THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

I. PURIFICATION AND CHARACTERIZATION OF BRIAREIN A, A CHLORINE CONTAINING DITERPENOID FROM THE GORGONIAN *BRIAREUM ASBESTINUM* (PALLAS)

II. ISOLATION AND CHARACTERIZATION OF TWO NEW CEMBRANOLIDES FROM THE GORGONIAN *EUNICEA SUCCINEA* (PALLAS): PEUNICIN AND EPIPEUNICIN

III. LOCATION OF METHYL GROUP IN METHYLGORGOSTANOL

A DISSERTATION

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CHUAN-YI CHANG

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APPROVED BY

Leon S Ciereszko

Michael A Chastain

F J Schmitz

[illegible]

S H. Wender

DISSERTATION COMMITTEE

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to my wife

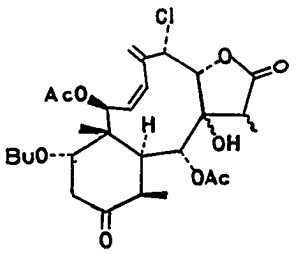
Emma

PURIFICATION AND CHARACTERIZATION OF BRIAREIN A, A CHLORINE CONTAINING DITERPENOID FROM THE GORGONIAN *BRIAREUM ASBESTINUM*

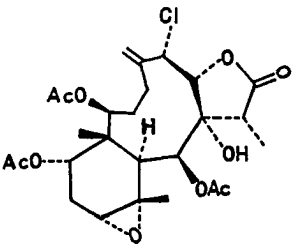
INTRODUCTION

The chlorinated diterpenoid compounds isolated from the gorgonian *Briareum asbestinum* (Pallas) have been studied for more than a decade[1,2]. They were first encountered during a study of the lipids in gorgonians from the Caribbean by Professor Leon S. Ciereszko[3] in 1960. These chlorinated diterpenoids were first separated into three compounds by Dr. R. W. Hyde[1], and were further studied by Dr. C. Bartholome[2]. Several possible structures were also suggested by Bartholome, but the correct structures were not known.

These chlorinated diterpenoids, "briareins", have unknown biological functions. Two structurally related compounds, "ptilosarcone" and "stylatulide" both have mild toxicity[4,5]. Their structures are shown below.



Ptilosarcone



Stylatulide

The purpose of this study is to determine the structure of briarein A, and possibly to assign the structures to the other related chlorinated compounds isolated from *B. asbestinum*.

RESULTS AND DISCUSSION

Briareins isolated from *B. asbestinum* (Pallas) were separated into five distinct spots on thin layer chromatography. Briarein A ($C_{30}H_{39}O_{13}Cl$) corresponds to the second spot on the thin layer chromatogram of the purified *B. asbestinum* benzene extract using silica gel H as adsorbent and chloroform-ethyl acetate (1:1) as the solvent.

Pure briarein A was recrystallized from ethyl acetate as colorless orthorhombic crystals with the space group of $P2_12_12_1$. The chlorinated diterpenoid, m.p. 251.5-253.5° (dec), is stable at room temperature.

The structure (Fig. 1) derived from single crystal X-ray analysis[6] contains a tricyclic nucleus (a five membered γ-lactone ring, a six- and a ten-membered carbocyclic ring), one chlorine, three methyl groups, five acetyl groups and an exomethylene group "conjugated" to a double bond with a conformational angle of 68.9°. No u.v. absorption indicating conjugation of these two double bonds (u.v., λ_{max}<210 nm) is observed.

On the basis of the X-ray analysis of briarein A, p.m.r. assignments can be made as in Table 1. The 100 MHz and 220 MHz p.m.r.'s are shown in Figures 2 and 3.

The six-membered ring in briarein A, in a chair form, is fused to the ten-membered ring at C-1 and C-10 (Fig. 4). Two protons on C-13 are assigned to the AB quartet at δ 2.35 (ax.) and 2.02 (eq.) (J=17).

Figure 1 : Absolute structure of briarein A

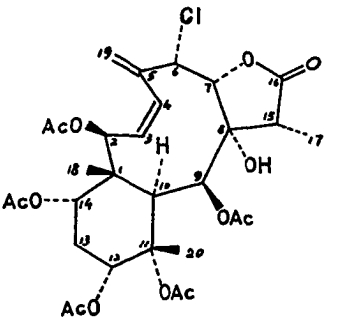


Table 1 : Chemical shifts and coupling constants for briarein A.

Chemical Shifts (δ)	Coupling Constants[a] (Hz)	Assignments
6.12	d, J=10	H-2
6.00	d, J=12	H-4
5.84	dd, J=12, 10	H-3
5.71	dd, J=3.4, 2.8	H-12 or H-14
5.60	d, J=1	H-19
5.58	s	H-9
5.48	d, J=1	H-19
5.12	dt, J=3.7, 1, 1	H-6
4.87	d, J=3.7	H-7
4.87	dd, J=3, 2.5	H-12 or H-14
3.83	s	H of -OH
3.14	q, J=7	H-15
3.10	s	H-10

Table 1 (continued)

Chemical Shifts (δ)	Coupling Constants[a] (Hz)	Assignments
2.35	ddd, J=17, 2.8, 2.5	H-13(ax)
2.24	s	H of Acetate
2.02	ddd, J=17, 3.4, 3	H-13(eq)
2.00	s	H of Acetate
1.98	s	"
1.98	s	"
1.97	s	"
1.54	s	H of $-CH_3$
1.40	s	"
1.34	d, J=7	H-17

[a]The abbreviations under "Coupling Constants" are the multiplicities as specified in the experimental section.

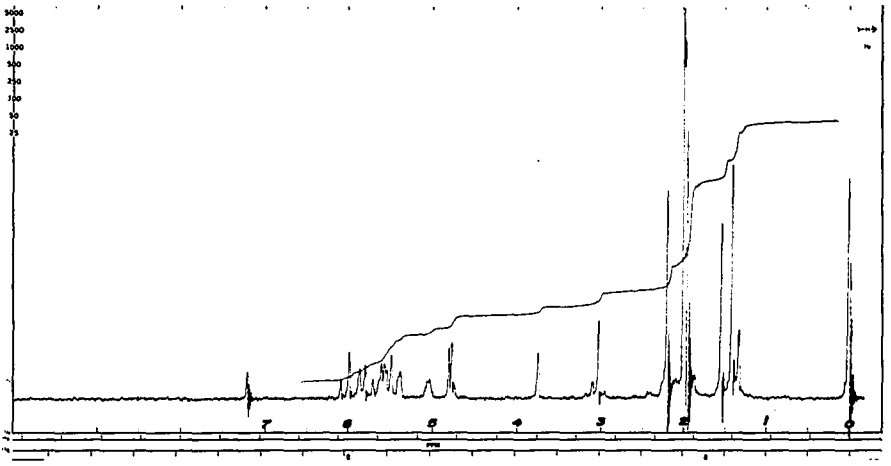


Figure 2 100 MHz p.m.r. spectrum of briarein A

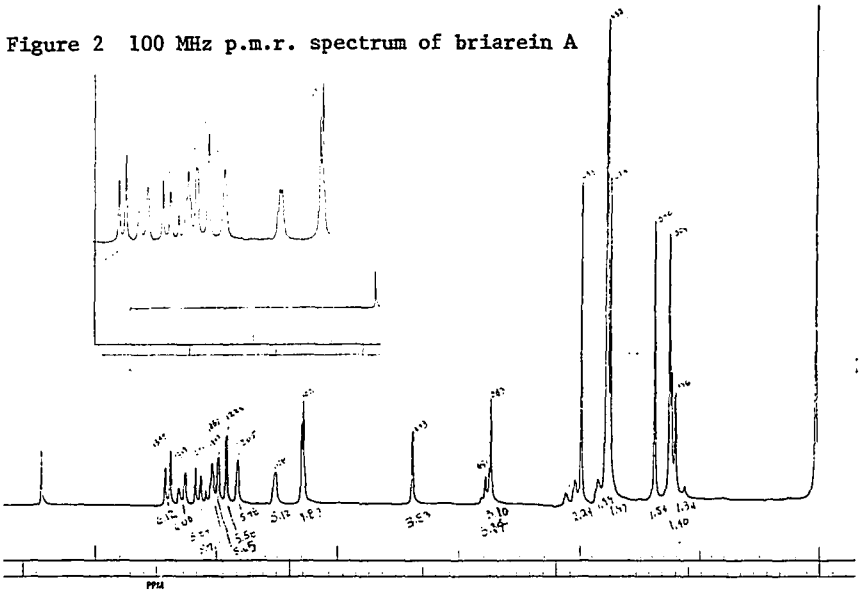
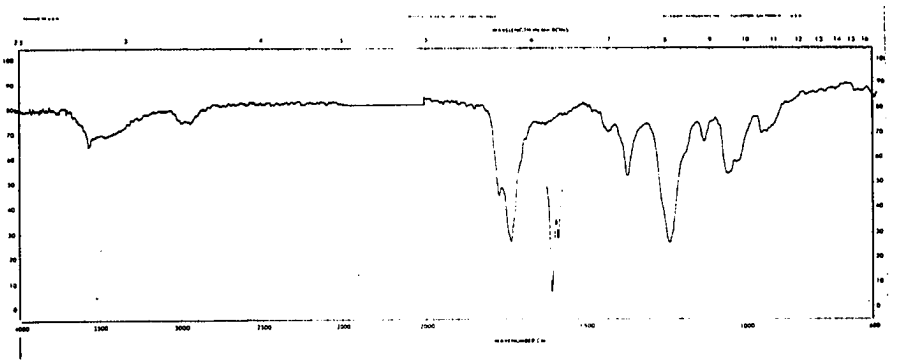
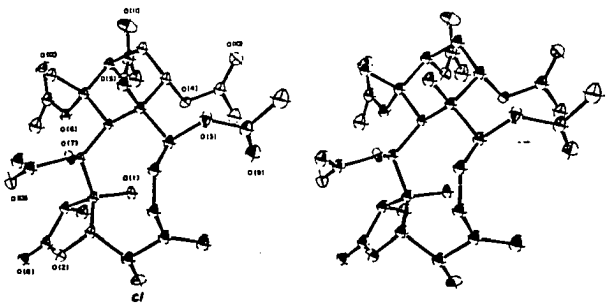


Figure 3 220 MHz p.m.r. spectrum of briarein A



I.r. spectrum of briarein A



Stereoview of briarein A

Figure 4

This AB quartet is further coupled to the protons on C-12 and C-14 (δ 5.71 and 4.87).

The noise decoupled c.m.r. spectrum (Fig. 5) shows all thirty carbons in the compound, including six carbonyl carbons (δ 176, 170, 169.4, 168.2, 168 and 168), four olefinic carbons (δ137, 130.5, 128 and 116.3), seven carbons bound to oxygen by a single bond (δ 85, 84, 80, 72, 71.5, 71 and 65), and a quaternary carbon (δ 46).

The signal centered at δ 176 is assigned to C-16. It is a typical carbonyl carbon signal for the γ-lactone[7]. The other five signals are assigned to the carbonyl carbon of the acetate. Under off-resonance decoupling (Fig. 6) one of the four olefinic carbon signals remains as a singlet (δ 137), two of the signals become doublets (δ 130.5 and 128), the fourth signal (δ 116.3) becomes a triplet. The above results lead to the following assignments: signal at δ 137 to C-5, signals at δ 130.5 and 128 to the C-3* and C-4*, and the signal at δ 116.3 to C-10. The signals centered at δ 85 and 80 are assigned to the C-8+ and C-11+, and the signal at δ 46 is assigned to C-1, due to the fact that these signals are unaffected by off-resonance decoupling.

Low resolution mass spectra of briarein A show that the molecular ion (M^+) is 642 (Fig. 7 and 8). Due to the presence of chlorine in the compound, the relative abundance of the M + 2 ion is higher than that of M + 1 ion. The loss of chlorine ion from the molecule is observed (m/e = 607) but it is not abundant. The presence of m/e = 36 is additional evidence for the presence of chlorine in the compound. It appears from the mass spectrum that the loss of acetate groups follows

*, + These assignments may be reversed.

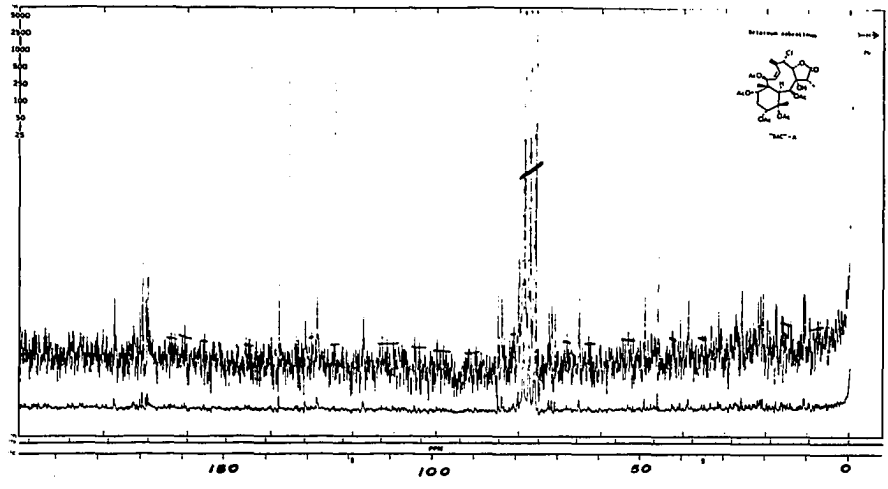


Figure 5 Noise decoupled c.m.r. spectrum of briarein A

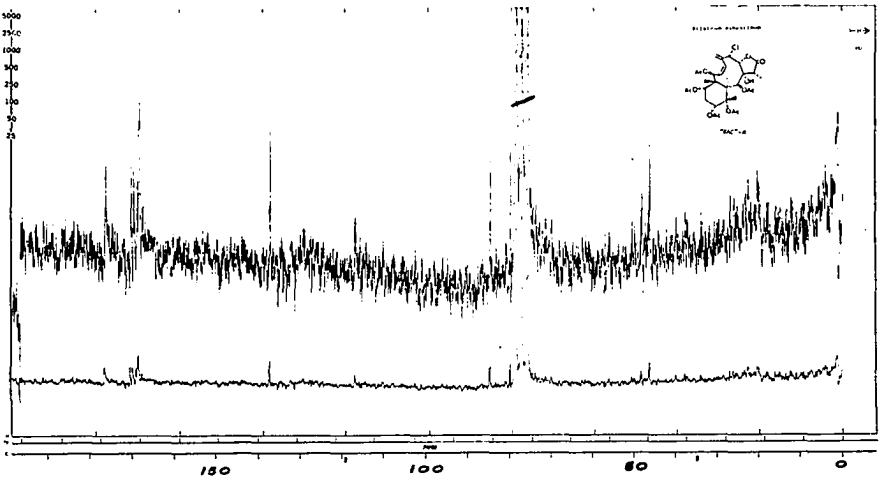


Figure 6 Off-resonance c.m.r. spectrum of briarein A

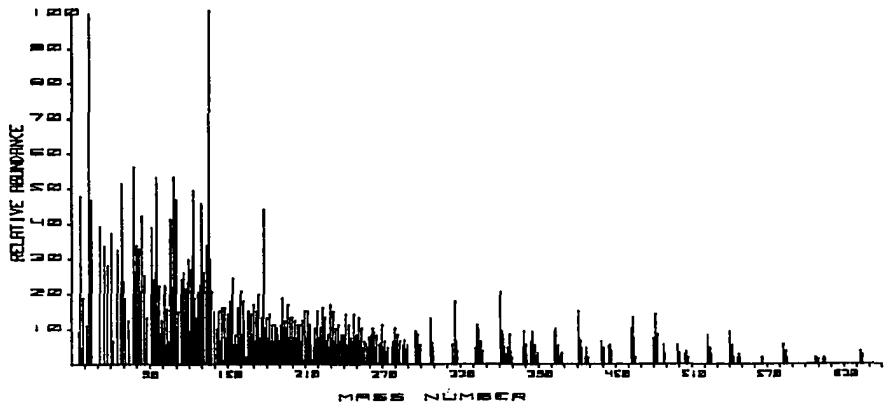


Figure 7 Low resolution mass spectrum of briarein A

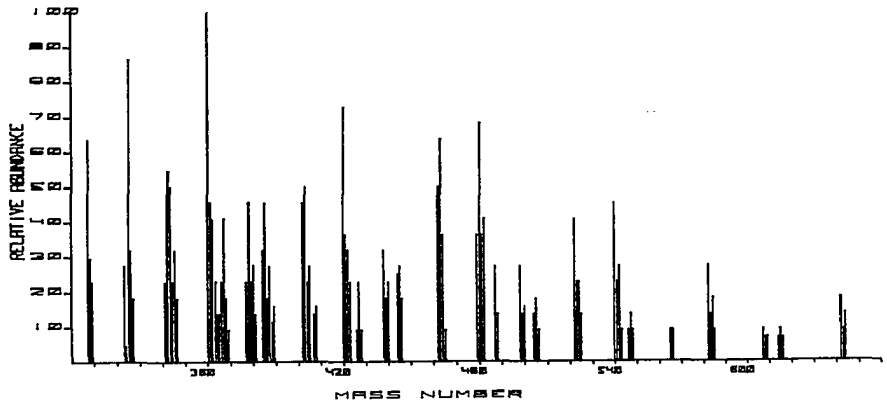


Figure 8 Partial mass spectrum of briarein A

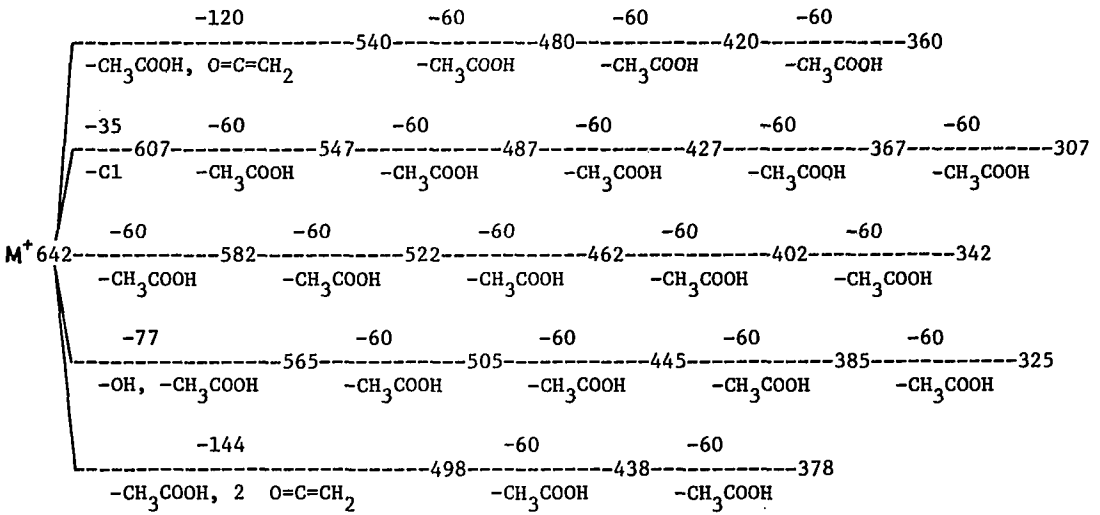


Figure 9 Five possible ways of removing the acetates in the briarein A

five different pathways (Fig. 9). This relevant portion of the mass spectrum is shown in Figure 8.

The m/e 540 fragment may involve a loss of CH_3COOH (M. Wt. 60) and a rearrangement fragment ($R\text{-}O\text{-}\overset{\overset{O}{\|}}{C}\text{-}CH_3 \longrightarrow R\text{-}OH + O{=}C{=}CH_2$, M. Wt. 42). The m/e 565 fragment most likely includes the loss of a hydroxyl group (M. Wt. 17) and an acetic acid molecule (M. Wt. 60). The m/e 498 fragment is due to the loss of one CH_3COOH and two $O{=}C{=}CH_2$.

Bartholome in his doctoral thesis[2] reported the existence of four briareins (A, B, C and D). The compound which I have discussed, corresponds to the second major spot on my thin layer chromatogram, and is very similar to the briarein A given in Bartholome's dissertation. By following his nomenclature system, I name my second compound as briarein A, and my first compound as briarein B, which is similar to Bartholome's briarein B. The major structure differences between my briareins and Bartholome's briareins[2] (Fig. 10) are the location of the double bond and the existence of the six membered ring. In my case, the existence of the six membered ring is supported not only by x-ray analysis[6] but also the c.m.r. spectrum (there is only one quaternary carbon with the δ value less than 60 p.p.m. from TMS).

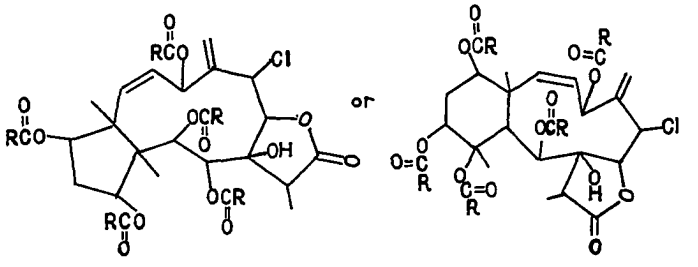
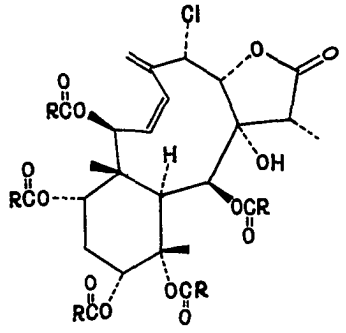


Figure 10 Structure of Bartholome's briarein A and B

The structure of my briarein B ($C_{32}H_{43}O_{13}Cl$) is shown below:

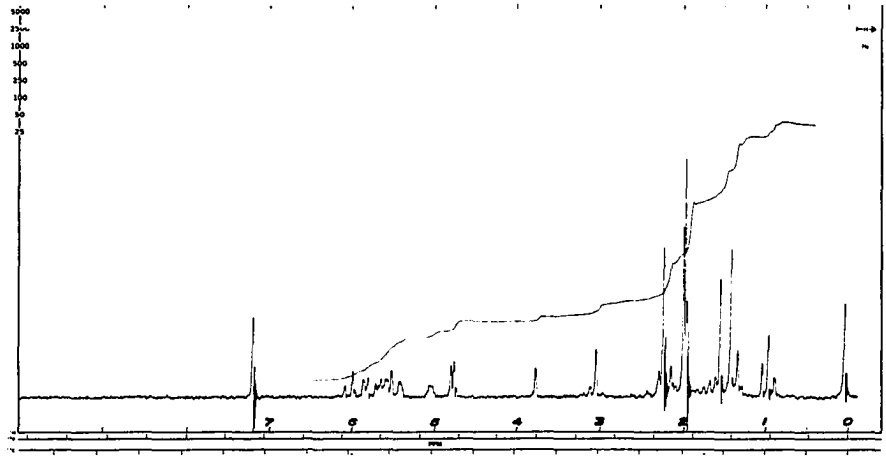


R = 1 $-C_3H_7$, 4 $-CH_3$

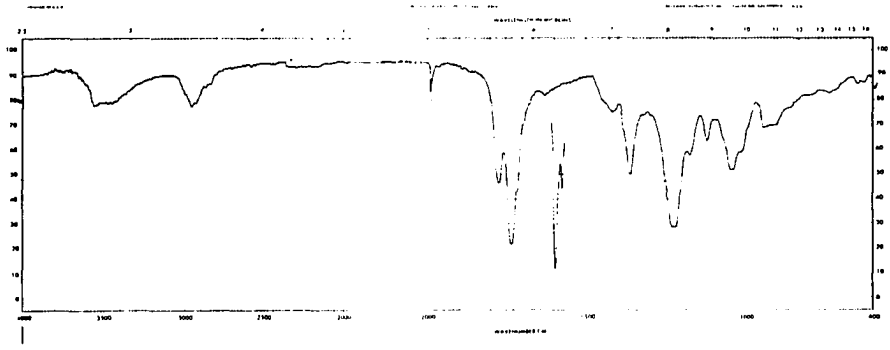
Since the p.m.r. spectrum (Fig. 11) of briarein B is very similar to that of briarein A (Fig. 2), a structure with the same type of nucleus found in briarein A is suggested for briarein B. The methyl triplet centered at δ0.95 in the p.m.r. spectrum of briarein B is assigned to the methyl group of the butyrate group. The signals of the protons on C-13 are shifted to δ2.21 and 2.00. This indicates that the possible location of the butyrate group in briarein B is at C-12 or C-14. The assignments of p.m.r. signals of briarein B are given in Table 2.

The briareins C and D of Bartholome's analysis of *B. asbestinum* are absent from my preparations. They may have been in the unanalyzed fractions.

Instead of briarein C and D, two new briareins (F and G) are observed. The p.m.r. spectrum of briarein G (fourth spot on tlc) (Fig. 12) has four acetate methyl signals at δ 1.94 (s), 1.96 (s), 2.02 (s), and 2.25 (s); four methyl signals at δ 0.9 (t, J=5), 1.37 (d, J=8), 1.50 (s), and 1.55 (s); and a singlet centered at 2.98 (1H) superimposed on a quartet (δ 3.06, 1H, J=8). This same pattern is found in briarein A



100 MHz p.m.r. spectrum of briarein B

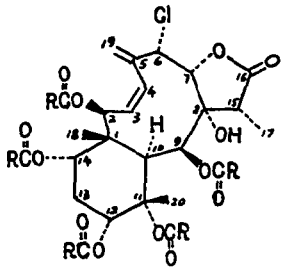


I.r. spectrum of briarein B

Figure 11

Table 2

Chemical shifts and coupling constants for briarein B.

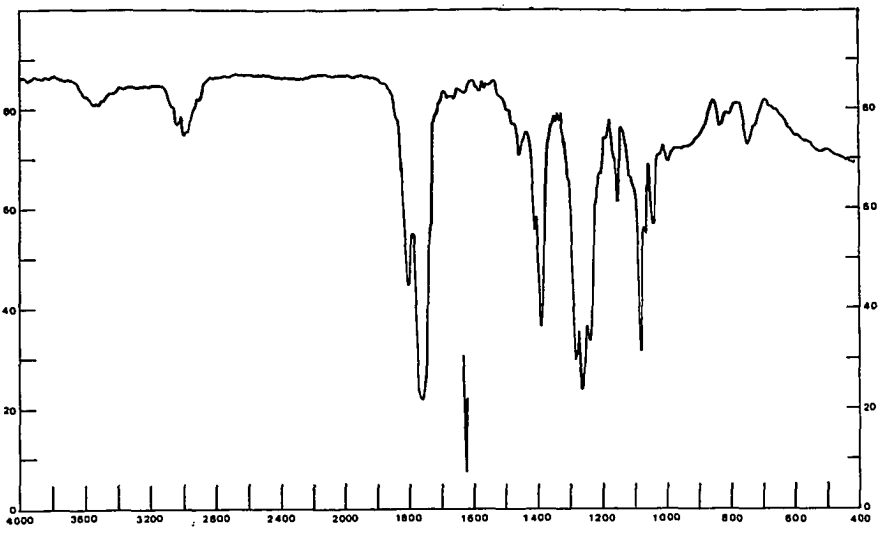


Chemical Shifts (δ)	Coupling Constants[a] (Hz)	Assignments
6.13	d, J=10	H-2
6.01	d, J=12	H-4
5.81	d, J=12, 10	H-3
5.73	dd, J=3.4, 2.8	H-12 or H-14
5.65	d, J=1	H-19
5.58	s,	H-9
5.48	d, J=1	H-19
5.13	dt, J=3.7, 1, 1	H-6
4.86	d, J=3.7	H-7
4.86	dd, J=3, 2.5	H-12 or H-14
3.83	s	H of -OH
3.12	q, J=7	H-15
3.09	s	H-10
2.23	s	H of Acetate
2.21	?	H-13
2.00	?	H-13

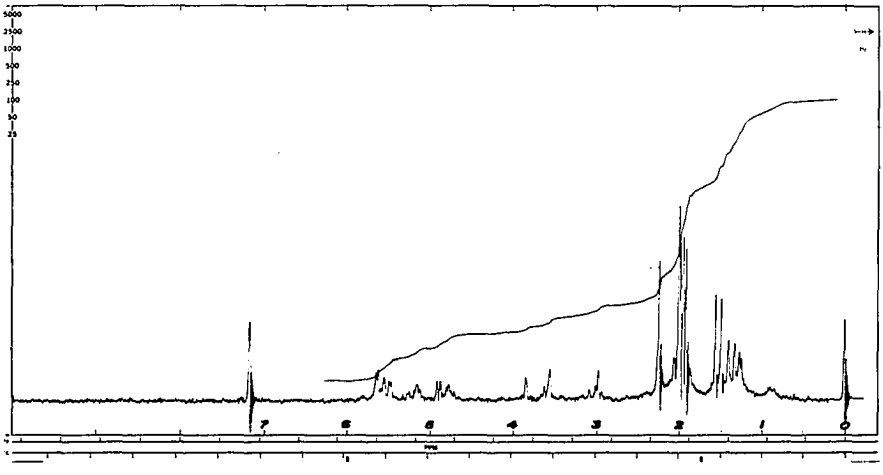
Table 2 (continued)

Chemical Shifts (δ)	Coupling Constants[a] (Hz)	Assignments
1.98	s	H of Acetate
1.96	s	"
1.95	s	"
1.51	m	H of $-CH_2-$
1.49	s	H of $-CH_3$
1.40	s	"
1.36	d, J=7	H-17
0.95	t, J=7	$-CH_3$ of BuO-

[a]The abbreviations under "Coupling Constants" are the multiplicities as specified in the experimental section.



I.r. spectrum of briarein G



100 MHz p.m.r. spectrum of briarein G

Figure 12

and B and is assigned in a similar manner. Unlike briarein A and B, briarein G has a second set of these protons, one singlet superimposed on a double doublet, centered at δ 3.56 (s, 1H) and 3.60 (dd, 1H, J=3, 11). This double doublet is coupled to the broad doublet centered at δ 5.21 (bd, 1H, J=11). Double irradiation at δ 5.21 collapses the double doublet into a doublet (J=4). The double doublet is tentatively assigned to a proton attached to a carbon bearing a hydroxyl group. The broad doublet is therefore assigned to a carbon next to the hydroxyl carbon and bearing an ester group.

A list of proton signals and their tentative assignments are given in Table 3. One of the possible structures of briarein G is also shown in Table 3.

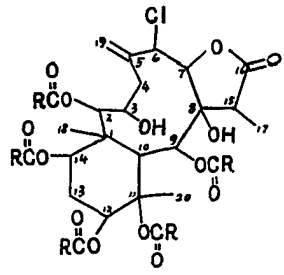
The p.m.r. spectrum of briarein F (third spot on tlc) shows three acetate methyls (δ 2.3, 2.04, and 1.96), possibly four methyl groups (δ 1.6, 1.4, 1.3, and 0.96), a doublet at δ 1.5 (d, 2H, J=8), a group of peaks (6H ?) in the region of 3 p.p.m. to 4 p.p.m., and two multiplets at δ 4.82 and 5.59 (Fig. 13).

Due to the limited supply of material and the lack of necessary 220 MHz n.m.r. spectrometer briarein F and G were not further studied. The i.r. and p.m.r. (100 MHz) spectra of briarein G and the p.m.r. (60 MHz) spectrum of briarein F are shown in Figure 12 and 13 respectively. A sample of briarein G has been sent to MIT for high resolution mass spectrum analysis.

All these "briarein" type compounds isolated so far (Fig. 14), have the same type of nucleus with the chlorine atom attached to C-6, exomethylene to C-5 and a hydroxyl group to C-8. The main difference is

Table 3

The chemical shifts and coupling constants for briarein G



R = 1 $-C_3H_7$, 4 $-CH_3$

Chemical Shifts (δ)	Coupling Constants[a] (Hz)	Assignments
5.64	d, J=3	H-19
5.55	s	H-9
5.48	d, J=3	H-19
5.21	d, J=11	H-2
5.14	dd, J=4, 6	H-6
4.99	d, J=4	H-7
4.80	m	H-4
4.77	t, J=4	H-4
3.84	s	H of -OH
3.60	dd, J=3, 11	H-3
3.56	s	H of -OH
3.06	q, J=8	H-15
2.98	s	H-10
2.25	s	H of Acetate
2.02	s	"

Table 3 (continued)

Chemical Shifts (δ)	Coupling Constants[a] (Hz)	Assignment
1.96	s	H of Acetate
1.94	s	"
1.55	s	H of $-CH_3$
1.50	s	"
1.37	d, J≈8	"
1.25	m ?	
0.90	t, J≈5	H of BuO-

[a]The abbreviations under "Coupling Constants" are the multiplicities as specified in the experimental section.

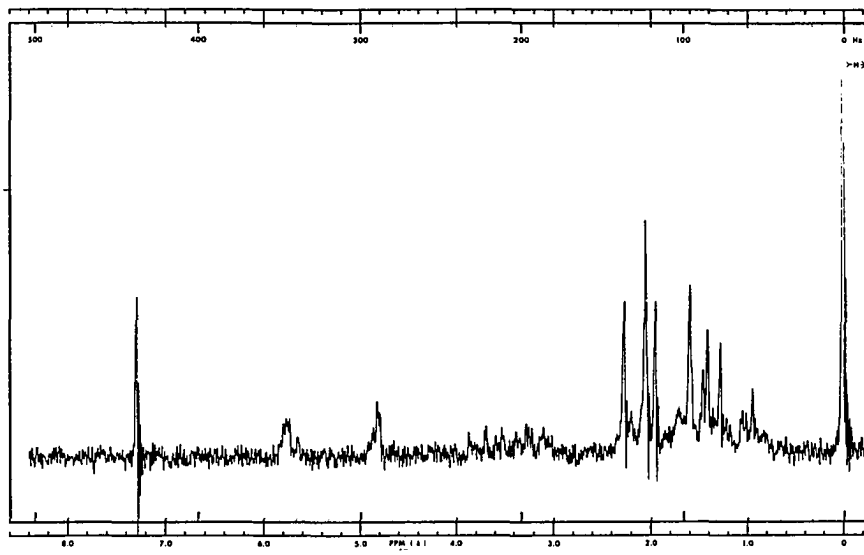
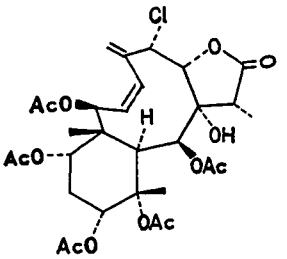


Figure 13 60 MHz p.m.r. spectrum of briarein F

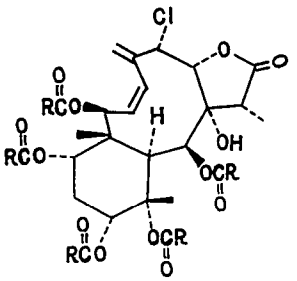
in the five substitution groups (refer to briarein A). The *cis* fused γ-lactone ring was only observed in the case of stylatulide (by x-ray analysis), otherwise they are all *trnas* fused. The structures of three of these eight known compounds were determined by single crystal x-ray diffraction (briarein A, E and stylatulide).

The absolute configurations of briarein A and E are shown in Figure 14.

Figure 14 The known briarein type compounds

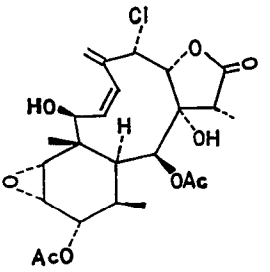


Briarein A

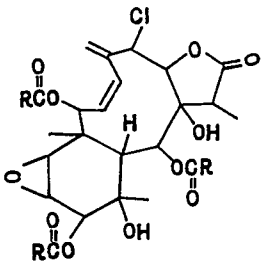


R = 1 $-C_3H_7$, 4 $-CH_3$

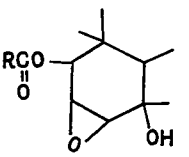
Briarein B



Briarein E[8]



or

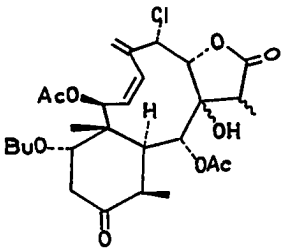


R = 1 $-C_3H_7$, 2 $-CH_3$ for C

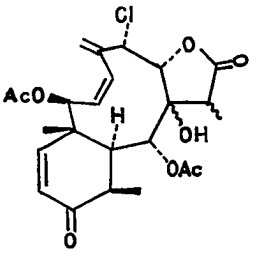
R = 1 $-CH_3$, 2 $-C_3H_7$ for D

Briarein C & D[2]

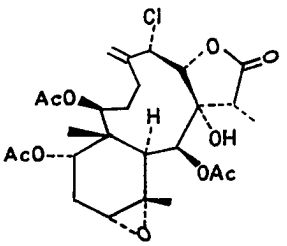
Figure 14 (continued)



Ptilosarcone*,4



Ptilosarcenone*,4



Stylatulide 5

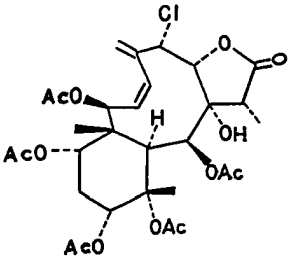
*The stereochemistry of hydroxyl and methyl group on the lactone ring is not known.

SUMMARY

Four chlorine containing diterpenoids briarein A, B, F and G have been isolated from a gorgonian of the suborder *Soleraxonia*, *Briareum asbestinum* (Pallas).

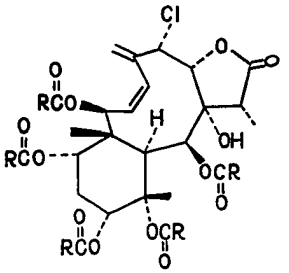
The structure of briarein A ($C_{30}H_{39}O_{13}Cl$), Figure A, determined by single crystal X-ray diffraction method contains a tricyclic nucleus, one chlorine, one hydroxyl group, three methyl groups, five acetate groups and an exomethylene group. The p.m.r. spectrum of briarein A was correlated to this structure.

The structure assigned to briarein B ($C_{32}H_{43}O_{13}Cl$), Figure B, has the same type of nucleus and substitution as briarein A, but one of the acetate group in briarein B is replaced by a butyrate group. The structure suggested for briarein G, Figure C, also has a saturated nucleus of the same type. The substitution groups are one chlorine, two hydroxyls, three methyls, four acetates, one butyrate and an exomethylene. The structure assignments of B and G were based on p.m.r. results.



Briarein A

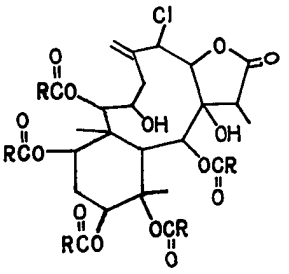
Figure A



R = 1 $-C_3H_7$, 4 $-CH_3$

Briarein B

Figure B



R = 1 $-C_3H_7$, 4 $-CH_3$

Briarein G

Figure G

EXPERIMENTAL

General procedure Melting points were taken on Kofler Micro Hot Stage (Arthur H. Thomas Co.) and are uncorrected.

Solvents were analytical reagent grade and were distilled prior to use.

Thin layer chromatography plates were prepared by coating 5x20 or 20x20 cm glass plates with E. Merck (Darmstadt) silica gel H to a thickness of 0.25 mm. The chromatograms were visualized with iodine vapor or 10% sulfuric acid spray.

Combustion analyses were performed by Dr. E. Meier, Stanford University.

Optical rotations were measured on a Gaertner L-320 polarimeter.

Ultraviolet spectra were measured in methanol (spectral grade, Fisher Scientific Co.) on a Cary model 118 (Varian) spectrometer.

Infrared spectra were recorded on a Beckman IR-8 spectrophotometer.

Low resolution mass spectra were obtained on a Hitachi Perkin-Elmer RMU-6 single focusing spectrometer.

Proton nuclear magnetic resonance spectra were obtained on a Varian T-60, XL-100 or 220 MHz spectrometers; carbon-13 nuclear magnetic

resonance spectra were obtained on a Varian XL-100 spectrometer. P.m.r. and c.m.r. chemical shifts are reported as δ values (p.p.m. from TMS). The multiplicites are reported as follows: s, singlet; d, doublet; dd, double doublet; ddd, double double doublet; dt, double triplet; t, triplet; q, quartet; bs, broad singlet; bd, broad doublet; bt, broad triplet and m, multiplet. The coupling constants are reported in Hertz (Hz).

Sample collection *Briareum asbestinum* (Pallas), a gorgonian, was collected by Professor L. S. Ciereszko in 1969, near South Cay, off Port Royal, Jamaica. The cleaned animals were drained, cut into small pieces and dried within 12 hours in a stream of warm air. The dried animals were stored in a plastic container until they were used in 1973.

Isolation of briareins The briareins can be isolated from *B. asbestinum* by two different methods. Both methods start with extraction using n-hexane then followed by benzene or ether.

The dried animal pieces (4.7 kg) were first extracted with n-hexane at room temperature. The defatted animal was then air dried and extracted with benzene in a continuous percolator-extractor[9]. The benzene extract was concentrated *in vacuo* to one half of the original volume and kept at room temperature. Colorless crystals precipitated from the concentrated extract (9.4 gm) and were washed with benzene and ether.

In the second method, instead of benzene extraction, an ether extraction was applied. Briareins were precipitated from the concentrated extract at 5°C (0.06 gm from 896 gm of animal) in a colorless crystalline form.

The separation of briareins Since the crude briarein preparation showed five spots on thin layer chromatogram (chloroform:ethyl ether,

1:1, silica gel H), a portion (0.3 gm) of crude briarein preparation was chromatographed on a thin layer mesh silica gel H column (2.6x29 cm) using chloroform-ethyl ether (4:5, v/v) as the solvent and ten ml fractions were collected (90 fractions at 10 ml/30 min.).

Briarein B (69 mg) was crystallized from the material obtained in fractions 21-25. Briarein A (150 mg) was collected from fractions 28-31. Briarein A recrystallized from ethyl acetate was utlized for single x-ray diffraction analysis. The crystals had the orthorhombic shape. A third compound (9 mg) was eluted from the column in fractions 40-42. These fractions were combined and further purified by preparative tlc on silica gel H (0.5 mm in thickness, 20x20 cm) using ethyl acetate-hexane (50:55, v/v) as the solvent. This solvent system separates the third component into five stripes. The fast moving stripe (R_f=0.28) was collected (4 mg). The p.m.r. of this compound showed a pattern different from Bartholome's briareins[2]. The name of briarein F was reserved for this compound. The fourth compound, briarein G (42 mg), was crystallized from the fractions 50 and 51. The fifth component (10 mg) from fractions 62-90 was a yellow glass like material. No analysis was done on this component.

Pure briarein A has $[\alpha]_D^{24} = -98.2^\circ$ (c 1.1, $CHCl_3$); R_f=0.26 (chloroform:ethyl ether, 1:1, silica gel H).

The i.r. spectrum (neat, KBr) exhibits absorption at 3520, 2950, 1765 (γ-lactone), 1730 and 1230 cm^{-1} (acetate), 1420, 1120 and 930 (=C=CH_2), 1370 (methyl), 1050 and 1020 cm^{-1}.

The p.m.r. spectra ($CDCl_3$) show signals at δ 6.12 (d, 1H, J=10,

AcO-  -C= , 6.00 (d, 1H, J=12, =  --), 5.84 (dd, 1H, J=12, 10, AcO-  -=C), 5.71 (dd, 1H, J=3.4, 2, -(AcO)-), 5.60 (d, 1H, J=1, =C=CH_2), 5.58 (s, 1H, -  (AcO)-  (OH)-), 5.48 (d, 1H, J=1, =C=CH_2), 5.12 (dt, 1H, J= 3.7,1,1, -  (Cl)-), 4.87 (dd, 1H, J=3, 2.5, AcO--), 4.87 (d, 1H, J=3.7, H-  =O), 3.83 (s, 1H, -OH), 3.14 (q, 1H, J=7,  =O), 3.10 (s, 1H, =C-H), 2.35 (ddd, 1H, J=17,2.8,2.5, $-CH_2-$), 2.02 (ddd, 1H, J= 17,3.4,3, $-CH_2-$), acetate methyls at 2.24 (s), 2.00 (s), 1.98 (s), 1.98 (s) and 1.97, and methyl signals at 1.54 (s), 1.40 (s) and 1.34 (d, J=7,  =O).

The c.m.r. spectrum ($CDCl_3$) shows signals for five acetate carbonyl carbons (δ 170, 169.4, 168.2, 168 and 168), one lactone carbonyl carbon (δ 176), four olefinic carbons (δ 137, 130.5, 128 and 116.3), two tertiary carbons bearing oxygen (δ 85 and 80), five secondary carbons bearing oxygen (δ 84, 72, 71.5,71 and 65), one quaternary carbon (δ 46), twelve alkyl carbons (δ 49.6, 40, 38.9, 33.3, 31.7, 27.8, 26.2, 22, 21.8, 21, 17.9 and 11.1).

Mass spectrum (70 eV) shows the m/e (rel. intensity), M^+, 642 (4), 643 (2, M + 1), 644 (3, M + 2), 615 (2), 614 (2), 613 (1.5), 609 (1.5), 608 (1.5), 607 (2), 585 (2), 584 (4), 583 (3), 582 (6), 566 (2), 565 (2), 548 (2), 547 (3), 546 (2), 543 (2), 542 (6), 541 (5), 540 (10), 506 (2), 505 (4), 504 (3), 500 (3.5), 499 (3), 498 (6). 488 (3), 487 (6), 482 (9), 481 (8), 480 (15), 479 (8), 465 (2), 464 (8), 463 (14), 462 (11), 446 (4), 445 (6), 444, (5.5), 440 (5), 439 (4), 438 (7), 428 (2), 427 (5), 426 (2), 423 (5), 422 (7), 421 (8), 420 (16), 408 (3.5), 407 (3), 405 (6), 404 (5), 403 (11), 402 (10), 389 (3.5), 388 (2.5), 387 (6), 386 (4),

385 (10), 384 (7), 381 (3), 380 (6), 379(5), 378 (10), 377 (5), 369 (2), 368 (4), 367 (9), 366 (5), 365 (3), 364 (3), 363 (5), 362 (9), 361 (10), 360 (22), 346 (4), 345 (7), 344 (5), 343 (11), 342 (12), 341 (5), 327 (4), 326 (7), 325 (19), 324 (1), 323 (6), 309 (5), 308 (7), 307 (14), 299 (6), 298 (6), 297 (9), 296 (8), 295 (10), 289 (6), 288 (4.5), 287 (8), 286 (5), 285 (6), 283 (7), 282 (4), 281 (9), 280 (6), 279 (11), 278 (7), 277 (6), 273 (5), 272 (4), 271 (7), 270 (5), 269 (12), 268 (7), 267 (6), 265 (9), 264 (5), 263 (10), 262 (11), 261 (9), 260 (6), 259 (9), 258 (5), 257 (7), 255 (7), 254 (5), 254 (11), 252 (7), 251 (14), 250 (8), 249 (9), 248 (8), 247 (15), 246 (7), 245 (8), 244 (8), 243 (12), 242 (6), 241 (15), 240 (8), 239 (9), 238 (5), 237 (9), 236 (7), 235 (15), 234 (6), 233 (11), 232 (7), 231 (7), 230 (8), 229 (18), 228 (5), 227 (9), 226 (7), 225 (14), 224 (11), 223 (17), 222 (8), 221 (11), 220 (8), 219 (16), 218 (7), 217 (11), 216 (5), 215 (1), 214 (6), 213 (12), 212 (8), 211 (16), 210 (8), 209 (16), 208 (1), 207 (13), 206 (7), 205 (12), 204 (6), 203 (12), 202 (8), 201 (13), 200 (8), 199 (14), 198 (7), 197 (14), 196 (8), 195 (18), 194 (11), 193 (13), 192 (11), 191 (20), 190 (12), 189 (7), 188 (8), 187 (11), 186 (7), 185 (12), 184 (7), 183 (12), 182 (7), 181 (15), 180 (8), 179 (14), 178 (11), 177 (47), 176 (8), 175 (14), 174 (21), 172 (8), 171 (16), 170 (7), 169 (18), 168 (9), 167 (15), 166 (8), 165 (16), 162 (13), 161 (19), 160 (9), 159 (22), 158 (8), 157 (17), 156 (6), 155 (9), 154 (6), 153 (26), 152 (18), 151 (19), 150 (14), 149 (15), 148 (8), 147 (17), 145 (17), 144 (7), 143 (16), 142 (11), 141 (11), 139 (16), 137 (22), 136 (32), 135 (100) base peak, 134 (24), 133 (36), 131 (28), 130 (17), 129 (49), 128 (24), 127 (22), 126 (14), 125 (14), 124 (20), 123 (53), 122 (33), 121 (29), 120 (10), 119 (32), 118 (25), 117 (23), 116 (12), 115 (28), 114 (10), 113 (26), 111 (16), 110 (10), 109

(50), 108 (33), 107 (57), 106 (23), 105 (44), 104 (7), 103 (17), 102 (6), 101 (24), 100 (10), 99 (14), 98 (10), 97 (24), 96 (16), 95 (57), 94 (28), 93 (26), 92 (22), 91 (42), 87 (14), 85 (27), 83 (45), 82 (22), 81 (35), 80 (12), 79 (36), 72 (28), 77 (60), 74 (10), 73 (13), 71 (20), 69 (25), 68 (55), 67 (25), 65 (35), 61 (7), 60 (40), 57 (30), 55 (36), 51 (42), 45 (50), 44 (16), 43 (99), 42 (8), 41 (12), 38 (20), 37 (5), 36 (51), and 35 (10).

Briarein A, in methanol, shows only strong end adsorption, (λ_{max} < 210 nm), in the ultraviolet.

Anal. Calcd. for $C_{30}H_{39}O_{13}Cl$: C, 56.03; H, 6.11; O, 32.34; Cl, 5.51; M. wt. 643.082.

Found: C, 55.68; H, 6.24; O, - ; Cl, - ; M. wt. 642 (low resolution mass spectrum).

M.p. 251.5-253.5°C (dec.)

Pure briarein B ($C_{32}H_{43}O_{13}Cl$) has $[\alpha]_D^{24} = -80.9^\circ$ (c. 0.45, $CHCl_3$); R_f=0.34 (chloroform:ethyl ether, 1:1, silica gel H); i.r. (neat, KBr), 3560, 2960, 1775, 1735, 1420, 1360, 1230, 1175, 1120, 1040, 1010, 940, 900, 740, and 640 cm^{-1}; 100 MHz p.m.r. ($CDCl_3$), δ 6.13 (d, 1H, J=10, RCOO-C(H)-C=), 6.01 (d, 1H, J=12, =C(H)-C(=)-), 5.81 (d, 1H, J=12, 10, RCOO-C-C(H)=), 5.73 (dd, 1H, J=3.4, 2.8, -C(H)(RCOO)-), 5.65 (d, 1H, J=1, >C=CH_2), 5.58 (s, 1H, -C(H)(RCOO)-C(OH)-), 5.48 (d,1H, J=1, >C=CH_2), 5.13 (dt, 1H, J=3.7, 1, 1, -C(H)(Cl)-), 4.86 (d, 1H, J=3.7, ), 4.86 (dd, 1H, J=3, 2.5, RCOO-C(H)<), 3.83 (s, 1H, -OH), 3.12 (q, 1H, J=7, ), 3.09 (s, 1H, >C-H), 2.23 (s, 3H, H of acetate),

2.21 (1H, $-CH_2-$), 2.00 (1H, $-CH_2-$), acetate methyls at 1.98 (s), 1.96 (s), 1.95 (s), 1.51 (m, 2H, $-CH_2-$), and methyl signals at 1.49 (s), 1.40 (s), 1.36 (d, J=7, ); and 0.95 (t, J=7, $-\underset{\underset{O}{\|}}{C}-CH_2-CH_2-\underline{CH_3}$); u.v. (MeOH) strong end absorption (λ_{max}<210 nm).

No mass spectrum is available due to the low volatility of briarein B.

Anal. Calcd. for $C_{32}H_{43}O_{13}Cl$: C, 57.27; H, 6.45; O, 31.0: Cl, 5.28.

M. wt. 671.736.

Found: C, 56.23; H, 6.52; O, 31.63, Cl, 5.62.

M.p. 214-216°C (dec.)

Pure briarein G has $[\alpha]_D^{24}$= + 28° (c. 0.25, $CHCl_3$); R_f=0.72 (70% ethyl acetate in n-hexane), R_f= 0.21 (ethyl acetate:n-hexane, 50:55, v/v) on silica gel H; i.r. (neat, KBr), 3500, 2960, 1800, 1750, 1420, 1390, 1280, 1260, 1240, 1150, 1080, 1040, 840 and 780 cm^{-1}; 100 MHz p.m.r. δ 5.64 (d, 1H, J=3, $\geq C=\underline{CH_2}$), 5.55 (s, 1H, $-(RCOO)\underset{\underline{H}}{C}-C(OH)-$), 5.48 (d, 1H, J=3, $\geq C=\underline{CH_2}$), 5.21 (d, 1H, J=11, $-\underset{\underline{H}}{C}(RCOO)-\underset{\underline{OH}}{C}-$), 5.14 (dd, 1H, J=4, 6, $-\underset{\underline{H}}{C}-Cl$), 4.99 (d, 1H, J=4, ), 4.8 (m, 1H, $-\underline{CH_2}-$), 4.77 (t. 1H, J=4, $-CH_2-$), 3.84 (s, 1H, -OH), 3.60 (dd, 1H, J=3, 11, $-C(RCOO)-\underset{\underline{H}}{C}(OH)-$), 3.56 (s, 1H, -OH), 3.06 (q, 1H, J=8, ), 2.98 (s, 1H, $\geq C-\underline{H}$), acetate methyls at 2.25 (s), 2.02 (s), 1.96 (s) and 1.94 (s), methyl groups at 1.55 (s), 1.50 (s) and 1.37 (d, J=8), 1.25 (m), and 0.9 (t, 3H, J=5, $-\underline{CH_3}$ of $-\underset{\underset{O}{\|}}{C}-CH_2-CH_2-\underline{CH_3}$).

Mass spectrum and elemental analysis have not been obtained.

M.p. 196-199°C (dec.)

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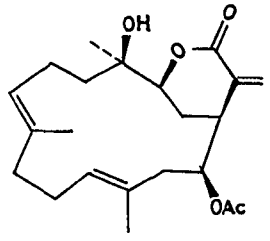
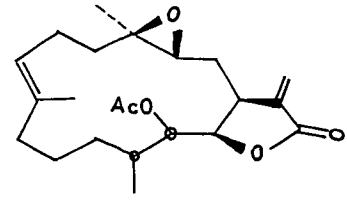
ISOLATION AND CHARACTERIZATION OF TWO NEW CEMBRANOLIDES FROM THE GORGONIAN *EUNICEA SUCCINEA* (PALLAS): PEUNICIN AND EPIPEUNICIN

INTRODUCTION

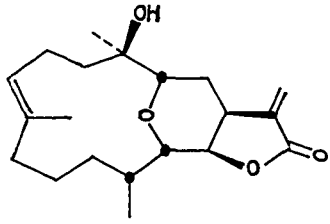
A systematic study of octocorals has led to the isolation of a series of cembranolides from gorgonians[1]. The structures of some of these 14-carbon ring compounds have been studied in detail[2]. The stereochemistry of the following compounds (Fig. 1) was determined by single crystal X-ray diffraction.



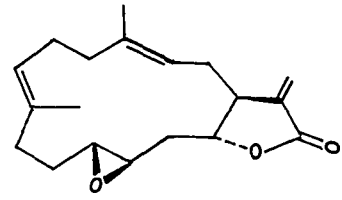
Crassin Acetate



Eupalmerin Acetate

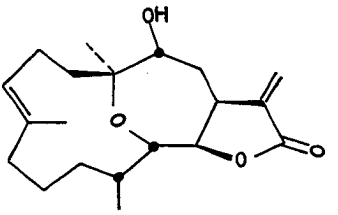


Eunicin

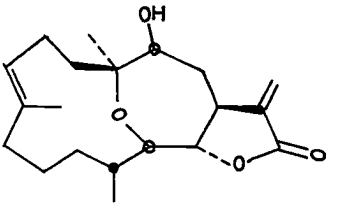


Lobophytolide[12]

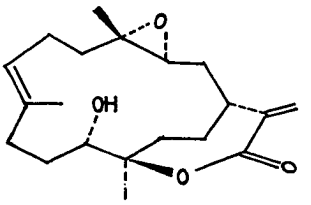
Figure 1



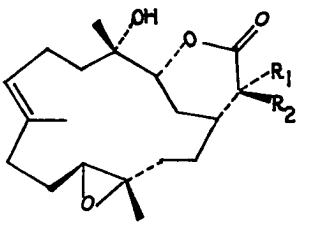
Jeunicin



2,3-bis-epijeunicin



Sinulariolide[23]



SLRN $R_1 + R_2$ = exomethylene[9]

DSLRN R_1 = H, R_2 = Me[9]

Figure 1 (continued)

All these compounds contain a lactone ring carrying an exo-methylene group, which has been suggested as essential for the biological activity of these cembranolides[1,2,10].

Peunicin ($C_{20}H_{26}O_4$) is a toxic compound. A solution of 5 p.p.m. in sea water will stop the swimming activity of larvae of the nudibranch *Phestilla sibogae* within 10 minutes, and kills and disintegrates the larvae within 24 hours[3].

This part of the dissertation deals with the molecular structure elucidation of two new cembranolides "peunicin" and "epipeunicin" isolated from the gorgonian *Eunicea succinea* (Pallas) collected off the Atlantic coast of Panama.

RESULTS AND DISCUSSION

Crystals isolated from n-hexane extract of *Eunicea succines* (Pallas), Panama, are separated into six distinct spots on thin layer chromatography (silica gel H, benzene : ethyl acetate, 3:1, v/v). Peunicin, the second fast moving spot on thin layer chromatogram, is the major component.

Peunicin is not stable in chloroform. It gradually becomes a white powderlike material insoluble in chloroform. Peunicin is stable in dry crystalline form or dissolved in pure ethanol for at least two years.

The i.r. spectrum (Fig. 2) of peunicin shows the existence of a γ-lactone (1770 cm^{-1}) and an α,β-unsaturated ketone (1675 cm^{-1}). The latter feature is also indicated by the strong u.v. absorption at λ_{max}^{MeOH} = 241.3 nm ($\varepsilon = 1.27 \times 10^4$).

In the noise decoupled c.m.r. spectrum (Fig. 4) twenty distinct signals are observed, including ones for two carbonyl carbons (δ 196 and 170), six olefinic carbons (δ 148.1, 137.5, 137.5, 134.0, 128.3 and 120.6), three sp^3 carbons bound to oxygen (δ 75.3, 60.8 and 58.0), and a purely tertiary carbon (δ 43.2).

The p.m.r. spectrum (Fig. 3) of peunicin shows two doublets

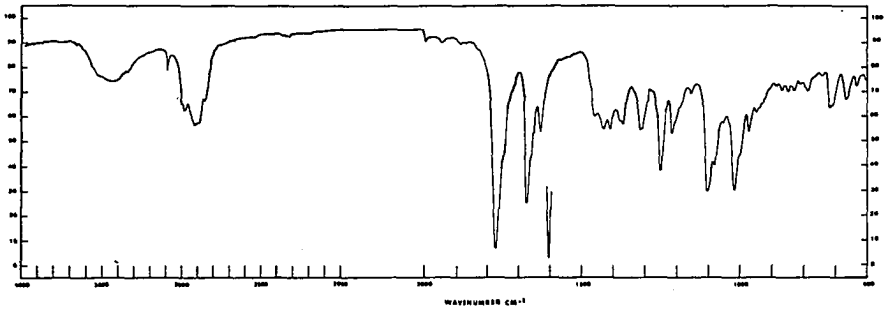


Figure 2 I.r. spectrum of peunicin

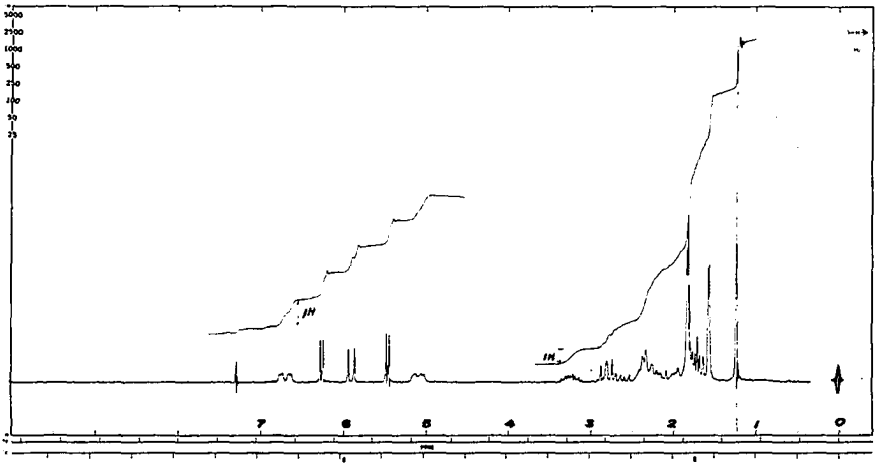
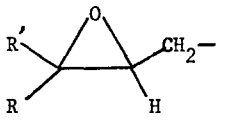


Figure 3 100 MHz p.m.r. spectrum of peunicin

at δ 5.46 (1H, J=3) and 6.25 (1H, J=3). These are typical exomethylene proton peaks conjugated to carbonyl group[3]. The double doublet at δ 2.8 (1H, J=6, 8) is assigned to a hydrogen attached to a carbon bearing an epoxide ring. Elementary analysis indicates the presence of four oxygens. The c.m.r. spectrum shows two $\rangle C{=}O$ groups and one $\rangle C{-}O{-}$ group and two $H{-}C{-}O{-}$ groups in peunicin. The lactone ring accounts for one $\rangle C{=}O$ group and one $H{-}C{-}O{-}$ group. The other $\rangle C{=}O$ is present in the α,β-unsaturated ketone. Thus, one oxygen remains that is singly bonded to two carbons, either in the form of an epoxide or another ether type linkage.

Double irradiation at δ 1.80 collapses the double doublet δ 2.8 to a sharp singlet. This result leads to the partial structure 1.

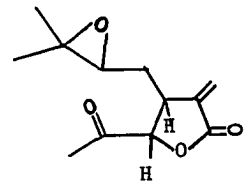


Partial structure 1

The two double doublets centered at δ 1.72 (1H, J=8, 8) and 1.74 (1H, J=4, 6) are assigned to the methylene protons attached to the carbon next to the epoxide carbon.

The broad singlet centered at δ 3.25 is correlated with the proton on the lactone ring next to the exomethylene group, since double irradiation at δ 3.25 causes the exomethylene protons to become singlets and the doublet at δ 5.9 become singlet. The doublet centered at δ 5.9

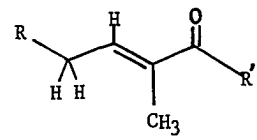
(1H, J=8) is therefore assigned to the hydrogen on the γ-carbon of lactone.



Partial structure 2

The coupling constant between these two lactone protons is 8 Hz, which indicates that these two protons are *cis* to each other. This configuration is supported by x-ray analysis.

Irradiation at δ 1.85 (d, 3H, J=-1.7) collapses the broad doublet centered at δ 6.7 (1H, J=10, -1.7) to a sharp double doublet (J=10, 3). This observation suggested the partial structure 3.



Partial structure 3

The second olefinic hydrogen gives a broad doublet at δ 5.1 (1H, J=12) and is attributed to a $-CH=C(CH_3)-$ unit. The exomethylene group in the lactone ring accounts for the third olefinic unit. These three olefinic unit assignments agree with the observations in the off-

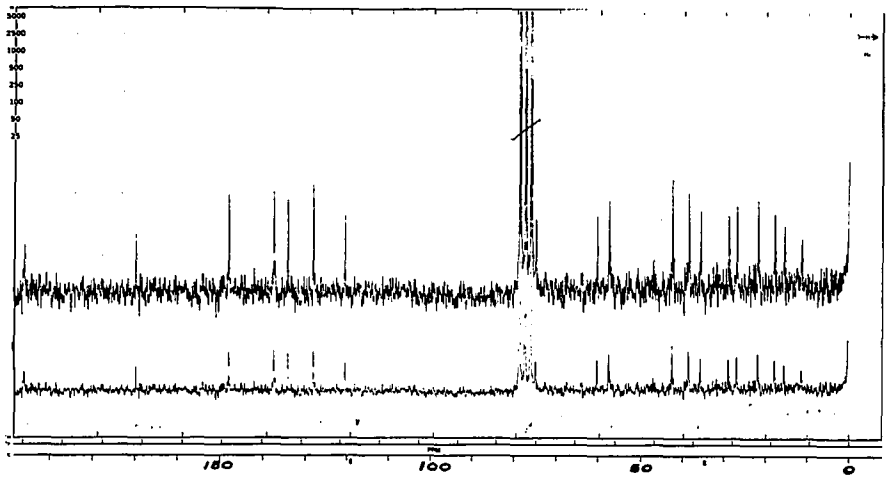


Figure 4 Noise decoupled c.m.r. spectrum of peunicin

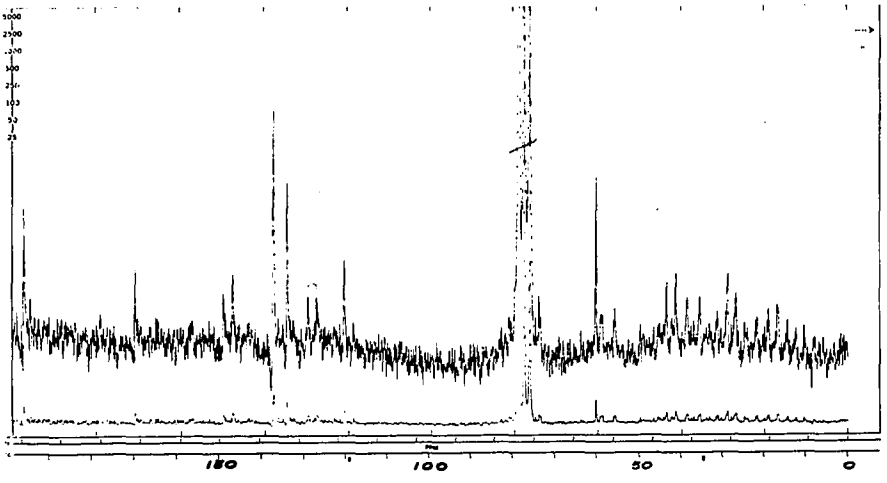
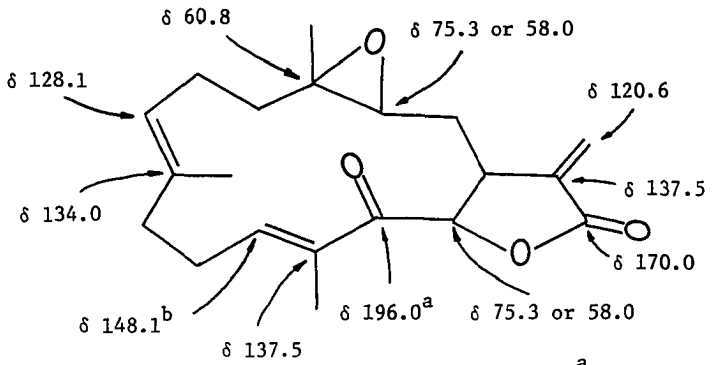


Figure 5 Off-resonance decoupled c.m.r. spectrum of peunicin

resonance decoupled c.m.r. spectrum. In the off-resonance c.m.r. spectrum, the two peaks at δ 137.5 and the peak at δ 134.0 remain as singlets, the peaks at δ 148.1 and 128.1 become doublets and the peak centered at δ 120.6 becomes a triplet (Fig. 5). The suggested assignments are shown in Figure 6.



[a] See reference No. 4

[b] See reference No. 5

Figure 6 C.m.r. assignments for peunicin

Peunicin is obtained from pure ethanol solution as clear orthorhombic crystals. The crystallographic data are given in Table 1. The stereoviews, bond distances, bond angles, final positional parameters, thermal parameters, torsion angles and least square plane are given in Figures 7 to 11 and Tables 2 and 3. No abnormal bond lengths and bond angles are observed. The lactone ring in peunicin is *cis* fused to the fourteen membered ring. The two olefinic units in the fourteen membered ring have the E configuration. The shortest intermolecular distance is 2.211 Å between hydrogen H-19 and H-7 ($-x, -\frac{1}{2} + y, -\frac{1}{2} - z$) and 2.267 Å

Table 1

Crystallographic data

Formula	$C_{20}H_{26}O_4$
Formula weight	330.41
Space group	$P2_12_12_1$
Cell dimensions	(at $-160^{\circ}C$)[a]
	a = 9.5920 (6) Å b = 11.634 (1) Å c = 15.686 (2) Å z = 4 V = 1750.50 Å^3
	(at $25^{\circ}C$) V = 1808.35 Å^3
Density	$\rho_{cal.}$ = 1.213
	$\rho_{obs.}$ = 1.223 (at $25^{\circ}C$, in CCl_4 and C_6H_{14})
Linear absorption coefficient	μ = 0.927

[a]Determined by least-squares fit to the $+2\theta$ and -2θ values of 48 reflections taken from all octants of reciprocal space.

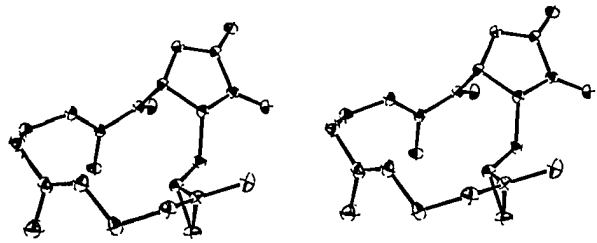


Figure 7 Stereoview of single molecule

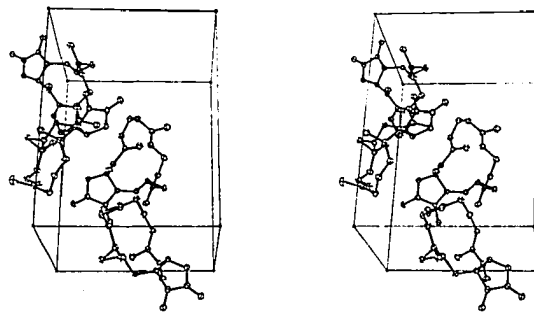


Figure 8 Stereoview of molecular packing.

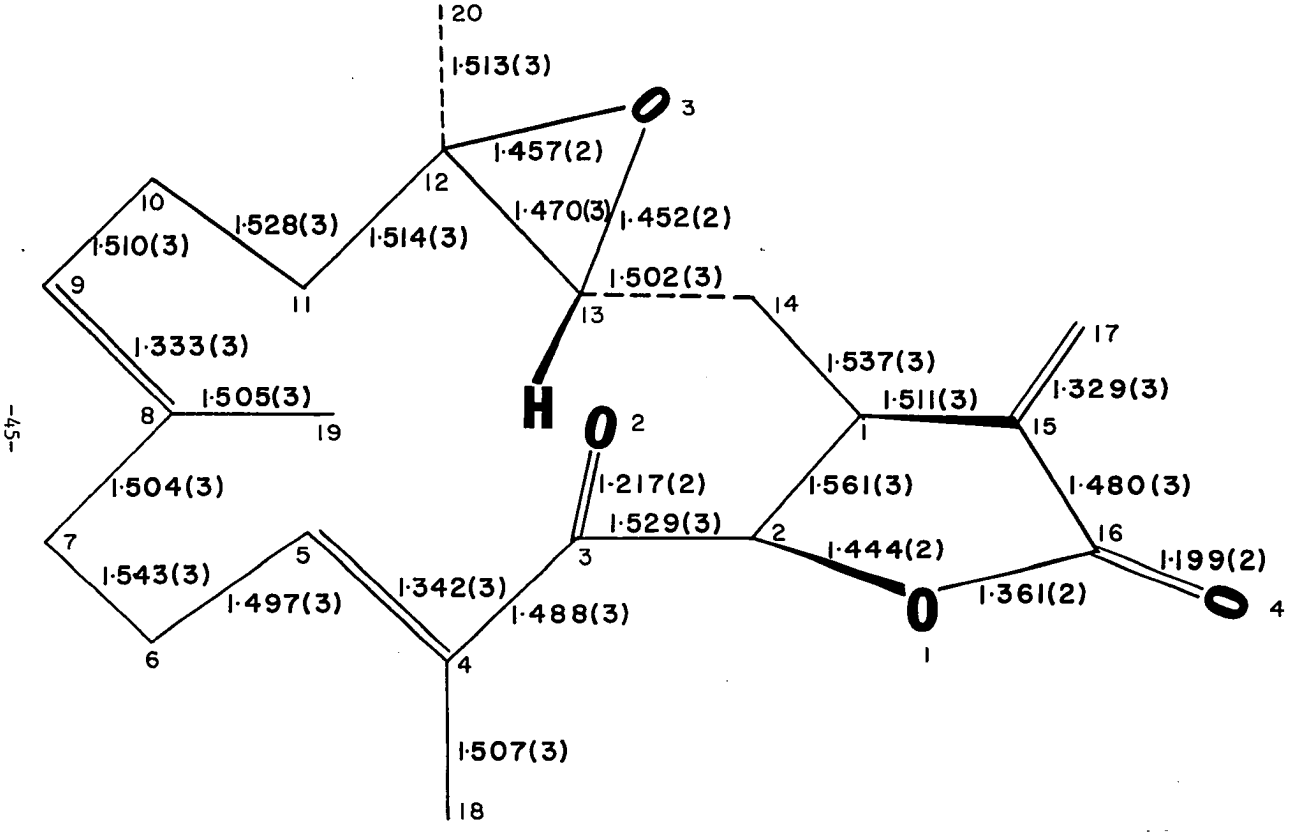


Figure 9 Bond distances and numbering system of peunicin

between H-19 and H-10 ($-\frac{1}{2} + x$, $-\frac{1}{2} - y$, $-z$). The distances between O-2, O-3 and O-4 to the hydrogens are also short (O-2 to H-19; 2.554 Å, O-3 to H-17; 2.534 Å, O-4 to H-17; 2.491 Å). These relatively short intermolecular distances between atoms have been observed in other terpenoids[6]. The short intermolecular distance between oxygen and hydrogen may serve to stabilize the crystal structure. The average distance between carbon and hydrogen atoms is 0.98 Å. The bond lengths of the epoxide ring are around 1.5 Å. A list of the epoxide dimensions in some of the cembranolides are given in Table 4. In all the cases the values are very close. The bond lengths, C-6 to C-5 and C-3 to C-4, are shorter than the other carbon-carbon bonds in the compounds. This is due to the resonance effect of the conjugation (Fig. 9). The conformational angle between C2-C3-C4-C5 is 41.0° (Fig. 11). This angle deforms the conformational angle (141.6°) of the α,β-unsaturated ketone, but does not affect the conjugation very much.

The γ-lactone ring in the peunicin is not flat (Fig. 12). Since the $O{=}C{-}C{=}CH_2$ system is essential for some of the antitumor agents[10], it will be of interest to see whether the configuration of the γ-lactone ring also affects the antitumor activity.

Some of the conformational angles between the hydrogens or hydrogen and methyl group are listed in Table 5. The calculated and observed "J" values are also included.

Epipeunicin crystallizes from n-hexane and benzene solution as colorless, plate-shaped crystals. Epipeunicin is even less stable than peunicin in air or chloroform. The i.r. and p.m.r. spectra (Fig. 13 and 14)

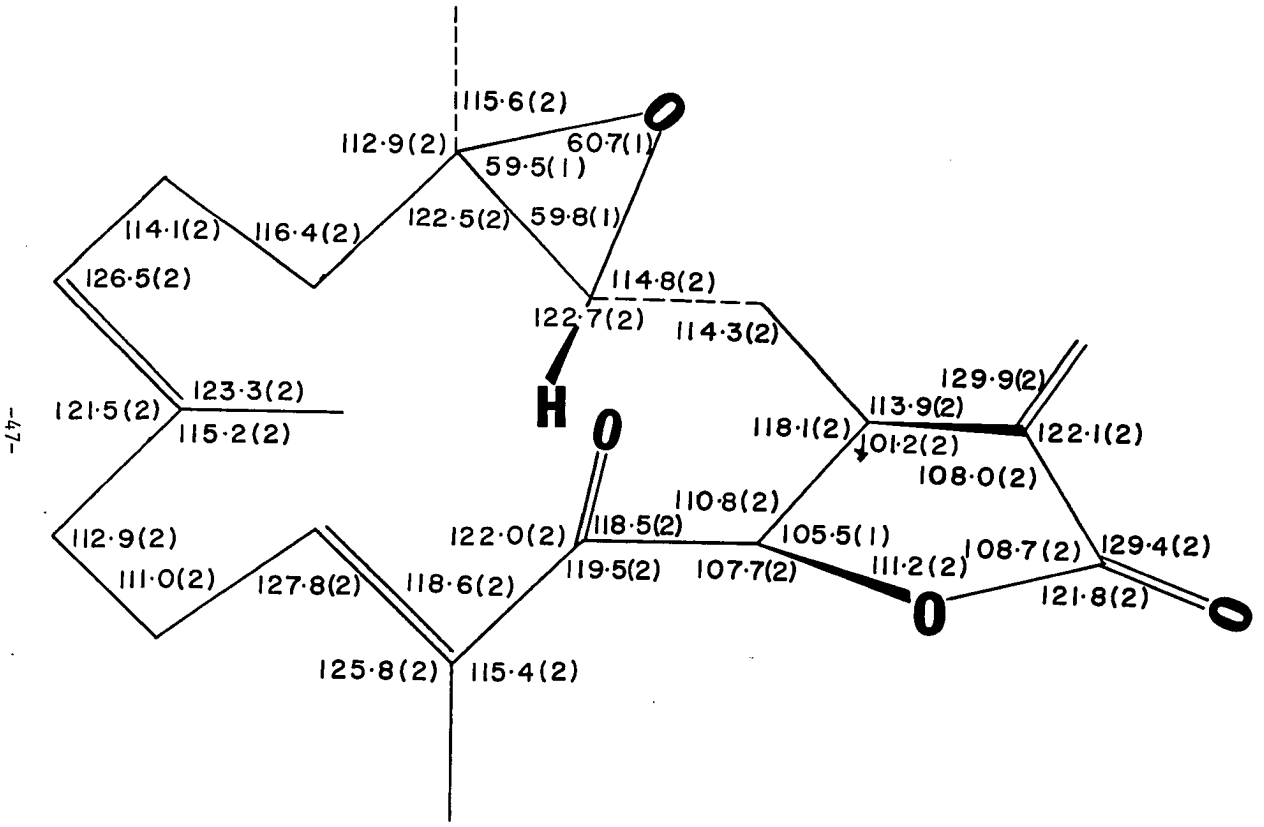


Figure 10 Bond angles of peunicin

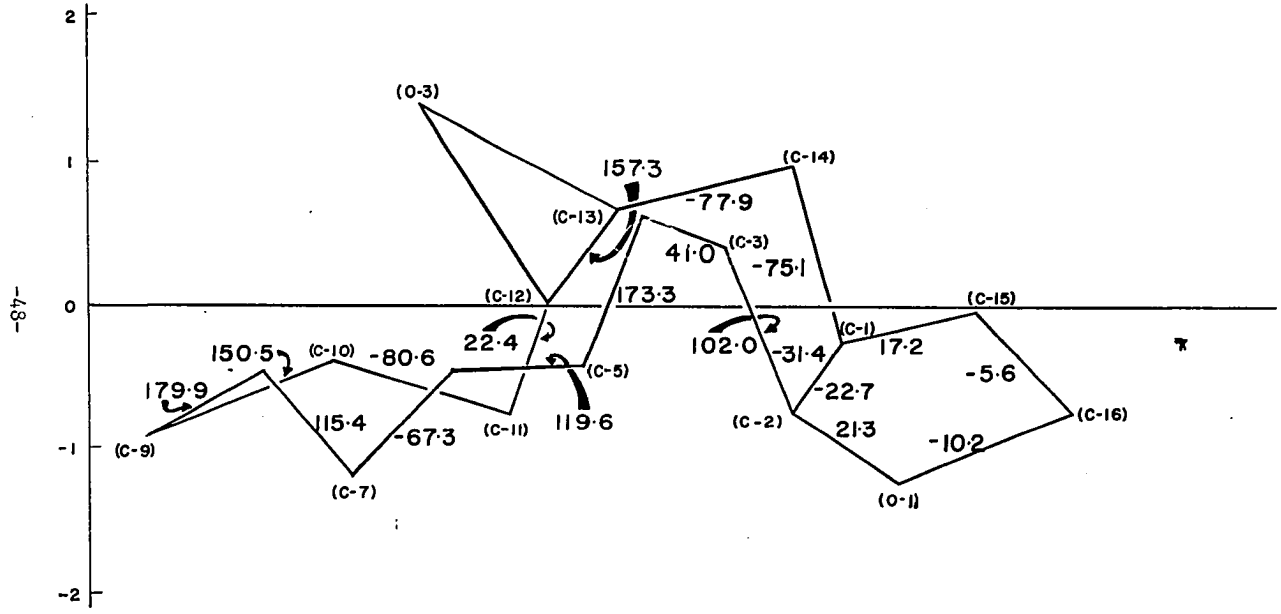


Figure 11 Torsion angles and least square plane of peunicin

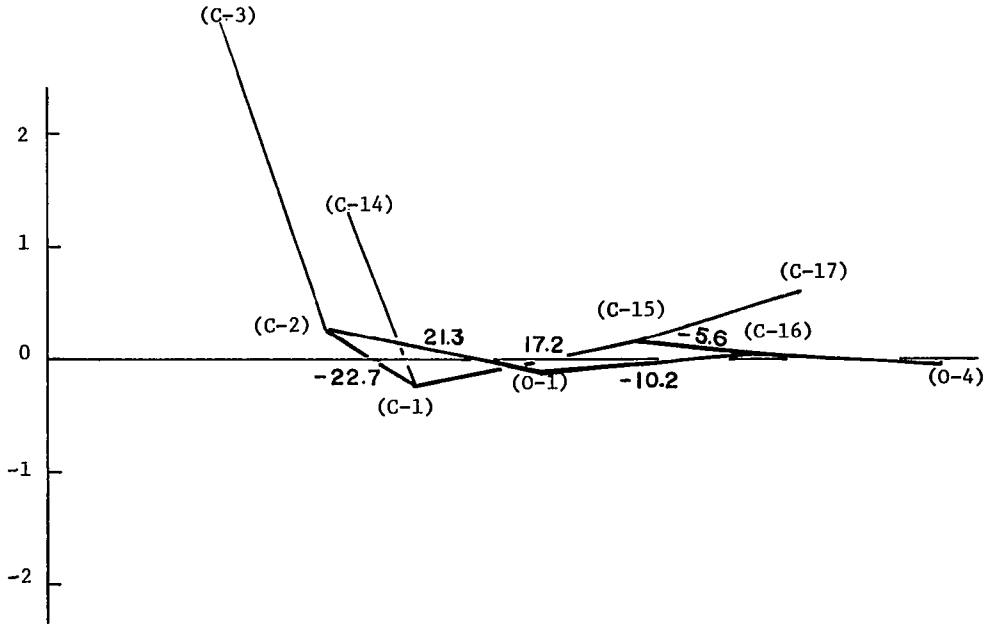


Figure 12 Torsion angles and least square plane of the γ-lactone ring in peunicin.

Table 2

Positional and anisotropic thermal parameters[a] for oxygen and carbon atoms

Atoms	X ($\times 10^4$)	Y ($\times 10^5$)	Z ($\times 10^5$)	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
O(1)	914(2)	32256(12)	6175(8)	327(8)	152(6)	180(6)	2(7)	37(6)	4(6)
O(2)	3045(2)	17258(14)	7461(9)	220(7)	309(8)	189(6)	-17(7)	-30(6)	2(6)
O(3)	-953(2)	-17338(12)	6041(9)	260(7)	147(6)	269(6)	5(6)	-52(6)	34(6)
O(4)	952(2)	40695(13)	18997(9)	341(8)	189(7)	235(7)	-37(7)	34(6)	-31(6)
C(1)	-143(2)	14156(17)	9428(12)	170(9)	178(8)	141(7)	7(8)	5(7)	8(7)
C(2)	745(2)	20838(16)	2723(12)	230(10)	136(8)	150(7)	14(8)	4(8)	-10(7)
C(3)	2194(2)	15508(17)	1853(11)	201(9)	176(9)	150(9)	-24(8)	19(7)	35(7)
C(4)	2506(2)	7975(17)	-5571(12)	157(8)	171(8)	190(9)	0(8)	26(8)	13(7)
C(5)	2023(2)	11043(17)	-13275(12)	196(9)	185(9)	185(9)	-2(8)	30(8)	-2(8)
C(6)	2324(2)	5513(18)	-21696(13)	238(10)	239(10)	160(9)	7(9)	33(8)	-13(8)
C(7)	973(2)	1070(19)	-25889(12)	256(10)	273(10)	162(9)	25(9)	-11(8)	4(8)
C(8)	326(2)	-8774(18)	-21099(13)	243(10)	217(10)	190(9)	9(9)	-39(8)	-27(8)
C(9)	-921(2)	-7800(18)	-17414(12)	238(10)	243(10)	181(9)	19(9)	-44(8)	0(8)

Table 2 (continued)

Atoms	X ($\times 10^4$)	Y ($\times 10^5$)	Z ($\times 10^5$)	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
C(10)	-1680(2)	-17007(19)	-12465(13)	241(10)	247(10)	221(10)	-9(9)	-33(8)	-44(8)
C(11)	-2642(2)	-12376(19)	-5501(13)	182(9)	239(10)	240(10)	-21(9)	-10(8)	0(8)
C(12)	-1956(2)	-8988(17)	2820(12)	185(9)	173(9)	194(9)	-1(8)	0(8)	39(7)
C(13)	-468(2)	-6059(16)	3415(12)	204(9)	146(8)	180(9)	3(8)	2(8)	18(7)
C(14)	119(2)	1200(16)	10476(12)	216(9)	149(8)	167(9)	-4(8)	-22(8)	3(7)
C(15)	141(2)	21074(17)	17390(12)	165(9)	160(8)	189(9)	25(8)	10(7)	-9(7)
C(16)	714(2)	32339(17)	14767(12)	218(9)	184(9)	177(9)	36(9)	22(8)	-13(7)
C(17)	-63(2)	18368(17)	25527(13)	224(9)	200(9)	187(9)	2(9)	14(8)	-1(8)
C(18)	3457(2)	-2039(19)	-3749(14)	223(10)	234(10)	254(10)	66(9)	-7(8)	13(9)
C(19)	1187(3)	-19580(21)	-20794(17)	289(12)	231(11)	472(14)	22(9)	70(11)	22(11)
C(20)	-2980(2)	-4371(21)	9308(14)	222(10)	319(12)	235(10)	-14(10)	44(9)	20(9)

[a]Thermal parameters ($\times 10^4$) are of the form:

$\exp\left[-2\pi^2(U_{11}h^2a^{*2} + U_{22}k^2b^{*2} + U_{33}l^2c^{*2} + 2U_{12}hka^*b^* + 2U_{13}hla^*c^* + 2U_{23}klb^*c^*)\right]$.

Calculated standard deviations for last digit are listed in parentheses.

Table 3

Positional and isotropic thermal parameters for hydrogens

Atoms[a]	X ($\times 10^4$)	Y ($\times 10^4$)	Z ($\times 10^4$)	B ($A^2 \times 10^2$)
H(1-1)	-118(2)	152(2)	77(1)	1.1(4)
H(2-1)	24(3)	213(2)	-29(1)	2.1(5)
H(5-1)	143(3)	184(2)	-137(2)	2.8(6)
H(6-1)	301(3)	-7(2)	-213(2)	2.7(6)
H(6-2)	279(3)	116(2)	-256(1)	1.7(5)
H(7-1)	118(3)	-13(2)	-318(1)	1.9(5)
H(7-2)	31(2)	76(2)	-261(2)	1.8(5)
H(9-1)	-146(2)	-6(2)	-177(1)	1.4(4)
H(10-1)	-100(3)	-225(2)	-102(2)	2.9(6)
H(10-2)	-225(3)	-218(2)	-163(1)	2.2(5)
H(11-1)	-304(3)	-186(2)	-40(1)	2.5(6)
H(11-2)	-311(3)	-61(2)	-79(1)	2.0(5)
H(13-1)	6(2)	-64(2)	-19(1)	1.7(5)
H(14-1)	115(3)	4(2)	109(1)	1.6(5)
H(14-2)	-33(2)	-13(2)	159(1)	1.2(4)
H(17-1)	13(3)	236(2)	301(1)	2.5(5)
H(17-2)	-43(3)	110(2)	272(2)	2.1(5)
H(18-1)	429(3)	3(3)	-4(2)	4.2(7)
H(18-2)	295(3)	-72(3)	1(2)	5.8(9)
H(18-3)	387(4)	-50(3)	-86(2)	5.8(9)
H(19-1)	157(4)	-212(3)	-267(2)	7.3(10)
H(19-2)	67(3)	-265(2)	-190(2)	4.3(7)
H(19-3)	197(4)	-186(3)	-168(2)	5.8(9)
H(20-1)	-381(3)	-98(3)	93(2)	3.7(7)
H(20-2)	-333(3)	34(2)	76(2)	2.6(6)
H(20-3)	-251(3)	-41(3)	151(2)	3.5(6)

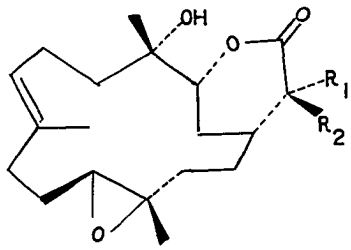
[a]Hydrogens are numbered as (carbon number - hydrogen number).
Calculated standard deviations for last digit are listed in parentheses.

Table 4

Epoxide dimensions for some of the cembranolides

	C-C	C-O	C-O-C	O-C-C
EPA[7]	1.469	1.450	60.8	59.6
Asperdiol[8]	1.474	1.467	60.3	59.7
SLRN (II)[9,a]	1.479	1.455	61.1	59.4
DSLRN (I)[9,a]	1.473	1.448	61.1	59.5
Peunicin	1.470	1.454	60.7	59.6

a

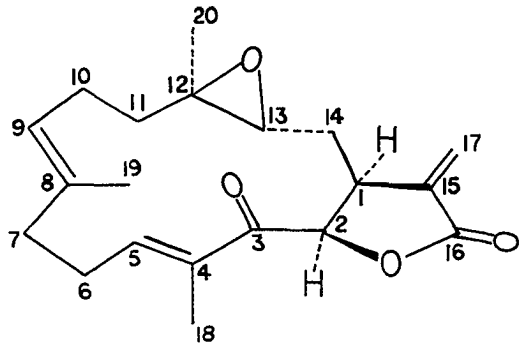


I R_1 = H, R_2 = Me

II $R_1 + R_2$ = exomethylene group

Table 5

Some calculated and observed coupling constants (J) for peunicin



Atoms	Angles[a]	Coupling Constants Calculated[b]	Observed
$H_{1-1}-C_1-C_2-H_{2-1}$	26.9	6.5	8
$H_{5-1}-C_5-C_6-H_{6-1}$	170.0	8.9	10
$H_{5-1}-C_5-C_6-H_{6-2}$	55.2	2.5	3
$H_{1-1}-C_1-C_{14}-H_{14-1}$	168.1	8.8	8
$H_{1-1}-C_1-C_{14}-H_{14-2}$	74.7	0.4	-
$H_{13-1}-C_{13}-C_{14}-H_{14-1}$	40.0	4.6	6

Table 5 (continued)

Atoms	Angles[a]	Coupling Constants Calculated[b]	Observed
$H_{13-1}-C_{13}-C_{14}-H_{14-2}$	18.8	7.3	8
$H_{9-1}-C_{9}-C_{10}-H_{10-1}$	92.1	0.3	-
$H_{9-1}-C_{9}-C_{10}-H_{10-2}$	154.9	7.4	~12
$H_{9-1}-C_{9}-C_{8}-C_{23}$	179.6	~0[c]	0
$H_{5-1}-C_{5}-C_{4}-C_{18}$	4.2	$0 \leq J \leq -3$[c]	-1.7

[a] The angles are absolute values.
[b] $J=8.5 \cos^2\theta - 0.3$, for $(0 \leq \theta < 90)$, $J=9.5 \cos^2\theta - 0.3$ for $(90 \leq \theta \leq 180)$. See reference 11.
[c] $J \propto \cos^2\theta$, $0 \leq J \leq -3$. See reference 11.

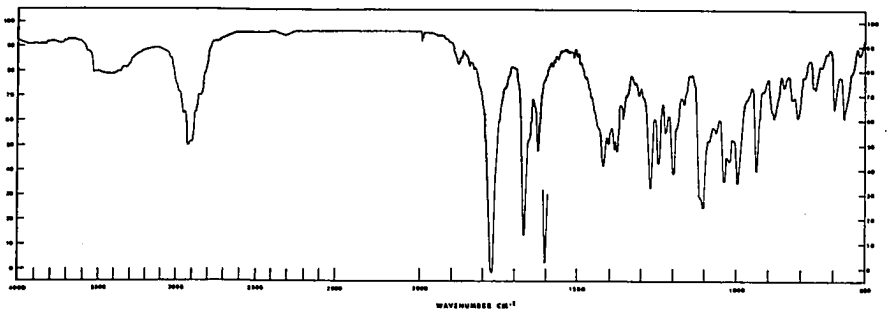


Figure 13 I.r. spectrum of epipeunicin

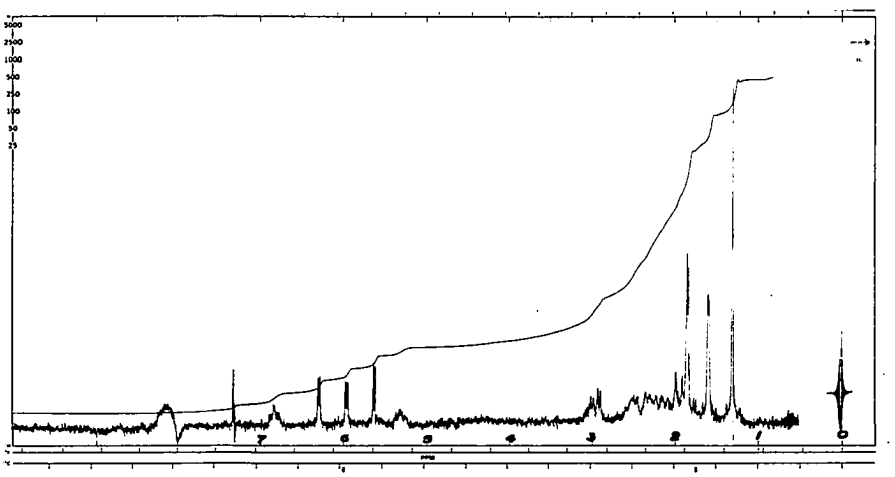


Figure 14 100 MHz p.m.r. spectrum of epipenicin

of epipeunicin are similar to those of peunicin (Fig. 2 and 3). This feature indicates that epipeunicin may have the same carbon skeleton as peunicin. In epipeunicin the doublet at δ 5.95 has a coupling constant of 2.2 (J=8 in peunicin). One of the exomethylene proton signals in the spectrum of epipeunicin occurrs at δ 5.63 (d, 1H, J=2) and the broad singlet for the proton at C-1 has shifted to δ 2.99 (m, 1H) and is coincident with the proton δ 2.95 (dd, 1H, J=3, 8) that is attached to the epoxide carbon. All of these differences indicate a possible configurational change at C-1 or C-2 relative to that found in peunicin. Some of the known examples of *trans* fused γ-lactones are given in Table 6. A summary of proton chemical shifts and decouplings of peunicin and epipeunicin are given in Table 7.

The low resolution mass spectrum (70 ev) of both compounds show closely related m/e fragments. There are differences in the relative abundances of m/e 27, 28, 31, 32, 82 (base peak), 205 and 206 (Fig. 15). The highly abundant m/e 82 fragment can be derived by the following mechanism:

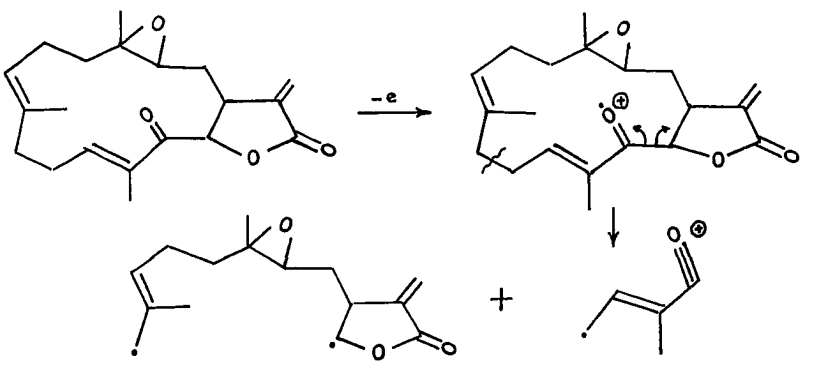


Table 6

P.m.r. data on natural products containing *trans* fused γ-lactone ring with small coupling constant

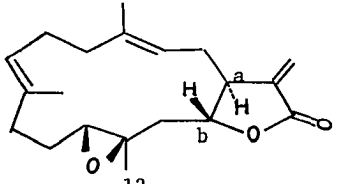
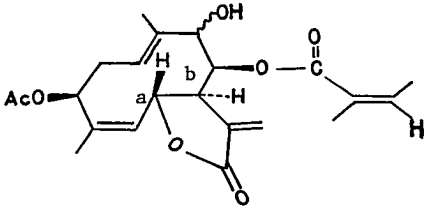
Compound	Chemical Shift (δ) and Coupling Constants (Hz)
 Lobophytolide[12]	H_a δ = 2.66 (m) H_b δ = 3.94 (q) $J_{a,b}$ = 5
 Eupatocunin[13]	H_a δ = 4.22 (dd) H_b δ = 6.7 (m) $J_{a,b}$ = 2.5

Table 6 (continued)

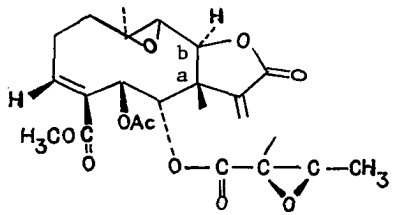
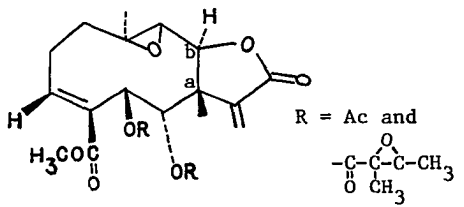
Compound	Chemical Shift (δ) and Coupling Constants (Hz)
 Enhydrin[14]	H_a δ = 3.0, (m) H_b δ = 6.8, (dd) $J_{a,b}$ = 1.4
 Maculatin[14]	H_a δ = 3.04, (m) H_b δ = 6.71 (dd) $J_{a,b}$ = 1.4

Table 7

Some p.m.r. coupling data for peunicin and epipeunicin[a]

Peunicin			Epipeunicin		
Signal Irradiated	Change Observed		Signal Irradiated	Change Observed	
6.25	5.46	affected	6.29	5.63	affected
5.90	3.25	affected			
5.46	6.25	affected	5.63	6.29	affected
3.25	6.25	d → s	2.94	6.29	d → s
	5.90	d → s		5.95	d → s
	5.46	d → s		5.63	d → s
	1.70	affected			
2.80	6.90	bd → bs			
	1.83	affected			
2.20	5.10	bd → bs			
1.85	6.70	bd → dd	1.84	6.84	bt → t
				2.95	dd, affected
1.80	2.80	dd → s			
1.58	5.10	affected	1.60	5.32	bt → t
	2.35	affected		2.95	affected

[a]Run on a Varian XL-100 spectrometer at 100 MHz in $CDCl_3$ solution using tetramethylsilane as internal standard. Abbreviations specified in the experimental section apply.

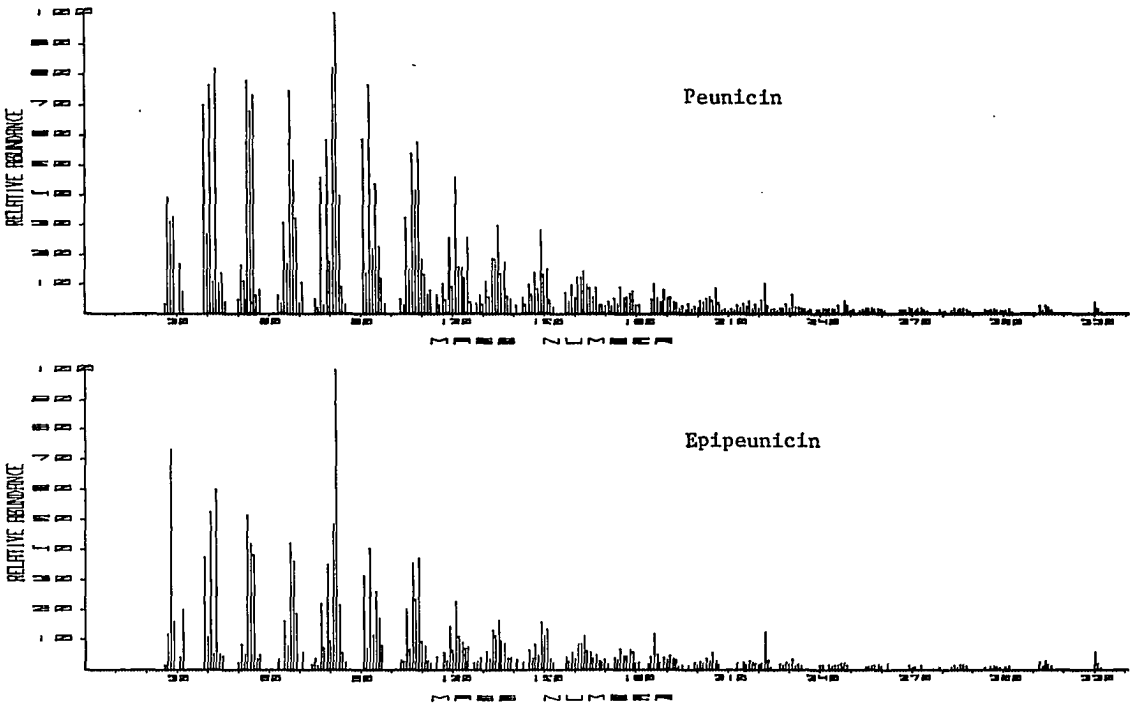
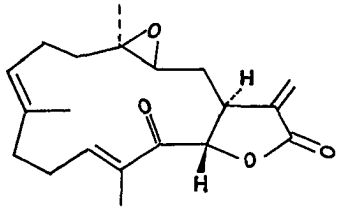
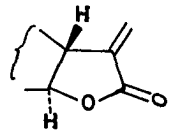


Figure 15 Low resolution mass spectrum of peunicin and epipeunicin

The actual mechanism is unknown. Due to the limited supply and the high instability of epipeunicin no further analysis is available. The structure suggested for epipeunicin is as follows.





SUMMARY

Two new epimeric cembranolides, peunicin and epipeunicin were isolated from the hexane extracts of the gorgonian *Eunicea succinea* (Pallas). The structure of peunicin was determined by the application of various spectral methods coupled with single crystal X-ray diffraction. Peunicin, Figure A, ($C_{20}H_{26}O_4$, m.p. 175-6°), a toxic compound, forms orthorhombic crystals, with cell dimensions a=9.5920 (6), b=11.634 (1), c=15.685 (2) A, $\alpha=\beta=\gamma=90^{\circ}$, Z=4. The space group is $P2_12_12_1$.

Epipeunicin (1 or 2) is suggested to have the same overall structure as peunicin, but is epimeric at one of the lactone ring fusion carbons

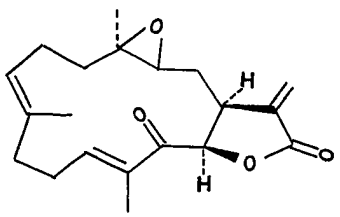
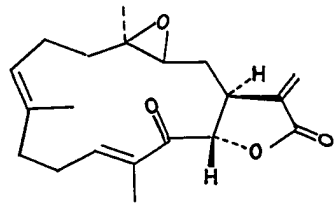
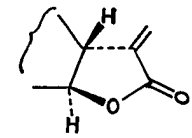


Figure A



1



2

Figure B

EXPERIMENTAL

General experimental conditions specified in section I apply with the following addition.

Preparation of peunicin and epipeunicin. The *Eunicea* were collected by hand in 30 feet of water off Buenaventura Island near the town of Portobelo, Panama, on 27 September, 1974 by Dr. Charles Birkeland* of Smithsonian Tropical Research Institute, Galeta, Panama.

The specimens were hung on a line to drip dry in the warm air exhaust of a window air conditioner. The cortex was stripped off the horny skeleton and dried further in an oven at 70°C for one hour. Drying was completed within 6 hours of collection. The dried animals were kept in plastic containers for three months before they were studied.

The dried, broken cortex was extracted in a Soxhlet extractor with n-hexane. On cooling the extracts deposited solids that were washed with hexane, dissolved in benzene and freed of pigments by chromatography on Florisil, using benzene as the eluting solvent. The benzene solution was concentrated and hexane was added. Colorless crystals were obtained by chilling to 5°C. The crystalline product was further purified by chromatography on a silica gel H column using a mixture of benzene and

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*Now at University of Guam.

ethyl acetate (4:1, v/v). The major fraction was recrystallized from absolute ethanol to obtain crystals, m.p. 175-6°C. for x-ray analysis.

The original hexane extract after the removal of peunicin, was further concentrated and kept at 5°C. Epipeunicin forms plate-shaped colorless crystals in chilled n-hexane - benzene. The purified epieunicin melts at 143-5°C.

X-ray data collection The x-ray reflection data were collected on a Nonius CAD-4 automatic diffractometer at -160°C [Unit-cell-dimension data (Table 1) (Cu $K\alpha_1$ radiation, λ=1.54051 Å) and integrated x-ray intensity data (Cu K_{α} radiation, λ=1.5418 Å, Ni-filtered)]. The mosaic spread of the crystal was 0.6°. The space group was determined uniquely as $P2_12_12_1$ from systematic absences. All 2059 independent reflections with $2\theta<144^{\circ}$ were measured by a θ-2θ scans technique with variable scan width $(0.9 + 0.09 \tan\theta)^{\circ}$. The maximum scan time was 60 seconds with 2/3 of the time used in scanning the peak and 1/6 each for the high and low background. There were 176 reflections with the net count less than $1.4\sigma(I)$, which were assigned intensities equal to the square root of the total count. An experimental weighting scheme was assigned to each amplitude as described in Reference 15. Lorentz and polarization corrections were applied to the data. No absorption correction was made.

Structure determination and refinement of peunicin. The structure of peunicin was solved by direct methods using the program MULTAN[16]. The phases for 220 normalized structure greater than 15., were used in generating an E map (merit=1.24, residuals=26.30). The structure factor was refined by the block-diagonal least-squares method (9x9) to an R

value of 0.038 for all data[17]. A final difference Fourier map was calculated in which all electron densities were between -0.15 to + 0.18 $eA^{\bullet 3}$. The atomic scattering factors for O and C atoms were taken from International Table for X-ray Crystallography (1962)[18]. The anomalous scattering factors for O atoms were those of Cromer and Liberman[19]. The scattering factors for hydrogen atoms were those of Stewart, Davidson and Simpson[20]. The absolute configuration of peunicin was determined by using the anomalous contribution of the oxygen atoms in the molecule. The data were collected and analyzed as described by Ealick, van der Helm and Weinheimer[21]. The results are summarized in Table 8. In twenty-six selected reflections, twenty-one pairs of reflections indicated the absolute configuration derived from peunicin and five pairs indicated the opposite configuration. The data crystal used in this study was 0.3x0.2x0.1 mm^3.[22]

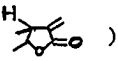
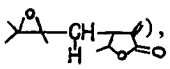
Pure peunicin had $[\alpha]_D^{24}$ + 93.6° (c 7.8, $CHCl_3$); R_f=0.55 (benzene:ethyl acetate, 3:1, silica gel H), R_f=0.73 (50% ether/chloroform, silica gel H); i.r. (neat, KBr) 3099, 3000, 2900, 2850, 1950, 1890, 1770, 1675, 1632, 1460, 1430, 1410, 1375, 1315, 1250, 1220, 1156, 1105, 1080, 1020, 970, 945, 885, 870, 850, 815, 790, 745, 715, 665, 630, 600 cm^{-1}; u.v. (MeOH) λ_{max}=241,3nm, ε_{max}=1.27x10^4, with an infection at 300 nm; 100 MHz p.m.r. ($CDCl_3$) δ 6.7 (bd, 1H, J=10, -1.7, H-C=C-C=O), 6.25(d, 1H, J=3, $\rangle C=CH_2$), 5.9 (d, 1H, J=8, O=C-C-O-C=O), 5.46(d, 1H, J=3, $\rangle C=CH_2$), 5.1(bd, 1H, J=~12, H-C=C$\langle$), 3.25(m, 1H, ), 2.8 (dd, 1H, J=8, 6, ), 2.8(m, 1H), 2.35(m, 4H), 1.9(m, 1H), 1.85(d, 3H, J=~1.7, $-CH_3$), 1.74(dd, 1H, J=4, 6, ), 1.72(dd, 1H, J=8,

Table 8

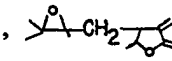
Comparison of observed and calculated Bijvoet differences

h	k	l	ΔIo	ΔIo/Io	ΔFc^2	Fc^2	$\Delta Fc^2/Fc^2$
1	14	3	-0.96	-1.6%	-553.74	7084.77	-7.8%
2	1	16	-1.43	-2.5%	6649.95	138035.99	4.8%
1	5	3	3.05	3.9%	5897.30	61227.48	9.6%
1	7	5	-7.38	-1.8%	-141721.20	4258327.00	-3.4%
1	5	18	-1.06	-1.4%	-1331.54	44673.42	-3.0%
1	8	3	-0.06	-0.3%	1219.38	16072.61	7.6%
1	8	6	-0.42	-0.6%	11204.23	232864.06	4.8%
1	10	8	0.45	0.6%	9883.37	242286.03	4.1%
2	3	5	10.14	1.3%	176194.90	5195878.20	3.3%
2	6	1	-2.06	-3.4%	-2936.36	64529.43	-4.5%
2	7	6	-0.78	-1.2%	-6522.54	149682.29	-4.4%
2	9	5	0.45	0.4%	16308.58	557635.50	2.9%
2	9	8	-0.96	-4.3%	-1542.99	27890.99	-5.5%
2	10	5	-1.71	-5.7%	-2433.39	47894.30	-5.1%
4	5	2	-0.66	-0.4%	19894.13	451161.83	4.4%
4	8	10	-0.26	-1.4%	-1186.23	14305.53	-8.3%
9	7	3	-1.68	-3.4%	-4806.20	58591.07	-8.2%
9	7	5	0.87	15.8%	159.93	593.44	26.9%
3	1	15	0.10	0.3%	3781.48	55464.96	6.8%

Table 8 (continued)

h	k	l	ΔIo	ΔIo/Io	ΔFc^2	Fc^2	$\Delta Fc^2/Fc^2$
3	4	8	0.46	1.0%	-2125.84	53398.63	-4.0%
3	3	8	1.47	6.8%	2216.08	12475.86	17.8%
3	7	10	-1.99	-3.2%	-6963.50	212120.58	-3.3%
3	12	4	0.31	1.7%	818.50	11541.60	7.1%
3	3	1	8.97	2.4%	32278.80	1039534.60	3.1%
1	5	1	-1.32	-3.5%	-180.90	11875.72	-1.5%
1	4	1	-1.73	-5.5%	-634.20	6752.35	-9.4%

The $\Delta I = I_{hkl} - I_{\bar{h}\bar{k}\bar{l}}$, $\Delta F_c^2 = F_{hkl}^2 - F_{\bar{h}\bar{k}\bar{l}}^2$,

8, ), 1.57(s, 3H, $-CH_3$), 1.24(s, 3H, $-CH_3$); c.m.r. ($CDCl_3$) δ 196, 170.0, 148.1, 137.5, 137.5, 134.0, 128.1, 120.6, 75.3, 60.8, 58.0, 43.2, 39.3, 36.3, 29.7, 27.7, 23.0, 18.6. 16.3, 12.1, for assignments see Figure 6; mass spectrum (70 eV) m/e (rel. intensity) M^+, 330 (4), 316 (1), 315 (2), 314 (2), 313 (1), 312 (2), 302 (1), 301 (1), 299 (1), 231 (6), 222 (10), 206 (9), 205 (4), 204 (6), 203 (5), 191 (5), 190 (5), 189 (8), 187 (5), 186 (10), 185 (5), 179 (8), 177 (5), 176 (5), 175 (10), 173 (5), 167 (9), 166 (7), 165 (9), 164 (10), 163 (14), 162 (12), 161 (12), 160 (5), 159 (10), 157 (7), 151 (15), 150 (13), 149 (28), 148 (8), 147 (14), 146 (6), 145 (10), 143 (5), 139 (5), 138 (6), 137 (17), 136 (13), 135 (30), 134 (18), 133 (19), 132 (5), 131 (11), 129 (6), 125 (26), 124 (12), 123 (15), 122 (16), 121 (46), 120 (9), 119 (26), 117 (10), 115 (6), 113 (8), 112 (6), 110 (18), 109 (57), 108 (42), 107 (54), 106 (15), 105 (32), 103 (5), 97 (12), 96 (23), 95 (43), 94 (22), 93 (76), 92 (14), 91 (60), 84 (9), 83 (40), 82 (100) base peak, 81 (82), 80 (17), 79 (58), 77 (46), 75 (5), 71 (10), 69 (32), 68 (52), 67 (74), 66 (17), 65 (30), 63 (6), 57 (8), 56 (6), 55 (73), 54 (68), 53 (11), 51 (16), 50 (5), 45 (13), 44 (10), 43 (82), 42 (10), 41 (76), 40 (26), 39 (69), 32 (7), 31 (16), 29 (32), 28 (30), 27 (40), 26 (3).

Anal. Calcd. for $C_{20}H_{26}O_4$: C, 72.70; H, 7.93; O, 19.37. Found: C, 72.92; H, 7.99; O, 19.19; M. Wt. 330.41.

M.p. 175-176°C.

Pure epipeunicin had $[\alpha]_D^{24}=-320^\circ$ (c.0.05, $CHCl_3$)*; $R_f=0.55$ (benzene:ethyl acetate, 3:1, silica gel H); i.r. (neat, KBr) 2960, 2910 2840, 2300 (weak), 1800 (weak), 1775, 1670, 1625, 1420, 1405, 1385, 1375,

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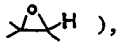
*$[\alpha]_D^{24}=-500^\circ$ (c.0.08, $CHCl_3$)

1355, 1305, 1270, 1245, 1225, 1200, 1165, 1105, 1065, 1040, 1020, 1000, 940, 882, 850, 830, 810, 755, 700, 665 cm^{-1}; u.v. (MeOH) λ_{max}=240 nm, ε_{max}≈1.37x10^4; p.m.r., 100 MHz ($CDCl_3$) δ 6.84(t, 1H, J=6, H-C=C-C=O), 6.29(d, 1H, J=2, $\rangle C=CH_2$), 5.95(d, 1H, J=2.2, O=C-C(H)-C-O-C=O), 5.63 (d, 1H, J=2, $\rangle C=CH_2$), 5.32(t, 1H, J=5, H-C=C$\rangle$), 2.99(m, 1H, ), 2.95 (q, 1H, J=3, 8, ), 2.5(m), 2.4(m), 2.2(m), 1.85(d, 3H, J=1, $-CH_3$), 1.55(d, 3H, J=1, $-CH_3$), 1.30(s, 3H, $-CH_3$); mass spectrum (70 eV) m/e (rel. intensity) M^+, 330 (6), 316 (1), 315 (2), 314 (2), 313 (1), 312 (2), 302 (1), 301 (1), 222 (12), 205 (5), 203 (3), 186 (11), 179 (6), 178 (7), 175 (7), 167 (5), 165 (6), 164 (6), 163 (11), 161 (8), 159 (6), 158 (6), 151 (13), 150 (11), 149 (16), 148 (5), 147 (8), 137 (9), 136 (9), 135 (17), 134 (11), 133 (13), 131 (6), 125 (7), 124 (7), 123 (9), 122 (11), 121 (22), 120 (6), 119 (14), 117 (6), 112 (3), 111 (8), 110 (9), 109 (37), 108 (23), 107 (36), 106 (7), 105 (18), 103 (3), 97, (8), 96 (17), 95 (26), 94 (12), 93 (40), 92 (7), 91 (31), 84 (5), 83 (22), 82 (100) base peak, 81 (49), 80 (9), 79 (35), 78 (7), 77 (22), 71 (6), 69 (19), 68 (36), 67 (42), 66 (8), 65 (16), 57 (5), 55 (38), 54 (42), 53 (51), 52 (5), 51 (8), 44 (5), 43 (60), 42 (5), 41 (52), 40 (11), 39 (37), 32 (20), 29 (16), 28 (73), 27 (12).

Due to the unstable nature of epipeunicin no combustion analysis was obtained.

M.p. 143-145°C

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22. Lists of structure factors (11 pages) for peunicin is included in Appendix A.

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Appendix A

Lists of Structure Factors for Peunicin (11 pages)

FO = Observed structure factor modulus

FC = Calculated structure factor modulus

AL = Phase angles

* = Unobserved reflections

L	FO	FC	AL
H=	0	K=	0
2	270	282	0
4	163	167	0
6	226	222	50
8	277	277	50
10	113	106	50
12	52	52	50
14	59	58	50
16	13	2	0*
18	37	38	50
H=	0	K=	1
1	237	242	25
2	132	135	75
3	15	19	25
4	453	471	25
5	85	84	75
6	219	213	75
7	61	61	25
8	61	61	75
9	90	88	75
10	12	2	25*
11	89	92	25
12	134	136	25
13	69	67	25
14	188	193	25
15	32	18	25
16	12	9	75*
17	42	37	75
18	11	8	75*
19	37	39	75
H=	0	K=	2
0	126	135	0
1	63	63	50
2	59	61	50
3	97	97	0
4	61	64	0
5	10	7	50*
6	40	37	50
7	120	123	0
8	63	70	0
9	73	68	0
10	96	97	0
11	47	44	50
12	49	52	50
13	134	138	50
14	99	97	50
15	39	36	50
16	78	77	50
17	12	4	0*
18	11	12	0*
19	37	34	50
H=	0	K=	3
1	250	255	75
2	186	192	25
3	250	251	75
4	217	222	75
5	93	87	75
6	232	231	75
7	57	58	75
8	63	61	75
9	93	90	25
10	91	96	25
11	13	7	25*
12	82	86	75
13	13	5	25*
14	81	84	75
15	124	126	25
16	55	54	75
17	39	42	25
18	17	13	75
19	28	31	25
H=	0	K=	4
0	229	228	50
1	352	345	0
2	148	147	50
3	44	47	50
4	59	59	0
5	159	154	0
6	11	13	0*
7	14	21	50
8	105	107	0
9	63	57	0
10	26	28	0
11	144	147	0
12	45	44	0
13	130	131	0
14	102	103	50
15	30	24	0
16	67	66	50
17	56	54	0
18	24	16	0
H=	0	K=	5
1	264	256	75
2	246	239	25
3	72	69	75
4	65	65	75
5	47	48	25
6	94	89	25
7	35	33	75
8	58	62	75
9	12	8	25*
10	109	111	75
11	80	81	75
12	26	26	25
13	46	41	75
14	29	29	75
15	63	65	25
16	33	37	75
17	105	102	25
18	57	55	25
H=	0	K=	6
0	241	242	0
1	68	70	50
2	180	178	0
3	116	105	0
4	97	97	50
5	127	122	0
6	255	253	50
7	57	57	50
8	76	77	0
9	38	43	50
10	102	100	50
11	48	51	50
12	88	92	0
13	53	55	50
14	37	38	0
15	41	43	0
16	53	51	0
17	53	53	50
H=	0	K=	7
1	34	33	25
2	126	124	25
3	65	68	25
4	90	89	75
5	16	5	25
6	22	24	25
7	31	33	75
8	204	205	75
9	35	38	75
10	56	52	75
11	14	21	25*
12	14	16	75*
13	81	82	25
14	13	15	25*
15	12	3	25*
16	10	3	25*
17	32	33	75
H=	0	K=	8
0	281	281	50
1	21	20	0
2	36	36	50
3	117	117	50
4	68	65	50
5	13	18	50*
6	19	14	0
7	21	20	0
8	124	126	0
9	41	40	50
10	70	72	0
11	87	85	50
12	19	16	0
13	28	29	50
14	48	48	50
15	24	20	0
16	16	0	0
H=	0	K=	9
1	30	30	75
2	13	12	75*
3	18	21	75
4	118	114	75
5	22	18	25
6	70	64	75
7	17	13	25
8	21	8	75
9	133	134	25
10	39	40	25
11	38	39	25
12	12	13	75*
13	12	1	75*
14	37	37	25
15	38	39	75
H=	0	K=	10
0	133	137	0
1	14	18	50*
2	102	102	50
3	170	175	50
4	40	38	0
5	112	114	50
6	57	56	0
7	156	157	50
8	24	21	50
9	13	6	50*
10	33	31	50
11	20	16	0
12	30	30	50
13	46	45	0
14	48	50	50
H=	0	K=	11
1	14	9	25*
2	108	108	75
3	62	61	75
4	75	74	25
5	13	10	75*
6	13	13	75*
7	86	84	25
8	13	9	75*
9	12	4	75*
10	20	20	25
11	25	24	25
12	26	26	75
H=	0	K=	12
0	137	136	0
1	13	8	0*
2	16	18	0
3	46	46	0
4	12	2	0*
5	57	58	50
6	32	30	50
7	12	22	0*
8	29	32	50

L	FO	FC	AL
9	11	3	50*
10	40	38	50
11	43	43	50
H=	0	K=	13
1	12	1	78*
2	30	29	25
3	16	16	75
4	31	31	25
5	57	58	75
6	11	2	25*
7	11	4	75*
8	10	0	73*
H=	0	K=	14
0	20	20	50
1	15	4	0
2	13	3	0
3	27	26	50
4	41	42	0
5	30	32	0
H=	1	K=	0
1	195	203	25
2	13	13	50
3	508	532	25
4	34	31	0
5	255	257	25
6	62	61	0
7	218	218	25
8	56	55	50
9	61	64	25
10	140	136	0
11	28	28	75
12	22	16	50
13	85	85	25
14	13	17	50*
15	55	53	75
16	13	7	50*
17	24	17	75
18	115	115	50
19	49	49	75
H=	1	K=	1
0	65	67	25
1	226	242	94
2	376	406	55
3	116	114	68
4	315	324	12
5	227	227	73
6	62	60	66
7	169	169	80
8	109	111	26
9	99	99	15
10	41	43	90
11	22	19	1
12	91	94	59
13	80	82	61
14	65	65	30

L	FO	FC	AL
15	94	97	66
16	13	16	58*
17	55	56	64
18	21	19	7
19	35	37	1
H=	1	K=	2
0	298	312	75
1	594	633	56
2	280	285	0
3	219	219	39
4	299	300	0
5	148	150	89
6	159	155	86
7	41	41	82
8	248	248	22
9	54	54	87
10	83	81	7
11	179	181	17
12	96	96	27
13	24	25	72
14	85	85	80
15	13	10	43*
16	57	57	85
17	39	42	69
18	36	33	62
19	43	43	90
H=	1	K=	3
0	123	125	25
1	177	177	35
2	53	52	54
3	50	52	51
4	179	181	3
5	102	103	40
6	64	65	76
7	26	26	88
8	176	170	97
9	40	37	48
10	150	150	84
11	96	97	73
12	86	88	71
13	122	125	79
14	22	21	88
15	41	40	88
16	47	48	25
17	12	16	85*
18	61	61	25
19	61	62	16
H=	1	K=	4
0	59	56	75
1	41	45	76
2	161	162	18
3	110	112	8
4	207	205	79
5	119	111	69
6	255	248	83

L	FO	FC	AL
7	58	55	9
8	21	25	72
9	35	36	24
10	82	85	67
11	82	80	16
12	26	34	83
13	17	13	58
14	154	156	26
15	28	30	0
16	51	51	23
17	44	44	82
18	54	54	27
H=	1	K=	5
0	136	138	25
1	54	52	18
2	24	23	99
3	78	78	56
4	207	201	16
5	114	107	23
6	57	54	32
7	56	53	20
8	77	80	85
9	55	56	78
10	76	79	13
11	116	118	89
12	27	25	22
13	93	95	56
14	81	83	76
15	50	51	26
16	58	57	83
17	27	30	16
18	71	71	63
H=	1	K=	6
0	26	22	25
1	126	120	22
2	171	163	57
3	165	164	19
4	189	179	44
5	67	67	22
6	50	48	30
7	63	62	92
8	36	35	33
9	33	39	55
10	83	85	49
11	97	94	62
12	56	60	9
13	31	30	67
14	78	76	71
15	42	43	5
16	47	47	47
17	52	54	82
H=	1	K=	7
0	32	32	25
1	95	96	65
2	41	37	38

L	FO	FC	AL
3	237	237	73
4	23	22	37
5	234	227	58
6	136	127	7
7	45	48	70
8	51	50	31
9	60	64	4
10	44	46	41
11	110	117	15
12	60	61	20
13	46	46	20
14	73	71	71
15	44	42	97
16	21	21	65
17	12	14	83
H=	1	K=	8
0	38	40	25
1	28	29	80
2	104	103	78
3	56	56	98
4	34	36	25
5	103	105	76
6	111	111	54
7	84	86	85
8	52	51	4
9	136	137	67
10	109	112	28
11	120	121	35
12	18	17	95
13	25	29	40
14	46	42	77
15	54	53	39
16	49	47	88
H=	1	K=	9
0	52	51	25
1	51	52	17
2	58	55	1
3	53	52	57
4	152	157	59
5	61	57	40
6	99	102	98
7	35	33	11
8	82	83	12
9	14	17	75*
10	54	56	68
11	28	24	1
12	12	17	74*
13	44	41	91
14	55	53	57
15	17	13	32
H=	1	K=	10
0	261	277	25
1	66	67	4
2	67	69	26
3	54	56	4

L	FO	FC	AL
4	45	50	84
5	29	27	75
6	14	16	41*
7	50	48	37
8	107	109	85
9	18	16	44
10	50	53	93
11	12	16	53*
12	27	28	77
13	41	41	28
14	16	16	59
H=	1	K=	11
0	20	25	75
1	37	36	54
2	18	24	41
3	42	40	25
4	28	28	65
5	14	17	32*
6	86	87	65
7	13	14	74*
8	19	7	81
9	43	45	10
10	11	9	52*
11	35	35	73
12	41	42	40
H=	1	K=	12
0	31	27	75
1	24	29	96
2	52	52	51
3	46	46	24
4	76	73	9
5	74	75	4
6	54	56	96
7	44	41	57
8	88	90	15
9	14	12	75
10	41	38	76
11	20	18	69
H=	1	K=	13
0	17	19	25
1	55	56	89
2	57	57	29
3	35	35	72
4	46	46	10
5	68	67	70
6	43	42	80
7	54	56	73
8	28	26	88
H=	1	K=	14
0	31	35	75
1	23	23	66
2	29	28	23
3	43	47	33
4	14	19	6
5	27	31	77

L	FO	FC	AL
H=	2	K=	0
0	101	105	0
1	37	37	75
2	377	389	50
3	234	239	75
4	36	36	0
5	119	121	25
6	53	53	0
7	15	17	75
8	10	4	0*
9	75	75	25
10	36	38	0
11	30	30	25
12	85	84	0
13	13	8	75*
14	127	132	50
15	72	74	25
16	25	23	50
17	58	62	75
18	11	9	50*
19	42	43	25
H=	2	K=	1
0	431	449	50
1	258	262	6
2	289	295	40
3	131	133	60
4	163	164	19
5	174	171	95
6	135	136	51
7	110	113	19
8	25	26	0
9	77	73	72
10	138	137	8
11	74	74	37
12	64	68	88
13	90	93	59
14	68	67	82
15	55	57	75
16	93	95	67
17	51	52	98
18	32	31	78
19	22	20	40
H=	2	K=	2
0	202	204	50
1	242	251	61
2	299	300	24
3	212	219	93
4	130	130	66
5	271	272	78
6	167	161	76
7	158	157	93
8	80	77	91
9	64	60	24
10	142	140	84
11	103	103	36

L	FO	FC	AL
12	26	23	73
13	58	56	44
14	41	41	71
15	75	77	28
16	25	14	6
17	57	55	5
18	35	37	26
19	41	41	12
H=	2	K=	3
0	225	227	50
1	216	222	55
2	127	128	1
3	338	345	46
4	68	69	45
5	237	237	63
6	180	182	39
7	110	111	52
8	57	60	92
9	148	145	2
10	62	63	29
11	145	145	12
12	40	41	85
13	114	112	99
14	67	69	12
15	27	26	64
16	38	38	35
17	15	17	86
18	34	31	6
H=	2	K=	4
0	196	195	0
1	152	152	51
2	167	165	0
3	246	250	46
4	159	158	76
5	137	131	66
6	138	139	20
7	15	13	49
8	52	52	1
9	59	60	0
10	78	76	56
11	47	46	27
12	103	102	29
13	129	132	1
14	32	30	49
15	80	79	53
16	53	53	92
17	16	13	85
18	10	11	98*
H=	2	K=	5
0	11	8	50*
1	44	38	99
2	47	46	5
3	69	64	29
4	217	208	67
5	81	83	93

L	FO	FC	AL
6	59	55	36
7	103	96	38
8	26	28	9
9	59	59	47
10	39	40	35
11	208	208	43
12	138	148	27
13	52	52	45
14	82	85	8
15	13	11	70*
16	52	54	89
17	10	2	95*
18	26	25	94
H=	2	K=	6
0	81	77	0
1	78	79	59
2	39	33	97
3	81	80	12
4	72	71	35
5	40	39	40
6	131	128	55
7	105	106	30
8	54	50	99
9	46	45	89
10	103	97	56
11	13	7	32*
12	31	29	83
13	41	40	46
14	17	21	41
15	49	48	80
16	20	14	90
17	34	34	84
H=	2	K=	7
0	102	102	50
1	12	7	50*
2	133	133	43
3	128	121	79
4	19	16	48
5	78	77	73
6	100	97	85
7	89	88	33
8	106	109	74
9	29	30	27
10	125	128	99
11	96	96	51
12	45	46	9
13	50	50	92
14	20	19	56
15	23	21	37
16	20	21	65
H=	2	K=	8
0	44	46	50
1	75	74	1
2	27	32	26
3	58	62	5

L	FO	FC	AL
4	122	123	36
5	83	87	88
6	84	84	47
7	143	147	4
8	18	17	18
9	88	93	96
10	138	139	7
11	18	17	25
12	19	24	91
13	42	45	62
14	31	30	13
15	21	19	13
16	47	48	41
H=	2	K=	9
0	83	84	0
1	51	53	24
2	108	109	18
3	119	118	52
4	64	65	38
5	135	135	61
6	70	68	88
7	127	131	66
8	61	63	43
9	19	24	17
10	40	45	70
11	59	59	97
12	21	20	2
13	34	35	3
14	46	47	36
15	21	22	55
H=	2	K=	10
0	95	98	0
1	51	53	33
2	93	93	60
3	121	122	57
4	62	59	13
5	74	75	6
6	36	39	24
7	38	35	88
8	13	4	11*
9	32	24	97
10	68	68	13
11	27	16	53
12	32	33	12
13	48	49	64
H=	2	K=	11
0	14	18	0*
1	142	146	14
2	50	53	72
3	63	64	17
4	35	35	32
5	98	95	11
6	85	86	77
7	65	64	87
8	32	30	52

L	FO	FC	AL
9	44	45	67
10	11	5	99*
11	34	35	46
12	13	12	30
H=	2	K=	12
0	20	18	0
1	59	64	77
2	75	79	42
3	24	24	89
4	37	35	36
5	12	8	41*
6	47	46	49
7	15	14	11
8	52	52	24
9	74	73	50
10	11	12	9*
H=	2	K=	13
0	109	108	50
1	27	27	55
2	12	11	34*
3	17	10	39
4	44	41	14
5	35	33	7
6	35	35	83
7	37	40	33
8	51	52	82
H=	2	K=	14
0	48	49	0
1	19	20	98
2	32	35	20
3	56	56	35
4	53	56	57
H=	3	K=	0
1	429	445	25
2	236	240	50
3	146	147	25
4	255	268	0
5	171	170	75
6	27	30	0
7	273	270	75
8	49	44	50
9	69	68	75
10	12	2	0*
11	72	71	75
12	12	9	50*
13	76	75	25
14	20	15	50
15	17	25	75
16	47	43	0
17	12	9	25*
18	29	30	50
19	13	15	75
H=	3	K=	1
0	272	278	75
1	369	377	86

L	FO	FC	AL
2	149	147	53
3	135	136	80
4	105	109	42
5	168	169	66
6	102	102	70
7	252	251	63
8	165	168	25
9	12	7	49*
10	93	92	5
11	84	85	7
12	32	30	14
13	154	158	27
14	49	54	67
15	73	75	41
16	41	43	71
17	36	38	44
18	26	27	86
H=	3	K=	2
0	179	184	25
1	333	341	93
2	154	152	55
3	121	124	38
4	110	110	94
5	46	46	87
6	138	136	66
7	165	163	58
8	147	151	95
9	31	30	80
10	38	37	3
11	48	51	44
12	85	84	24
13	28	27	9
14	48	49	75
15	90	89	2
16	37	41	17
17	40	39	65
18	28	31	57
H=	3	K=	3
0	359	372	25
1	157	159	91
2	151	152	31
3	26	30	52
4	89	88	71
5	78	76	78
6	175	171	71
7	103	100	98
8	51	51	92
9	109	112	7
10	136	135	61
11	98	99	55
12	94	94	21
13	142	145	32
14	44	48	18
15	40	42	35
16	69	70	32

L	FO	FC	AL
17	11	6	30*
18	32	30	90
H=	3	K=	4
0	411	414	75
1	69	69	20
2	45	43	30
3	79	80	77
4	142	144	49
5	63	60	7
6	145	144	29
7	105	106	41
8	79	75	9
9	62	60	90
10	30	28	48
11	44	44	39
12	16	12	91
13	68	68	38
14	59	60	53
15	12	3	54*
16	21	18	88
17	24	20	81
18	15	13	29
H=	3	K=	5
0	131	124	25
1	122	122	2
2	175	177	79
3	98	97	15
4	111	108	8
5	60	63	60
6	108	109	30
7	47	46	91
8	63	69	37
9	55	54	52
10	122	123	39
11	118	121	78
12	14	13	55*
13	42	40	71
14	110	111	79
15	82	81	62
16	19	15	1
17	10	9	11*
H=	3	K=	6
0	48	49	25
1	194	191	34
2	147	141	89
3	92	85	48
4	34	28	91
5	45	39	45
6	122	121	5
7	133	131	66
8	22	22	1
9	65	67	90
10	62	59	57
11	14	12	28*
12	59	60	3

L	FO	FC	AL
13	76	76	4
14	29	29	43
15	20	16	99
16	72	73	23
17	13	14	26
H=	3	K=	7
0	50	47	25
1	13	19	3*
2	71	72	79
3	64	63	78
4	38	41	49
5	89	90	59
6	39	38	61
7	77	80	64
8	57	55	76
9	47	47	88
10	104	106	91
11	28	29	24
12	13	10	96*
13	50	48	24
14	37	37	73
15	42	41	59
16	56	55	21
H=	3	K=	8
0	14	20	75*
1	67	65	85
2	63	59	94
3	51	51	10
4	107	104	77
5	56	57	69
6	114	112	80
7	63	66	90
8	51	56	10
9	30	34	82
10	49	48	41
11	29	34	12
12	29	27	56
13	49	50	99
14	64	65	25
15	63	62	48
H=	3	K=	9
0	234	238	25
1	96	97	25
2	108	110	28
3	52	56	43
4	96	93	30
5	76	81	16
6	45	46	83
7	37	41	18
8	72	74	83
9	20	25	91
10	90	94	75
11	19	20	30
12	26	17	59
13	40	39	64

L	FO	FC	AL
14	20	21	14
H=	3	K=	10
0	70	65	75
1	40	39	43
2	72	66	56
3	43	42	79
4	46	49	55
5	48	52	69
6	61	60	36
7	18	19	89
8	90	92	51
9	30	27	29
10	70	68	29
11	26	32	77
12	53	54	47
13	21	20	39
H=	3	K=	11
0	98	103	75
1	49	48	54
2	81	80	63
3	13	13	72*
4	71	70	29
5	40	40	17
6	19	13	3
7	52	50	63
8	132	133	35
9	11	10	87*
10	79	78	34
11	51	49	76
H=	3	K=	12
0	39	39	25
1	44	46	89
2	32	31	50
3	41	38	44
4	50	51	17
5	26	29	90
6	25	25	65
7	63	60	76
8	20	23	44
9	31	35	45
10	44	44	4
H=	3	K=	13
0	44	46	75
1	22	21	73
2	37	40	71
3	63	62	62
4	59	59	53
5	38	40	28
6	38	40	59
7	14	16	45
H=	3	K=	14
0	19	21	25
1	31	33	98
2	39	39	22
H=	4	K=	0

L	FO	FC	AL
0	142	138	0
1	112	113	25
2	40	39	0
3	39	39	75
4	27	27	0
5	84	82	25
6	77	77	50
7	32	33	75
8	95	100	0
9	43	40	75
10	48	44	0
11	25	19	75
12	25	22	0
13	31	28	75
14	19	23	50
15	38	42	25
16	73	73	50
17	35	36	75
18	35	31	0
H=	4	K=	1
0	233	243	50
1	176	177	10
2	188	189	38
3	61	57	71
4	22	18	88
5	111	110	96
6	105	99	86
7	11	10	1*
8	216	219	4
9	12	19	22*
10	107	100	93
11	48	46	21
12	172	179	31
13	12	6	99*
14	48	49	29
15	23	19	62
16	17	19	56
17	11	11	40*
18	20	20	72
H=	4	K=	2
0	78	80	0
1	186	187	65
2	139	136	92
3	97	95	39
4	99	97	64
5	116	116	43
6	102	101	44
7	12	17	34*
8	108	105	30
9	73	74	4
10	76	78	85
11	55	59	3
12	126	128	42
13	67	66	66
14	94	95	5

L	FO	FC	AL
15	21	19	14
16	38	37	56
17	35	35	50
18	51	50	99
H=	4	K=	3
1	22	20	45
2	95	91	93
3	49	47	30
4	138	137	18
5	122	120	85
6	21	20	87
7	24	24	17
8	86	87	10
9	17	13	36
10	28	26	48
11	38	38	76
12	61	65	52
13	71	78	9
14	61	69	91
15	23	22	81
16	60	61	65
17	20	21	21
18	29	31	68
H=	4	K=	4
0	97	93	50
1	134	135	4
2	83	84	45
3	162	162	4
4	126	122	49
5	147	148	99
6	73	75	5
7	95	91	12
8	44	43	44
9	35	40	90
10	98	97	86
11	135	132	52
12	43	50	18
13	36	35	85
14	27	27	71
15	28	27	59
16	20	20	89
17	10	14	43*
H=	4	K=	5
0	12	9	50*
1	57	59	91
2	133	130	22
3	90	91	95
4	69	71	19
5	36	33	23
6	68	64	46
7	45	49	73
8	107	108	51
9	128	132	93
10	73	76	48
11	80	83	63

L	FO	FC	AL
12	29	33	69
13	49	47	45
14	114	112	92
15	29	30	50
16	25	20	98
17	59	60	23
H=	4	K=	6
0	124	121	50
1	80	82	17
2	94	95	63
3	79	75	85
4	103	105	65
5	154	156	83
6	125	128	82
7	53	55	6
8	68	69	98
9	59	59	65
10	110	115	95
11	93	95	94
12	126	125	90
13	46	45	39
14	98	98	59
15	54	55	8
16	26	27	39
H=	4	K=	7
0	179	179	50
1	75	74	17
2	110	107	48
3	25	26	82
4	110	114	18
5	80	79	21
6	78	79	97
7	81	82	83
8	59	61	86
9	72	73	6
10	97	97	14
11	12	9	91*
12	26	23	41
13	77	73	91
14	59	61	45
15	19	16	15
H=	4	K=	8
0	37	41	0
1	46	49	76
2	94	93	91
3	102	100	45
4	108	114	56
5	95	94	44
6	27	28	74
7	45	44	93
8	60	57	43
9	43	40	49
10	54	54	15
11	48	47	13
12	24	29	65

L	FO	FC	AL
13	45	45	68
14	28	31	10
15	52	52	73
H=	4	K=	9
0	83	80	0
1	45	47	42
2	39	44	98
3	138	142	48
4	58	54	72
5	13	6	53*
6	55	55	6
7	37	42	46
8	69	73	59
9	127	127	39
10	38	38	63
11	42	38	44
12	22	21	10
13	11	12	86*
14	16	13	52
H=	4	K=	10
0	62	63	50
1	104	106	27
2	33	32	76
3	48	44	29
4	67	67	39
5	80	80	17
6	13	3	50*
7	33	36	77
8	75	78	78
9	26	23	8
10	46	44	54
11	28	28	33
12	33	31	83
H=	4	K=	11
0	13	17	0*
1	58	61	95
2	23	22	15
3	59	62	61
4	47	48	63
5	12	8	41*
6	17	13	36
7	23	23	13
8	38	41	45
9	19	20	21
10	42	41	50
11	25	28	47
H=	4	K=	12
0	56	58	50
1	72	70	38
2	26	22	91
3	51	48	83
4	38	38	96
5	65	64	63
6	30	31	31
7	30	31	84

L	FO	FC	AL
8	49	51	9
9	16	19	21
H=	4	K=	13
0	14	5	0
1	27	30	21
2	44	46	7
3	70	69	27
4	44	42	3
5	51	52	76
6	59	58	93
H=	5	K=	0
1	11	15	75*
2	14	7	50
3	249	260	75
4	11	17	50*
5	12	14	75*
6	12	16	50*
7	39	39	75
8	110	112	50
9	109	112	25
10	32	30	0
11	45	48	25
12	36	36	50
13	215	221	25
14	43	42	50
15	50	50	75
16	11	2	0*
17	60	59	75
H=	5	K=	1
0	11	7	75*
1	93	87	95
2	134	136	89
3	92	96	59
4	24	20	18
5	109	111	12
6	111	113	95
7	74	75	88
8	41	42	22
9	126	123	90
10	71	70	23
11	55	58	64
12	61	61	52
13	42	50	58
14	60	61	30
15	22	27	71
16	37	40	4
17	25	26	64
H=	5	K=	2
0	336	336	25
1	101	98	50
2	92	87	87
3	216	218	35
4	73	76	61
5	131	128	36
6	86	83	1

L	FO	FC	AL
7	55	55	7
8	81	81	80
9	88	89	12
10	24	26	90
11	68	71	83
12	41	40	35
13	36	39	93
14	17	19	91
15	43	40	89
16	58	57	17
17	27	25	44
H=	5	K=	3
0	27	24	25
1	49	45	32
2	117	118	87
3	95	95	13
4	133	130	55
5	49	54	24
6	100	100	83
7	145	144	47
8	116	118	93
9	13	14	14*
10	26	26	54
11	64	65	63
12	38	39	20
13	34	36	89
14	74	77	33
15	30	30	72
16	33	35	43
17	36	35	94
H=	5	K=	4
0	75	78	75
1	185	183	35
2	51	49	63
3	37	37	76
4	135	135	72
5	93	93	91
6	30	29	23
7	32	33	76
8	13	21	2*
9	53	51	90
10	111	111	29
11	86	87	93
12	29	25	46
13	65	68	76
14	12	10	76*
15	54	56	74
16	22	19	85
17	36	36	30
H=	5	K=	5
0	44	45	75
1	81	79	45
2	44	46	32
3	37	32	50
4	40	45	62

L	FO	FC	AL
5	58	57	54
6	53	57	8
7	67	69	1
8	49	50	10
9	63	61	40
10	63	66	8
11	26	27	0
12	68	68	8
H= 5 K= 6			
13	56	54	85
14	65	64	79
15	48	52	47
16	10	14	37*
H= 5 K= 6			
0	51	53	25
1	83	83	39
2	80	78	64
3	204	205	75
4	40	40	46
5	74	76	59
6	110	111	54
7	105	108	79
8	53	51	67
9	30	32	38
10	54	53	47
11	107	106	25
12	40	39	12
13	78	80	17
14	11	9	69*
15	57	56	99
H= 5 K= 7			
0	68	73	75
1	48	44	95
2	60	63	56
3	112	113	75
4	29	26	75
5	59	55	47
6	56	57	11
7	33	32	94
8	72	73	35
9	93	93	89
10	34	35	35
11	31	34	15
12	96	97	46
13	14	13	8
14	44	38	90
15	53	54	43
H= 5 K= 8			
0	36	44	25
1	55	53	19
2	147	150	28
3	141	144	28
4	97	96	41
5	107	110	31
6	83	88	62
7	35	37	85

L	FO	FC	AL
8	37	34	26
9	62	62	64
10	46	45	47
11	30	30	76
12	27	28	84
13	25	25	81
14	35	33	0
H= 5 K= 9			
0	25	22	25
1	50	53	8
2	104	103	47
3	66	67	44
4	32	26	53
5	36	38	45
6	39	37	41
7	48	51	41
8	12	11	90*
9	20	22	34
10	19	17	75
11	50	48	7
12	76	72	45
13	9	9	95*
H= 5 K= 10			
0	28	29	75
1	60	60	39
2	59	61	39
3	40	40	87
4	69	72	74
5	35	30	22
6	32	30	53
7	45	49	68
8	36	31	29
9	11	5	85*
10	78	75	30
11	42	44	84
H= 5 K= 11			
0	16	13	25
1	41	36	3
2	35	35	24
3	22	23	30
4	32	30	97
5	26	23	20
6	39	43	83
7	33	30	12
8	22	14	97
9	46	44	20
10	77	79	1
H= 5 K= 12			
0	37	37	25
1	37	36	86
2	30	32	91
3	24	25	74
4	34	32	90
5	28	29	1
6	41	40	97

L	FO	FC	AL
7	34	31	80
H= 5 K= 13			
0	50	54	25
1	56	53	89
2	44	45	4
3	29	31	6
H= 6 K= 0			
0	143	139	50
1	50	48	25
2	32	32	50
3	79	77	75
4	116	117	50
5	12	10	25*
6	45	47	50
7	46	43	25
8	169	170	0
9	44	42	25
10	111	113	0
11	38	38	75
12	39	37	0
13	27	29	25
14	71	69	50
15	15	11	75
16	51	51	50
17	29	27	25
H= 6 K= 1			
0	15	2	50
1	61	61	89
2	140	141	24
3	45	48	91
4	47	48	53
5	69	72	83
6	158	158	47
7	81	83	89
8	100	104	58
9	77	81	90
10	104	106	75
11	77	79	20
12	59	59	5
13	56	61	43
14	31	32	93
15	33	32	91
16	42	41	93
H= 6 K= 2			
0	12	1	50*
1	190	191	49
2	198	200	3
3	113	111	62
4	26	29	41
5	89	92	61
6	135	136	75
7	111	112	79
8	104	108	73
9	84	84	13
10	52	52	76

L	FO	FC	AL
11	62	60	4
12	95	96	64
13	68	70	9
14	26	21	93
15	36	35	6
16	68	65	45
H= 6 K= 3			
0	30	30	0
1	33	30	62
2	108	105	56
3	54	59	42
4	18	17	53
5	87	87	81
6	53	54	70
7	143	147	7
8	79	78	55
9	29	32	1
10	99	104	16
11	63	60	1
12	65	63	15
13	24	24	34
14	15	14	79
15	32	31	75
16	10	4	11*
H= 6 K= 4			
0	84	86	0
1	82	81	5
2	90	91	85
3	92	95	2
4	29	30	64
5	43	42	62
6	96	101	81
7	57	56	63
8	114	115	51
9	13	2	51*
10	27	24	46
11	147	148	44
12	13	20	41*
13	16	14	85
14	33	33	35
15	75	74	67
16	35	33	92
H= 6 K= 5			
0	48	48	50
1	117	120	57
2	46	49	63
3	71	70	72
4	41	44	68
5	43	42	47
6	133	137	81
7	115	118	28
8	80	83	88
9	27	31	42
10	60	58	86
11	59	59	48

L	FO	FC	AL
12	24	23	82
13	12	9	6*
14	17	19	24
15	14	13	61
H=	6	K=	6
0	73	72	0
1	64	65	42
2	45	46	49
3	93	92	28
4	52	54	55
5	72	73	38
6	50	51	26
7	74	75	30
8	67	70	73
9	40	41	57
10	62	65	98
11	54	57	95
12	17	18	72
13	62	61	86
14	72	73	48
H=	6	K=	7
0	27	25	0
1	40	36	71
2	85	84	93
3	21	24	4
4	103	104	34
5	54	53	45
6	98	99	62
7	53	49	71
8	47	44	79
9	56	59	50
10	46	47	40
11	72	72	33
12	52	52	8
13	18	19	66
14	16	21	22
H=	6	K=	8
0	78	84	0
1	63	69	36
2	86	87	12
3	99	99	44
4	44	41	52
5	50	55	50
6	48	50	19
7	32	33	96
8	78	81	59
9	67	64	54
10	41	37	79
11	38	37	74
12	38	38	47
13	30	31	73
H=	6	K=	9
0	31	31	0
1	69	73	30
2	34	31	6

L	FO	FC	AL
3	49	47	2
4	13	5	4*
5	30	32	72
6	17	22	74
7	43	44	73
8	12	12	4*
9	27	25	12
10	20	16	56
11	31	27	84
H=	6	K=	10
0	15	9	50
1	52	51	1
2	18	13	56
3	72	72	96
4	25	20	83
5	85	81	94
6	18	16	4
7	24	26	13
8	19	20	7
9	26	27	85
10	46	48	25
H=	6	K=	11
0	12	12	50*
1	15	13	14
2	39	39	8
3	44	48	78
4	59	61	0
5	19	22	63
6	19	18	18
7	10	7	98*
8	56	56	21
H=	6	K=	12
0	37	39	50
1	54	54	58
2	22	22	10
3	11	14	66*
4	10	19	2*
5	36	37	39
H=	7	K=	0
1	79	77	25
2	27	29	0
3	28	27	75
4	25	23	50
5	45	47	75
6	71	72	0
7	184	184	75
8	70	71	0
9	94	92	75
10	43	38	0
11	48	52	75
12	68	72	0
13	76	75	25
14	41	41	0
15	27	30	75
H=	7	K=	1

L	FO	FC	AL
0	19	9	25
1	30	26	17
2	49	48	57
3	84	83	48
4	63	64	69
5	109	111	51
6	48	47	18
7	72	73	61
8	107	113	85
9	13	17	8*
10	45	46	7
11	78	82	4
12	12	16	7*
13	39	38	88
14	51	48	10
15	19	19	87
H=	7	K=	2
0	99	97	25
1	49	50	32
2	81	83	99
3	92	87	19
4	39	38	59
5	32	33	22
6	71	70	91
7	49	49	52
8	78	81	79
9	50	49	34
10	40	45	77
11	53	55	30
12	72	72	22
13	28	29	90
14	43	42	80
15	21	23	31
H=	7	K=	3
0	108	116	75
1	63	64	66
2	51	51	69
3	75	75	91
4	70	71	55
5	135	140	77
6	75	73	28
7	81	81	85
8	116	116	28
9	90	91	94
10	120	125	39
11	29	25	87
12	49	48	85
13	39	41	32
14	46	46	75
15	36	37	54
H=	7	K=	4
0	83	81	75
1	55	55	17
2	72	73	36
3	73	78	46

L	FO	FC	AL
4	83	84	15
5	53	57	19
6	112	119	28
7	79	83	40
8	18	22	25
9	28	23	32
10	50	49	23
11	25	30	40
12	84	84	66
13	38	38	8
14	71	72	77
15	54	53	93
H=	7	K=	5
0	13	13	75*
1	110	113	81
2	174	179	21
3	114	116	58
4	54	54	97
5	71	73	64
6	45	46	66
7	38	36	91
8	70	71	64
9	26	26	80
10	72	75	60
11	30	31	82
12	19	21	17
13	23	27	0
14	22	20	53
H=	7	K=	6
0	47	46	25
1	52	50	49
2	84	86	48
3	14	11	59*
4	38	39	92
5	79	79	20
6	36	39	33
7	62	61	87
8	12	8	75*
9	22	20	82
10	84	82	73
11	32	29	40
12	48	47	50
13	67	68	26
H=	7	K=	7
0	14	22	25*
1	46	43	3
2	108	113	21
3	35	34	20
4	49	50	11
5	58	59	36
6	39	43	73
7	42	38	36
8	68	71	93
9	32	31	6
10	65	64	89

L	FO	FC	AL
11	26	25	62
12	63	65	71
H=	7	K=	8
0	23	19	25
1	14	17	26*
2	64	67	11
3	80	80	24
4	28	30	80
5	53	51	97
6	64	66	81
7	33	31	10
8	39	37	49
9	48	49	27
10	35	35	78
11	47	48	19
H=	7	K=	9
0	41	44	25
1	29	26	56
2	30	31	63
3	13	15	22*
4	27	24	81
5	59	62	95
6	50	49	14
7	45	45	39
8	33	32	14
9	10	8	6*
10	40	40	16
H=	7	K=	10
0	82	80	75
1	32	29	99
2	17	13	79
3	41	43	98
4	43	42	54
5	28	28	63
6	25	22	10
7	11	11	84*
8	12	23	27
H=	7	K=	11
0	53	53	25
1	10	9	13*
2	26	26	49
3	34	33	61
4	50	51	94
5	10	18	4*
H=	8	K=	0
0	13	12	0*
1	112	114	75
2	101	108	50
3	36	39	75
4	33	38	0
5	13	0	19*
6	67	67	50
7	32	30	75
8	47	47	50
9	33	26	75

L	FO	FC	AL
10	28	26	0
11	50	49	25
12	23	23	0
13	54	52	25
14	22	28	50
H=	8	K=	1
0	117	121	0
1	67	67	55
2	28	31	97
3	24	27	50
4	82	88	83
5	41	43	51
6	130	136	75
7	13	14	25*
8	37	36	62
9	19	24	41
10	12	10	56*
11	50	50	16
12	12	15	25*
13	70	72	90
14	16	18	4
H=	8	K=	2
0	18	15	0
1	58	57	55
2	69	69	86
3	13	14	63*
4	120	125	29
5	19	16	97
6	25	23	62
7	24	24	27
8	31	38	23
9	68	69	39
10	46	48	28
11	11	14	55*
12	32	31	43
13	47	45	44
14	31	32	7
H=	8	K=	3
0	83	81	0
1	91	92	21
2	124	128	53
3	112	112	8
4	37	34	28
5	42	42	39
6	92	88	12
7	68	66	84
8	39	40	66
9	62	61	45
10	64	61	0
11	73	73	54
12	11	4	5*
13	102	102	53
14	15	19	97
H=	8	K=	4
0	28	30	0

L	FO	FC	AL
1	73	72	8
2	84	85	68
3	116	116	56
4	47	48	36
5	37	36	65
6	57	56	86
7	23	20	29
8	50	49	10
9	25	27	34
10	56	55	76
11	55	56	45
12	53	52	71
13	40	37	47
H=	8	K=	5
0	65	64	50
1	103	110	36
2	115	114	45
3	24	24	48
4	17	19	24
5	48	42	48
6	19	18	4
7	48	48	77
8	49	47	22
9	43	44	84
10	18	20	88
11	53	55	90
12	15	15	47
13	19	16	18
H=	8	K=	6
0	13	15	0*
1	41	41	66
2	44	44	70
3	47	44	98
4	46	46	91
5	41	42	87
6	12	9	67*
7	51	47	78
8	54	54	7
9	22	21	3
10	52	51	14
11	11	14	5*
12	16	10	79
H=	8	K=	7
0	25	23	50
1	36	32	56
2	63	65	18
3	30	32	61
4	73	76	13
5	52	54	62
6	51	52	39
7	20	20	26
8	28	26	58
9	50	48	40
10	98	96	38
11	16	18	8

L	FO	FC	AL
H=	8	K=	8
0	48	48	0
1	12	12	87*
2	104	104	9
3	29	28	51
4	68	66	64
5	20	24	48
6	36	34	29
7	18	20	98
8	20	20	34
9	46	46	23
H=	8	K=	9
0	77	76	50
1	18	18	48
2	27	29	28
3	41	40	82
4	11	9	47*
5	32	29	80
6	16	13	50
7	10	13	57*
8	73	71	90
H=	8	K=	10
0	24	21	0
1	43	41	33
2	35	33	39
3	67	69	49
4	10	7	50*
5	10	10	32*
H=	9	K=	0
1	24	22	25
2	62	67	0
3	55	59	25
4	13	10	50*
5	13	11	25*
6	111	108	0
7	32	31	75
8	12	12	50*
9	43	44	75
10	11	15	50*
11	24	23	75
12	25	22	50
13	17	20	25
H=	9	K=	1
0	104	103	25
1	48	44	14
2	13	5	37*
3	118	120	27
4	24	22	3
5	123	122	26
6	48	44	42
7	28	28	16
8	80	77	76
9	18	20	3
10	50	47	11
11	48	47	81

L	FO	FC	AL
12	70	71	57
H=	9	K=	2
0	90	87	25
1	66	70	86
2	24	26	40
3	13	4	98*
4	67	67	53
5	61	62	69
6	60	59	96
7	16	15	75
8	84	79	20
9	68	67	90
10	11	12	65*
11	44	43	11
12	27	25	55
H=	9	K=	3
0	25	30	25
1	13	11	44*
2	57	57	30
3	22	25	20
4	46	42	58
5	49	48	37
6	51	52	74
7	13	18	31*
8	37	36	41
9	44	44	79
10	50	52	25
11	34	30	54
12	52	52	47
H=	9	K=	4
0	13	1	26*
1	43	44	67
2	49	48	39
3	57	58	54
4	44	43	12
5	85	84	77
6	59	58	81
7	64	64	94
8	83	80	91
9	17	18	74
10	75	73	4
11	15	13	87
H=	9	K=	5
0	20	16	75
1	57	58	87
2	53	53	42
3	52	50	71
4	89	91	71
5	41	42	87
6	25	16	96
7	34	34	61
8	42	41	46
9	35	32	46
10	18	17	77
11	46	49	22

L	FO	FC	AL
H=	9	K=	6
0	13	1	75*
1	31	28	23
2	68	66	77
3	84	82	15
4	52	52	99
5	25	24	24
6	36	36	50
7	35	35	63
8	31	32	11
9	25	22	38
10	19	21	0
H=	9	K=	7
0	100	102	75
1	67	68	12
2	47	45	26
3	74	76	10
4	43	43	51
5	24	24	51
6	31	30	46
7	38	33	82
8	55	56	28
H=	9	K=	8
0	63	61	25
1	14	16	13
2	63	64	52
3	29	29	22
4	10	9	44*
5	44	42	51
6	30	29	50
7	53	53	41
H=	9	K=	9
0	38	37	75
1	10	6	0*
2	14	16	52
3	42	40	67
4	52	50	46
H=	10	K=	0
0	158	159	0
1	46	48	25
2	34	33	50
3	34	33	75
4	45	41	50
5	48	48	25
6	55	55	50
7	45	43	75
8	11	2	0*
9	35	40	25
10	35	36	0
H=	10	K=	1
0	88	87	0
1	52	52	14
2	57	56	23
3	33	33	60
4	98	97	27

L	FO	FC	AL
5	34	30	88
6	46	45	34
7	11	3	97*
8	45	41	74
9	10	16	59*
10	26	24	41
H=	10	K=	2
0	47	45	50
1	20	25	25
2	70	70	64
3	25	24	6
4	57	55	42
5	12	9	64*
6	35	32	81
7	11	15	91*
8	74	74	93
9	25	27	16
10	48	47	0
H=	10	K=	3
0	12	21	0*
1	12	13	59*
2	18	17	65
3	17	18	67
4	35	36	66
5	11	9	44*
6	14	11	92
7	65	69	2
8	50	50	59
9	32	34	18
10	44	46	23
H=	10	K=	4
0	32	30	50
1	41	43	93
2	52	51	38
3	56	56	63
4	30	28	78
5	36	36	69
6	42	42	56
7	46	46	53
8	14	14	95
9	10	11	29*
H=	10	K=	5
0	46	49	50
1	57	58	65
2	68	69	20
3	30	29	42
4	50	52	89
5	42	43	20
6	52	52	88
7	28	30	26
8	10	12	23*
H=	10	K=	6
0	68	69	0
1	43	45	46
2	35	37	87

L	FO	FC	AL
3	36	37	16
4	40	41	11
5	20	20	88
6	123	123	46
7	34	35	43
H=	10	K=	7
0	11	12	0*
1	33	32	36
2	31	30	28
3	61	62	19
4	32	31	43
5	34	35	51
H=	10	K=	8
0	10	15	50*
1	18	16	26
H=	11	K=	0
1	49	47	25
2	41	42	0
3	26	24	75
4	10	8	50*
5	10	5	25*
6	37	40	50
7	13	14	75
H=	11	K=	1
0	11	2	75*
1	28	30	80
2	62	62	66
3	11	11	79*
4	32	34	82
5	60	57	59
6	11	17	21*
7	47	46	88
H=	11	K=	2
0	70	68	75
1	30	29	67
2	48	49	68
3	11	2	97*
4	16	19	49
5	40	38	81
6	27	23	59
7	64	66	58
H=	11	K=	3
0	63	62	75
1	10	8	68*
2	35	34	54
3	21	21	82
4	23	27	20
5	60	60	76
6	44	45	65
H=	11	K=	4
0	18	12	25
1	10	9	65*
2	98	101	34
3	32	32	35
4	13	16	71

L	FO	FC	AL
5	27	28	88
H=	11	K=	5
0	44	45	25

L	FO	FC	AL
1	27	29	74
2	26	22	57
3	12	16	57

L	FO	FC	AL
4	19	23	78
H=	12	K=	0
0	63	65	0

LOCATION OF METHYL GROUP IN METHYLGORGOSTANOL

INTRODUCTION

Gorgosterol was first discovered by Bergmann[1] in 1943, and its structure was solved in 1970[2]. This C_{30} sterol has a cyclopropane moiety at position 22, 23 in the side chain. Several other sterols carrying cyclopropane moiety have been reported[3]. Methylgorgostanol, a C_{31} sterol, is one of these.

Methylgorgostanol (M. Wt. 442) was first reported by Steudler[4] in his comprehensive study of sterols from various sea animals. This sterol is the major component (18.93%) of the sterols isolated from the zooxanthellae of *Briareum asbestinum* (Pallas), collected at Enrique reef off La Parguera, Puerto Rico. However, methylgorgostanol makes up only 2.28% of the total sterols of *B. asbestinum*. The major sterol in *B. asbestinum* is gorgosterol[4] (26.99%). It is likely that the animal and its symbiotic algae, the zooxanthellae, have an integrated sterol synthesis system.

Since the possible number of structural isomers for a C_{31} sterol with cyclopropyl substituted side chain is twenty[5], it is the purpose of the research reported in this section to reconfirm the location

of the methyl group in the methylgorgostanol isolated from *Briareum* and its zooxanthellae.

RESULTS AND DISCUSSION

The animal used in this study was the gorgonian *Briareum asbestinum* (Pallas) from Jamaica, collected by Professor Leon S. Ciereszko in 1966. This animal contains a small amount of 442 sterol and a large quantity of gorgosterol. Since these two sterols have very close gas chromatographic retention times, it was necessary to chromatograph a sample several times to obtain a pure sample. A small amount of g.c. pure 442 sterol was isolated and the low resolution mass spectrum was obtained (Fig. 1). The M^+ ion is 442. The base peak is m/e 330, which corresponds to cleavage across the cyclopropane ring[2,4]. The overall fragmentation pattern is similar to the one obtained by Steudler[4] from g.c./m.s. analysis. The only difference between the two patterns is the relative abundance of the ion fragments in the high m/e region. This may be due to the high temperature in the g.c./m.s. connecting line (det. 260°C) in Steudler's study. In my study the solid sample was introduced into the ionization chamber directly with a solid sample probe, and the sample was vaporized at 100°C inside the chamber. The difference may be also due to different instrumental conditions. A sample of 4-methylgorgostanol was prepared by synthesis from gorgosterol and analyzed on the same mass spectrometer to further prove the structure of 4-methyl gorgostanol. Pure gorgosterol was used as the starting material. The

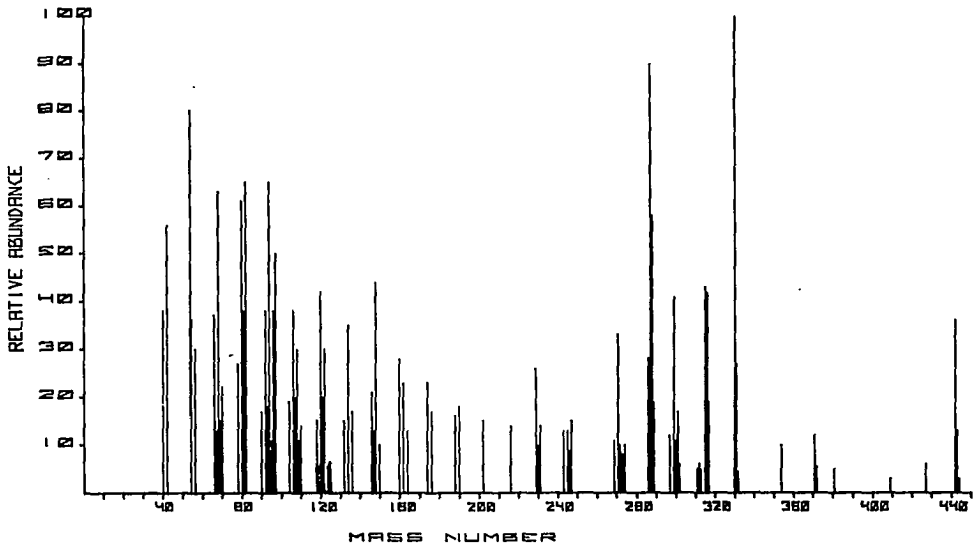
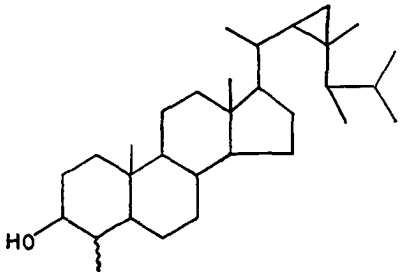


Figure 1 Mass Spectrum of 442 Sterol

gorgosterol was first converted to gorgost-4-en-3-one by treating with CrO_3/H_2SO_4 in acetone followed by base. This α,β-unsaturated ketone was then subjected to reductive methylation using $Li/NH_3/THF$ followed by addition of CH_3I. The methylated ketone was reduced by $LiAlH_4$ to the corresponding alcohol, 4-methylgorgostanol. The product was obtained as a yellow powder. After several recrystallizations from MeOH, a white powder was obtained and used for mass spectrum analysis. The result is shown in Figure 2.

There is no significant difference between the fragmentation of the synthetic and naturally occurring 442 sterol. This evidence strongly suggested that the extra methyl group in the methyl gorgostanol is located at the 4 position. The suggested structure of the 442 sterol is shown in the following:



The stereochemistry of the 4-methyl group in the 442 sterol is under investigation.

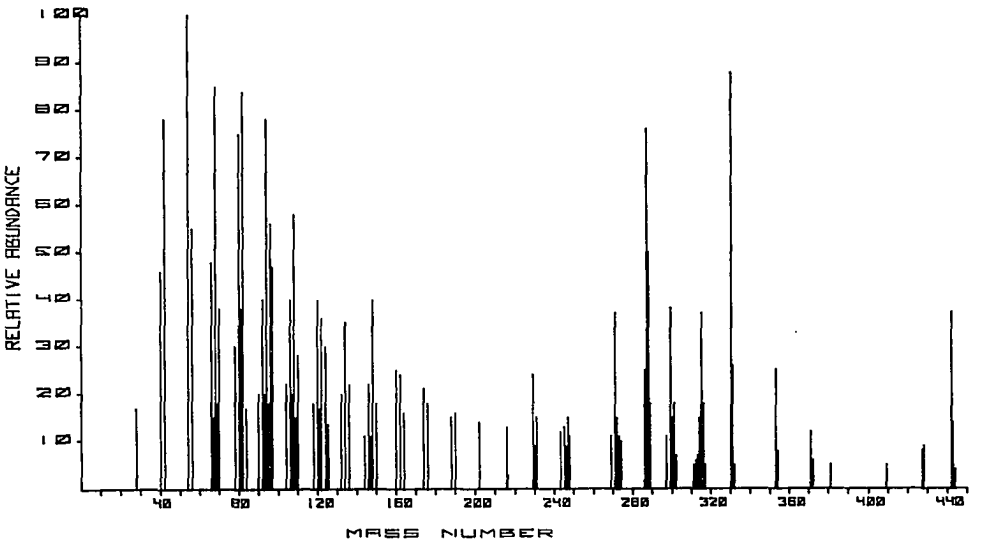


Figure 2 Mass Spectrum of 4-methylgorgostanol

SUMMARY

4-Methylgorgostanol was synthesized from gorgosterol. The mass spectrum of the synthetic compound is virtually identical to that of the naturally occurring 442 sterol isolated from *Briareum asbestinum* (Pallas). It is therefore suggested that the 442 sterol is 4-methylgorgostanol. The stereochemistry of the 4-methyl group is not known.

EXPERIMENTAL

General experimental conditions specified in section I apply with the following addition.

Analytic and preparative gas chromatography were carried out on a Varian 1200 instrument using a 2% OV-17 column (6xl/8" OD for analytic work and an 8xl/4" for preparative work, OV-17 on 80-100 mesh Gas Chrom Q, Applied Science Lab., Inc.). The operating conditions were: injection port 280°C, column 260°C, detector 300°C, helium flow rate 20 ml/min..

High performance liquid chromatography[6] was carried out on a Chromatec LC3000 (Chromatec. Inc.) equipped with R.I. (R401) and U.V. detector (Waters Assoc.) and a μBondapak C_{18} / Porasil B (Water Assoc.) column. The mobile phase was methanol-chloroform-water (85:6:9, v/v).

The mass spectra were taken on a LKB-9000 mass spectrometer (LKB Instruments, Inc.), at Oklahoma State University.

Preparation of sterol from *Briareum asbestinum* (Pallas)

The *B. asbestinum* was collected by Professor Ciereszko in 1966 at South Cay near Port Royal, Jamaica and was dried by the same method used in section I.

The lipids were isolated from the animal by hexane extraction.

The hexane extract was concentrated on the rotary evaporator to dryness. The dried lipid fraction was saponified by dissolving the lipids into ten times of 15% potassium hydroxide in 85% methanol and was heated to reflux on a steam bath without any special care for five hours. At the end of the reflux the mixture was concentrated on a rotary evaporator to about 100 milliliters. The concentrated saponified mixture was then diluted with ten volumes of saturated sodium chloride solution and extracted exhaustively with diethyl ether in a liquid-liquid extractor. The ether extract was washed with saturated sodium chloride until the solution was neutral.

Preparation of 442 sterol and gorgosterol Bromination[7] was used to separate the unsaturated sterols from the saturated sterols. The unsaturated sterol fraction (containing gorgosterol) was debrominated by sodium iodide in absolute alcohol as mentioned by Schoenheimer[8]. The saturated and unsaturated sterols were then acetylated by dissolving the sterols in pyridine and acetic anhydride[9]. The sterol acetates were then separated by h.p.l.c.[6]. The fractions from h.p.l.c. were collected and deacetylated by reduction with $LiAlH_4$ and were analyzed by g.c. and m.s.. In some cases the gorgosterol was isolated directly from a preparative g.c. column.

Preparation of 4-methyl gorgostanol from gorgosterol Gorgosterol (30 mg) was dissolved in acetone (10-15°C) and chromium trioxide reagent was added as described by Djerassi, *et al.*[6]. The gorgost-5-en-3-one so obtained was heated on the steam bath in methanol/10% potassium hydroxide for 5 minutes and then neutralized with acetic acid to give

gorgost-4-en-3-one (15 mg).

According to the procedure of Stork *et al.*[11], gorgost-4-en-3-one (15 mg) was dissolved in dry tetrahydrofuran (THF) and was added dropwise to Li/NH_3 solution (0.1 g Li in 50 ml dry NH_3). The mixture was then stirred for 10 minutes after the addition was complete. Fifty milliliters of dry THF was added and the resulting solution was heated under reflux. Then ten grams of methyl iodide was added and the mixture heated under reflux for one hour. After one hour the solution was cooled to room temperature, diluted with water, and extracted with chloroform. The chloroform extract containing the 4-methylgorgostanone was dried. The crude 4-methylgorgostanone was reduced with $LiAlH_4$ to 4-methylgorgostanol and was purified by preparative gas chromatography.

The naturally occurring 4-methylgorgostanol has m.p. 209-11°C; mass spectrum (70 eV) m/e (rel. intensity) M^+, 442 (36), 427 (6), 409 (3), 381 (5), 372 (6), 371 (12), 354 (10), 332 (5), 331 (27), 330 (100) base peak, 317 (19), 316 (42), 315 (43), 313 (5), 312 (6), 311 (5), 302 (6), 301 (17), 300 (11), 299 (41), 297 (12), 289 (19), 288 (58), 287 (90), 286 (28), 274 (10), 273 (8), 272 (10), 271 (33), 269 (11), 247 (15), 246 (9), 245 (13), 243 (13), 231 (14), 230 (10), 229 (26), 216 (14), 202 (15), 190 (18), 188 (16), 176 (17), 174 (23), 164 (13), 162 (23), 160 (28), 150 (10), 148 (44), 147 (13), 146 (21), 136 (17), 134 (35), 132 (15), 125 (5), 124 (4), 122 (30), 121 (20), 120 (42), 118 (15), 110 (14), 109 (11), 108 (30), 107 (20), 106 (38), 104 (19), 97 (50), 96 (38), 95 (11), 94 (65), 93 (18), 92 (38), 90 (17), 82 (65), 81 (38), 80 (61), 78 (27), 70 (22), 69 (15), 68 (63), 67 (13), 66 (37), 56 (30), 54 (80),

42 (56), 40 (38).

The mass spectrum of synthetic 4-methyl gorgostanol at 70 eV shows m/e (rel. intensity) M^+, 442 (37), 428 (9), 427 (7), 409 (5), 381 (5), 372 (6), 371 (12), 354 (8), 353 (25), 332 (5), 331 (26), 330 (88), 317 (5), 316 (18), 315 (37), 314 (15), 313 (7), 312 (6), 311 (5), 302 (7), 301 (18), 300 (15), 299 (38), 297 (11), 289 (18), 288 (50), 287 (76), 286 (25), 274 (10), 273 (11), 272 (15), 271 (37), 269 (11), 248 (11), 247 (15), 246 (9), 245 (13), 243 (12), 231 (15), 230 (9), 229 (24), 216 (13), 202 (14), 190 (16), 188 (15), 176 (18), 174 (21), 164 (16), 162 (24), 160 (25), 150 (18), 148 (40), 147 (11), 146 (22), 144 (11), 136 (22), 134 (35), 132 (20), 125 (14), 124 (30), 122 (36), 121 (17), 120 (40), 118 (18), 110 (28), 109 (15), 108 (58), 107 (20), 106 (40), 104 (22), 97 (47), 96 (56), 95 (18), 94 (78), 93 (20), 92 (40), 90 (20), 84 (17), 82 (84), 81 (38), 80 (75), 78 (30), 70 (38), 69 (18), 68 (85), 67 (15), 66 (48), 56 (55), 54 (100) base peak, 42 (78), 40 (46), 28 (17).

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