (4), and Tridachiapyrones

H#	3	4	5	6	9	10	11	12	13
H-7	1.89 br s	1.41 br s	6.60 br s	6.63 br s	5.64 br s	5.61 br s	5.50 br s	5.47 br s	5.16 br s
H-9		5.39 br s			5.69 br s	5.71 br s	3.0 br s	3.16 br s	3.22 br s
H-10	2.37 q 7.2								
H-11	2.40 br s	2.80 br s			2.78 br s	2.80 br s	3.40 br s	2.99 q 2.0	5.64 br s
H-13	5.37 br d 8	5.26 br d 8	4.96 br d 8	4.99 br d 8	4.97 br d 9.8	5.07 br d 9.5	5.41 br d 9.2	4.42 dq 7, 2	5.19 br d 9.8
H-14	3.45 dq 8, 7	3.45 dq 8, 7	3.39 dq 8, 7	3.37 dq 8, 7	3.16 dq 9.8, 6.5	3.20 dq 9.5, 7	3.50 m	2.78 quintet 7	3.42 dq 9.8, 7
H-15									
H-16	2.47 m	2.47 m	2.32 m	2.41 m	2.34 m	2.11 m	2.46 m	2.48 m	2.42 m
Me-17	1.05 t 7	1.04 t 7	0.99 t 7	1.01 t 7	0.98 t 7.1	0.88 t 7	1.05 t 7	1.03 t 7.2	1.03 t 6.9
Me-18	1.83 s	1.84 s	1.85 s	1.84 s	1.80 s	1.81 s	1.85 s	1.86 s	1.82 s
Me-19	1.95 s	1.95 s	1.85 s	1.84 s	2.08 s	2.10 s	2.02 s	2.25 s	2.07 s
Me-20	1.20 s	1.11 s	1.63 s	1.66 s	1.43 s	1.44 s	1.17 s	1.45 s	1.31 s
Me-21	1.24 s	1.20 s	1.94 br s	1.95 br s	1.79 br s	1.80 br s	1.95 br s	1.53 br s	1.77 br s
Me-22	1.05 d 7.2	1.61 br s	1.72 s	1.72 s	1.69 br s	1.75 br s	1.32 s	1.56 s	1.65 br s
Me-23	1.83 br s	1.58 br s	1.70 br s	1.67 br s	1.46 br s	1.44 br s	1.58 br s	4.92 t, 2 4.84 t, 2	1.65 br s
Me-24	1.17 d 7	1.16 d 7	1.17 d 7	1.08 d 7	0.75 d 6.5	1.05 d 7	1.20 d 7	1.13 d 7	1.12 d 7
OMe	3.95 s	3.94 s	3.96 s	3.93 s	3.97 s	3.98 s	3.98 s	3.94 s	3.92 s

*Spectra were recorded at 300 MHz in $CDCl_3$ with TMS as internal standard, assignments were established by ^{1}H decoupling, ^{1}H difference decoupling, and NOEDS experiments and by analogy to the reported values[8] for 1, 2, 3, and 4. All values are given in δ units.

Table 2. ^{13}C NMR Data* of Tridachione (1)[8] and 9,10-Deoxytridachione (2)[8]

C#	1	2
1	161.0 s	161.7 s
2	97.8 s	98.8 s
3	181.8 s	181.8 s
4	118.4 s	120.0 s
5	160.1 s	161.1 s
6	55.3 s	59.6 s
7	129.0[a] d	124.3[†] d
8	132.5[b] s	134.9[††] s
9	54.7 d	122.4[†] d
10	60.5 s	132.2[††] s
11	46.7 d	47.6 d
12	128.5[b] s	131.0[††] s
13	131.6[a] d	127.8[†] d
14	31.2 t	26.9 t
15	c	e
16	6.1 q	6.8 q
17	11.6 q	12.2 q
18	c	e
19	c	e
20	c	e
21	c	e



1

*^{13}C NMR spectra were recorded at 20 MHz and assignments were made by using J^r values. a,b,†,†† These assignments may be interchanged. [c] Assignments were not made for the following signals, δ 21.8 (q), 21.5 (q), 20.2 (q), 12.9 (q), 12.0 (q). [e]Assignments were not made for the following signals, δ 22.3, 21.5, 13.8, 13.7. All spectra were obtain- in $CDCl_3$.

Table 2[a]. ^{13}C NMR Data* of Crispatone (3), Crispatene (4), 5, 6, and 9

C#	3	4	5	6	9
1	162.20 s	162.17 s	161.85 s	161.94 s	161.90 s
2	99.84 s	99.45 s	99.95 s	100.02 s	96.95 s
3	181.06 s	181.46 s	180.27 s	180.54 s	181.10 s
4	120.94 s	126.34 s	121.94 s	122.16 s	119.79 s
5	157.42 s	159.95 s	158.05 s	158.05 s	161.20 s
6	42.45 s	40.68 s	48.13 s	48.62 s	59.51 s
7	37.63 d	36.76 d	146.21 d	146.78 d	124.19 d
8	31.92 s	31.88 s	132.19[a] s	132.20[a] s	137.71 s
9	215.51 s	129.29 d	186.05 s	186.03 s	122.94 d
10	49.14 d	143.25 s	133.35[a] s	133.28[a] s	134.10 s
11	51.32 d	58.16 d	156.33 s	156.13 s	45.52 d
12	137.04 s	137.67 s	134.27[a] s	133.71[a] s	136.08 s
13	126.88 d	126.46 d	130.19 d	130.58 d	128.70 d
14	46.02 d	46.07 d	46.10 d	46.46 d	59.67 d
15	211.95 s	212.28 s	210.91 s	210.26 s	212.21 s
16	33.81 t	33.65 t	33.75 t	34.58 t	34.29 t
17	c	e	f	g	h
18	6.82 q	6.76 q	6.97 q	7.03 q	6.72 q
19	10.87 q	12.92 q	12.68 q	12.70 q	12.24 q
20	c	e	f	g	h
21	c	e	f	g	h
22	c	e	f	g	h
23	c	e	f	g	h
24	c	e	f	g	h
OMe	55.36 q	55.08 q	55.45 q	55.94 q	55.38 q

*Spectra were recorded at 75.4 MHz in $CDCl_3$, multiplicities were established by DEPT experiments, assignments were made by analogy to the reported values for 3 and 4, TMS was used as internal standard, and the values are given in δ units.[a] Assignment may be interchanged. [c] These signals, 16.8(q), 16.0(q), 14.0(q), 11.4(q), 10.87(q), and 7.9(q), were not assigned . [e]These signals, 16.59(q), 13.6(q), 13.35(q), 12.9 (q), 10.7(q), and 7.9(q) were not assigned . [f]These signals, 24.2(q), 15.8(q), 16.7 (q), 17.6(q), 9.0(q), and 7.6(q), were not assigned. g These signals, 24.5(q), 15.9 (q), 16.5(q), 17.5(q), 9.2(q), 7.8(q), were not assigned. [h] These signals, 22.28(q), 21.6(q), 16.1(q), 14.2(q), 27.0(q), 8.0(q), were not assigned.

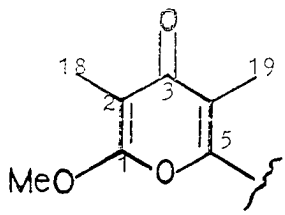
absorptions were observed at 1715 and 1660 cm^{-1} (carbonyls); (d) the ^{13}C NMR data indicated the presence of 25 carbons including two carbonyl carbons, [212.28 ppm (C-15, s); 181.46 (C-3, s)], and one sp^2 carbon deshielded by two oxygen substituents, [162.17 ppm (C-1, s)]. The ^1H NMR spectrum (Figure 5) exhibited 7 methyl singlets, one methyl doublet, and one methyl triplet (see Table 1). The ^{13}C NMR spectra (Figure 6) contained signals for 9 methyls, one sp^3 methylene, 3 sp^3 methines and 2 sp^2 methines, and 8 quaternary carbons in addition to the two carbonyl carbons. The spectral properties of compound 4 (see Tables 1a, 2a and experimental) are identical to those of crispatene isolated earlier by Faulkner[8] and Ireland. Therefore, this metabolite was identified as crispatene.

Structureelucidationof Tridachiapyrone-B (5) and Isotridachiapyrone-B (6):

Fraction C6 of Scheme 1 was chromatographed by HPLC with a reverse-phase C_{18} column and MeOH-H_2O (75:25) as eluent to yield 7 fractions. The third of these fractions was resolved by HPLC using a reverse-phase C_{18} column and Me-OH-H_2O (42:58) as a mobile phase to give two pure compounds, 5 (1.5 mg) and 6 (1.8 mg), as colorless oils. A molecular formula $C_{25}H_{32}O_5$ for 5 was derived from the following considerations: (a) the low resolution mass spectrum of 5 showed a molecular ion at m/z 412; (b) the ^{13}C NMR spectra of 5 indicated the presence of 25 carbons including 3 carbonyl carbons, (180.27 ppm, C-3), (186.05, C-9), and (210.9, C-15); (c) the ^1H NMR spectrum of 5 (Figure 7) contained signals

*assignment was made by analogy to crispatone (3) (see Table 2).

for 32 non-exchangeable protons including a methyl signal deshielded by a single oxygen, (3.96 ppm, OMe); (d) the IR spectrum of 5 displayed two carbonyl bands at 1710 and 1650 cm^{-1}, and two bands at 1660 and 1590* cm^{-1} due to a γ-pyrone. The ^{13}C NMR spectra of 5 revealed the existence of 9 methyls, one sp^3 methylene, one sp^3 methine and 2 sp^2 methines, and 9 quaternary carbons, in addition to the three carbonyl carbons. The ^{13}C NMR data (C-1 to C-5) (see Table 2a) and ^1H NMR signals at 1.85 ppm (s, 6H) and 3.96 (s, 3H) together with the IR bands at 1660 and 1590 cm^{-1} and UV band at 248 nm suggested the existence of the α-methoxy-β,β'-dimethyl-γ-pyrone ring system (partial structure A).

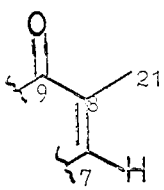


A

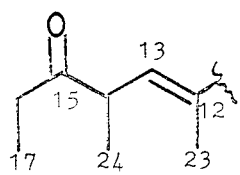
The ^1H NMR spectrum of 5 showed a down-field proton signal at 6.60 ppm (H-7) which was coupled (J = 1.5 Hz) to the methyl signal at 1.94 ppm (Me-21). The chemical shift of H-7 suggested that it could be at β position of an α,β-unsaturated carbonyl system (partial structure B). The side chain structural feature in 5 was established by ^1H decoupling

*assignment was made by analogy to compound 3.

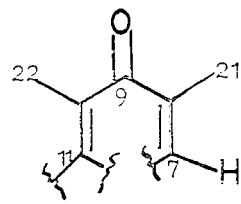
experiments. Irradiation of the multiplet at 2.32 (H-16) collapsed the methyl triplet at 0.99 ppm (Me-17) and also sharpened the ^{1}H signal at 3.39 ppm (H-14) (long-range "W"-type coupling). Considerations of the chemical shift of H-16 together with the mutual coupling between H-16 and Me-17 suggested a terminal ethyl ketone group. The olefinic proton signal at 4.96 ppm (H-13) was coupled (J = 1.4 Hz) to the vinylic methyl signal at 1.70 ppm (Me-24) and also to the methine proton signal at 3.39 ppm (H-14) which was in turn coupled (J = 7 Hz) to the methyl doublet signal at 1.17 ppm (Me-23). Therefore, a 1,3-dimethyl-1-hexenyl-4-one partial structure C was formulated.



B



C



D

The carbonyl band at 1650 cm^{-1} in the IR spectrum and the ^{13}C NMR signal at 186.05 ppm (C-9, s) suggested a cross conjugated ketone as in partial structure D. The methyl signal at 1.72 ppm (Me-22) was linked to C-10 on the basis of its chemical shift and by applying biogenetic rules. The lack of any coupling between the Me-20 signal (1.63 ppm) and any other ^{1}H signals suggested that Me-20 could be connected to a quaternary carbon (C-6). Further evidence for placing a methyl group (Me-20) at C-6 was the observation of an Overhauser enhancement between Me-20 and H-7. Moreover, biogenetic rules supported this assignment. The side chain partial structure C was connected to C-11, because of the

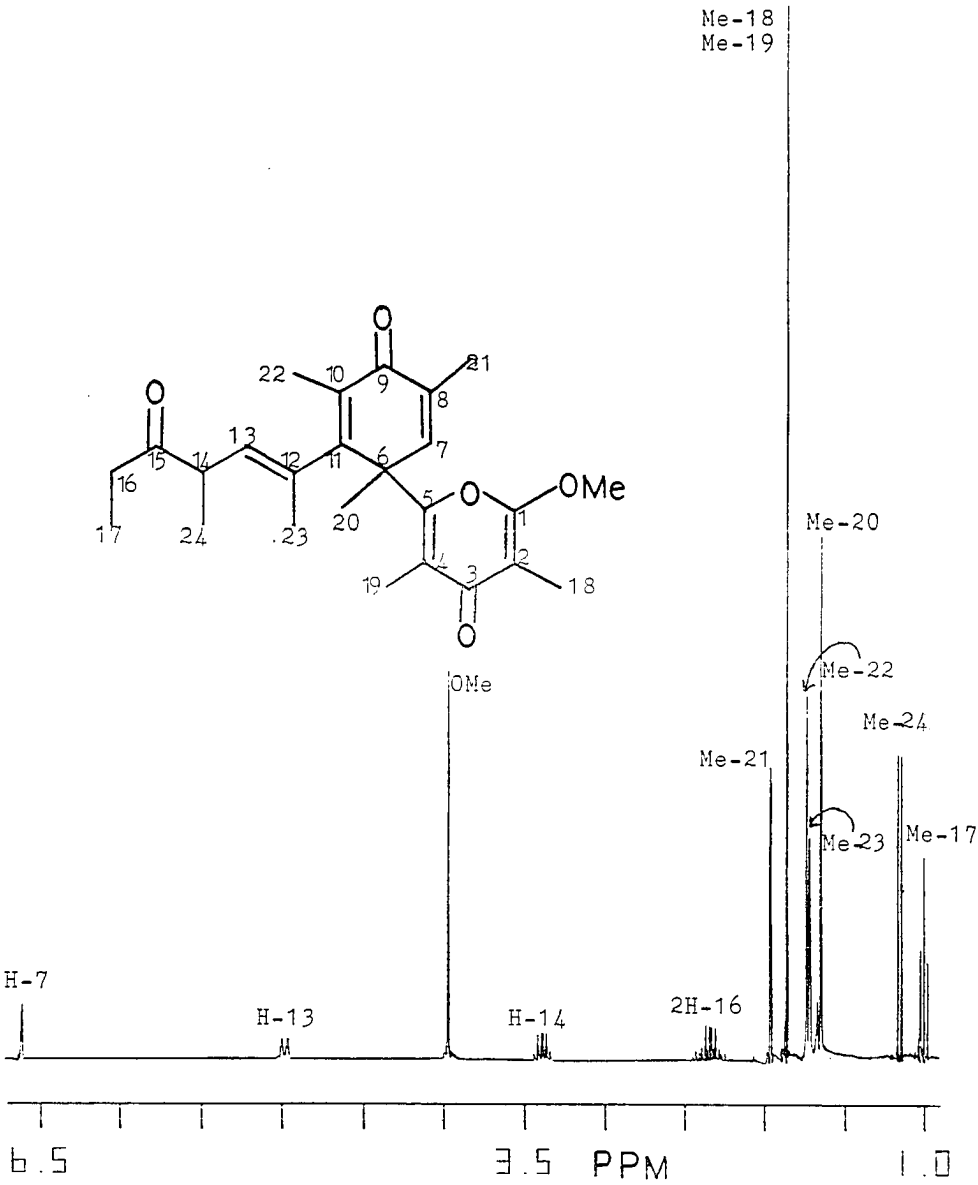


Figure 7 . 300 MHz proton NMR spectrum of 5 in $CDCl_3$.

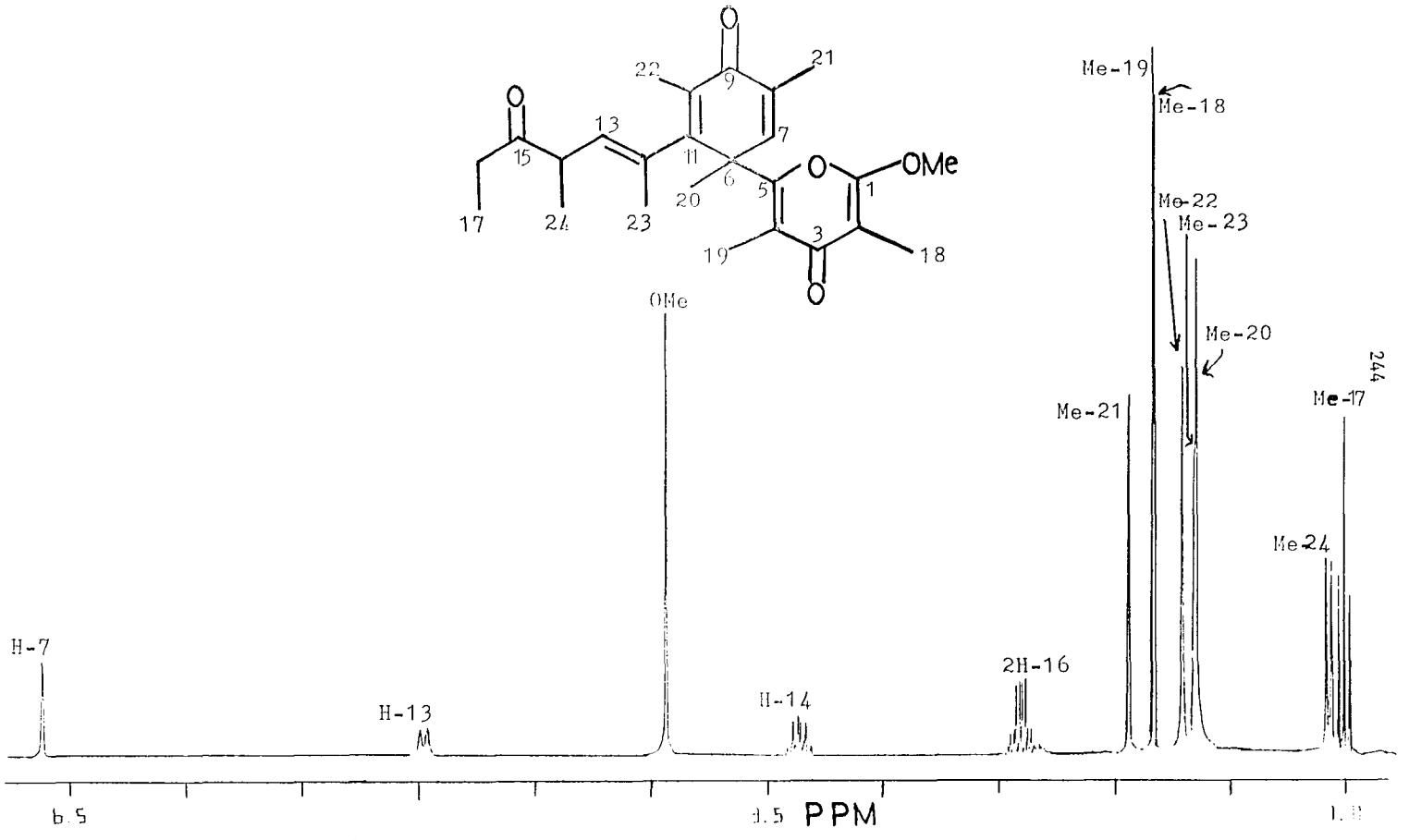


Figure 8 . 300 MHz proton NMR spectrum of 6 in $CDCl_3$.

observation of an NOE effect between H-13 and Me-20 and also biogenetic reasons. The observed UV band at 248 nm (ε = 11900) was assigned to the γ-pyrone ring and to the cross-conjugated α,β-unsaturated carbonyl system C-7 to C-11. This suggested that the double bond at C-12,13 was not conjugated with the α,β-unsaturated carbonyl system (C-9,10,11) since such an extended conjugated system would be expected to absorb at longer wave lengths. This conclusion was supported by the observation of an NOE effect between H-13 and Me-20.

The relative stereochemistries at C-6 and C-14 were not determined.

Compound 6 had the same molecular formula as 5. The IR spectrum of 6 was also identical to that of 5. The ^{1}H NMR spectrum of 6 (see Figure 8 and Table 1a) was almost identical to that of 5 but for the two signals appearing at 2.41 ppm (H-16) and 1.08 (Me-23). Furthermore, the ^{13}C NMR spectra of 6 (see Table 2a) and 5 are virtually identical. This led to the conclusion that 6 is an epimer of 5 in regard to the configuration at C-14.

The geometry of the double bond at C-12,13 was assigned as E on the basis of the Overhauser enhancement observed between Me-23 and H-14 and the absence of any NOE between Me-23 and H-13. Irradiation of the Me-21 caused H-7 to enhance which further confirmed the assignment of Me-21.

Structure elucidation of Tridachiapyrone-E (7):

Fraction B17 of Scheme 1 was chromatographed by reverse phase HPLC to yield 14 fractions. The fourteenth of these fractions contained tridachiapyrone-E (7), 0.8 mg as a colorless oil. The molecular formula $C_{22}H_{28}O_4$ of 7 was derived from the following spectral data: (a) the low

resolution mass spectrum exhibited a molecular ion at m/z 356; (b) the ^{1}H NMR spectrum (Figure 9) showed signals for 28 non-exchangeable protons including one for a methyl deshielded by a single oxygen, (3.96 ppm, OMe); (c) the IR spectrum showed bands at 1690 cm^{-1} (CO), 1660, and 1585 cm^{-1} (γ-pyrone). The IR band at 1690 cm^{-1} together with the UV absorption at 225 nm suggested a conjugated carbonyl group. The ^{1}H NMR signals at 1.84 ppm (Me-16, s), 1.98 (Me-17, s), and 3.96 (OMe, s) suggested the existence of the α-methoxy-β,β'-dimethyl-γ-pyrone ring system. Two other methyl singlet signals at 1.19 (Me-19) and 1.21 ppm (Me-18) and the methine proton signal at 1.41 ppm (H-7) were assigned to a cyclopropyl ring by analogy to crispatene (4) (see Table 1). Comparison of the ^{1}H NMR data of 7 with the data of the known crispatene (4) (see Tables 1 and 1a), led to the conclusion that 7 possessed the same bicyclic ring system and the α-methoxy-β,β'-dimethyl-γ-pyrone ring system as in crispatene (4) and that 7 and 4 differ only in the structure of the side chain at C-11. The structure of the side chain in 7 was confirmed by ^{1}H decoupling experiments. Irradiation of the doubly allylic methine proton signal at 3.52 ppm sharpened the terminal methylene proton signals at 5.72 (H-21', s) and 6.21 (H-21, s) and also sharpened the methyl signal at 1.58 ppm (Me-20, s). A mutual coupling ($J < 1$ Hz) was found between H-21 and H-21' signals which confirmed that H-21 and H-21'; were part of an exocyclic terminal methylene group. The chemical shifts of H-21 and H-21' required that the terminal methylene group must be the terminus of an α,β-unsaturated carbonyl system. The methyl triplet signal at 1.15 ppm and the quartet signal at 2.80 were assigned to a terminal ethyl ketone moiety. The lack of coupling between H-7 and

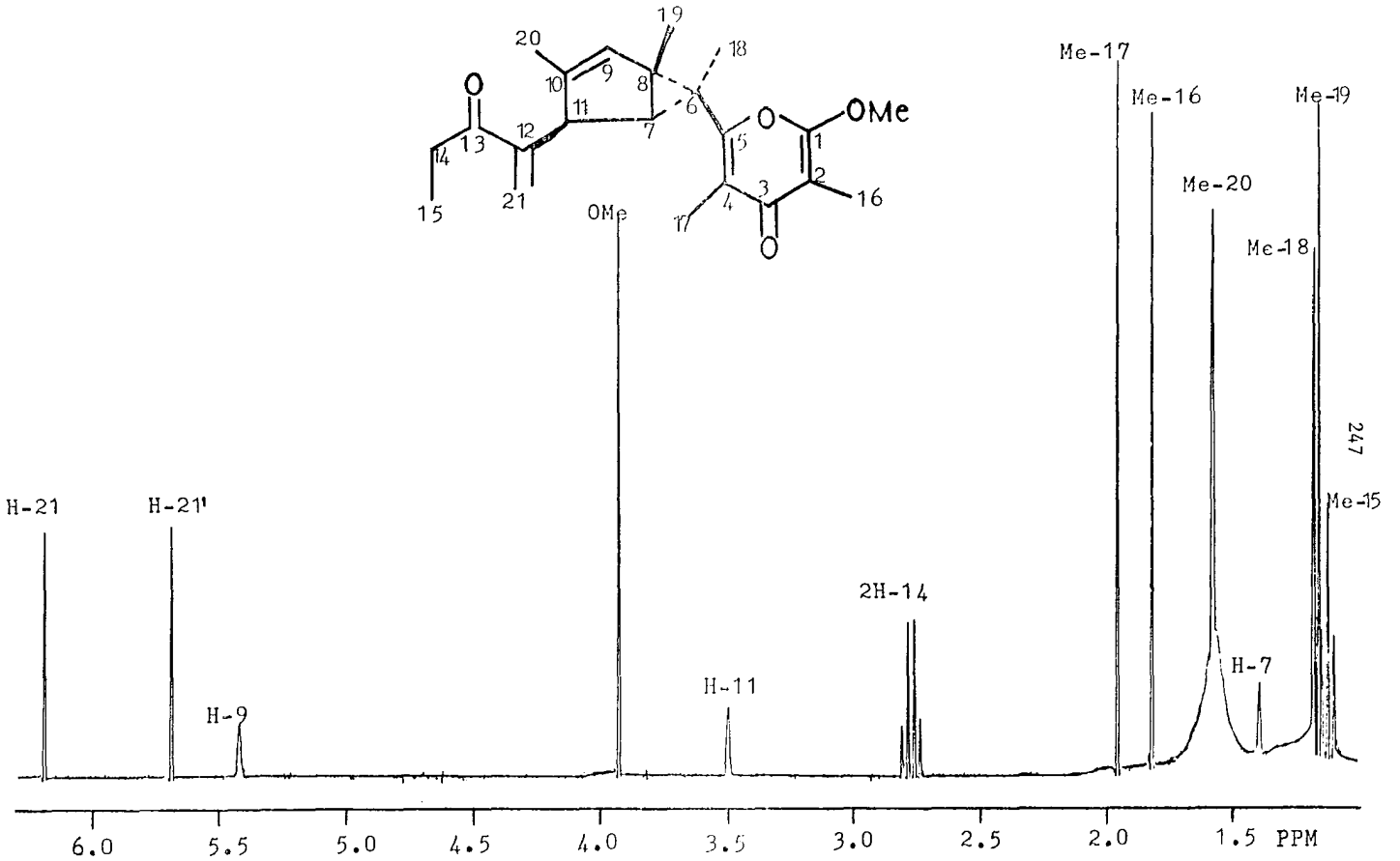


Figure 9. 300 MHz proton NMR spectrum of 7 in $CDCl_3$.

H-11 protons was attributed to the dihedral angle of near 90° between H-11 and H-7 as found in crispatene (4). This provided evidence that the stereochemistry about the bicyclic ring system is the same as for crispatene (4). A small W-coupling (J < 1 Hz) was found between H-7 and Me-18 which supported the assigned trans orientation between H-7 and Me-18. Therefore, the structure of 7 is completely elucidated.

Structure elucidation of Tridachiapyrone-F (8):

Fraction A22 of Scheme 1 was chromatographed on a short column of silica gel to give 5 fractions. The most polar fraction (fraction 5) was subjected to HPLC with silica gel to affort 5 fractions. The third of these fractions was purified by HPLC using a silica gel column and hexane-acetone (65:35) as eluent to produce 1.2 mg of tridachiapyrone-F (8) as a white powder. The molecular formula $C_{22}H_{28}O_5$ of 8 was obtained from the following spectral data: (a) the low resolution mass spectrum exhibited the molecular ion at m/z 372; (b) the ^1H NMR spectrum (Figure 10) displayed signals that accounted for 28 non-exchangeable protons including that for a methyl group deshielded by a single oxygen, (3.96 ppm, OMe); (c) the IR spectrum showed a carbonyl band at 1725 cm^{-1}, an α,β-unsaturated carbonyl band at 1690 cm^{-1}, and two bands typical for a γ-pyrone at 1665 and 1595 cm^{-1}. Comparison of the ^1H NMR data of 8 with that of 3 (see Tables 1 and 1a) showed close similarities which led to the conclusion that compound 8 contained the α-methoxy-β,β'-dimethyl-γ-pyrone ring system which was connected to a bicyclic ring system as in crispatone (3). Compounds 8 and 3 differed in the structure of the side

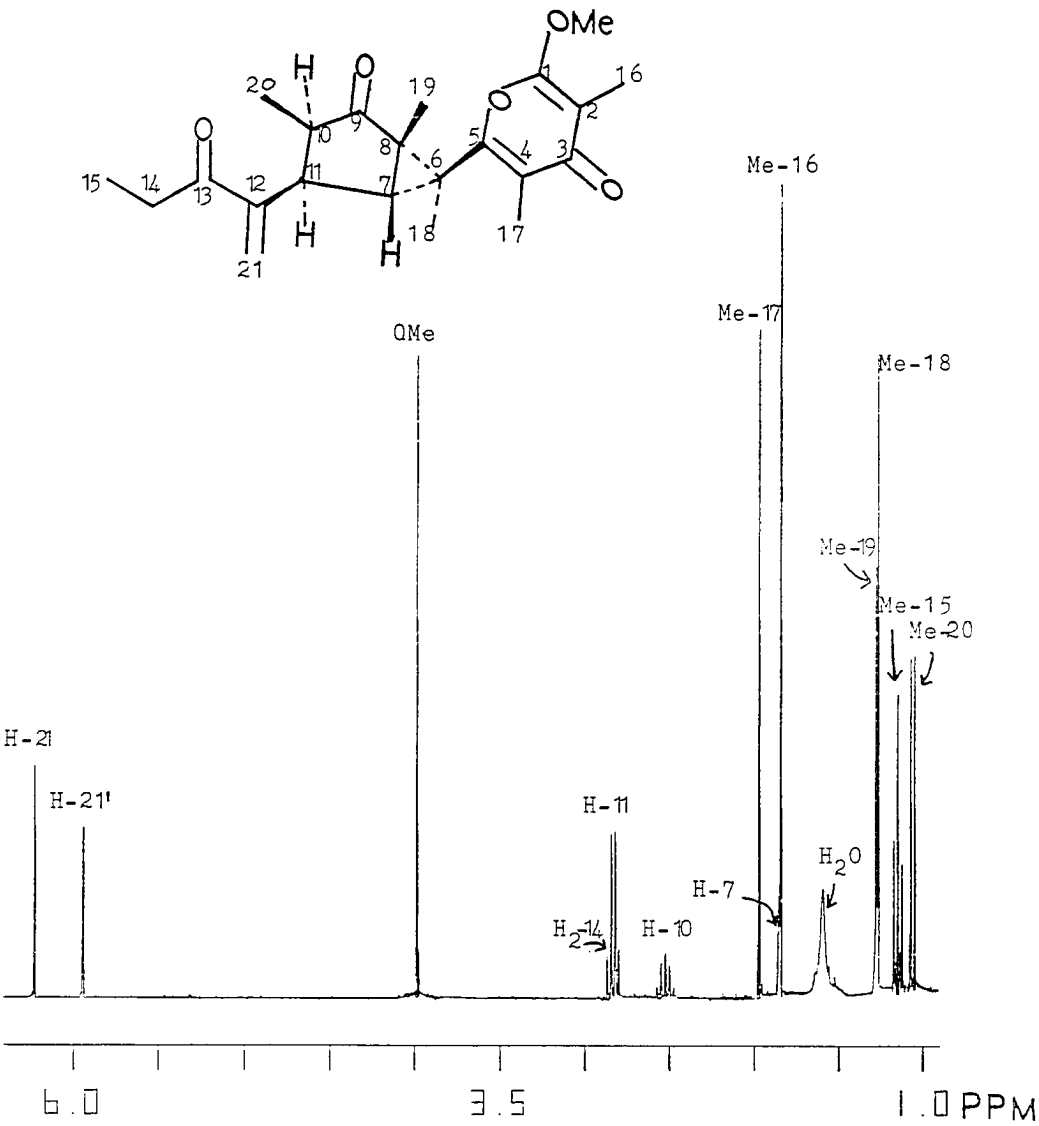


Figure 10. 300 MHz proton NMR spectrum of 8 in $CDCl_3$.

chain at C-11 and in the configuration at C-10. The side chain structure in 8 was established by ^1H decoupling experiments and by the presence of the IR band at 1690 cm^{-1} which confirmed the α,β-unsaturated carbonyl group (C-13). Moreover, the UV band at 225 nm supported this assignment. The same side chain structure was assigned to 8 as was confirmed for 7. The ^1H chemical shifts associated with the side chain for the two compounds were almost identical (see Table 1). The allylic methine proton signal at 2.80 ppm (H-11) was coupled (J = 8.2 Hz) to the methine proton signal at 2.50 ppm (H-10) which was in turn coupled (J = 7.1 Hz) to the methyl doublet signal at 1.05 ppm (Me-20). The 8.2 Hz coupling between H-10 and H-11 suggested that these protons are cis. The absence of any coupling between H-7 and H-11 protons suggested a dihedral angle near 90° between these protons as found in crispatone (3). The stereochemistry about the bicyclic ring system (C-6, C-7, and C-8) is assumed to be the same as in crispatone (3) because of the similarities in the ^1H NMR chemical shifts of H-7, Me-18, and Me-19 in 8 and in crispatone (3) (see Table 1).

Structure elucidation of Tridachiapyrone-A (9):

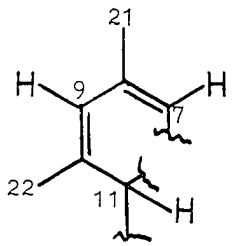
Silica gel chromatography by HPLC of the combined fractions of B5 and B6 (see Scheme 1) yielded two fractions. The second of these fractions was purified again by HPLC using a reverse-phase C_{18} column and MeOH-H_2O (73:27) as a mobile phase to yield a pure tridachiapyrone-A (9), 1.5 mg, as a colorless oil. The molecular formula $C_{25}H_{34}O_4$ was deduced from the following spectral data: (a) the low resolution mass spectrum exhibited a molecular ion at m/z 398; (b) the ^1H NMR spectrum

(see Figure 11) displayed signals for 34 non-exchangeable protons; (c) the ^{13}C NMR spectra indicated the presence of 25 carbons including two carbonyl carbons, [181.1 ppm (C-3, s); 212.0 (C-15, s)], and one other sp^2 carbon deshielded by two oxygen substituents, [161.9 ppm (C-1, s)]*. The ^{13}C NMR spectra revealed the existence of 9 methyls, one sp^3 methylene, 2 sp^3 methines and 3 sp^2 methines, and 8 quaternary carbons in addition to the two carbonyl carbons. The molecular formula of 9 correlates to the molecular formula of the known 9,10-deoxytridachione (2) plus an extra "propionate" unit. The IR data of 9 indicated the presence of one CO band at 1715 cm^{-1} together with the γ-pyrone bands at 1660 and 1585 cm^{-1}*. Comparison of the ^1H and ^{13}C NMR data (see Tables 1, 1a, 2, and 2a) of 9 with that of 9,10-deoxytridachione (2) suggested that both possessed the same carbon framework except that 9 contained an additional propionate moiety which was connected to the side chain to form a 1,3-dimethyl-4-oxo-1-hexenyl moiety. The presence of the latter side chain structural feature in 9 was confirmed by ^1H decoupling experiments. Irradiation of the multiplet at 2.34 ppm (H, H-16) collapsed the triplet at 0.98 (Me-17) to a singlet. This suggested that these protons (H-16) and (Me-17) could be assigned to a terminal ethyl ketone moiety. Furthermore, a small W-coupling ($J < 1$ Hz) was observed between (H-14) and (H-16). Thus, the C-14, C-15, and C-16 connections were confirmed. In the ^1H NMR spectrum (Figure 11) of 9, the methyl doublet signal at 0.98 ppm (Me-23) was coupled ($J = 6.8$ Hz) to the methine proton signal at 3.16 ppm (H-14), which was in turn coupled ($J = 9.5$ Hz) to

*assignment was made by analogy to crispatone (3).

the vinylic proton signal at 4.96 ppm (H-13). The ^{13}C NMR data (C-1 to C-5) (see Table 2a) and the ^{1}H NMR signals at 1.80 ppm (s, 3H), 2.08 (s, 3H), and 3.97 (s, 3H) together with the IR bands at 1660 and 1590 cm^{-1} suggested the presence of the α-methoxy-β,β'-dimethyl-γ-pyrone ring system*.

The cyclohexadiene ring structural feature in 9 was established by ^{1}H decoupling experiments. Irradiation of the doubly allylic methine proton signal at 2.78 ppm (H-11) sharpened the vinylic proton signal at 5.69 (H-9) which was in turn coupled (J ≈ 1.5 Hz) to the vinyl methyl signal at 1.69 ppm (Me-22). Moreover, a small W-coupling (J ≈ 1 Hz) was observed between H-11 and Me-22. The vinylic proton signal at 5.64 ppm (H-7) was coupled (J ≈ 1.5 Hz) to the vinylic methyl signal at 1.79 ppm (Me-21). Consequently, two double bonds were established. The UV band at 253 nm (ε = 12000) suggested a conjugated diene system for these two double bonds (see partial structure A).



A

Further evidence for this partial structure, is the similarity of the observed ^{1}H NMR chemical shifts of H-7, H-9, H-11 in 9 and 2 and Me-21 vs Me-19 and Me-22 vs Me-20 in 9 vs 2 (see Tables 1 and 1a). The methyl singlet signal at 1.42 ppm (Me-20) was assigned to a quaternary carbon (C-6) and C-6 was linked to C-11 by analogy with the triachione structure. Similarly, C-5 at the pyrone ring was bonded to C-6. NOE

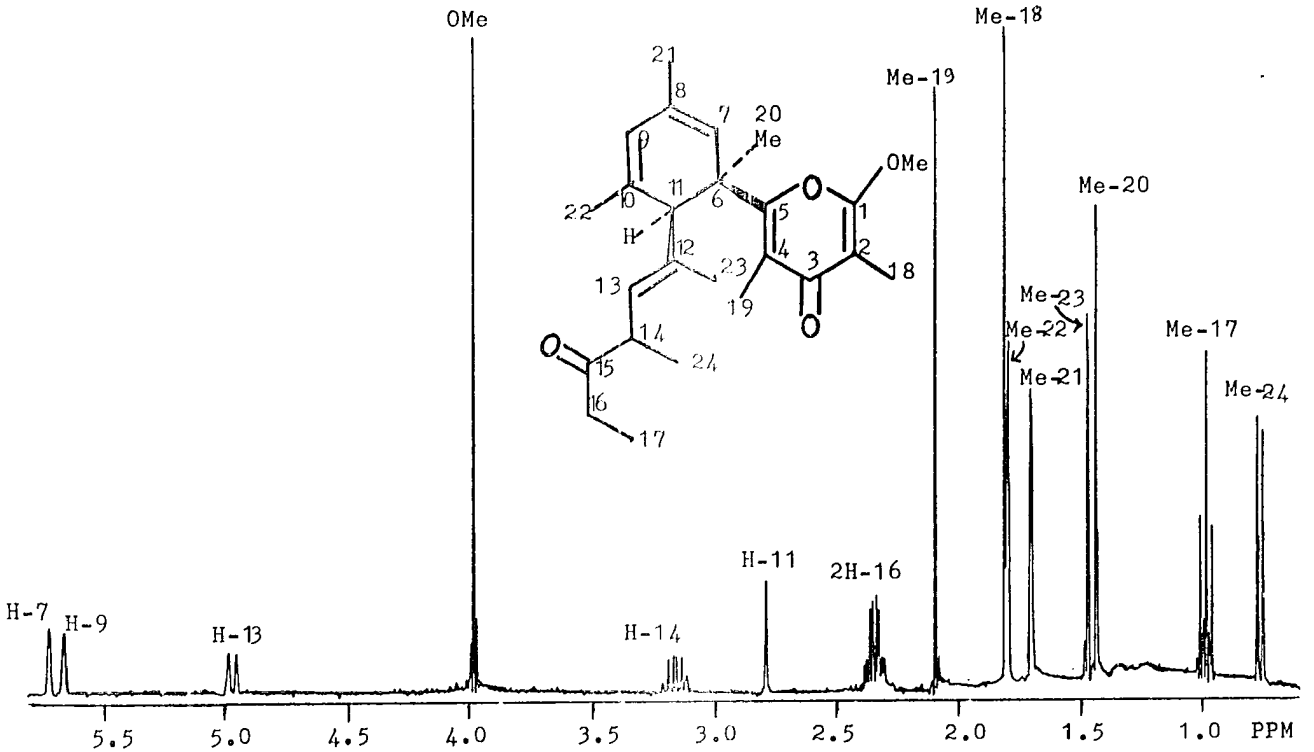


Figure 11. 300 MHz proton NMR spectrum of 2 in $CDCl_3$.

experiments supported this assignement: irradiation of Me-20 enhanced both H-7 and H-11. This also provides evidence for the cis configuration between Me-20 and H-11. Furthermore, ^{13}C data confirmed the proposed structure for 9 (see Table 2a).

The E geometry of the double bond at C-12,13 was assigned because irradiation of Me-23 signal caused an Overhauser enhancement of the H-14 signal and no enhancement was observed between Me-23 and H-13. The configuration at C-11 was assumed to be the same as the one observed for 2 on the basis of the similarities of the ^1H NMR chemical shifts for H-11 in 9 and 2 (see Tables 1 and 1a). The configuration at C-14 was not determined.

Structure elucidation of Isotridachiapyrone-A (10):

Silica gel chromatography using HPLC of the combined fractions B5 and B6 of Scheme 1 produced two fractions. The first fraction was purified by reverse-phase HPLC to yield pure isotridachiapyrone-A (10), 1.1 mg, as a colorless oil. The molecular formula $C_{25}H_{34}O_4$ was obtained from the following considerations: (a) the low resolution mass spectrum showed the molecular ion at m/z 398; (b) the ^1H NMR spectrum (Figure 12) displayed signals that accounted for 34 non-exchangeable protons including a methyl singlet signal deshielded by a single oxygen, [3.98 ppm (OMe)]; (c) the IR spectrum showed bands at 1715 cm^{-1} (CO), 1660, and 1585 cm^{-1} (γ-pyrone group)*. Comparison of the ^1H NMR spectrum of 10 with that of 9 (see Table 1a) showed close resemblance except for some minor differences associated with the side chain. Moreover, ^1H NMR

*assignment was made by analogy to other compounds in this series.

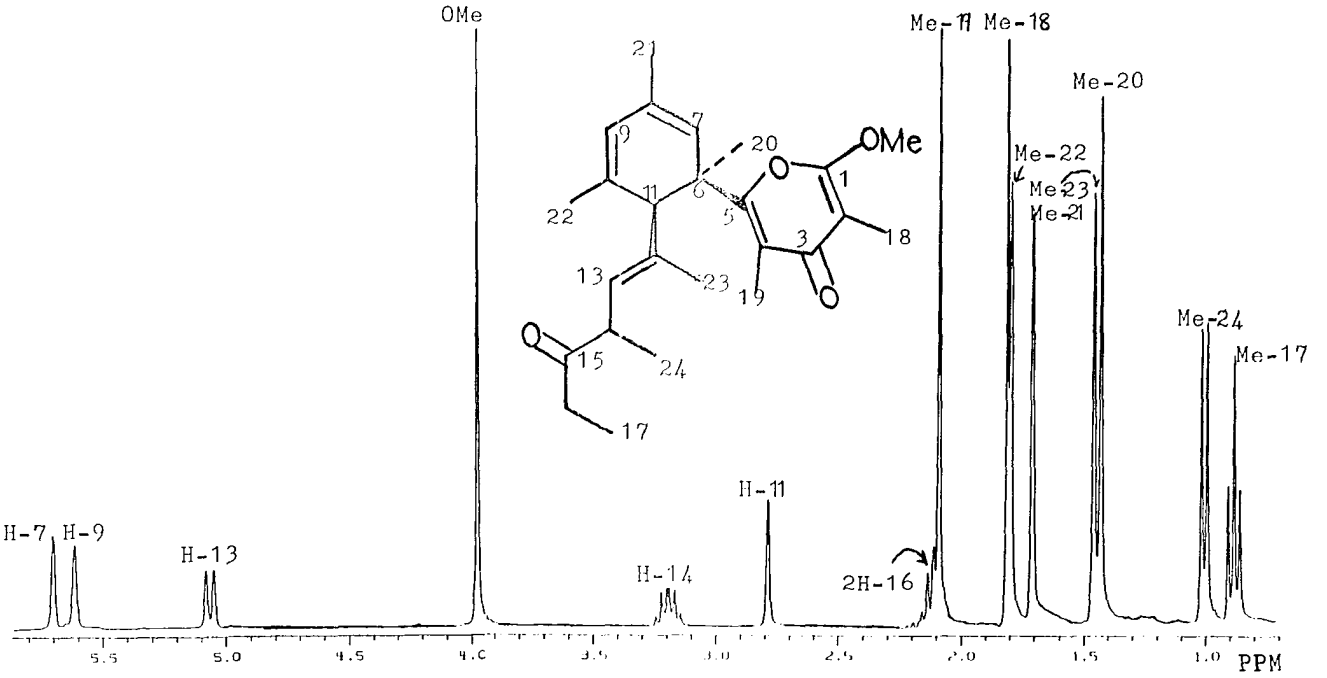


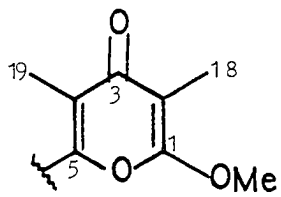
Figure 12. 300 MHz proton NMR spectrum of 10 in $CDCl_3$.

decoupling experiments established the ^1H connectivities in the side chain structure and confirmed the assignment of the ^1H signals due to the cyclcohexadiene ring system. This led to the conclusion that 9 and 10 are epimeric in respect to their configuration at C-14.

The E. geometry of the double band at C-12,13 was established by the observation of an NOE effect between Me-24 and H-14 and the lack of any NOE between Me-24 and H-13. The configuration at C-14 was not determined.

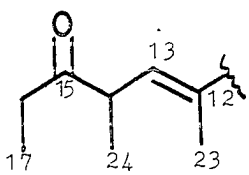
Structure elucidation of Tridachiapyrone-C (11):

Fraction C6 of Scheme 1 was chromatographed by HPLC using a reverse-phase C_{18} column and MeOH-H_2O (75:25) as eluent to yield 7 fractions. The fifth of these fractions contained 1.5 mg of tridachiapyrone-C (11) as a colorless oil. The molecular formula $C_{25}H_{34}O_5$ was obtained from the following spectral data: (a) the low resolution mass spectrum showed a molecular ion at m/z 414; (b) the ^1H NMR spectrum (Figure 13) exhibited signals for 34 non-exchangeable protons including an epoxide proton signal, (3.0 ppm, H-9),* and a methyl signal deshielded by a single oxygen, (3.98 ppm, OMe)*; (c) the IR data showed absorptions at 1715 cm^{-1} (CO), and 1655, 1585 cm^{-1} (γ-pyrone)*. The ^1H NMR signals at 1.85 ppm (Me-18, s), 2.02 (Me-19, s), and 3.98 (OMe, s) together with the IR bands at 1655 and 1585 cm^{-1} and the UV absorption at 254 nm suggested the presence of γ-pyrone ring system (partial Structure A).



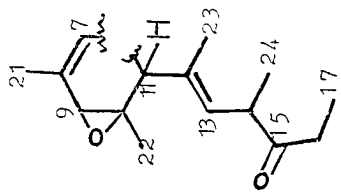
A

The side chain structural feature in 11 was established by ^1H decoupling experiments. Irradiation of the multiplet signal at 2.46 ppm (H-16) changed the methyl triplet signal at 1.05 ppm (Me-17) into a singlet and also sharpened H-14 signal at 3.50 ppm. The mutual coupling between H-16 and Me-17 signals and the chemical shift of H-16 suggested a terminal ethyl ketone moiety in 11. Irradiation of the olefinic proton signal at 5.41 ppm (H-13) collapsed the multiplet signal at 3.50 ppm (H-14) into a quartet and removed small allylic couplings (J ≈ 1 Hz) from H-11 and Me-23 signals. The H-14 signal was also coupled (J = 7 Hz) to the methyl doublet signal at 1.20 ppm (Me-24). Thus, the 1,3-dimethyl-4-oxo-1-hexenyl partial structure B was built.



B

The allylic methine proton signal at 3.40 ppm (H-11) was found to be coupled (J ≈ 1 Hz) to the epoxide proton signal at 3.0 ppm (H-9) (W-coupling) and also coupled (J ≈ 1 Hz) to the olefinic proton signals at 5.41 ppm (H-13) and 5.50 (H-7). The epoxide proton resonating at 3.0 ppm (H-9) was found to couple (J ≈ 1 Hz): (a) to the olefinic proton appearing at 5.50 ppm (H-7) (allylic coupling), to the vinylic methyl at 1.95 ppm (Me-21), and to the allylic proton at 3.40 ppm (H-11) (W-coupling). Irradiation of the vinylic methyl signal at 1.95 ppm (Me-21) confirmed the presence of a long-range coupling between H-9 and Me-21 signal and removed a small allylic coupling from H-7 signal. The methyl singlet signal at 1.32 ppm (Me-22) was connected to C-10 on the basis of the observation of an NOE effect between Me-22 and both H-9 and H-11. Therefore, partial structure B was extended into partial structure C.



C

The methyl group causing the singlet signal at 1.17 ppm (Me-20) was placed on a quaternary carbon (C-6) because of the absence of any coupling between the Me-20 signal and any other ^{1}H signal, and also for biogenetic reasons. C-11 was linked to C-6 and C-6 to C-7 on the basis of a long-range coupling observed between H-11 and H-7. Furthermore, the

*assignment was made by analogy to tridachione (1).

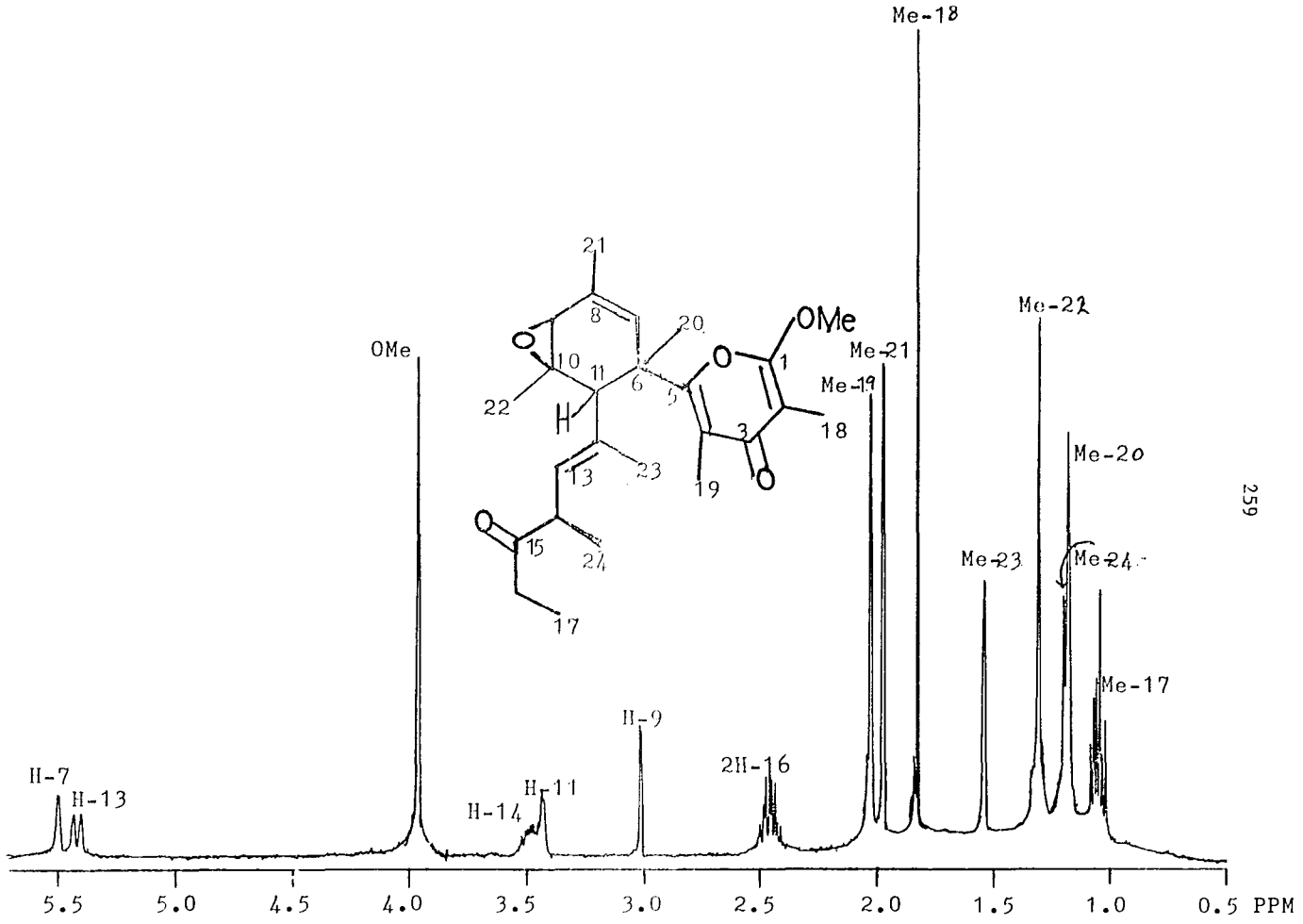


Figure 13. 300 MHz proton NMR spectrum of 11 in $CDCl_3$.

observation of an NOE effect between Me-20 and H-7 supported the C-6 and C-7 connection. C-5 of partial structure A was connected to C-6 by applying biogenetic rules.

Neither the stereochemistry of the epoxide group at C-9,10, nor the configuration at C-6 was determined. However, the lack of an NOE effect between Me-20 and H-11 and the downfield shift which is observed for H-11 in 11 compared to that of tridachione (1) (see Tables 1 and 1a) suggested that Me-20 and H-11 are trans to each other, unlike the observed configuration for tridachione (1). The geometry of the double bond at C-12,13 was established as E on the basis of the observation of an NOE effect between Me-23 and H-14 and the absence of any NOE between Me-23 and H-13. The configuration at C-14 in 11 was assumed to be the same as in crispatene (4) on the basis of the similarities of the ^{1}H NMR chemical shifts observed for the side chain in 11 and 4 (see Table 1a).

Structure elucidation of Tridachiapyrone-D (12):

Fraction C6 of Scheme 1 was chromatographed by HPLC using a reverse-phase C_{18} column and MeOH-H_2O (75:25) to give 7 fractions. Evaporation of the seventh fraction left 0.7 mg of pure tridachiapyrone-D (12) as a colorless oil. The molecular formula $C_{25}H_{34}O_6$ for 12 was established by the analysis of its high resolution mass spectrum (obs, 430.2421; requires 430.2355). The IR spectrum of 12 showed a broad band (OH) at 3450 cm^{-1}, a carbonyl band at 1712, and two bands for a γ-pyrone at 1660 and 1585 cm^{-1}. The ^{1}H NMR spectrum (Figure 14) exhibited signals for 33 non-exchangeable protons and one exchangeable proton (OH). Comparison of the ^{1}H NMR data of 12 with those of 11 led to the

conclusion that 12 and 11 had the same carbon skeleton, but that 12 had an additional hydroxyl group at C-13 and had a terminal methylene group joined to C-12 instead of a vinyl methyl (Me-23) at C-12. The side chain moiety in 12 was established by ^1H decoupling and ^1H difference decoupling (DDS) experiments. Irradiation of the multiplet at 2.48 ppm (H-16) collapsed the triplet at 1.03 ppm (Me-17) into a singlet. Furthermore, a small W-coupling ($J < 1$ Hz) was found between H-14 and H-16. Considerations of the chemical shift of H-16 together with the mutual coupling between H-16 and Me-17 led to the suggestion that Me-17 and H-16 were in a terminal ethyl ketone moiety. The methine proton signal at 2.75 ppm (H-14) was coupled ($J = 7$ Hz) to the methyl doublet signal at 1.13 ppm (Me-23) and to the one-proton doublet of quartets at 4.42 ppm (H-13) which was in turn coupled ($J = 2.1$ Hz) to the terminal methylene proton signals at 4.84 ppm (H-23') and 4.92 (H-23). Moreover, the H-13 signal was found to have a 2.1 Hz W-coupling with H-11 (2.99 ppm). The allylic methine proton signal at 2.99 ppm (H-11) was established to be coupled ($J = 2.1$ Hz) to the terminal methylene proton signals at 4.92 and 4.84 ppm. Furthermore, a W-coupling ($J = 2.1$ Hz) was confirmed between H-11 and the epoxide proton at 3.16 ppm (H-9). The epoxide proton signal was also coupled ($J < 1$ Hz) to the vinylic methyl signal at 1.53 ppm (Me-21) and to the vinylic proton signal at 5.47 ppm (H-7). From the above discussion, a partial structure A was formulated.

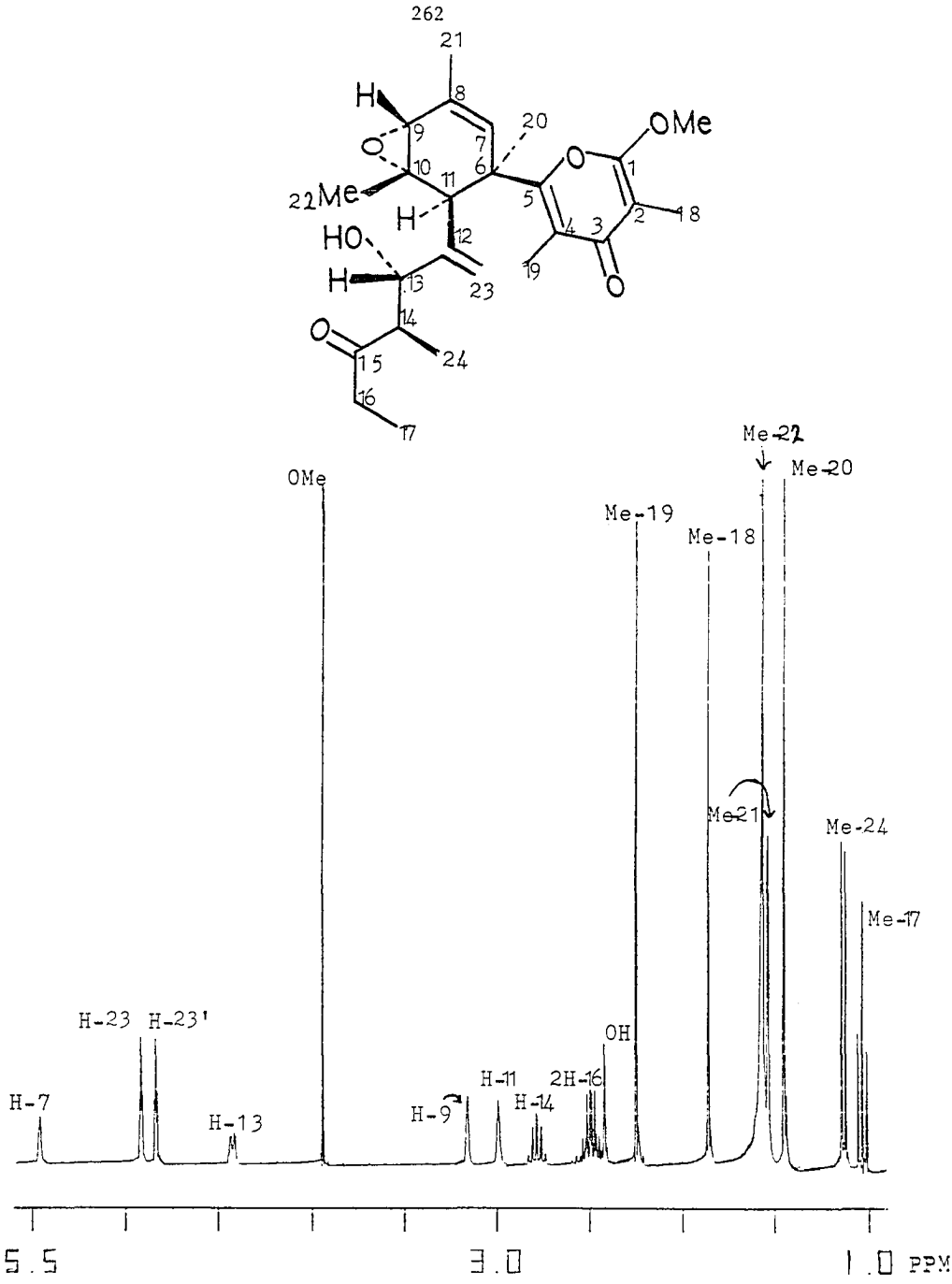
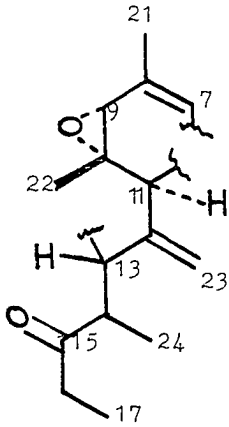


Figure 14. 300 MHz proton NMR spectrum of 12 in $CDCl_3$.



A

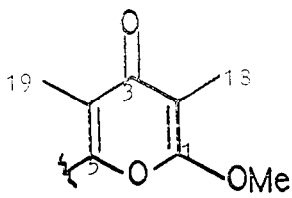
Consideration of the chemical shift at H-13 (4.42 ppm) required that C-13 also bear a hydroxyl group. The remaining structural features in 12 were assumed to be similar to those of tridachione (1) on the basis of the similarities of the ^1H chemical shifts observed for 12 and 1 (see Table 1).

The stereochemical features in 12 were established by NOE experiments. The Z configuration of the epoxide group at C-9,10 was confirmed by the Overhauser enhancement between Me-22 and H-9. The cis configuration of Me-20 and H-11 was also established by an NOE experiment (irradiation of Me-20 enhanced H-11 and vice versa). The configuration at C-14 in 12 was assumed to be identical to that of 11 on the basis of the similarities of the ^1H chemical shifts of Me-23, H-16, and Me-17 observed in 12 and 11 (see Table 1a).

Structure eludication of Tridachiapyrone-G (13):

Fraction C2 of Scheme 1 was chromatographed by HPLC using a reverse-phase C_{18} column and MeOH-H_2O (7:3) as eluent to yield 9 fractions. The eighth of these fractions was subjected to HPLC with a reverse-phase C_{18} column and MeOH-H_2O (8:2) as a mobile phase to afford

3 fractions. The second of these fractions contained 0.2 mg of tridachiapyrone-G (13) as a colorless oil. The molecular formula $C_{25}H_{34}O_4$ was obtained from the following spectral data: (a) the low resolution mass spectrum exhibited a molecular ion at m/z 398; (b) the ^1H NMR spectrum (Figure 15) showed signals for 34 non-exchangeable protons including a methyl signal deshielded by a single oxygen, [3.92 ppm (OMe)]; (c) the IR spectrum displayed bands at 1715 cm^{-1} (CO), 1660, and 1585 cm^{-1} (γ-pyrone group).* The ^1H NMR signals at 1.82 ppm (Me-18, s), 2.07 (Me-19, s) and 3.92 (OMe, s) together with the IR bands at 1660 and 1585 cm^{-1} and UV absorption at 250 nm suggested the existence of the α-methoxy-β,β'-dimethyl-γ-pyrone* ring system (partial structure A).

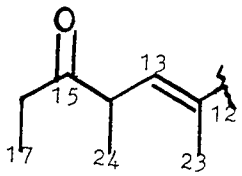


A

The side chain structural feature in 13 was confirmed by ^1H decoupling experiments. Irradiation of the multiplet at 2.42 ppm (H-16) collapsed the methyl triplet at 1.03 ppm (Me-17) into a singlet and also sharpened H-14 signal (W-coupling). Considerations of the chemical shift of H-16 and the mutual coupling between H-16 and Me-17 suggested a terminal ethyl ketone group. The olefinic proton signal at 5.19 ppm (H-13) was found to be coupled ($J \simeq 1.1$ Hz) to the vinylic methyl signal at

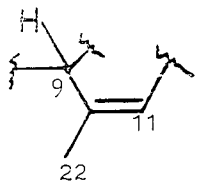
*assignment was made by analogy to other compounds in this series.

1.65 ppm (Me-23) and also to the methine proton signal at 3.42 ppm (H-14) which was in turn coupled (J = 7 Hz) to the methyl doublet signal at 1.12 ppm (Me-24). Thus, the 1,3-dimethyl-4-oxo-1-hexenyl partial structure B was constructed.



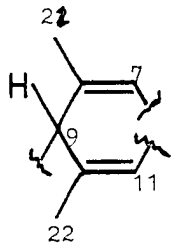
B

Two isolated double bonds were established in 13. The downfield proton signal at 5.64 ppm (H-11) was coupled (J ≈ 1 Hz) to the vinylic methyl signal at 1.65 ppm (Me-22) and also to the triply allylic proton signal at 3.22 ppm (H-9). Consequently, partial structure C was confirmed.



C

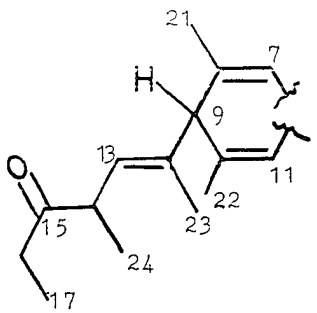
Irradiation of the vinylic methyl signal at 1.77 ppm (Me-21) sharpened the olefinic proton signal at 5.16 ppm (H-7) and also sharpened the proton signal due to H-9. Therefore, partial structure C was extended into D.



D

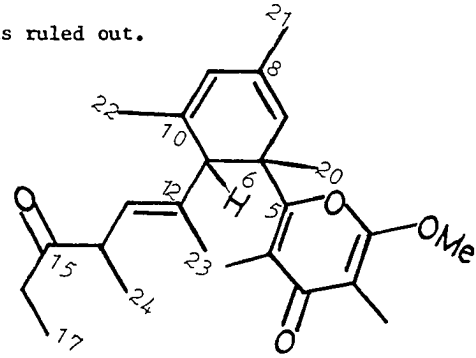
Irradiation at the 3.22 ppm signal, H-9 of partial structure D, removed small allylic coulings from the olefinic proton signals at 5.64 ppm (H-11) and 5.19 (H-13) and also sharpened the signal at 1.65 ppm (Me-22 or Me-23) and 1.77 (Me-21). This established the C-9,12 connection and enlarged partial structure D into E.

E



The absence of any coupling between the Me-20 signal (1.31 ppm) and any other ^{1}H signals suggested that Me-20 could be connected to a quaternary carbon (C-6). This quaternary carbon (C-6) was also linked to C-7, C-11 and C-5 on the basis of biogenetic reasons. However, two other possible structures like F and G would satisfy the presented data. The observed UV band for 13 at 250 nm (ε = 6000) was assigned to the γ-

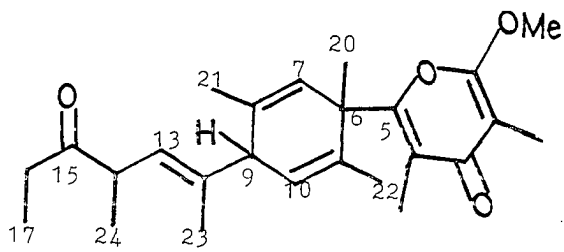
pyrone ring system as found in other compounds in this series. If, however, a structure like F is considered, the UV band at 250 nm should have an ε=12000 as found in compounds 2, 9, and 10. Therefore, structure F was ruled out.



F

The observed small coupling (J ≈ 1 Hz) between the two signals at 3.22 ppm (H-9) and 5.64 could be due to either an allylic type coupling (proposed structure) between H-9 and H-11, or could be a vicinal type coupling between the signals at 3.22 ppm (H-9) and 5.64 (H-10 in structure G). However, if structure G is to be considered the dihedral angle between H-9 and H-10 (structure G) must be near 90° in one conformation and that must be the predominant conformation.

The relative stereochemistries at C-9, C-11, and C-14 were not determined.



G

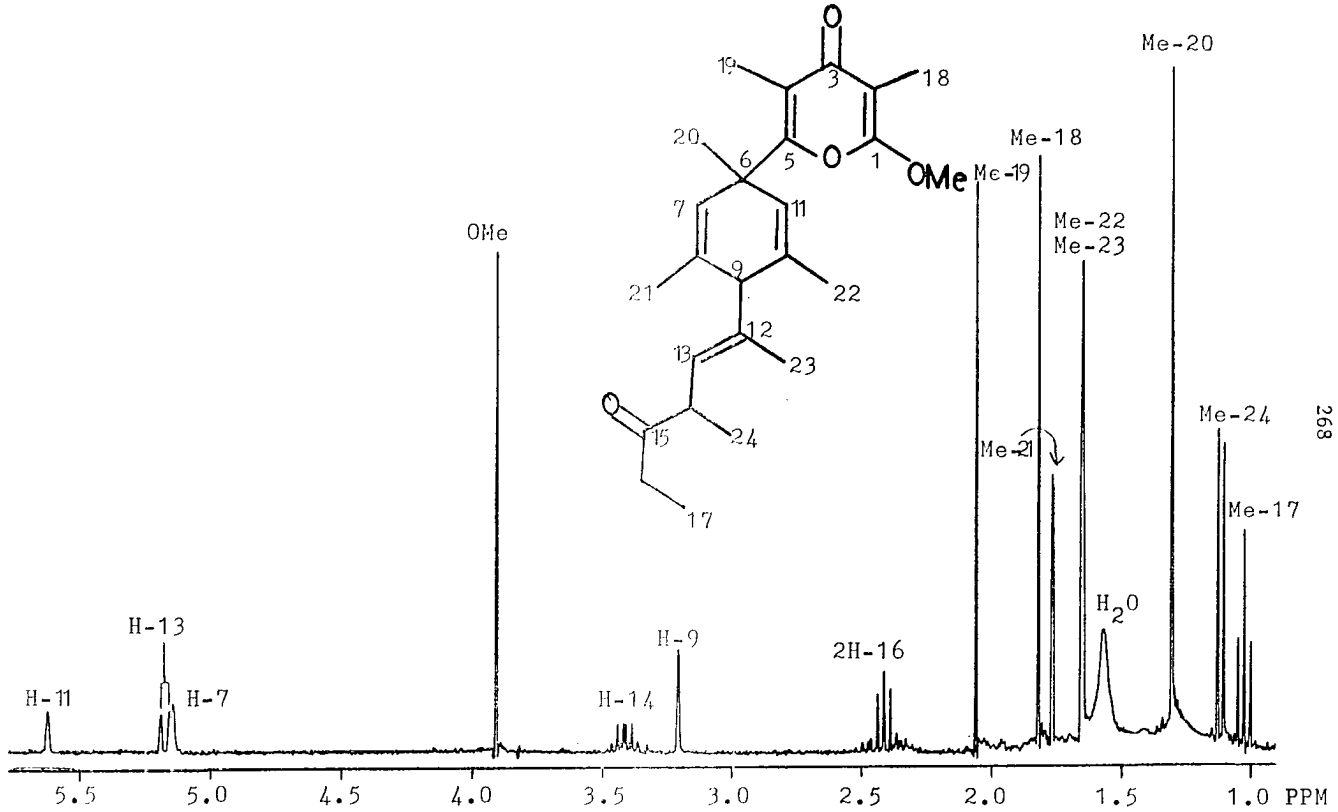
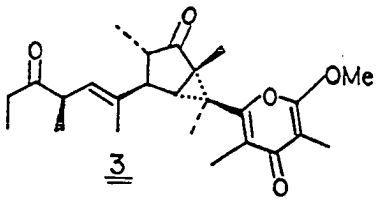


Figure 15. 300 MHz proton NMR spectrum of 13 in $CDCl_3$.

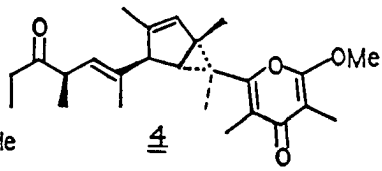
Summary

Two known compounds and nine new compounds have been isolated from *Tridachia crispata*. The known natural products, crispatone (3) and crispatene (4), have been isolated previously from *Tridachia crispata*, collected from the Caribbean.[7]

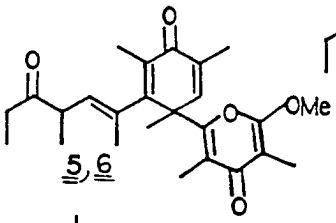
The epimeric metabolites 5 and 6 have new carbon skeletons. Compound 7 possesses the same substituted ring system as crispatene (4), but 7 has one less propionate unit in the side chain. Compound 8 is structurally similar to 7, but has a ketone in the 5-membered-ring rather than a double bond. The C-14 epimeric compounds 9 and 10 have the same substituted ring system as the known 9,10-deoxytridachione (2), but they bear an additional propionate unit at C-14. Compound 11 possesses the same substituted ring system as in the known tridachione (1), but has an extra propionate unit at C-14. Compound 12 is similar to the known tridachione (1), but in 12 the side chain is longer by one propionate unit , there is a hydroxyl group at C-13, and there is a terminal methylene group at C-12,23. Compound 13 is similar to 9 and 10, but the side chain is attached to C-9 rather than C-11 and there is a 1,4-cyclohexadiene ring instead of the conjugated 1,3-cyclohexadiene found in 9 and 10 .



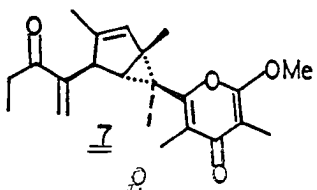
3



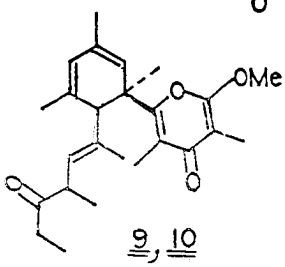
4



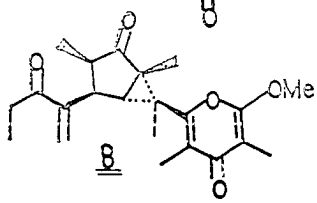
5, 6



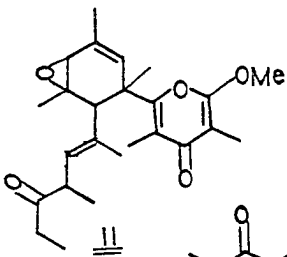
7



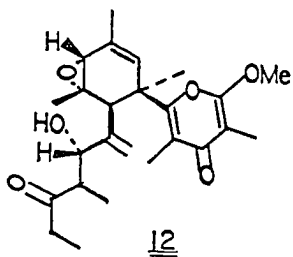
9, 10



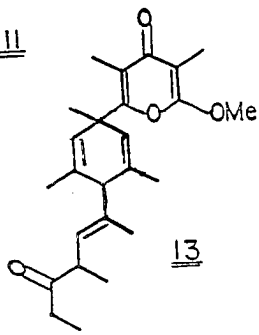
8



11



12



13

Experimental

^1H NMR spectra were recorded at 300 MHz and ^{13}C spectra at 75.4 MHz on a Varian XL-300 spectrometer; chemical shifts are reported in parts per million (δ) downfield from internal tetramethylsilane. IR spectra were recorded on a Perkin-Elmer Model 298 spectrophotometer. UV spectra were measured with a Perkin-Elmer Lambda 3-spectrophotometer. Low resolution mass spectra were recorded on a Hewlett-Packard 5985B mass spectrometer; high resolution mass spectra were taken on a CEC (DuPont, Monrovia, CA) 110 instrument. The chromatographic adsorbent used was Brinkmann silica gel 60(230-400 mesh). Thin layer chromatograms were run on precoated Macherey-Nagel polygram silica gel G/UV_{254} (0.25 mm) plates. A sulfuric acid-vanillin $\frac{180}{1}$ spray was used to visualize TLC plates. Altex 9.6 mm X 25 cm preparative silica gel (5μ particles, LiChrosorb 60) and Adsorbosphere 9.6 mm X 25 cm reverse-phase C_{18} (5μ particles) columns were used for HPLC separations.

Extraction and Partition Procedure: Freshly thawed nudibranchs, *Tridachia crispata* (80 animals), collected and shipped frozen from Discovery Bay, Jamaica, in May of 1979, were allowed to soak in acetone (4000 mL) for 24 h. The acetone solution was concentrated and the concentrate diluted with H-20 and extracted with either (2 X 3000 mL). The ether-soluble portion (6.5 g) of the acetone extracts was chromatographed extensively to yield 10 compounds (see Scheme 1).

Isolation of Crispatone (3): The ether-soluble fraction (6.5 g) of acetone extracts of homogenized *Tridachia crispata* (80 animals) was chromatographed on a column of silica gel [elution started with acetone-hexane (6:94)] and the amount of acetone was increased stepwise to yield 24 fractions. The twenty-second of these fractions (70 mg) was rechromatographed on a short column of silica gel using hexane-acetone (6:4) as eluent and collecting 5 fractions. The most polar fraction (fraction 5, 20 mg) was subjected to HPLC with a silica gel column and hexane-acetone (65:35) as eluent to afford 5 fractions. The second of these fractions was further purified by HPLC with a reverse-phase C_{18} column and MeOH-H_2O (75:25) to give 12 mg of pure crispatone (3) as a colorless oil. Compound 3 has the following spectral data: IR ($CHCL_3$) 2990, 2970, 2930, 2870, 1735, 1712, 1660, 1590, 1460, 1420, 1380, 1330, 1240, 1160, 880 cm^{-1}; for ^1H and ^{13}C NMR data see Tables 1a and 2a; low resolution mass spectrum (70 eV) m/z (relative intensity) 414.2 (M^+, (4), 348.9 (16), 289.1 (4), 277.1 (25), 249.1 (15), 233.1 (11), 203.1 (14), 194.0 (16), 189.1 (26), 159.1 (16), 155.0 (21), 149.0 (44), 125.1 (29), 115.1 (28), 111.1 (44), 109.1 (49), 97.2 (52), 91.1 (53), 83.1 (89), 77.1 (40), 69.1 (85), 57.1 (100), 55.2 (58); HRMS, obs: 414.24325, $C_{25}H_{34}O_5$ requries 414.2406.

Isolation of Crispatene (4): Fraction C3 (15 mg) (see Scheme 1) was subjected to HPLC with a reverse-phase C_{18} column and MeOH-H_2O (8:2) as eluent to obtain 10 fractions. Evaporation of the tenth fraction left 6.2 mg of pure crispatene 4 as a colorless oil. Compound 4 possesses the following spectral properties: IR (neat) 2970, 2930, 2870, 1715, 1660, 1615, 1590, 1460, 1405, 1340, 1255, 1170, 1110, 1050,

990, 890 cm^{-1}; for ^{1}H and ^{13}C NMR data see Tables 1a and 2a; low-resolution mass spectrum (70 eV) m/z (relative intensity) 398.2 [M^{+}, (35)], 383.3 (28), 342.2 (23), 341.2 (65), 313.1 (56), 281.1 (15), 273.1 (10), 253.2 (56), 237.1 (47), 225.2 (36), 182.1 (40), 159.1 (39), 145.0 (33), 133.0 (54), 129.0 (4), 125.1 (26), 119.1 (41), 115.1 (47), 105.1 (57), 91.1 (71), 85.1 (20), 83.1 (79), 57.1 (100).

Isolation of Tridachiapyrone-B (5) and Isotridachiapyrone-B (6): Fraction C6 (35 mg) of Scheme 1 was chromatographed by HPLC with a reverse-phase C_{18} column and MeOH-H_2O (75:25) as eluent to give 7 fractions. The third of these fractions was resolved by HPLC using a reverse-phase C-18 column and MeOH-H_2O (42:58) as a mobile phase to afford two pure compounds, 5 (1.5 mg) and 6 (1.8 mg), as colorless oils. Compound 5 has the following spectral data: UV (MeOH) λ_{max} 248 nm (ε = 11900); IR ($CHCL_3$) 3020, 2995, 2925, 2880, 1710, 1660, 1650, 1635, 1600, 1590, 1450, 1400, 1370, 1310, 1250, 1160, 1025, 975, 880 cm^{-1}; for ^{1}H and ^{13}C NMR data see Tables 1a and 2a; low resolution mass spectrum (70 eV) m/z (relative intensity) 412.1 [M^{+}, (6)], 356.1 (20), 355.2 (4), 327.1 (12), 253.1 (12), 241.1 (15), 214.1 (13), 213.1 (43), 183.1 (17), 182.1 (91), 155.0 (10), 153.1 (10), 142.0 (15), 128.2 (13), 115.2 (16), 105.1 (15), 91.1 (33), 83.1 (87), 57.1 (100). Compound 6 has the following spectral data: UV (MeOH) λ_{max} 248 nm (ε = 11900); IR data are identical to that of 5 (see above); for ^{1}H and ^{13}C NMR data see Tables 1a and 2a; low resolution mass spectrum is identical to that of 5 (see above).

Isoaltion of Tridachiapyrone-E (7): Fraction B17 of Scheme 1 was chromatographed by reversed phase HPLC [elution with MeOH-H_2O (7:3)] to

yield 14 fractions. The fourteenth of these fractions contained tridachiapyrone-E (7), 0.8 mg as a colorless oil. Compound 7 has the following spectral data: UV (MeOH) λ_{max} 255, 225 (ϵ = 5800, 7500); IR (neat) 2980, 2950, 2860, 1690, 1660, 1585, 1460, 1415, 1380, 1330, 1260, 1160, 1170, 1040, 990, 870 cm^{-1}; for ^1H NMR data see Table 1; low resolution mass spectrum (70 eV) m/z (relative intensity) 356.2 [M^+, (17)], 341.2 (13), 299.2 (20), 267.1 (13), 239.2 (25), 213.1 (17), 185.1 (32), 182.1 (82), 157.0 (16), 153.1 (33), 141.0 (16), 129.0 (25), 119.2 (31), 115.1 (31), 105.1 (17), 97.1 (26), 91.1 (35), 83.1 (54), 81.1 (16), 77.0 (25), 69.1 (33), 65.1 (14), 57.1 (100).

Isolation of Tridachiapyrone-F (8): The twenty-second fraction (70 mg) of Scheme 1 was chromatographed on a short column of silica gel [elution with hexane-acetone (6:4)] to give 5 fractions. The most polar fraction (fraction 5, 20 mg) was subjected to HPLC with a silica gel column and hexane-acetone (65:35) as eluent to afford 5 fractions. The third of these fractions was purified by HPLC with a silica gel column and hexane-acetone (65:35) as a mobile phase to produce 1.2 mg of tridachiapyrone-F (8) as a white powder. Compound 8 has the following spectral data: UV (MeOH) λ_{max} 255, 225 nm (ϵ = 5800, 7400); IR ($CHCL_3$) 2960, 2850, 1725, 1690, 1665, 1595, 1450, 1390, 1370, 1325, 1220, 1050, 990, 920 cm^{-1}; for ^1H NMR data see Table 1; low resolution mass spectrum (70 eV) m/z (relative intensity) 372.8 [M^+, (35)], 357.7 (8), 315.7 (14), 297.5 (12), 283.5 (13), 269.4 (15), 255.6 (23), 241.5 (22), 227.5 (29), 220.4 (12), 201.4 (36), 189.3 (28), 182.3 (32), 173.3 (31), 171.4 (19), 161.3 (24), 159.3 (23), 155.3 (20), 153.3 (16), 134.2 (56), 133.2

(36), 128.4 (31), 115.3 (45), 105.2 (44), 91.2 (100), 77.2 (41), 57.2 (78).

Isolation of Tridachiapyrone-A (9): The combined fractions B5 and B6 (6 mg) from Scheme 1 were chromatographed by HPLC with a silica gel column and hexane-acetone (75:25) as eluent to produce 2 fractions. The second of these fractions was purified by HPLC using a reverse-phase C_{18} column and MeOH-H_2O (73:27) as a mobile phase to afford pure tridachiapyrone-A (9), 1.5 mg, as a colorless oil. Compound 9 has the following spectral data: UV (MeOH) λ_{max} 253 nm (ϵ = 11900); IR ($CHCL_3$) 2990, 2930, 2860, 1715, 1660, 1600, 1585, 1460, 1410, 1375, 1315, 1250, 1165, 1100, 990 cm^{-1}; for ^1H and ^{13}C NMR data see Tables 1a and 2a; low resolution mass spectrum (70 eV) m/z (relative intensity) 398.2 [M^+, (7)], 383.3 (21), 341.1 (15), 313.1 (41), 273.1 (3), 267.2 (6), 253.1 (18), 237.1 (17), 225.1 (14), 199.1 (14), 179.1 (22), 171.1 (18), 155.1 (26), 153.0 (11), 149.0 (28), 143.1 (22), 135.1 (12), 129.0 (40), 125.1 (17), 119.2 (22), 115.1 (41), 111.2 (26), 105.1 (35), 97.1 (28), 91.1 (48), 85.1 (31), 83.1 (84), 73.0 (42), 69.1 (44), 57.1 (100).

Isolation of Isotridachiapyrone-A (10): The combined fractions B5 and B6 of Scheme 1 were subjected to HPLC with silica gel and hexane-acetone (75:25) as eluent to give two fractions. The first fraction was purified by HPLC using a reverse-phase C_{18} column and MeOH-H_2O (75:25) as a mobile phase to afford pure isotridachiapyrone-A (10), 1.1 mg, as a colorless oil. Compound 10 has the following spectral properties: UV (MeOH) λ_{max} 255 nm (ϵ = 11800); IR (neat) 2980, 2915, 2860, 1715, 1660, 1600, 1585, 1460, 1405, 1325, 1310, 1250, 1160, 985 cm^{-1}; for ^1H NMR data see Table 1a; low resolution mass spectrum (70 eV) m/z (relative

intensity) 398.2 [M^+, (20)], 384.2 (52), 341.2 (35), 313.2 (100), 297.2 (17), 281.2 (14), 273.1 (6), 259.1 (21), 253.2 (34), 237.2 (29), 225.1 (22), 209.1 (11), 197.1 (17), 179.1 (23), 155.1 (24), 153.0 (9), 129.0 (19), 115.1 (20), 105.1 (21), 91.1 (25), 83.1 (25), 57.1 (28).

Isolation of Tridachiapyrone-C (11): Fraction C6 (35 mg) of Scheme 1 was chromatographed by HPLC using a reverse-phase C_{18} column and MeOH-H_2O (75:25) as eluent to afford 7 fractions. The fifth of these fractions contained 1.5 mg of tridachiapyrone-C (11) as a colorless oil. Compound 11 has the following spectral properties: UV (MeOH) λ_{max} 254 nm (ε = 5890); IR (neat) 2990, 2930, 2860, 1715, 1655, 1600, 1585, 1460, 1410, 1380, 1330, 1240, 1170, 980 cm^{-1}; for ^1H NMR data see Table 1a; low resolution mass spectrum (12 eV) m/z (relative intensity) 414.1 [M^+, (3)], 399.2 (2), 357.3 (5), 329.2 (5), 289.2 (1), 273.1 (5), 246.2 (16), 245.2 (100), 233.1 (3), 227.3 (21), 199.2 (16), 170.1 (31), 155.0 (9), 153.0 (5), 142.1 (8), 125.1 (22), 57.2 (10).

Isolation of Tridachiapyrone-D (12): A 35 mg sample of fraction C6 (see Scheme 1) was chromatographed by HPLC using a reverse-phase C_{18} column and MeOH-H_2O (75:25) to give 7 fractions. Evaporation fo the seventh fraction left 0.7 mg of pure tridachiapyrone-D (12) as a colorless oil. Compound 12 showed the following spectral properties: UV (MeOH) λ_{max} 255 nm (ε = 5900); IR (neat) 3450, 2980, 2860, 1712, 1660, 1600, 1585, 1460, 1415, 1380, 1375, 1260, 1180, 1110, 980, 900, 885 cm^{-1}; for ^1H NMR data see Table 1a; high-resolution mass spectrum, observed m/z (composition, interpretation, calculated millimass) 430.24209 ($C_{25}H_{34}O_6$, M^+, 430.2355), 412.2235 ($C_{25}H_{32}O_5$, M^+-H_2O, 412.2250), 299.16785 ($C_{19}H_{23}O_3$, $M^+-C_6H_{11}O_3$, 299.16472), 153.05363 ($C_8H_9O_3$, M^+-

$C_{17}H_{25}O_3$, 153.05517), 141.09030 ($C_8H_{13}O_2$, M^+-$C_{17}H_{21}O_4$, 141.09156), 123.07868 ($C_8H_{11}O$, M^+-$C_{17}H_{23}O_5$), 123.08099), 115.07465 ($C_6H_{11}O_2$, M^+-$C_{19}H_{23}O_4$, 115.07591), 97.06567 (C_6H_9O, M^+-$C_{19}H_{25}O_5$, 97.06534), 85.06498 (C_5H_9O, M^+-$C_{20}H_{25}O_5$, 85.06534).

Isolation of Tridachiapyrone-G (13): Fraction C2 of Scheme 1 was chromatographed by HPLC using a reverse-phase C_{18} column and MeOH-H_2O (7:3) as eluent to yield 9 fractions. The eighth of these fractions was subjected to HPLC with a reverse-phase C_{18} column and MeOH-H_2O (8:2) as a mobile phase to afford 3 fractions. The second of these fractions contained 0.2 mg of tridachiapyrone-G (13) as a colorless oil. Compound 13 has the following spectral properties: UV (MeOH) λ_{max} 250 nm (ε = 5900); IR (neat) 2985, 2910, 2845, 1715, 1660, 1600, 1585, 1455, 1400, 1320, 1300, 1250, 1155, 985 cm^{-1}; for ^1H NMR data see Table 1a; low resolution mass spectrum (70 eV) m/z (relative intensity) 398.8 [M^+, (17)], 342.7 (20), 341.6 (79), 313.6 (29), 273.5 (7), 253.5 (47), 237.4 (47), 225.4 (37), 197.4 (31), 193.3 (29), 182.3 (60), 165.3 (29), 159.3 (44), 155.3 (70), 153.3 (27), 149.2 (57), 145.2 (39), 143.3 (46), 132.2 (45), 129.2 (53), 128.3 (39), 125.3 (40), 121.3 (59), 119.3 (47), 115.2 (53), 107.3 (53), 97.3 (56), 91.2 (84), 85.2 (36) 83.2 (100), 69.2 (100), 57.2 (87).

Bibilography

1. Marcus, E.; Marcus, E. *Stud. Trop. Oceanogr. 1967, 6(11)*, 154.

2. Faulkner, D. J.; Ireland, C. "Marine Natural Products Chemistry," Edited by Faulkner, D. J., Fenical, W. H., pp. 23-34, Plenum Press, New York (1977); Fenical, W.; Sleeper, H. L; Paul, V. J.; Stallard, M. O.; Sun, H. H. *Pure Appl. Chem. 1979, 51*, 1865.

3. Stallard, M. O.; Faulkner, D. J. *Comp. Biochem. Physiol. 1974, 49B*, 25.

4. Burreson, B. J.; Scheuer, P. J.; Finer, J.; Clardy, J. *J. Am. Chem. Soc. 1975, 97*, 4763.

5. French, R. K.; Greene, R. W.; Bystrom, B. J. *J. Cell. Biol. 1969*, *42*, 404.

6. Ireland, C; Faulkner, D. J.; Solheim, B. A; Clardy, J. *J. Am. Chem. Soc. 1978, 100*, 1002.

7. Ireland, C.; Faulkner, D. J.; Finer, J. S.; Clardy, *Ibid. 1979*, *101*, 1275.

8. Ireland, C; Faulkner, D. J. *Tetrahedron. 1981, 37*, suppl, 233.

9. Ireland, C; Scheuer, P. J. *Science, 1979, 205*, 922.

CHAPTER III

Introduction

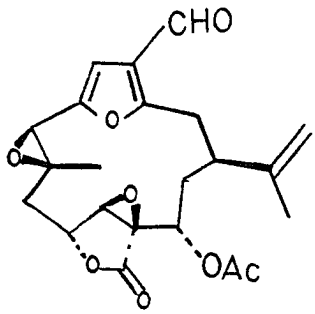
Chemical investigation of the organic extracts of gorgonians started in 1896, when Drechsel isolated diiodotyrosine from *Gorgonia cavollini* [1] This was followed by the isolation of dibromotyrosine from the gorgonian, *Primnoa lepadifera*, by Mörner[2] in the early 1900's.

By 1958, researchers[3,4,5,6] had isolated antibiotic and toxic compounds from non-sterol extracts from gorgonians. Several review papers[7,8,9] have summarized the chemistry of metabolites obtained from Gorgonacea. The metabolites include sesquiterpenoids, diterpenoids, and prostaglandins. Among the numerous diterpenoid metabolites are a small group which may be classified as furanocembranoids. These modified cembranoids and a few closely related derivatives are listed in Figure 1. These compounds are reviewed here because a new metabolite isolated from *Leptogorgia setacea* in this work is a member of this furanocembranoid group.

The sea whip coral, *Leptogorgia setacea* (pallas), is a marine invertebrate in the family Gorgoniidae. It is found in the Gulf of Mexico.[4] This gorgonian attaches to shell fragments or other solid objects by a tuft of stolons. It is sometimes washed ashore in great tangled masses especially after a storm. *Leptogorgia setacea* does not

FIGURE 1

Furanocembranoids and Related Derivatives



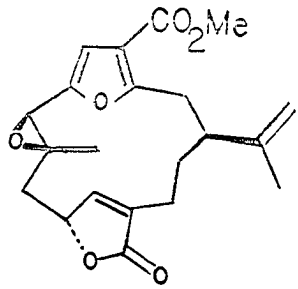
Lophogorgia[10,11]

L. cuspidata[10]

L. rigida[10]

L. chilensis[10]

1 lophotoxin



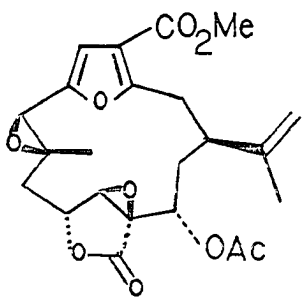
L. alba[10]

L. cuspidata[10]

L. rigida[10]

L. chilensis[10]

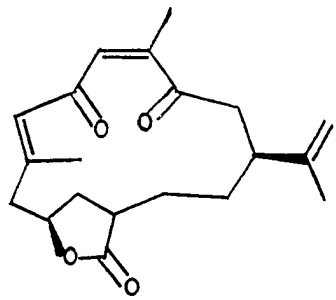
2 pukalide[13]



L. rigida[10]

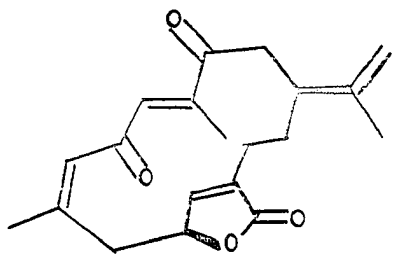
3

FIGURE 1 (continued)



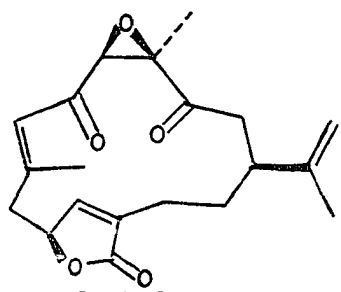
Lophogorgia alba[11]

4 lophodione



L. alba[11]

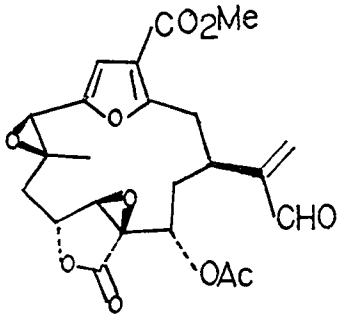
5 isolophodione



L. alba[11]

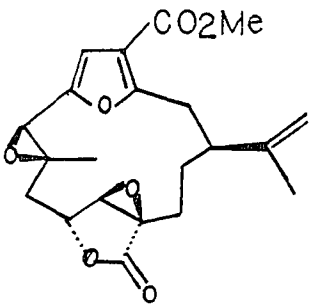
6 epoxylophodione

FIGURE 1 (continued)



Pseudopterogorgia cf. Kallos[12]

7



*Leptogorgia setacea**

8 11β,12β-epoxypukalide

*This work

contain the symbiotic zooxanthellae found in many other reef coelenterates (L.S. Ciereszko, personal communication).

Chemical investigation of *Leptogorgia setacea* was initated in the late 1950's by Ciereszko who reported the isolation[4] of a lactone compound. However, the structure was not elucidated. This chapter describes results of a reinvestigation of this problem in collaboration with Professor Ciereszko with the objective of purifying and determining the structure of this lactone.

RESULTS AND DISCUSSION

Structure elucidation of 11β,12β-Epoxypukalide (8):

Our investigation of the gorgonian *Leptogorgia setacea* (pallas), the spaghetti of shrimp fishermen[4] collected from Mustang Island, in the Gulf of Mexico led to the isolation of 8. *Leptogorgia* is often washed up on the beach after storms blowing in from the south. The collected animals were dried in the sun. Drying was rapid as the animals are slender and are comprised largely of calcite spicules. The cortex of the dried, yellow *Leptogorgia* was stripped off and ground in a blender. The ground cortex was extracted in a large continuous extractor with redistilled n-pentane. The precipitate, obtained by allowing the extract to stand at room temperature for a week, was filtered off on a sintered glass funnel, washed with pentane and extracted with chloroform. Crystals formed readily when pentane was added to the chloroform solution. After standing overnight and washing with pentane, 6 mg of 11β,12β-epoxypukalide (8) was obtained as white crystalline needles.

The structure of 11β,12β-epoxypukalide (8), was elucidated by spectral analysis. A molecular formula of $C_{21}H_{24}O_7$ was established for

8 by high resolution mass spectrometry (see Experimental). The ^{13}C NMR spectrum of 8 (Figure 2) revealed the existence of 21 carbons in agreement with this formula. This composition indicated 10 degrees of unsaturation in 8. Infrared bands at 1710, 1070, and 1225 cm^{-1}, UV absorbtion at 238 nm (ε = 5424), ^{13}C NMR resonances at 164.03 ppm (C-18,s), and 51.39 (C-21,q), and a three-proton singlet in the ^{1}H NMR spectrum (all ^{1}H NMR data discussed below are in $CDCL_3$) (Figure 3) at 3.78 ppm (Me-21) established the presence of an α,β-unsaturated carbomethoxy group. Consideration of the ^{13}C NMR absorptions at 160.04 ppm (C-3,s), 114.5 (C-4,s), 148.27 (C-6,s), and 107.55 (C-5,d) permitted the formulation of a furan function with a carbomethoxy group in a β position by analogy with literature precedents.[13] A resonance in the ^{1}H NMR spectrum at 6.48 ppm (H-5) could be assigned to a proton in the β' position on the furan ring which was also α,α'-dialkylated. This same structural feature is present in pukalide.[13] An IR band at 1782 cm^{-1} together with a ^{13}C NMR signal at 170.20 ppm (C-20,s) and a ^{1}H NMR resonance at 4.73 ppm (H-10), were consistent with the presence of an α,β-epoxy-γ-lactone group as found in micromelin[14] and linderane.[15] Four of the heteroatom deshielded ^{13}C NMR signals at 62.57 ppm (C-12,s), 63.36 (C-11,d), 56.31 (C-8,s), and 55.63 (C-7,d) could be assigned to two trisubstituted epoxides,[11] with corresponding ^{1}H NMR signals at 4.01 ppm (H-11), and 4.05 (H-7).

Careful analysis of the ^{1}H NMR decoupling data in $CDCL_3$ and in C_6D_6 (Tables 2 and 3), resulted in the construction of additional partial structures (Figure 4). Irradiation of the broad singlet at 6.48 ppm, corresponding to the proton on partial structure A, resulted in a

removal of small allylic coupling (J ≈ 1 Hz) from the broad singlet at 4.05 ppm due to H-7 of partial structure B and vice versa. Consequently, C-6 of partial structure A must be joined to C-7 of partial structure B (see Figure 4).

The connectivities between C-7, C-8, and C-9 as shown in partial structure B were established from the results of irradiation of the methyl signal at 1.14 ppm (Me-19). This sharpened the signals at 2.04 ppm (H-9') and 4.05 (H-7) and conversely, irradiation at 2.04 ppm and 4.05 sharpened the methyl signal at 1.14 ppm (Me-19). Thus, a W-coupling between Me-19 and both H-7 and H-9' was indicated. Carbon 9 was linked to C-10 on the basis of mutual ^{1}H coupling (see Tables 2 and 3 and Figure 4). Thus, the C-7,8,9 and 10 connectivity in partial structure B was completely established.

A strong IR band at 890 cm^{-1} considered together with a ^{13}C NMR signal at 113.45 ppm (C-16,t), suggested a terminal methylene group. A 3-proton broad signal at 1.75 ppm (Me-17) was found to be coupled (J = 1.9 Hz) to two terminal methylene proton signals at 5.11 ppm (H-16') and 4.91 (H-16). Thus, the presence of an isopropenyl group, partial structure C, (Figure 4) in $\underline{8}$ was postulated.

Partial structure D was formulated from the following data: The chemical shift of the methine singlet at 4.01 ppm (H-11) suggested an oxygen substituent. Since this signal was a slightly broadened singlet, it was assumed that C-11 was flanked by a non-protonated carbon (C-12). A very slight coupling (J ≈ 1 Hz) was found between H-11 and H-13 which was attributed to W-coupling across a quarternary carbon, as shown on partial structure D. The connectivity sequence C-13, C-14, C-1, and C-2

in partial structure D followed from vicinal proton couplings, see Figure 4, all of which were detected but some of which were not resolved into first order patterns. A small allylic coupling ($J < 1$ Hz) was found between H-1 and H-16 of partial structure C. This proved that the isopropenyl group is tied to structure D at C-1 (see Figure 4). Since the chemical shifts of the signals (^1H NMR spectrum in $CDCL_3$) at 3.05 ppm (H-2) and 2.95 (H-2') were close, the results of ^1H decoupling experiments in $CDCL_3$ at H-2 and H-2' were not definite. For this reason, ^1H decoupling experiments were also performed in C_6D_6 (see the chemical shift difference between H-2 and H-2' in the ^1H NMR spectrum of 8 in C_6D_6, Figure 5). The results of these experiments are shown in Table 3. The carbon bearing the allylic proton signals at 3.06 ppm (H-2) and 2.95 (H-2') was bonded to partial structure A at C-3. This was proved by NOE experiments which showed that H-2, H-2' proton signals were enhanced by irradiation of the methoxy signal, H-21 (see Table 4). A very small coupling ($J < 1$ Hz) was found between H-10 and H-11. The small value of this vicinal coupling was attributed to the dihedral angle between H-10 and H-11 being near 90° and provided evidence for joining these carbons. A very similar situation is found for lophotoxin[11] where the coupling between H-10 and H-11 is also very small. Accordingly, C-10 and C-11 are bonded.

On the basis of the above evidence, partial structures A to D could be linked together to give partial structure F (see Figure 4). In partial structure F, six unsaturation sites were accounted for. The two epoxides, the lactone carbonyl carbon, and two bonds to C-12 were not accounted for yet. The two epoxides could be assigned to carbons 7,8

and 11,12. The carbonyl carbon could be connected to C-12 and to the oxygen at C-10 to form a γ-lactone. This assignment is consistent with the biogenetic considerations and with the observed chemical shift of H-10. Therefore, this accounted for all ten degrees of unsaturation in 8. The α,β-epoxy-γ-lactone[14,15] feature was in full agreement with the spectral data of 8. This same functional group has been found in several natural products, including 3,[10] lophotoxin,[11] micromelin,[14] and linderane.[15]

The proposed structure of 8 received support from nuclear Overhauser enhancement difference spectroscopy (NOEDS) experiments (see Table 4). Since irradiation of the Me-19 protons enhanced the signals of both H-5 and H-11, all three of these groups must be generally directed towards one face of the carbocyclic ring. Accordingly, the orientation of the epoxide groups were assigned as β, like in lophotoxin.[11] The trans configuration of the 7,8 epoxide was established by confirmation of an NOE between H-7 and H-9' and the lack of NOE between H-7 and Me-19. The trans assignment between H-10 and H-11 was supported by recent ^{1}H NMR data for some synthetic α,γ-dialkylated α,β-epoxy-γ-lactones which showed less than 1 Hz couplings in the trans arrangement of the β-epoxy and γ-lactone methine protons, and 2-3 Hz coupling in the cis arrangement.[16] The relative stereochemistry at C-1 is assigned as the one observed for pukalide[13] and lophotoxin,[11] since the chemical shifts of H-1 and C-1 in pukalide[13] and in 8 were very close.

The high resolution mass spectrum of 8 is fully consistent with the proposed structure (see Scheme 1).

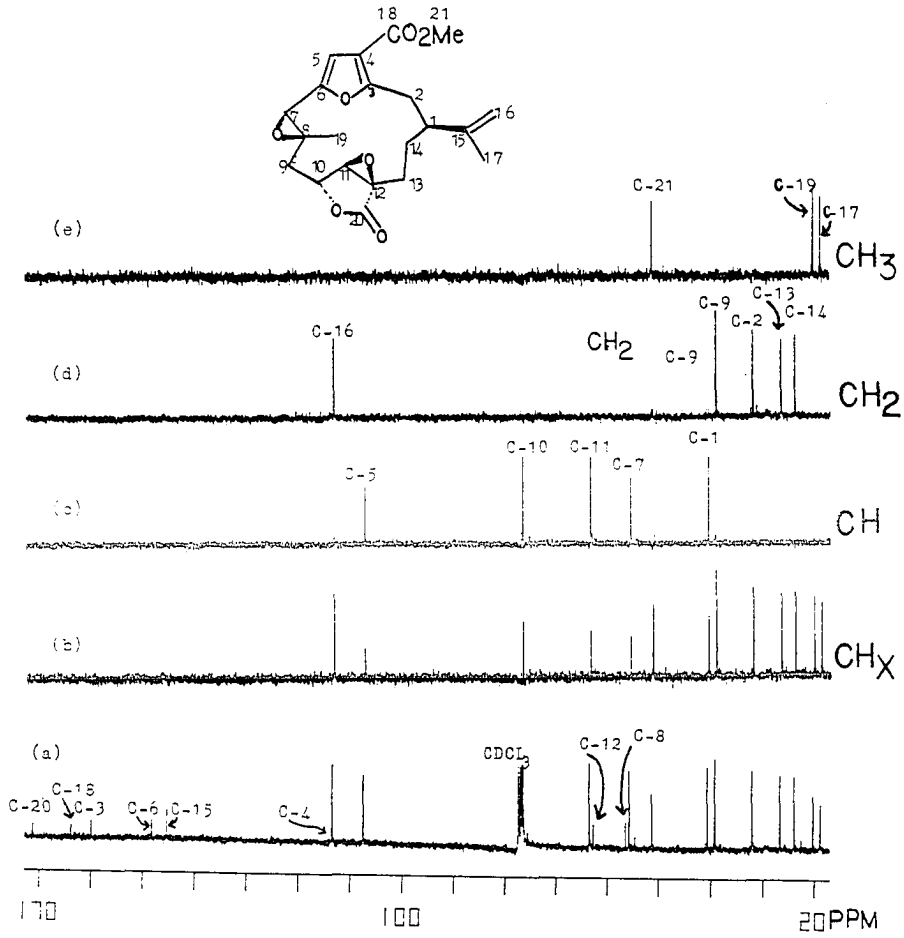
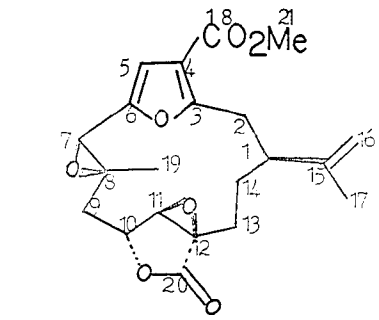


Figure 2. (a) 75.4 MHz broadband ^{1}H decoupled ^{13}C NMR spectrum of 11β,12β-epoxypukalide (8) in $CDCl_3$. (b) all protonated carbons. (c) methine carbons. (d) methylene carbons. (e) methyl carbons. Spectra b-e were recorded in $CDCl_3$ and resulted from a DEPT experiment.



11β,12β-epoxypukalide (8)

Figure 3. 300 MHz ^{1}H NMR spectrum of 8 in $CDCl_3$.

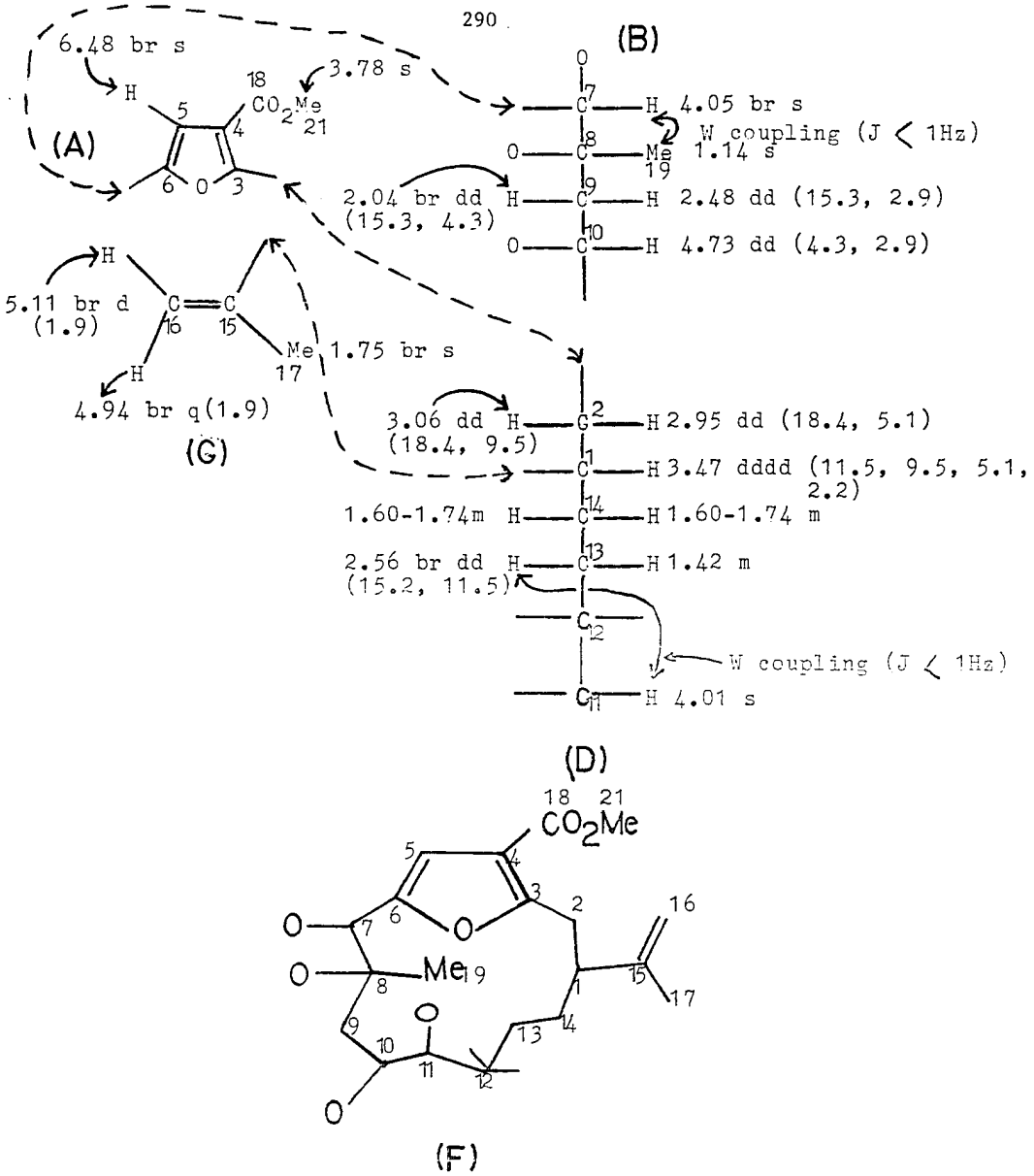
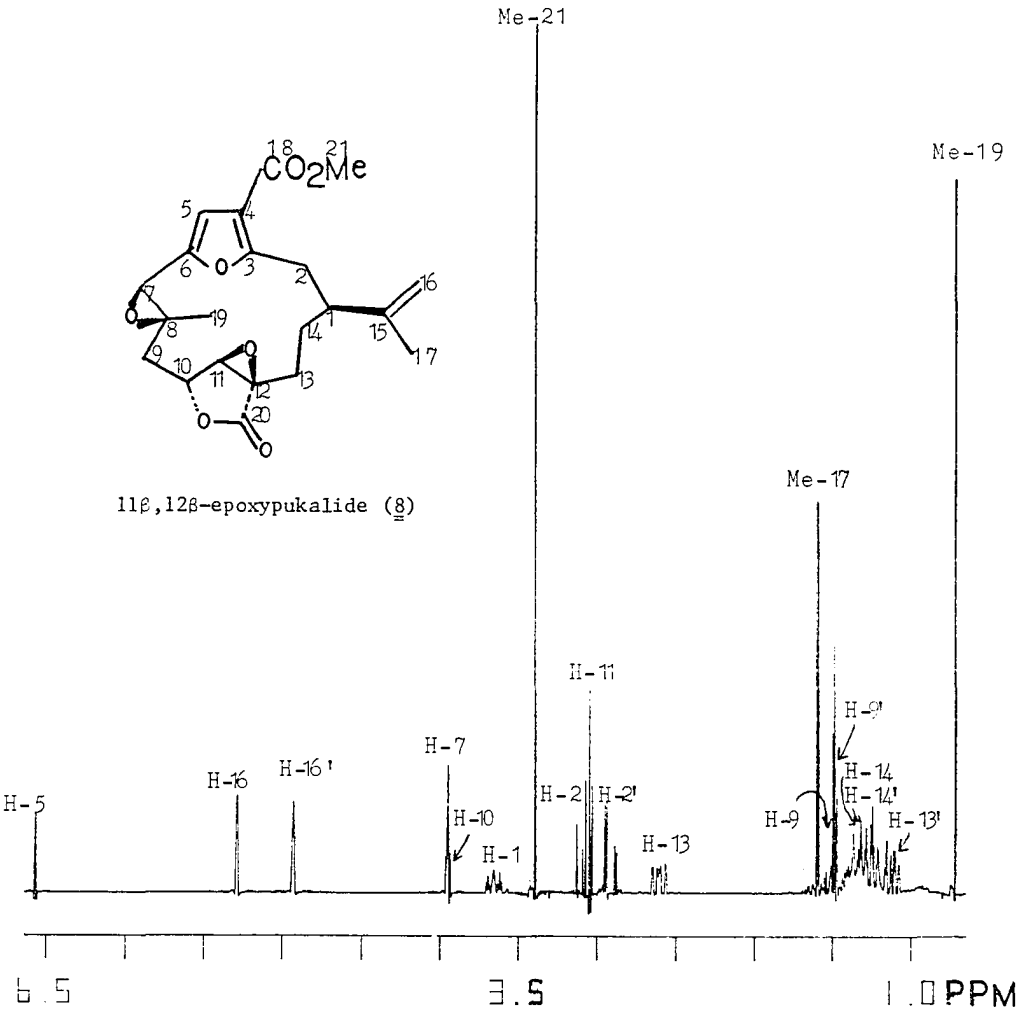


FIGURE 4

Partial Structures for 8



11β,12β-epoxypukalide (8)

Figure 5. 300 MHz ^{1}H NMR spectrum of 8 in C_6D_6

Table 1. NMR Data of 11ß,12ß-epoxypukalide (8)

C#	^{13}C*	^{1}H chem shift[a], ppm, multiplicity (J, Hz)	^{1}H chem shift[b], ppm, multiplicity (J, Hz)
1	40.68 d	3.47 dddd (11.5, 9.5, 5.1, 2.2).	3.65 ddt (11.9, 10.5, 3.4).
2	32.13 t	3.06 dd (18.4, 9.5). 2.95 dd (18.4, 5.1).	3.09 dd (18.3, 11.9). 2.90 dd (18.3, 3.4).
3	160.04 s		
4	114.50 s		
5	107.55 s	6.48 br s.	6.56 br s.
6	148.27 s		
7	55.63 d	4.05 br s.	3.95 br s.
8	56.31 s		
9	39.27 t	2.48 dd (15.3, 2.9). 2.04 br dd (15.3, 4.3).	1.51 dd (16.5, 3.5). 1.44 dd (16.5, 4).
10	76.84 d	4.73 dd (4.3, 2.9).	3.95 dd (4, 3.4).
11	63.36 d	4.01 s.	3.03 s.
12	62.57 s		
13	26.71 t	2.56 br dd (15.2, 11.5). 1.42 m.	2.59 ddd (16, 10.3, 2.2). 1.11 ddd (16, 8.2, 2).
14	23.99 t	1.60-1.74 m. 1.60-1.74 m.	1.31 m. 1.24 m.
15	145.40 s		
16	113.54 t	5.11 br d (1.9). 4.94 br q (1.9).	5.28 br d (1.8). 4.92 br q (1.8).
17	18.90 q	1.75 br s.	1.58 br s.
18	164.03 s		
19	20.30 q	1.14 s.	0.71 s.
20	171.20 s		
21	51.39 q	3.78 s.	3.39 s.

* The ^{13}C NMR spectrum was recorded in $CDCl_3$ at 75.40 MHz. Multiplicities were obtained by DEPT and assignments were made by comparison[3] to models. The δ values are given in ppm downfield from TMS. a) The ^{1}H NMR spectrum was recorded in $CDCl_3$ at 300 MHz. Assignments were established by spin-decoupling experiments. b) The ^{1}H NMR spectrum was recorded in C_6D_6 at 300 MHz and assignments were established by spin-decoupling experiments.

Table 2. Results of ^{1}H decoupling* with 11β,12β-epoxypukalide (8)

Irradiated proton, chemical shift, ppm, multiplicity (J, Hz)	effect of ^{1}H decoupling
H-5, 6.48 br s	H-7, 4.05 br s ⟶ sharpened
H-16, 5.11 br d (1.9)	H-16', 4.94 br q (1.9) ⟶ q (1.9)
	Me-17, 1.75 br s ⟶ sharpened
	H-1, 3.47 dddd (11.5, 9.5, 5.1, 2.2) ⟶ sharpened
H-16', 4.94 br q (1.9)	H-16, 5.11 br d (1.9) ⟶ sharpend
	Me-17, 1.75 br s ⟶ sharpened
H-10, 4.73 dd (4.3, 2.9)	H-9, 2.48 dd (15.3, 2.9) ⟶ d (15.3)
	H-9', 2.04 br dd (15.3, 4.3) ⟶ br d (15.3)
	H-11, 4.01 s ⟶ sharpened
H-7, 4.05 br s	H-5, 6.48 br s ⟶ s
	Me-19, 1.14 s ⟶ sharpened
H-11, 4.01 s	H-10, 4.73 dd (4.3, 2.9) ⟶ sharpened
	H-13, 2.56 br dd (15.2, 11.5) ⟶ sharpened
H-1, 3.47 dddd (11.5, 9.5, 5.1, 2.2)	H-2, 3.06 dd (18.4, 9.5) ⟶ d (18.4)
	H-2', 2.95 dd (18.4, 5.1) ⟶ d (18.4)
	H-16, 5.11 br d (1.9) ⟶ sharpened
	H-14, & H-14', 1.60-1.74 m ⟶ a
H-13, 2.56 br dd (15.2, 11.5)	H-11, 4.01 s ⟶ sharpened
	H-13', 1.42 m ⟶ a
	H-14, & H-14', 1.60-1.74 m ⟶ a
H-9, 2.48 dd (15.3, 2.9)	H-9', 2.04 br dd (15.3, 4.3) ⟶ br d (4.3)
	H-10, 4.73 dd (4.3, 2.9) ⟶ d (4.3)
H-9', 2.04 br dd (15.3, 4.3)	H-9, 2.48 dd (15.3, 2.9) ⟶ d (2.9)
	H-10, 4.73 dd (4.3, 2.9) ⟶ d (2.9)
	Me-19, 1.14 s ⟶ sharpened
Me-17, 1.75 br s	H-16, 5.11 br d (1.9) ⟶ d (1.9)
	H-16', 4.94 br q (1.9) ⟶ d (1.9)
H-14, & H-14', 1.60-1.74 m	H-1, 3.47 dddd (11.5, 9.5, 5.1, 2.2) ⟶ a
	H-13', 1.42 m ⟶ a
	H-13, 2.56 br dd (15.2, 11.5) ⟶ br d (15.2)
H-13', 1.42 m	H-13, 2.56 br dd (15.2, 11.5) ⟶ br d (11.5)
	H-14, & H-14', 1.60-1.74 m ⟶ a
Me-19, 1.14 s	H-9', 2.04 br dd (15.3, 4.3) ⟶ dd (15.3, 4.3)
	H-7, 4.05 br s ⟶ sharpened

* ^{1}H decoupling experiments were performed in $CDCl_3$ at 300 MHz with TMS as internal standard. The values are given in ppm downfield from TMS. [a]Change in multiplicity.

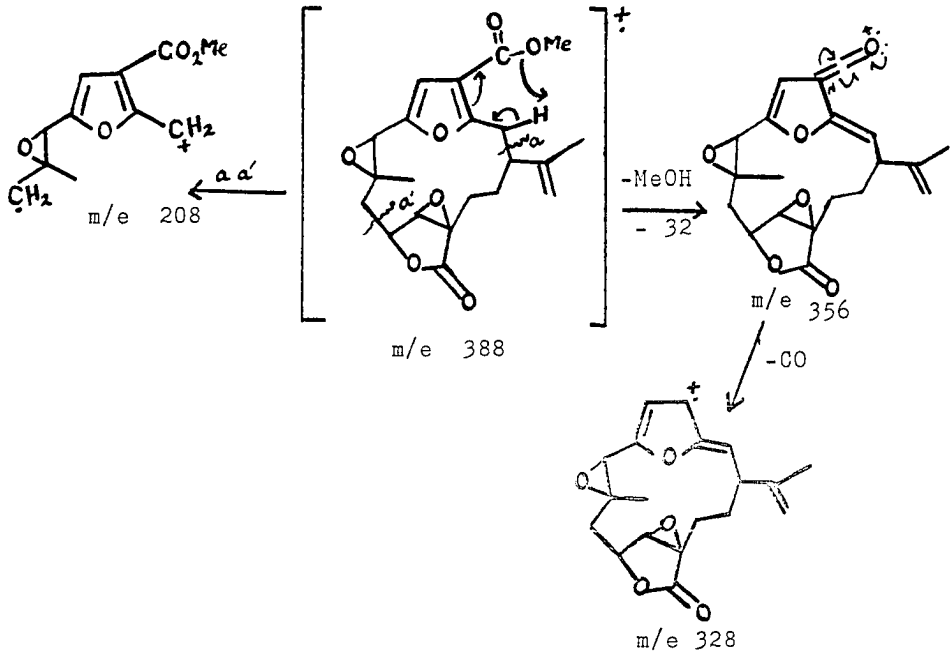
Table 3. Results of ^{1}H decoupling* with 11β,12β-epoxypukalide (8)

Irradiated proton, chemical shift, ppm, multiplicity (J, Hz)	effect of ^{1}H decoupling
H-5, 6.56 br s.	H-7, 3.95 br s → sharpened.
H-16, 5.28 br d (1.8).	H-16', 4.92 br q (1.8) → q (1.8).
	Me-17, 1.58 br s → sharpened.
	H-1, 3.65 ddt (11.9, 10.5, 3.4) → sharpened.
H-16', 4.92 br q (1.8).	H-16, 5.28 br d (1.8) → sharpened.
	Me-17, 1.58 br s → sharpened.
H-7, 3.95 br s.	H-5, 6.56 br s → s.
	Me-19, 0.71 s → sharpened.
H-10, 3.95 dd (4, 3.5).	H-9, 1.51 dd (16.5, 4) → d (16.5).
	H-9', 1.44 dd (16.5, 3.5) → d (16.5).
H-1, 3.65 ddt (11.9, 10.5, 3.4).	H-16, 5.28 br d (1.8) → sharpened.
	H-2, 3.09 dd (18.3, 11.9) → d (18.3).
	H-2', 2.90 dd (18.3, 3.4) → d (18.3).
	H-14, 1.31 m → s.
	H-14', 1.24 m → s.
	H-13, 2.59 ddd (16, 10.3, 2.2) → sharpened.
H-2, 3.09 dd (18.3, 11.9).	H-2', 2.90 dd (18.3, 3.4) → d (3.4).
	H-1, 3.65 ddt (11.9, 10.5, 3.4) → dt (10.5, 3.4).
H-2', 2.90 dd (18.3, 3.4).	H-2, 3.09 dd (18.3, 11.9) → d (11.9).
	H-1, 3.65 ddt (11.9, 10.5, 3.4) → ddd (11.9, 10.5, 3.4).
H-11, 3.03 s.	H-13, 2.59 ddd (16,10.3, 2.2) → sharpened.
H-13, 2.59 ddd (16, 10.3, 2.2).	H-11, 3.03 s → sharpened.
	H-13', 1.11 ddd (16, 8.2, 2) → dd (8.2, 2).
	H-14, 1.31 m → s.
	H-14', 1.24 m → s.
	H-1, 3.65 ddt (11.9, 10.5, 3.4) → sharpened.
Me-17, 1.58 br s.	H-16, 5.28 br d (1.8) → d (1.8).
	H-16', 4.92 br q (1.8) → d (1.8).
H-14, 1.31 m.	H-1, 3.65 ddt (11.9, 10.5, 3.4) → s.
	H-13, 2.59 ddd (16, 10.3, 2.2) → br d (16).
	H-13', 1.11 ddd (16, 8.2, 2) → br d (16).
H-13', 1.11 ddd (16, 8.2, 2).	H-13, 2.59 ddd (16, 10.3, 2.2) → dd (10.3, 2.2).
	H-14, 1.31 m → s.
	H-14', 1.24 m → s.
Me-19, 0.71 s.	H-7, 3.95 br s → sharpened.
	H-9, 1.51 dd (16.5, 4) → sharpened.

*^{1}H decoupling experiments were performed in C_6D_6 at 300 MHz with Me_4Si as internal standard. The values are given in ppm downfield from TMS. [a]Change in multiplicity.

Table 4. Results of NOEDS experiments with 11β,12β-epoxypukalide (8) in $CDCl_3$ solution at 300 MHz with TMS as internal standard.

Irradiated proton, chem shift , ppm	proton (s) enhanced
H-5, 6.48	H-7, 4.05
H-7, 4.05	H-5, 6.48
	H-9', 2.04
H-10, 4.73	H-11, 4.01
	H-9, 2.48
H-11, 4.01	H-10, 4.73
	H-19, 1.14
Me-21, 3.78	H-2, 3.06
	H-2', 2.95
H-9', 2.04	H-9, 2.48
	H-7, 4.05
	H-10, 4.73
Me-17, 1.75	H-2, 3.06
	H-2', 2.95
	H-16', 4.94
Me-19, 1.14	H-11, 4.01
	H-5, 6.48



Scheme 1. Possible Mass Spectral Fragmentation Pathways for 8

Experimental

Collection and Isolation. *Leptogorgia setacea* was collected in the surf near the Jetty on Mustang Island near Port Aransas, Texas, as it was being washed up onto the beach following a storm. The animals were freed of adhering debris, washed in sea water, drained and spread out on a clean concrete walkway to dry in the sun. Drying was rapid as the animals are very slender and are comprised largely of calcite spicules.

The cortex of the dried, yellow *Leptogorgia* was stripped off and ground in a blender. The ground cortex was extracted in a large continuous extractor with redistilled n-pentane. The precipitate, obtained by allowing the extract to stand at room temperature for a week, was filtered off on a sintered glass funnel, washed with pentane and extracted with chloroform. Crystals formed readily when pentane was added to the chloroform solution. After standing overnight and washing with pentane, 6 mg of white crystalline needles* were obtained from 920 g of gorgonian cortex. 11β,12β-epoxypukalide 8 has the following spectral properties: $[\alpha]_D^{25}$ -5.33 (c .6, MeOH); UV (EtOH) λ_{max} 238 nm (ε = 5424); IR ($CHCL_3$) 3090, 3030, 2950, 2860, 1782, 1708, 1640, 1575, 1440, 1380, 1350, 1285, 1255, 1210, 1070, 900, 890, 860, 820 cm^{-1}; for ^1H NMR and ^{13}C NMR see

*Only 6 mg of pure 1 were isolated. Unfortunately, no mp is available because it was not taken at the outset and after prolonged exposure to solvents for spectral measurements, the sample had decomposed.

Table 1; high-resolution mass spectrum, observed m/z (composition, interpretation, calculated millimass) 388.15316 ($C_{21}H_{24}O_7$, M^+, 388.15221), 373.12938 ($C_{20}H_{21}O_7$, M^+-Me, 373.12873), 356.12796 ($C_{20}H_{20}O_6$, M^+-MeOH, 356.12599), 328.13280 ($C_{19}H_{20}O_5$, M^+-MeOH-CO, 328.13108), 313.11019 ($C_{18}H_{17}O_5$, M^+-MeOH-CO-Me, 313.10760), 208.07579 ($C_{11}H_{12}O_4$, M^+-$C_{10}H_{12}O_3$, 208.07356), 168.04149 ($C_8H_8O_4$, M^+-$C_{13}H_{16}O_3$, 168.04226), 167.07055 ($C_9H_{11}O_3$, M^+-$C_{12}H_{13}O_4$, 167.07082), 167.03361 ($C_8H_7O_4$, M^+-$C_{13}H_{17}O_3$, 167.03444).

Bibliography

1. Dreschsel, E. *Zeitschrift fur Biologie 1896, 33*, 85.

2. Mörner, C. T. *Hoppe-Seyler's Z. Physiol. Chem. 1913, 88*, 138.

3. Burkholder, P. R.; Burkholder, L.M. *Science 1958, 127*, 1174.

4. Ciereszko, L. S.; Sifford, D. H.: Weinheimer, A. J. Ann. *N.Y. Acad. Sci. 1960, 90*, 917.

5. Ciereszko, L. S. *Trans. N.Y. Acad. Sci. 1962*, (2) *24*, 502.

6. Buck, J. D.; Meyers, S. P. *Limnol. and Oceanog. 1965, 10*, 385.

7. Ciereszko, L. S.; Karns, T. K. B. In "Biology and Geology of Coral Reefs", Vol. II: Biology 1, Jones, O. A. and Endean R., Eds.; Academic Press: New York, *1973* pp. 183-203.

8. Tursch, B. *Pure and Appl. Chem. 1976, 48*, 1.

9. Tursch, B.; Braekman, J. C.; Daloze, D.; Kaisin, M. In "Marine Natural Products Chemistry, Chemical and Biological Perspectives," Vol. II. Scheuer, P. J., Ed.; Academic Press: New York, *1978*, pp. 247-296.

10. Fenical, W; Okuda, R. K. ; Bandurraga, M. M.; Culver, P.; Jacobs, R. S. *Science 1981, 212*, 1512.

11. Bandurraga, M. M.; McKittrick, B; Fenical, W; Arnold, E.; Clardy, J. *Tetrahedron 1982, 38*, 305.

12. Look, S. A. Ph.D. Thesis, Univ. of California, 1983.

13. Missakian, M. G.; Burreson, B. J.,; Scheuer, P. J. *Tetrahedron 1975, 31*, 2513.

14. Cassady, J. M.; Ojima, N.; Chang, C. J.; McLaughlin, J. L. *Lloydia 1979, 42*, 274.

15. Joshi, B. S.; Kamat, V. N.; Govindachari, T. R.; *Tetrahedron 1967, 23*, 261.

16. Boeckman, R. K., Jr. and Thomas, E. W. *J. Am. Chem. Soc. 1979, 101*, 987.