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I. TWO NEW PROSTAGLANDINS: 15-EPI-PGA_2 AND ITS ACETATE, METHYL DIESTER FROM THE GORGONIAN PLEXAURA HOMOMALLA (ESPER)

II. TWO NOVEL MARINE STEROIDS: Δ^5-3,11, DIHYDROXY-9,11-SECO-GORGOSTEN-9-ONE (I) AND ITS 5α, 6α-EPOXIDE (II) FROM THE GORGONIAN PSEUDOPTEROGORGIA AMERICANA (GMELIN).

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APPROVED BY

DISSERTATION COMMITTEE

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I. TWO NEW PROSTAGLANDINS: 15-EPI-PGA_2 AND ITS ACETATE, METHYL DIESTER FROM THE GORGONIAN PLEXAURA HOMOMALLA (ESPER)

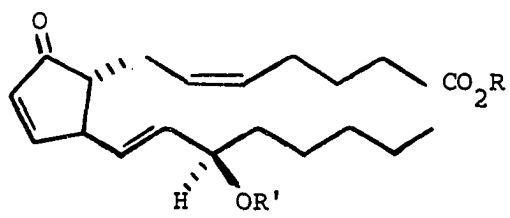
INTRODUCTION

This work formed a part of the research on natural products of a class of sea animals known as gorgonians. The program was supported by the National Institutes of Health through Training Grant HE-05675 from the National Heart Institute.

A number of whole dried gorgonians were extracted and examined for their total extractables and for unique spectral features of the crude extract. The crude hexane extract of a single gorgonian specimen collected by Dr. Robert E. Middlebrook proved to have a large amount of extractables with interesting NMR absorptions. Dr. Frederick M. Bayer of the Institute of Marine Science of Miami, Florida, identified the specimen as the gorgonian, Plexaura homomalla (Esper). Examination of additional material led to the identification of 15-epi-prostaglandins from this gorgonian.

RESULTS AND DISCUSSION

We recently reported[1,2,3] the occurrence of two novel prostaglandin derivatives in a sea animal, the gorgonian, Plexaura homomalla (Esper) in high concentration. The air dried cortex of the animal contains 0.2% 15-epi-PGA_2 (I, M^+ 334), figure 1 and 1.3% of its acetate, methyl diester (II, M^+ 390), figure 2. This is the first reported occurrence of prostaglandins in a marine organism, and the only known natural source for practical quantities of these potentially useful compounds, Previously, prostaglandins have been found only in very low concentrations widely dispersed in mammalian tissue with human seminal plasma the richest source for prostaglandins at a level of 300 µgm/ml.[4]

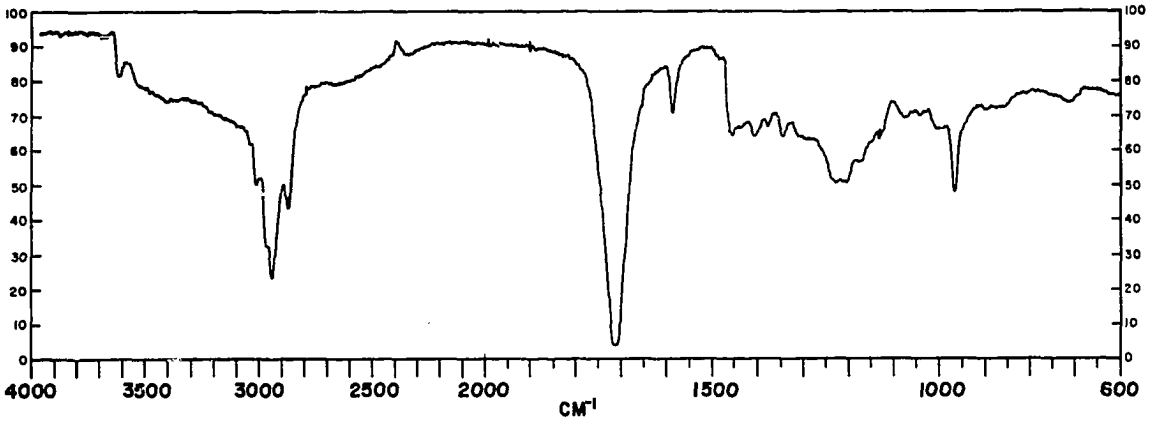


I R,R' = H

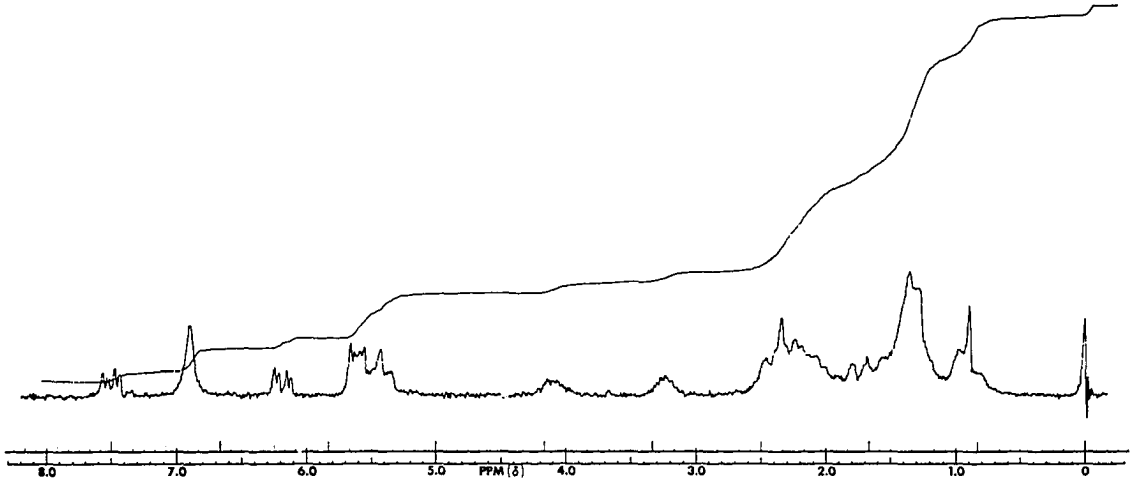
II R = Me,R' = Ac

Figure 1
Spectra of 15(R)-Hydroxy-9-Oxo-5-Cis,10,13-Trans-Prostatrienoic Acid (I)

IR Spectrum ($CHCl_3$) of I



NMR Spectrum ($CDCl_3$) of I



Mass Spectrum of I

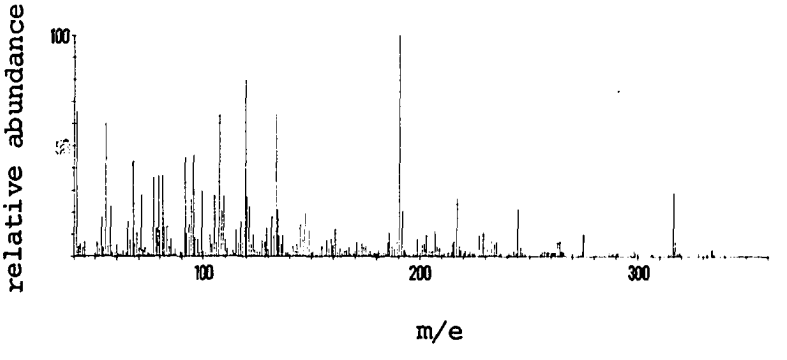
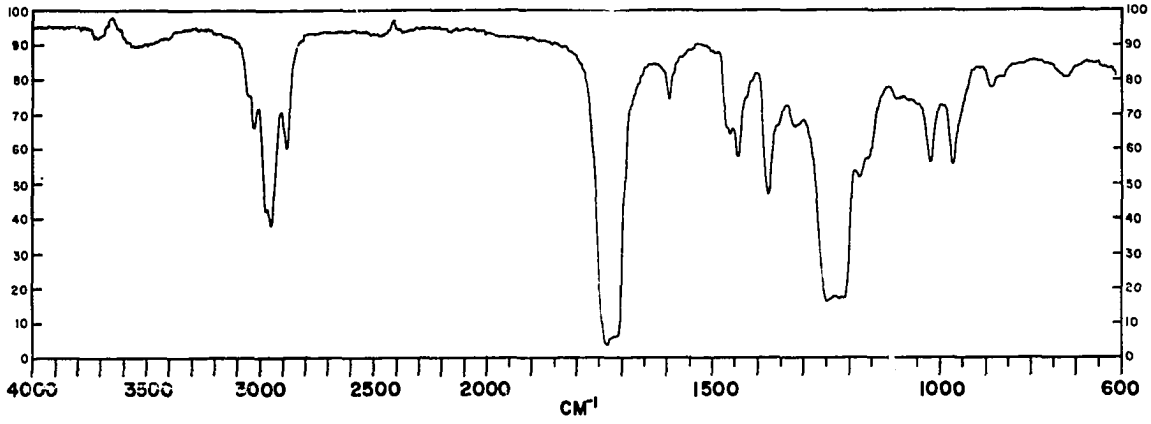
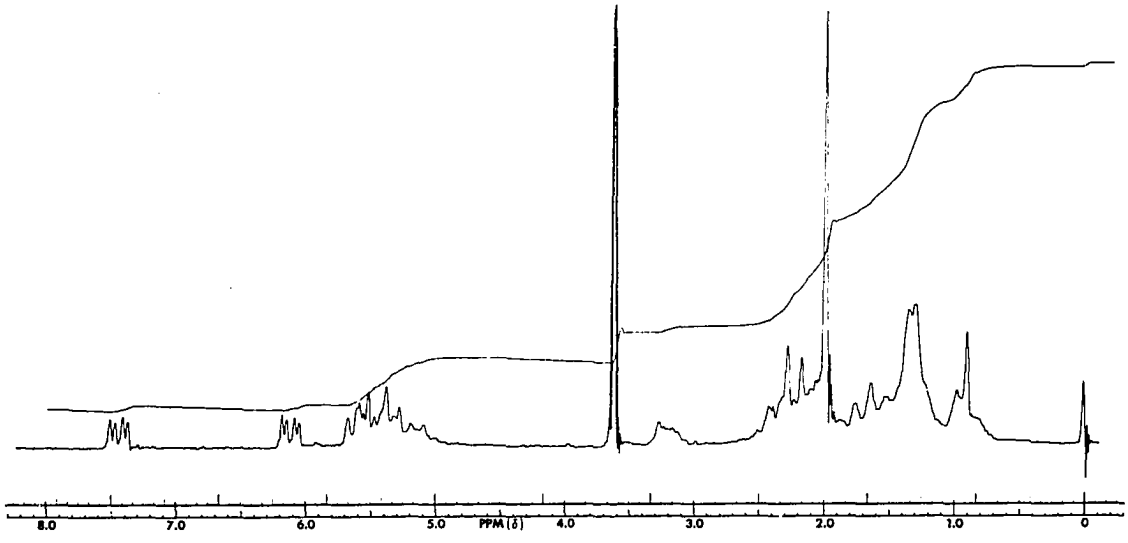


Figure 2
Spectra of Methyl 15-(R)-Acetoxy-9-Oxo-5-Cis,10,13-Trans-Prostatrienoate (II)

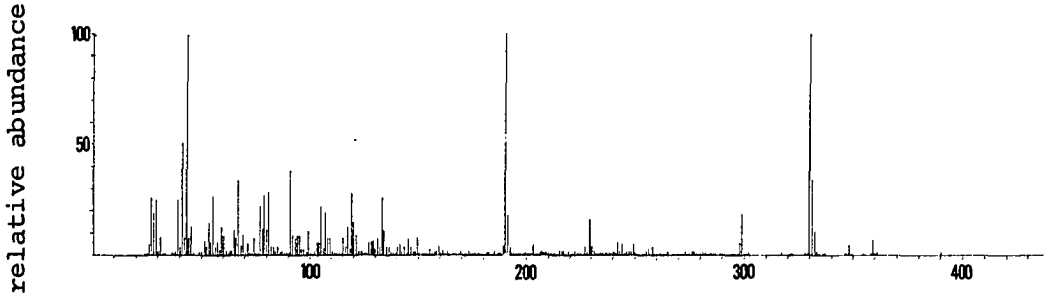
IR Spectrum ($CHCl_3$) of II



NMR Spectrum (CCl_4) of II



Mass Spectrum of II



In the early 1930's human seminal plasma was found to stimulate smooth muscles in the intestine and female reproductive tract.[5,6] Not until 1960, with the isolation and structure proof of PGE_1 and PGF_1 from sheep glands, were these activities associated with a new class of hormones, later named prostaglandins.[7] Since 1960 hundreds of papers have appeared showing a wide variety of biological activities associated with the sixteen previously known naturally occurring prostaglandins.[8] One member of this group, PGA_2, has shown cardiovascular activity as well as activity in smooth muscle. The 15-epi-PGA_2 (I) and its diester (II) are epimeric with PGA_2 at the allylic alcohol (acetate) center and possess neither of these activities.[9,10] The 15-epi-prostaglandins may yet be found to have characteristic biological activities since their presence in such high concentration in a relatively simple animal suggests a basic function for these compounds in the gorgonian. Their high concentration makes these 15-PGA_2 derivatives attractive as synthetic intermediates for prostaglandins with demonstrated biological activities.

Plexaura homomalla collected off the coast of Florida was air dried, and the ground cortex extracted with hexane at room temperature. The hexane extract was chromatographed (1:5 ratio) on silicic acid (Silicar CC-7) and eluted with a maximum flow rate in large fractions with solvent of increasing polarity; i.e., 0, 20, 40, 60, and 100% ethyl acetate in benzene. The diester II was eluted in the 20% ethyl acetate fraction. This fraction was chromatographed (1:50 ratio) on silicic acid (Silicar CC-4) using 8% ethyl acetate in benzene as solvent. Taking 50 ml. fractions, the liquid diester (II) was recovered in fractions 6 through 12 (R_{Dye} = 0.88

on TLC 40% EtOAc/Bz). The 60 and 100% ethyl acetate fractions contained 15-epi-PGA_2. These fractions were rechromatographed on silicic acid (Silicar CC-4, 1:100 ratio) using 20% ethyl acetate in benzene as solvent. By taking 100 ml. fractions, the liquid 15-epi-PGA_2 was recovered in fractions 10 through 17 (R_f = 0.52 on TLC, 1% HOAc/EtOH).

The diester (II) was shown by high resolution mass spectrometry to have the composition $C_{23}H_{34}O_5$. Its mass spectrum showed a normal methyl ester cleavage (M - 31), loss of acetic acid (M - 60), and a combination of these fragmentations. The base peak (mass = 190) suggests a McLafferty cleavage of the side chain alpha to the ketone after prior loss of acetic acid. This cleavage involves the formal transfer of a vinylic hydrogen atom. The IR spectrum of II shows carbonyl absorptions at 1735 cm^{-1} for the acetate and methyl ester absorptions and 1710 cm^{-1} for the conjugated cyclopentenone. The optical rotary dispersion curve shows a strong positive Cotton effect at 228 mμ due to this conjugated system. On hydrogenation of II in ethyl acetate using palladium on carbon at 1 atmosphere the hexahydrodiester (III, M^+396) was formed, figure 3. It was purified by chromatography on silicic acid (Silicar CC-7) using 5% ethyl acetate in benzene as solvent, to give an oil showing only one peak by gas chromatography. The mass spectrum and combustion data are consistent with the chemical composition $C_{23}H_{40}O_5$.

The mass spectrum of III reflects the following fragmentation scheme.

SOME MASS SPECTRAL FRAGMENTATIONS OF
THE HEXAHYDRODIESTER (III)

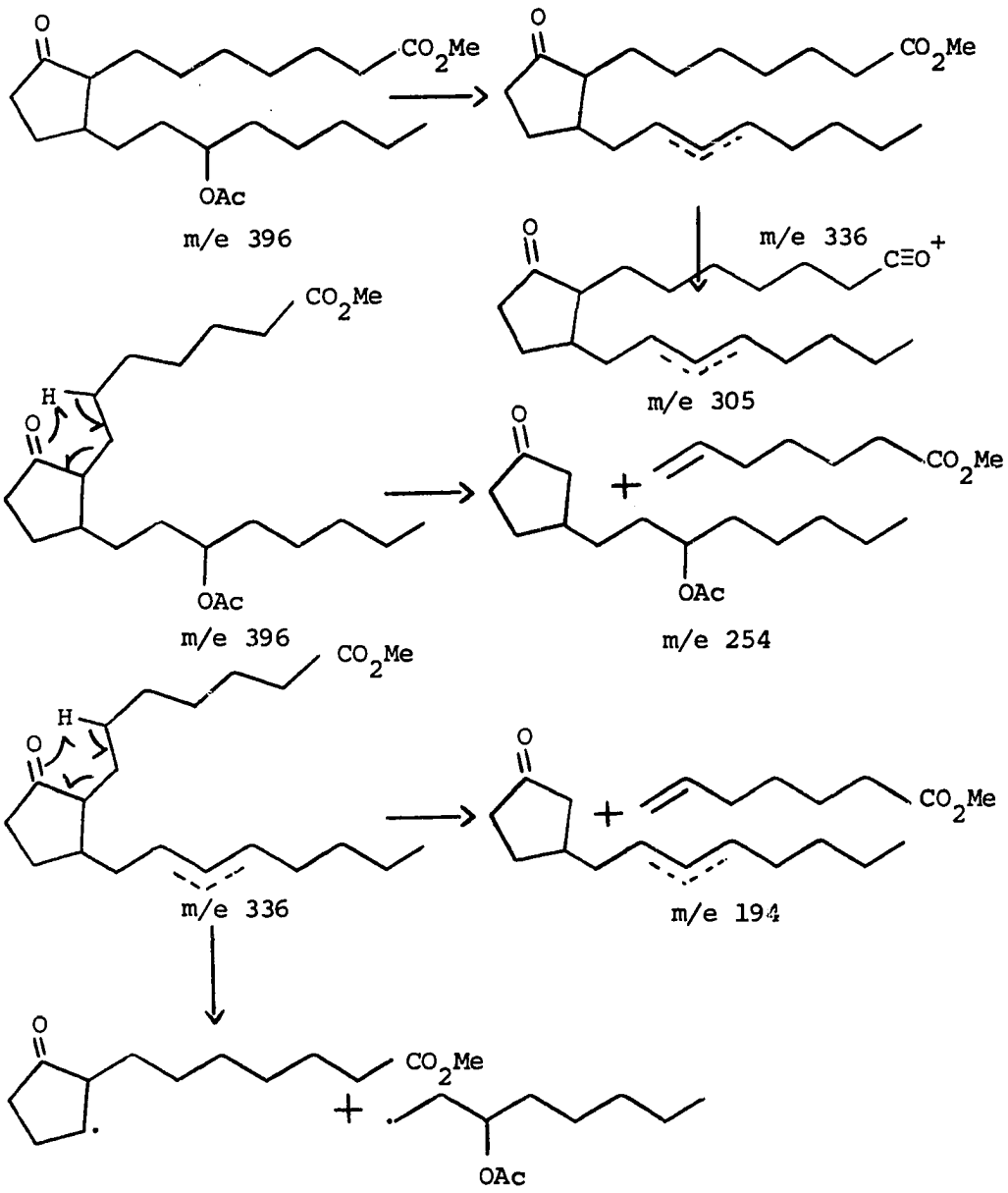
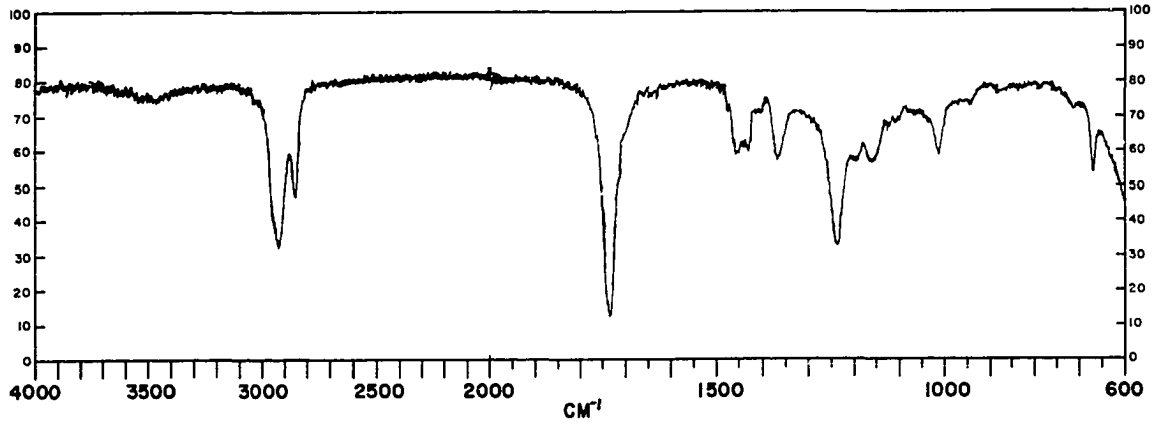
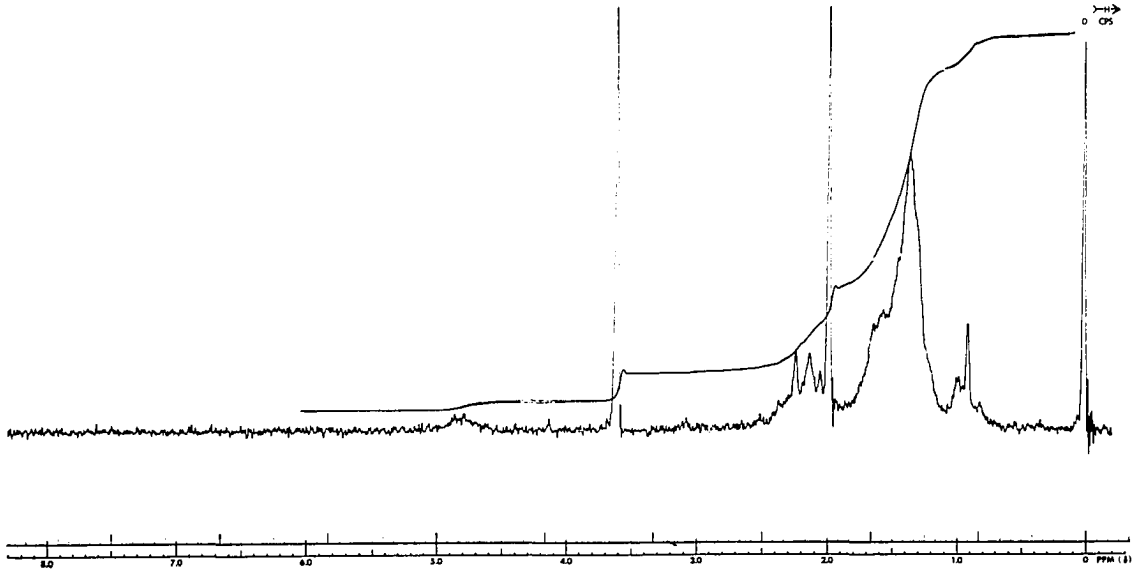


Figure 3
Spectra of Methyl 15(R)-Acetoxy-9-Oxo-Prostanoate (III)

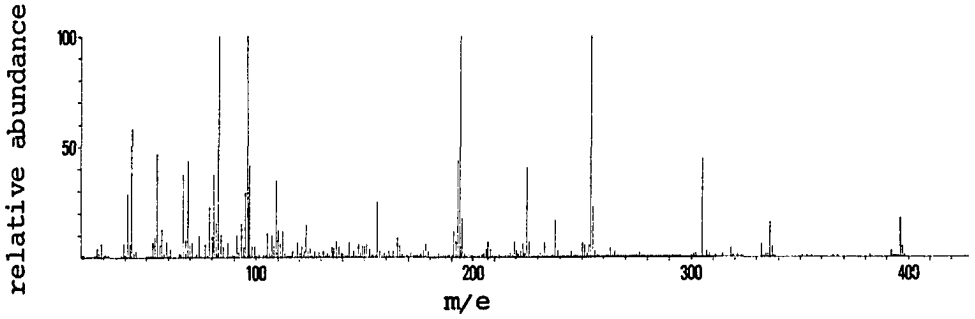
IR Spectrum (film) of III



NMR Spectrum (CCl_4) of III



Mass Spectrum of III



The hexahydrodiester (III) gave a parent ion of 396 and fragments of both 336 (M - 60) due to the loss of acetic acid and 305 (M - 60 - 31) due to the loss of acetic acid along with the normal methyl ester cleavage. Large base peaks for the McLafferty cleavage of the alpha side chain of III are observed differing by 60 mass units, reflecting a loss of acetic acid. The hexahydrodiester also fragmented with complete loss of the beta side chain (M - 171). The IR spectrum of III showed a single carbonyl absorption at 1735 cm^{-1} in the IR and an absorption at 300 mμ (ε = 30.7) in the UV confirming the presence of the five membered ring ketone.

Hydrogenation showed the diester (II) to have three carbon-carbon double bonds. Comparisons of the IR and UV spectra of II and III show that one of the double bonds is conjugated with the five membered ring ketone group. The presence of a second double bond in the alpha side chain is indicated by the McLafferty cleavages of $C_6H_9CO_2Me$ from II and of $C_6H_{11}CO_2Me$ from III. The NMR spectrum of II showed two sharp methyl singlets at δ1.98 (acetate) and 3.61 (methoxyl), and a third methyl group at 0.89 as a perturbed triplet characteristic of a normal alkyl chain terminus.

The position of the third double bond relative to the cyclopentenone system was determined by decoupling of NMR spectrum of II. A pair of double doublets at δ6.12 and 7.44 (J=2,6) characterizes the unsubstituted conjugated double bond (C-10,11) of the cyclopentenone system. The multiplicity of these absorptions clearly indicates monosubstitution at C-12; i.e., the beta side chain. Decoupling these signals showed them to be coupled to a single proton at δ3.22 (J=2). This proton (H-12) was further shown to be coupled to the protons of a second double bond (H-13,14) absorbing on the downfield side of the vinyl proton envelope at δ5.2-5.7. The diallylic

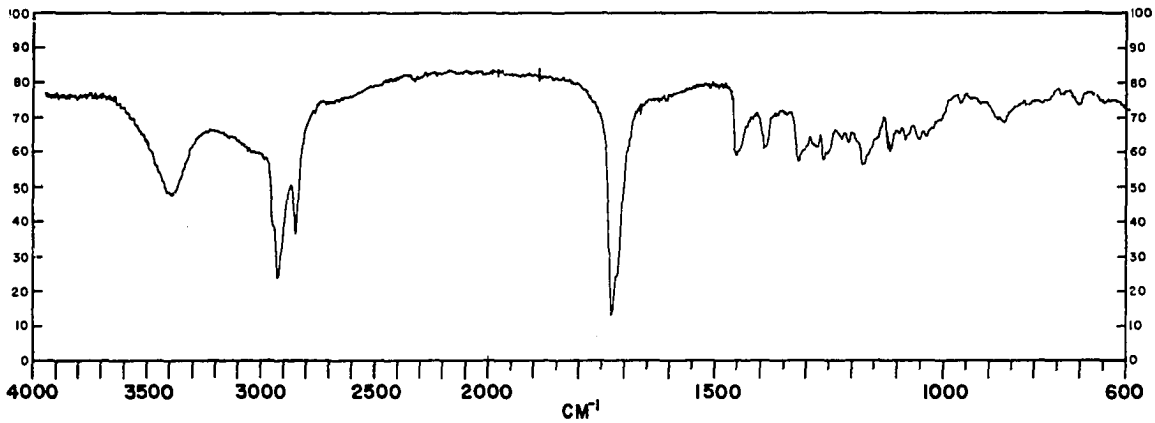
nature of the C-12 center locates the third double bond in the beta side chain adjacent to C-12. The partially resolved multiplet at δ5.15 is the allylic proton under oxygen of the acetate function of II which shifted to δ4.80 upon hydrogenation of II to III. On hydrolysis of III in methanolic KOH the corresponding hydroxy acid (IV, M^+340) was formed, figure 4. It was purified by chromatography on silicic acid (Silicar CC-7) using 30% ethyl acetate in benzene as solvent. The mass spectrum and combustion data support the chemical composition $C_{20}H_{36}O_4$. The proton under the resulting secondary alcohol oxygen in IV occurs at δ3.63 reflecting characteristic NMR spectral shifts for this C-15 hydrogen as part of the allylic acetate in II, the acetate in III and the alcohol function in IV.

The data presented account for the seven degrees of unsaturation and the five oxygens of II. Chemical evidence for the positions of the 13,14 double bond and the allylic acetate, as well as the length of the two side chains follows. Evidence for the detla-five position of the isolated double bond was obtained from neutral permanganate oxidation of II. The oxidation mixture was treated with diazomethane and its components were examined by gas chromatography. Dimethyl glutarate was identified by peak enhancement. The only other major component was not identified, but was probably methyl alpha-ketoheptanoate.

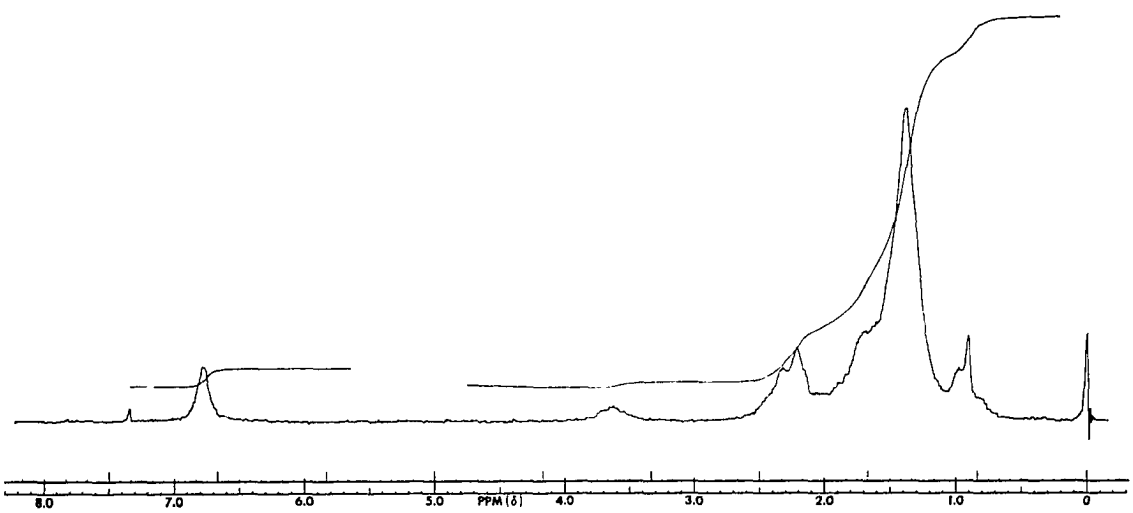
The location of the allylic acetate of II was confirmed chemically by a Baeyer-Villiger oxidation of methyl 9,15-diketoprostanoate (V, M^+354, figure 5). The diketone (V) was made by Jones oxidation of the methyl ester of IV and purified on silicic acid (Silicar CC-7) using 5% ethyl acetate in benzene as solvent. The recovered oil showed only one peak by gas chromatography. The Baeyer-Villiger oxidation products of V were

Figure 4
Spectra of 15(R)-Hydroxy-9-Oxo-Prostanoic Acid (IV)

LR IR Spectrum (KBr) of IV



NMR Spectrum ($CDCl_3$) of IV



Mass Spectrum of IV

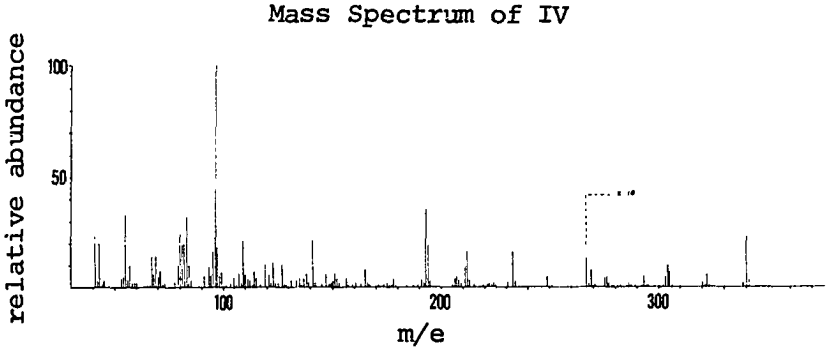
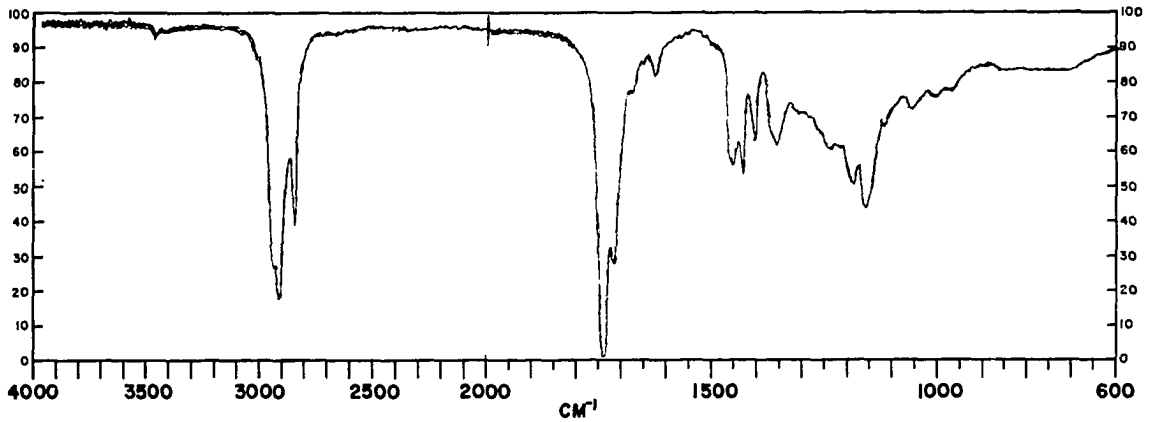
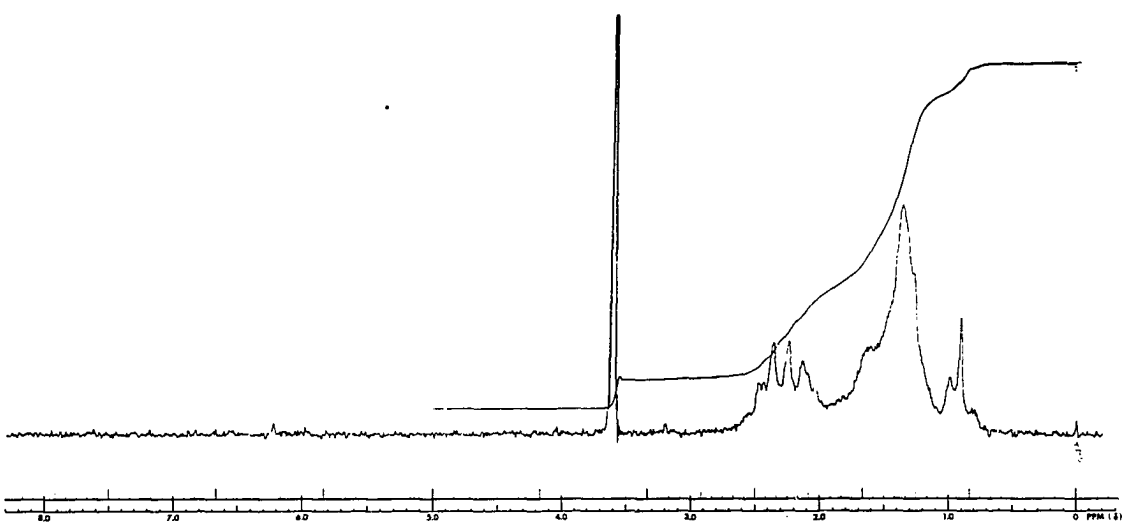


Figure 5
Spectra of Methyl 9,15-Dioxo-Prostanoate (V)

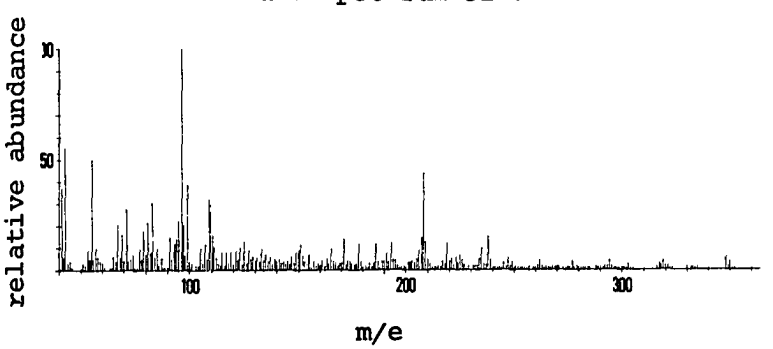
IR Spectrum (film) of V



NMR Spectrum (CCl_4) of V



Mass Spectrum of V



saponified in aqueous NaOH. The neutral fraction was separated from the acid fraction by continuous extraction with ether. After careful acidification, the acids were recovered from the aqueous mixture by continuous extraction with ether. The saponification afforded 1-pentanol and hexanoic acid from the neutral and acid fractions. Each was identified by gas chromatography (the acid as its methyl ester) by comparison with authentic compounds on three different columns.

The free hydroxy acid, 15-epi-PGA_2 (I, M^+334) was also isolated from the crude hexane extract of Plexaura homomalla and was converted into II (M^+390) by mild acetylation with acetic anhydride and pyridine in dilute benzene solution. After seven days, TLC showed the acetylation to be almost complete. The IR and NMR spectra as well as chromatographic properties of the reaction product were identical with those of the natural occurring II. Compound IV was obtained on hydrogenation of I and was shown to be identical with that originating from the diester (II) by mmp, specific roation, IR and NMR spectroscopy, R_f in TLC and retention time of methyl ester in gas chromatography.

These data are completely consistent with a structure for compound I identical with that of the mammalian hormone PGA_2. Compound I displayed NMR and IR (thick film) spectra nearly identical with that of authentic PGA_2.[11] However, I was inactive in the physiological blood pressure lowering test in dogs, while PGA_2 was active in side by side tests.[9] This showed that I was not PGA_2 but diasteromeric with PGA_2 at one or more centers. The structure for PGA_2 and I possesses five positions at which isomers are possible, namely the five and thirteen double bonds and the asymmetric centers at C-8, C-12, and C-13.

The geometries of the double bonds of I were shown to be identical with those of PGA_2 by selective hydrogenation. Compound I was selectively hydrogenated with 5% rhodium on carbon in ethyl acetate at a pressure of 50 psig hydrogen. By analyzing the hydrogenation mixture at various times before complete saturation of I, it was shown that the conjugated 10,11-double bond was reduced at a faster rate than the two carbon-carbon double bonds in the side chains. Hydrogenation of the 5,6-double bond was observed to be faster than the 13,14-double bond as determined by the disappearance of the respective vinyl absorptions in the NMR spectra of partially hydrogenated I.

These partially hydrogenated samples of I were also analyzed quantitatively by infrared spectrophotometry. The trans double bond absorption (970 cm^{-1}) associated with Δ^{13} in PGA_2^{12} and present in I was found to disappear on hydrogenation at the same rate (within experimental error) as the δ5.61 NMR ($CDCl_3$) absorption. Since the 970 cm^{-1} is the only trans olefin absorption in the IR spectrum of I, the remaining delta-five double bond must have a cis configuration. The IR spectrum of I as well as that of PGA_2 does not show a cis double bond absorption (CH, out-of-plane) in the 730-675 cm^{-1} region. The partial hydrogenation of the cis delta five double bond of I was accompanied by the disappearance of a 1350 cm^{-1} absorption in its IR spectrum and its NMR spectrum ($CDCl_3$) absorption at δ5.43. Since these absorptions are shown by both PGA_2 and I, it is concluded that the side chain double bonds of I have the same geometry as those in PGA_2.

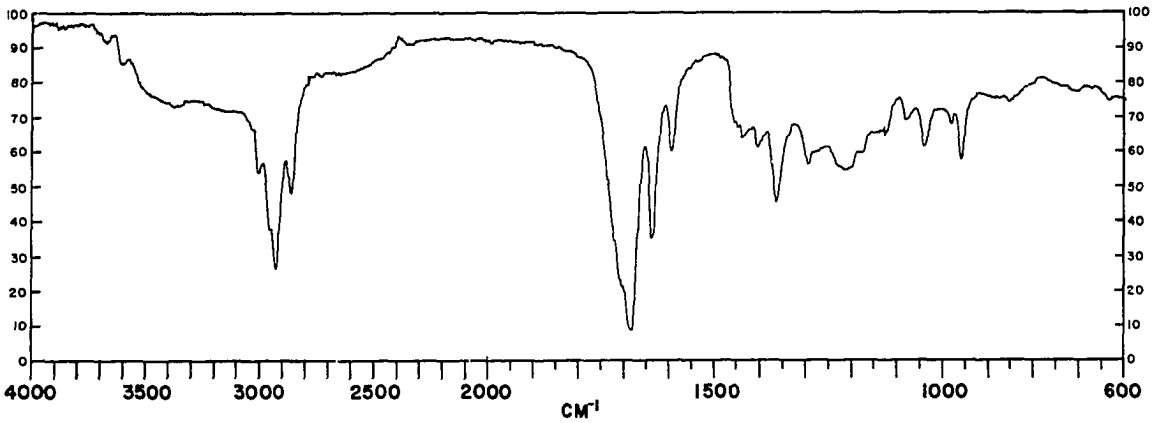
The ORD curves of PGA_2, I, and II all show strong positive Cotton effects, which have been correlated with the configuration at C-12 for the

"A" series prostaglandins.[13] In the "E" series (a cyclopentanone) negative Cotton effects are observed and are correlated with the configuration at C-8. The remaining asymmetric centers of the PGE prostaglandins are remote from the absorbing ketone chromophore and have little effect. The saturated prostaglandin derivatives III and IV both show negative Cotton effects suggesting the same configurations for I, II, III, IV and PGA_2 at C-8. It has been shown that 8-iso-prostaglandins isomerize in weak base (NaOAc) to normal prostaglandins.[14] The ORD curves for the 8-iso-PGE prostaglandins have positive Cotton effects while those of normal PGE's have negative. Compound IV obtained from hydrogenation of I, having no history of base treatment, is identical with IV recovered from prolonged treatment with KOH in methanol, verifying the configuration at C-8 and the trans relationship of the two side chains.

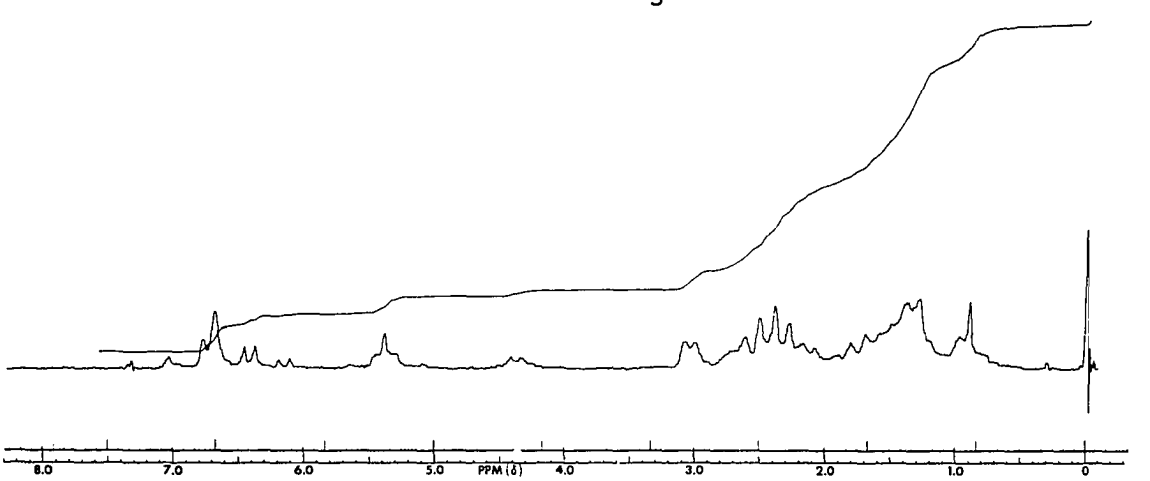
The opposite configuration of the asymmetric center at C-15 of I is the only remaining possibility for the difference of I and PGA_2 that was demonstrated in the blood pressure lowering tests in dogs.[9] The (R) - configuration of this center was demonstrated both by the formation of the (-) 15-epi-PGB_2 (VI), figure 6, by isomerization of I with NaOH in methanol, and by an ozonolytic degradation. Although VI exhibits the same TLC (silica gel H) mobility as the starting material (I), I and VI are easily separated by column chromatography (Silicar CC-4) using 20% ethyl acetate in benzene as solvent. The isomerization of the "A" series prostaglandins to the "B" series is well known,[15] however, the sign of rotation had not been established for known PGB configurations at C-15 and NMR data for the series was also unavailable. The NMR spectrum ($CDCl_3$) of VI showed signals characteristic for a hydroxy acid prostaglandin derivative and a trans coupling

Figure 6
Spectra of 15(R)-Hydroxy-9-Oxo-5-Cis-$\Delta^{8,12}$-13-Trans-Prostatrienoic Acid (VI)

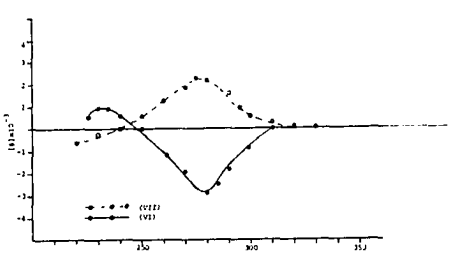
IR Spectrum ($CHCl_3$) of VI



NMR Spectrum ($CDCl_3$) of VI



CD Curves (MeOH) of VI and VII



constant (J=16) for the H-13 and H-14 protons of its 13,14-double bond. The H-13 proton (δ6.90) showed little or no allylic coupling with H-15; however, the H-14 vinyl proton (δ6.27) was further coupled with the H-15 proton (J=5). The prostaglandin PGB_2 (VII, figure 6 CD curve) was made from a small authentic sample of PGE_2 by treatment with NaOH in methanol. Initial elimination of water gave PGA_2 which isomerized under the reaction conditions to PGB_2. Since PGB_2 is epimeric with VI, the same chromatographic procedure was used to recover a small sample of PGB_2. The CD curve of VI (negative Cotton effect) is nearly the mirror image of that obtained for VII as expected for an epimeric pair.

Ozonolysis of the diester (II) followed by Jones oxidation of the ozonide[16] gave monomethyl glutarate (VIII, figure 7) and (+) alpha-acetoxy heptanoic acid (IX, figure 8). These compounds were separated by column chromatography using 15% ethyl acetate in benzene as solvent. The former had the same NMR ($CDCl_3$) spectrum as authentic monomethyl glutarate:[17] δ3.70 (methoxyl), 1.8 to 2.7 (6 protons), and 10.93 (one exchangeable acid proton). On treatment with diazomethane VIII was converted to its dimethyl ester which showed the same retention time as known material by gas chromatography. Alpha-acetoxy heptanoic acid (IX) was rechromatographed using 13% ethyl acetate in benzene as solvent. The NMR spectrum ($CHCl_3$) of IX showed resolved absorptions at δ0.90 perturbed triplet methyl, 5.05 (1H, t, J=6.5) and 8.46 (carboxyl proton). It showed a positive specific rotation in chloroform.

On hydrolysis of IX in aqueous NaOH, (-)-hydroxy heptanoic acid (X, figure 9) was obtained.[18] The product, a solid, had a melting range of 51-56.5°C and specific rotation of -17° in chloroform at 25°. On re-

Figure 7
NMR Spectrum of Monomethyl Glutarate (VIII)

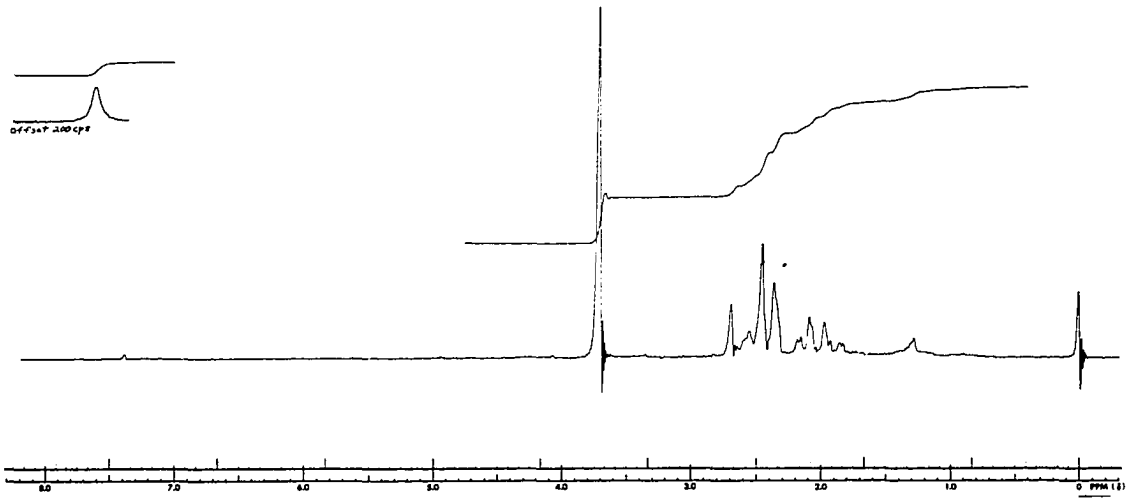


Figure 8
NMR Spectrum of α-Acetoxy Heptanoic Acid (IX)

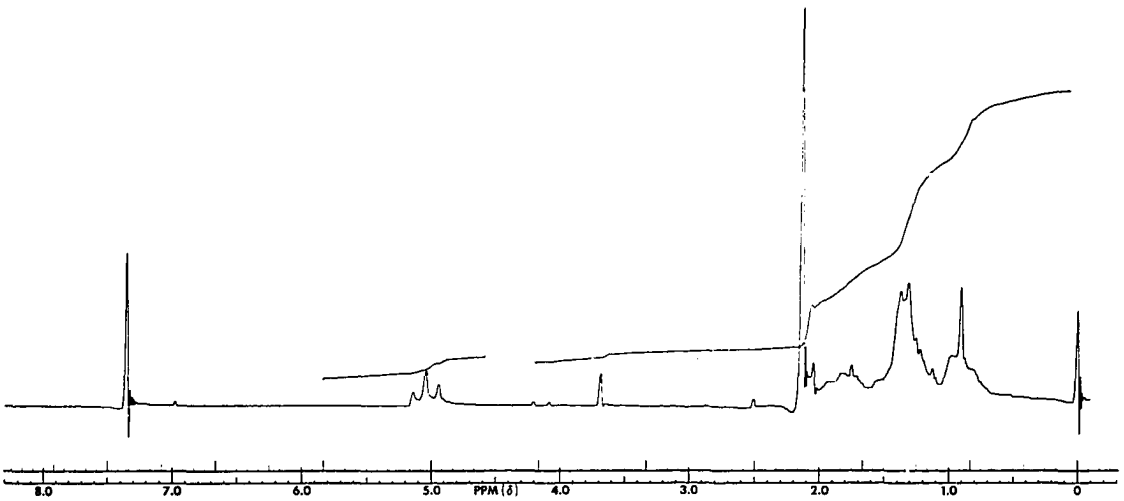
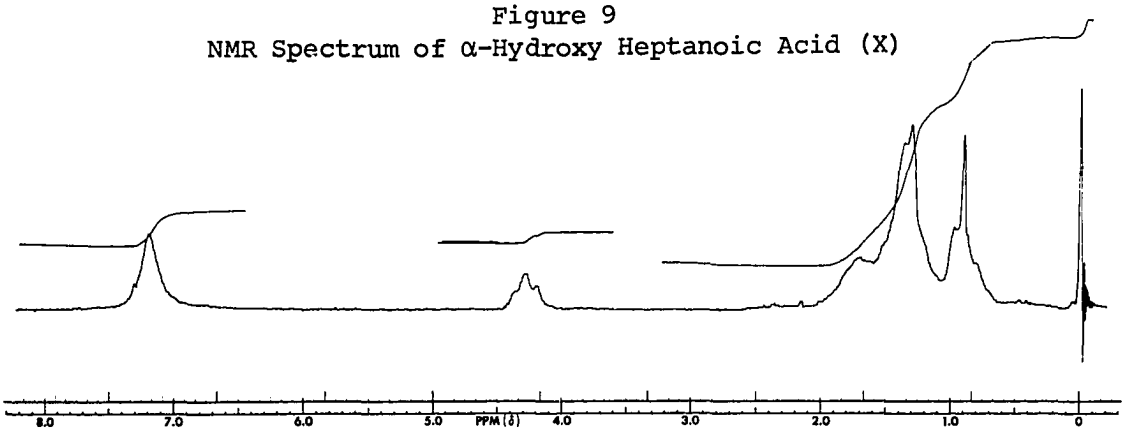


Figure 9
NMR Spectrum of α-Hydroxy Heptanoic Acid (X)



crystalization from benzene-pentane, the melting point was raised to 63.5-65^{o}C with a specific rotation of -13^{o} in chloroform at 25^{o}C. These high rotations were troublesome since the reported values (6^{o}, 6.9^{o} in chloroform) for the optical antipode of this acid were significantly lower suggesting that X was not α-hydroxy heptanoic acid.[18] The literature values in chloroform were correlated with specific rotations for the sodium salt of this acid in NaOH solution.[19] The identity of X was, however, verified by its NMR and mass spectra as well as by combustion data that support a chemical composition of $C_7H_{14}O_3$. Its NMR shows characteristic absorption δ0.92 (perturbed triplet methyl), 4.28 (1H,m) and 7.19 (two exchangeable protons). The parent ion of X was absent in its mass spectrum, with the largest peaks occurring at 128 (loss of water), 101 (loss of carboxyl group), and 83 (a combination of these fragmentations). The recrystallized hydroxy acid (mp=63.5-65^{o}) had a CD curve (CH_3OH) showing three peaks at [α] 265 = -910^{o}, [α] 250 = +780^{o}, and [α] 216 = -16,500^{o}. In 0.1N HCl the peaks were reduced in size with the 216 mμ peak reduced to -14,900^{o}. Earlier attempts to recover X by ozonolysis involved the same procedure for forming the ozonide, but its oxidation was performed in acetic acid using 30% hydrogen peroxide as reported in earlier prostaglandin ozonolyses.[19,20] The product acids were chromatographed as their methyl esters. After separation and basic hydrolysis only a small yield of alpha-hydroxy heptanoic acid was recovered (6.7%). This product was nearly racemic as shown by its CD peaks at 216 mμ of -2,313^{o} (CH_3OH). Similar attempts also fail to obtain a suitable product for measuring its rotation for comparison with literature values. In an attempt to improve reaction yields, the reaction time for oxidation of the diester

ozonide was reduced to 2.5 hours from the earlier reaction times of 18 hours. On work-up, a large yield of alpha-acetoxy heptanal (52% of theoretical) was recovered. Its NMR showed resolved absorptions at δ0.92 (perturbed triplet methyl), 2.19 (acetate methyl), 5.03 (1H, t, J=5) and 9.56 (1H, S, aldehyde proton). It was purified by silicic acid chromatography (Silicar CC-7) using 17% ethyl acetate in benzene as solvent. On oxidation with Jones reagent alpha-acetoxy heptanoic acid was recovered as shown by its NMR spectrum identical with that of IX first described. Since the hydrogen peroxide oxidation of the ozonide allows appreciable amounts of aldehyde to build up in the acetic acid solution, it is not surprising that appreciable racemization of the C-15 center occurs by enolization of the aldehyde intermediate under these acidic conditions.

EXPERIMENTAL

All melting points were corrected by adding 1.5°C and were taken on a Thomas Hoover, Capillary Melting Point Apparatus. All solvents were distilled before using.

NMR spectra were taken on Varian A-60 as well as 100 and 220 MHz Varian spectrometers using tetramethylsilane (TMS) as an internal reference. Chemical shifts were reported in δ-valves (ppm from TMS). The multiplicity of the signals are denoted by the symbols: s, singlet; d, doublet; dd, doubled doublet; t, triplet; q, quartet; m, multiplet. Coupling constants are reported in Hz.

Carbon hydrogen analyses were carried out by the Alfred Bernhardt Laboratories, Mulheim, West Germany and Chemalytics Incorporated, Tempe, Arizona.

Collection of Plexaura homomalla: The gorgonian, Plexaura homomalla (Esper), was collected on the coral reefs off the Florida coast. The base of the gorgonian is firmly embedded in the ocean's coral bottom and must be cut from the reef with a tool such as lopping shears.

Collection Work-Up: The animal is made up of a solid protein skeleton and an outer soft cortex that is easily removed from the protein skeleton after air drying. The drying was accomplished in several days at room temperature. After removing the cortex by hand, it was ground to a fine mesh size using a mechanical blender. The resulting ground

material was then ready for extraction.

Extraction of Plexaura homomalla: The ground cortex was extracted with distilled hexane at room temperature. This was accomplished by placing a known amount of the ground animal in a large conical filter funnel equipped with filter paper. Hexane was passed over the finely divided cortex until the resulting solution showed no color. The solvent from the extraction was removed on a rotary evaporator. The last traces of solvent were removed using a high vacuum Cenco pump system until a constant weight was observed. Care was taken in the final step as the crude extract tended to foam at reduced pressures. Table I shows the results of several hexane extractions of ground Plexaura homomalla.

TABLE I

HEXANE EXTRACTION OF FRESHLY DRIED PLEXAURA HOMOMALLA AT ROOM TEMPERATURE

Weight of Gorgonian	Weight of Hexane Extractables	% Hexane Extractables
94	9.8	10.4
106	9.0	8.5
450	43.0	9.6

The above extractions were done on freshly dried specimens; however, after air drying the intact animals on a laboratory bench top for two and one-half months at a room temperature of approximately 90°F a low yield of extractables was obtained (5.5%). The remaining whole animals were worked up as earlier described and then stored in a cold

room (4°C) in sealed plastic bags. The data in Table II suggest that the content of extractables can be maintained under these conditions.

TABLE II

HEXANE EXTRACTIONS OF PLEXAURA HOMOMALLA
AFTER 2.5 MONTHS DRYING AT ROOM TEMPERATURE

Weight of Gorgonian	Weight of Hexane Extractables	Time in Cold Room	% Hexane Extractables
385	21.0	0	5.5
334	16.3	2.5 months	4.9
283	12.9	6 months	4.7
443	20.0	7.5 months	4.5

Fractionation of the Hexane Extract of Plexaura Homomalla by Column Chromatography: A 2.5 inch diameter column was packed with 489 grams of silicic acid (Silicar CC-7) using benzene as solvent for the packing procedure. Eighty-three and one-half grams of crude extract was placed on the column in a benzene solution and washed into the column with benzene. The crude extract was then eluted from this column using known volumes of solvents of increasing polarity. The solvents were allowed to flow as fast as possible through the column. The results of this separation are shown in Table III.

TABLE III

FRACTIONATION OF THE HEXANE EXTRACT OF PLEXAURA HOMOMALLA ON A SILICIC ACID COLUMN 1:5 RATIO BY WEIGHT

Volume of Fraction in Mls.	Solvent	Weight of Fractions in Grams	% of Extract Eluted
4000	Benzene	46.5	54.7
1000	20% EtOAc/Bz	11.0	12.9
1000	40% EtOAc/Bz	15.0	17.7
1000	60% EtOAc/Bz	3.5	4.1
1000	EtOAc	1.0	1.2
Extract Weight= 85.0 grams		Percent Recovered=	90.6

A second separation was done using 500 grams of column support and 102 grams of hexane extract. These results are presented in Table IV.

TABLE IV

FRACTIONATION OF THE HEXANE EXTRACT OF PLEXAURA HOMOMALLA ON A SILICIC ACID COLUMN 1:5 RATIO BY WEIGHT

Volume of Fraction in Mls.	Solvent	Weight of Fractions in Grams	% of Extract Eluted
4000	Benzene	55.0	53.9
1000	20% EtOAc/Bz	2.5	2.5
1000	40% EtOAc/Bz	35.5	34.4
2000	60% EtOAc/Bz	6.5	6.4
1000	EtOAc	0.5	0.5
Extract Weight= 102 grams		Percent Recovered=	97.7

Thin Layer Chromatography Data on Hexane Extract of Plexaura homomalla: The crude hexane extracts of Plexaura homomalla and chromatographic fractions thereof were analyzed by TLC using two systems. The first system, TLC_1, used 0.17 mm silica gel H plates, a solvent of 40:60 ethyl acetate in benzene and a reference dye, Sudan Yellow (R_f = 0.78). The reference dye was chromatographed along with each sample and the movement of the components was compared to the movement of the dye. The following TLC spots were observed for the crude extract and the fractions presented in Table IV.

Crude hexane extract : R_{dye} = 0, .17, .31, .52, .70, .88, 1.2

Fraction 1 : R_{dye} = .60, .70, .83, 1.1

Fractions 2, 3 : R_{dye} = .31, .57, .86

Fractions 4, 5 : R_{dye} = 0, .21, .34

These same fractions of the hexane extract were also analyzed on silica gel H plates, but using 1:99 acetic acid in ethyl acetate as solvent (TLC_2).

Fraction 1 : R_f = 0.60, 0.74, 0.86

Fraction 2 : R_f = 0.74

Fraction 3 : R_f = 0.52, 0.66, 0.74

Fraction 4 : R_f = 0.52

Fraction 5 : R_f = 0.52

Isolation of 15(R)-Hydroxy-9-Oxo-5-Cis,10,13-Trans-Prostatrienoic Acid (I): Seventy grams of silicic acid (CC-4) was packed into a 1.25" diameter column as a benzene slurry. After preparing the column, it was washed with approximately 500 ml. benzene. A 0.7397 gram sample, fraction 4 of Table 3, was introduced to the column with benzene and chromatographed using 20% ethyl acetate in benzene as solvent. The flow rate was 3 ml./min. and 100 ml. fractions were taken.

Fraction	Weight of Fraction	Fraction	Weight of Fraction
1,2,3	0.0097	10	0.0152
4	0.0053	11	0.0302
5	0.0143	12	0.0599
6	0.0560	13	0.0907
7	0.0348	14	0.0642
8	0.0053	15	0.0350
9	0.0060	16	0.0358
		17	0.0221
		Stopped	

Fractions 10 through 17 contained I by TLC (R_f = 0.52) using 1:99 acetic acid : ethyl acetate as solvent and silica gel H plates.

The mass spectrum is consistent with the structure of I with peaks at m/e 334, 316, 245 and 190; ORD spectrum $[\alpha]_{254}$ = 7.216° (peak), $[\alpha]_{212}$ = -6,455° (trough); UV max (CH_3OH) 217 mμ; NMR spectrum shows peaks at δ7.50 (1, dd, J=2,6 Hz), 6.18 (1, dd, J=2,6 Hz), 5.61 (2,m), 5.43 (2,m),

4.10 (1,m), 3.24 (1,m) and 0.90 (3, perturbed triplet). The IR spectra of I shows peaks at: IR (film) 1705, 1585, 1480, 1455, 1435, 1405, 1375, 1345, 1305, 1220, 1175, 1145, 1140, 1045, 1005, 960, 835, 745 cm^{-1} and IR ($CDCl_3$) 1710, 1590, 1480, 1440, 1410, 1380, 1350, 1235, 1215, 1175, 1080, 1045, 1010, and 970 cm^{-1}.

Percentage 15(R)-Hydroxy-9-Oxo-5-Cis,10,13-Trans-Prostatrienoic Acid (I) Found in Dry Cortex of Plexaura homomalla (Esper): Fractions 4 and 5 of Table IV were combined. These fractions represent 6.9% of the hexane extract. The material was chromatographed on a 70 gram silicic acid column as described before. A sample (1.3118 grams) of the combined fractions was chromatographed using 20% ethyl acetate in benzene as solvent. Fifty milliliter fractions were taken and flow rate of 3 ml./min. was used.

Fraction	Weight of Fraction	Fraction	Weight of Fraction
1 - 16	not taken	25	0.600
17	0.0084	26	0.0438
18	0.206	27	0.0376
19	0.0339	28	0.0303
20	0.0411	29	0.0242
21	0.0603	30	0.0406
22	0.0608	31	0.0202
23	0.0730	32	0.0201
24	0.0668		
		Stopped	

A total of 0.5411 grams of material was recovered in fraction 17 through 30. This represented 41.2% of the chromatographed sample. Gas chromatography of the methyl esters of this material shows 15(R)-hydroxy-9-oxo-5-cis,10,13-trans-prostatrienoic acid to be present to the extent of 75.8%. The following table shows the results of the gas chromatography of the combined fractions 17 through 30 as their methyl esters.

TABLE V

GAS CHROMATOGRAPHIC ANALYSIS OF THE METHYL ESTERS OF FRACTIONS 17-30 CONTAINING 15-EPI-PGA_2 FROM PLEXAURA HOMOMALLA

Peaks	Retention Time	Percent of Peak
1	6.2 min.	1.3
2	9.5	1.7
3	12.5	75.8
4	15.0	1.7
5	20.2	19.2
		Total 99.7

Conditions: 3' x 1/8" glass column packed with 3% JXR coated on Chromosorb W, flow rate 95 ml./min., and flame ionization detector. Temperatures: Inlet 200°C, Column 185°C, and Detector 230°C.

A standard mixture of fractions 17 - 30 (0.0483 grams) and 0.0353 grams of methyl 15(R)-acetoxy-9-prostanoate (III) was weighed out and reacted with diazomethane. The methyl ester formation should increase the weight of the sample of 15-epi-PGA_2 to 0.0504 grams or 58.8% of this mixture.

The concentration of the methyl ester of 15-epi-PGA_2 in this mixture should be .588 x 75.8% or 44.6% by the previous gas chromatographic analysis in Table V. However, the gas chromatographic analysis of this mixture showed its concentration to be 39.9%. These results suggest that there are non-volatile components in the sample of fractions 17 - 30 that further reduced the concentration of 15-epi-PGA_2 to 75.8% x 39.9/44.6 or 68% by weight of fractions 17 - 30. The concentration of 15-epi-PGA_2 in the air dried cortex of Plexaura homomalla is then 0.2% based on its hexane extractables and the chromatographic procedures just described for its purification.

Isolation of Methyl 15-(R)-Acetoxy-9-Oxo-5,Cis,10,13-Trans-Prostatrienoate (II): Seventy grams of silicic acid (CC-4) was packed into a 1.25" diameter column as a benzene slurry. After preparing the column, it was washed with approximately 500 ml. benzene. A 1.4990 grams sample, fraction 2 of Table III, containing the diester was chromatographed on this column using 8% ethyl acetate in benzene as solvent. A flow rate of 2 ml./min. was used and 50 ml. fractions were taken.

Fraction	Weight of Fraction	Fraction	Weight of Fraction
1 - 4	Not taken	9	0.1735
5	Not taken	10	0.1087
6	0.0325	11	0.0860
7	0.1641	12	0.0488
8	0.2633	13	0.0494

Fractions 6 - 12 contain the prostaglandin diester by TLC_1 (R_d = 0.88). The following spectral data were taken for the acetate, methyl diester prostaglandin (II).

High resolution mass spectrum (m-60=3302187; i.e., $C_{21}H_{30}O_3$): The mass spectrum showed peaks at m/e of 390, 359, 330 and 190 (base peak); ORD spectrum (CH_3OH) $[\alpha]_{247}$ = 6740° (peak), $[\alpha]_{218}$ = -5154° (trough) UV max (MeOH) 215 mμ,ε= 9,300.

The IR spectra of II show peaks at: IR (film) 1735, 1710, 1585, 1455, 1435, 1370, 1310, 1240, 1165, 1015, 965, 885, 810 and 720 cm^{-1} and IR ($CHCl_3$) 1730, 1710, 1585, 1455, 1435, 1370, 1310, 1240, 1205, 1170, 1145, 1015, 965 and 880 cm^{-1}.

The NMR spectrum (CCl_4) of II shows peaks at δ7.44 (1,dd, J=2,6 Hz), 6.12 (1, dd, J=2,6 Hz), 5.48 (4 proton vinyl envelop), 5.14 (1,m), 3.61 (3,S), 1.98 (3,S), and 0.89 (3, perturbed triplet).

Percentage Methyl 15(R)-Acetoxy-9-Oxo-5-Cis,10,13-Trans-Prostatrienoate (II) Found in Dry Cortex of Plexaura homomalla (Esper): Fractions 2 and 3 from Table IV were combined. They represent 36.8% of the crude hexane extract. This material was chromatographed on a 70 gram silicic acid (CC-4) column using 8% ethyl acetate in benzene as solvent. A flow rate of 2 ml./min. was used and 50 ml. fractions were taken.

Fraction	Weight of Fraction	Fraction	Weight of Fraction
1,2,3	0.0022	10	0.0678
4	0.0013	11	0.0324
5	0.0080	12	0.0688
6	0.0056	13	0.2178
7	0.0412	14	0.1015
8	0.1218	15	0.0087
9	0.0902		

A solvent change at fraction 12 to 20% in ethyl acetate in benzene was made to elute the slower moving components (F_{dye} = 0.52).

Fractions 7 - 11 were combined and weighed 0.3595 grams. This sample represents 41.2% of fractions 2 and 3 of Table IV or 15.2% of the total hexane extract or 1.5% of the air dried cortex. Gas chromatography of this sample showed the diester concentration to be 91.4% of the mixture. A table of these results is shown below:

TABLE VI

GAS CHROMATOGRAPHY OF FRACTIONS 7-11
CONTAINING METHYL 15(R)-ACETOXY-9-OXO-
5-CIS,10,13-TRANS-PROSTATRIENOATE

Peaks	Retention Time (Min.)	Percent of Peak
1	9.6	0.7
2	11.4	1.5
3	15.5	91.4
4	20.0	5.5
5	23.4	1.0

Conditions: 3' x 1/8" glass column packed with 3% JXR coated on Chromosorb W, flow rate of 95 ml./min., and flame ionization detector. Temperatures: Inlet = 200°C, Column = 185°C, and Detectors = 230°C.

These results reduce the diester concentration in the air dried cortex of Plexaura homomalla to 1.3%. A sample of methyl 15(R)-acetoxy-9-oxo-prostanoate (III, 96.6%) was analyzed using the same gas chromatographic conditions. The results showed the sample to contain 96.6% III (R.T. = 18.1 minutes) and an impurity 3.4% (R.T. = 15.4 minutes). A standard mixture of this material (0.0927 grams) 70.1%, and (0.0395 grams) 29.9% fractions 7 - 11 was weighed out and gas chromatographed. It was found that the impurity in the sample of III overlapped with the diester (II) and peak 4 of the diester (II) sample overlapped with III. The calculated and observed gas chromatographed results of this mixture are shown below. This suggests that there are no non-volatile components present in the diester (II) sample and its concentration in the air dried cortex is 1.3%.

TABLE VII

GAS CHROMATOGRAPHIC ANALYSIS OF THE STANDARD MIXTURE DIESTERS II AND III

Peaks	% of Mixture Calculated	% of Mixture Found
1	.21	.59
2	.45	1.77
3	29.70	27.92
4	69.26	69.12
5	.29	.59

Methyl 15(R)-Acetoxy-9-Oxo-Prostanoate (III): A sample of II (0.455 grams) was shown by gas chromatography to be 85% pure. This sample was hydrogenated using 0.023 grams of 5% palladium on carbon as catalyst in 20 ml. ethyl acetate as solvent. After three hours reaction time at 1 atmosphere hydrogen the reaction was stopped. The catalyst was removed by filtration and the solvent was removed on a rotary evaporator. The resulting oil was chromatographed on 25 grams of silicic acid (CC-7) in a 3/4" diameter column using 5% ethyl acetate in benzene as solvent. Fifteen ml. fractions were taken at a flow rate of 0.75 ml./min. was used. Compound III started eluting in fraction 12 (97%, a trace). Fractions 14 through 18 were combined to obtain a sample (0.050 grams) containing only one peak by gas chromatography. An additional 0.200 grams of III was eluted in fractions 19 through 28. The gas chromatographic analysis used was system 1 (10', 2.5% SE 30 on Chromosorb W packed glass column, 90# argon gas flow, inlet and column temperature 185°C, argon detector). The retention times for derivatives of III where available,

will be divided by the retention time of III and reported as a decimal.

Anal. Calcd for $C_{23}H_{40}O$: C, 69.70%; H, 10.10%; O, 20.20%; found C, 69.64%; H, 10.18%; O, 20.18%.

The mass spectrum is consistent for the structure with peaks at m/e 396, 336, 254, and 225. ORD spectrum (CH_3OH), $[\alpha]_{313} = -1047°$ (trough), $[\alpha]_{274} = 1431°$ (peak); UV max (hexane) 300 mμ ($\varepsilon = 30.7$); $[\alpha]_D^{25} = -11$ (C = 2.24 $CHCl_3$).

The IR spectrum (film) showed peaks at 1735, 1455, 1430, 1405, 1370, 1240, 1200, 1160, and 1015 cm^{-1}. The NMR spectrum (CCl_4) showed resolved peaks at δ4.80 (1,m), 3.59 (3, S), 1.97 (3, S) and 0.91 (3, perturbed triplet).

15(R)-Hydroxy-9-Oxo-Prostanoic Acid (IV): A sample of fractions 4 and 5 from Table IV (1.7206 grams) was chromatographed on 70 grams of silicic acid (CC-4) in a 1.25" diameter column using 20% ethyl acetate in benzene as solvent. A flow rate of 1 ml./min. was used and 100 ml. fractions were taken. Fraction 9 (0.1781 grams) was analyzed by gas chromatography to be 90.1% 15-epi-PGA_2 (I). A portion of this material (0.1318 grams) was hydrogenated in 30 ml. ethyl acetate using 5% rhodium on carbon (0.1203 grams) as catalyst. The reaction was carried out in Parr hydrogenator at 50 psig hydrogen for 8 hours. The product was filtered and the ethyl acetate removed on a rotary evaporator. The resulting oil was chromatographed on 70 grams of silicic acid (CC-7) in a 1.25" diameter column using 30% ethyl acetate in benzene as solvent. A flow rate of 2 ml./min. was used and 100 ml. fractions were taken. Fractions 4 through 7 (0.076, 0.0419, 0.0225, and 0.0154 grams) were combined. A total of 0.0874 grams of IV (an oil) was collected. On

standing it crystallized to a solid, mp 65.5-69.5°C. On recrystallization from pentane-ether the melting point was raised to 71.5-72.5°C.

15(R)-hydroxy-9-oxo-prostanoic acid was also obtained from compound III. Chromatographed III (0.2234 grams) was stirred for 18 hours in 10 ml. MeOH containing 0.30 grams KOH. After this time the solution was acidified to a pH of approximately 2. The volume of the reaction solution was then reduced to about 3 ml. on a rotary evaporator. Chloroform (50 ml.) and 50 ml. water were added while transferring the reaction product to a separatory funnel. The aqueous phase was extracted 3 times with 50 ml. portions of chloroform. The chloroform extracts were combined and dried over sodium sulfate. The solvent was removed on a rotary evaporator yielding an oil 0.1825 grams. Using the column chromatographic techniques described before, IV was collected in fractions 4 through 6 (0.0059, 0.1029, and 0.0611 grams). A total of 0.1640 grams of IV was recovered and solidified on standing. After recrystallization from pentane-diethylether the melting point was 68-70°C. The mixed melting point with the product from the hydrogenation of I was 70-71.5°C. The spectra, as well as optical rotation, of compound IV from both of the above routes were identical.

Anal. Calcd for $C_{20}H_{36}O_4$: C, 70.59%; H, 10.59%; found: C, 70.65%; H, 10.56%.

The mass spectrum is consistent for the structure with peaks at m/e 340, 212, 211, and 194. The ORD spectrum (CH_3OH), $[\alpha]_{312} = -1419°$ (trough), $[\alpha]_{273} = 1820°$ (peak); $[\alpha]_D^{25} = -32$ ($\varepsilon = 0.79$, $CHCl_3$).

The IR spectra have peaks at: IR ($CHCl_3$) 1730, 1715, 1460, 1405, 1375, 1275, 1230, 1210, 1155, 1120, 1085, 1000, 965, 940, and 895 cm^{-1};

IR (KBr) 1730, 1720, 1465, 1405, 1330, 1290, 1270, 1220, 1185, 1175, 1155, 1125, 1095, 1065, 1050, 1020, 975, 925, 880, 790, and 715 cm^{-1}; the NMR spectrum ($CDCl_3$) shows resolved peaks at δ6.86 (two exchangeable protons), 3.63 (1,m) and 0.89 (3, perturbed triplet).

Base Treatment of 15(R)-Hydroxy-9-Oxo-Prostanoic Acid (IV): Compound IV (0.119 grams) obtained from the hydrogenation of 15-epi-PGA_2 (II) was stirred in 10 ml. methyl alcohol containing 0.30 grams KOH for 100 hours. The solvent volume was reduced to approximately 5 ml. on a rotary evaporator. The product was then acidified to a pH of approximately 4. The product was transferred to a separatory funnel with 50 ml. quantities of chloroform and water. The water phase was extracted with three 50 ml. portions of chloroform. After drying over anhydrous sodium sulfate, the chloroform was removed yielding an oil. After a chromatography as described earlier 0.0892 grams of IV were recovered. After recrystallization, a melting point of 70.0-71.5°C was obtained. The NMR spectrum and optical rotation of the product were identical to those of the starting material. The mixed melting point with the starting material was 71-72°C.

Methyl 15(R)-Hydroxy-9-Oxo-Prostanoate: Compound IV (0.3756 grams) made from the saponification of III was dissolved in 25 ml. ether. Approximately 15 ml. of diazomethane in ether solution (an excess) was added to IV and stirred for 5 mintues. The excess diazomethane was then destroyed with 1.5 ml. acetic acid. The ether solution was transferred to a separatory funnel with ether washings. The ether solution was then extracted with five 50 ml. portions aqueous saturated $NaHCO_3$. After drying over anhydrous sodium sulfate, the ether was

removed yielding 0.3304 grams of the hydroxy methyl ester, R_{Dye} = 0.60 (silica gel H plates and 40% ethyl acetate in benzene as solvent). Gas chromatography, System 1, R.T. (methyl ester)/R.T. (III) = 0.86.

The IR spectrum (film) showed peaks at 3460, 1735, 1450, 1430, 1405, 1360, 1310, 1255, 1190, 1160, 1120, 1060, 1005, 960 and 715 cm^{-1}.

The NMR spectrum (CCl_4) showed resolved absorptions at δ3.61 (3, S), 3.60 (1, m) and 0.90 (3, perturbed triplet).

Methyl 9,15-Dioxo-Prostanoate (V): Compound V (0.3304, .93 moles) was dissolved in 25 ml. of acetone (distilled over $KMnO_4$). After cooling the solution to 0°C, 0.19 ml. of Jones Reagent (0.51 moles) was added and the resulting mixture stirred at 0°C for 30 minutes. After this time 0.4 ml. (1.1 moles) more Jones Reagent was added and stirred for an additional 15 seconds. Ethanol (5 ml.) was added. The resulting mixture was transferred to a separatory funnel with washings totaling 30 ml. ether and 25 ml. water. The aqueous layer was extracted with eight 30 ml. amounts of ether. After drying over anhydrous sodium sulfate, the combined ether extractions yielded on removal of solvent, an oil (0.300 grams). Thin layer chromatography on silica gel H plates using 40% ethyl acetate in benzene as solvent showed incomplete oxidation with the starting material occurring at R_{Dye} = 0.60 and the product occurring at R_{Dye} = 0.85. The reaction product was chromatographed on 30 grams of silicic acid (CC-7) in a 1" diameter column using 5% ethyl acetate in benzene as solvent. A flow rate of 2 ml./min. was used and 40 ml. fractions were taken. Fractions 6 through 13 contained the reaction product, R_{Dye} = 0.85.

Fractions	Weight of Fraction	Fractions	Weight of Fraction
6	0.0059	10	0.0237
7	0.0316	11	0.0132
8	0.0531	12	0.0048
9	0.0315	13	0.0036

Total weight of the oil (V) was 0.1735 grams

The mass spectrum is consistent with the structure with peaks at m/e 352, 321, 238, 208, 186, 165, 133, 109, and 96. Gas chromatography system 1, R.T. (V)/R.T. (III) = 0.77. The IR spectrum (CCl_4) showed peaks at 1740, 1715, 1625, 1455, 1430, 1405, 1360, 1240, 1190, 1160, 1120, 1060, 1005, and 965 cm^{-1}.

The NMR spectrum (CCl_4) shows peaks at δ3.60 (3, S), 2.6 - 2.0 (9 protons), and 0.90 (3, perturbed triplet).

Baeyer-Villiger Oxidation of Methyl 9,15-Dioxo-Prostanoate (V): The diketone (V), 0.1735 grams (0.5 m moles) was dissolved in 7.4 ml. methylene chloride. Anhydrous disodium hydrogen phosphate (2.72 grams) was added and the mixture was cooled to 0°C with magnetic stirring in a 25 ml. round bottom flask. In another flask 7.4 ml. of methylene chloride and 1.9 ml. (13.68 mm) trifluoroacetic anhydride were cooled to 0°C. To the latter, 0.37 ml. (11.85 m moles) 90% H_2O_2 was carefully added. The resulting solution of trifluoroperacetic acid was transferred with small methylene chloride washings to a pressure equalized addition funnel. This solution was added drop-wise to the stirred mixture containing V with the reaction temperature held at 0°C. The addition required 30 minutes. After this time the mixture was allowed to warm to room temper-

ature and was stirred an additional hour.

The salts formed were filtered off and washed with two 30 ml. portions of methylene chloride. The filtrate and washings were combined. The methylene chloride solution was extracted first with 5% sodium bisulfite solution (30 ml.), then 5% sodium carbonate, and was then dried over magnesium sulfate overnight. The methylene chloride was revmoved on a rotary evaporator yielding 0.1045 grams of an oil. Its TLC showed a single spot whose R_{dye} = .74 (silica gel H plates and 40% ethyl acetate in benzene as solvent). Gas chromatography (system 1) revealed two peaks in a ratio of approximately 80:20. The IR and NMR spectra were consistent with the expected mixture of products.

The Baeyer-Villiger oxidation product (0.1045 grams) was stirred in 4 ml. water containing 0.32 grams NaOH. The saponification was continued for 48 hours at room temperature. The product was transferred to a continuous extractor with three 2 ml. water washes and three 10 ml. ether washings. The aqueous phase was continuously extracted with ether for 4 days. The resulting ether solution was found by gas chromatography using three different systems to contain a single component, amyl alcohol. After acidification of the aqueous saponification mixture, it was extracted again with ether for 4 days. After esterification with diazomethane, the acid fraction was analyzed by gas chromatography. It was found to contain methyl hexanoate by retention time on three different columns.

TABLE VIII

GAS CHROMATOGRAPHIC ANALYSIS OF AMYL ALCOHOL
A DEGRADATION PRODUCT OF COMPOUND I

System	Retention Time (Min.) Amyl Alcohol	B-V Neutral	Conditions
1	7.6	7.6	5' x 1/8" column, 20% FFAP on Chromosorb W; 76 ml./min. flow rate; temperature: 65°C column, 185°C inlet.
2	3.4	3.4	5' x 1/8" column, 20% DEGS; 105 ml./min. flow rate; temperature: 65°C column, inlet 190°C, detector flame 200°C.
3	3.9	3.9	5' x 1/8" column, 15% Carbowax on Chromosorb W; 99 ml./min. flow rate; temperature: 65°C column, injector 185°C, flame detector 220°C.

TABLE IX

GAS CHROMATOGRAPHIC ANALYSIS OF METHYL HEXANOATE
A DEGRADATION PRODUCT OF COMPOUND I

System	Retention Time (Min.) Methyl Hexanoate	B-V Acid as Methyl Ester	Conditions
1	3.0	3.0	5' x 1/8" column, 15% Carbowax, 82 ml./min. flow rate; temperature: 70°C column, 200°C inlet, 230°C flame detector.
2	7.6	7.6	5' x 1/8" column, 20% FFAP; 90 ml./min. flow rate; temperature: 50°C column, inlet 185°C, 210°C detector.
3	5.2	5.2	5' x 1/8" column, 20% DEGS: 102 ml./min. flow rate; temperature: 50°C column, 215°C inlet, 230°C detector.

Oxidation of Methyl 15(R)-Acetoxy-9-Oxo-5,Cis,10,13-Trans-Prostatrienoate (II): Compound II (0.421 grams) and $KMnO_4$ (1.2 grams) were stirred in 25 ml. acetone (distilled over $KMnO_4$) and 25 ml. water for 4 hours at 0°C and an additional 20 hours at 25°C. The purple color was gone after this time and the only color remaining was that of the suspended MnO_2. The MnO_2 was filtered off and most of the acetone removed on a rotary evaporator. The resulting aqueous solution was extracted 5 times with 25 ml. portions of ether. The ether extracts were combined and dried over anhydrous sodium sulfate. After removing the ether, 1.20 grams of neutral oils were recovered. The aqueous reaction products were acidified and again extracted with ether in the same manner as above. The ether solution was dried and the ether removed to yield an oil. After reaction with excess diazomethane 0.24% grams of oil were recovered as methyl ester. This mixture was analyzed by gas chromatography (5' x 1/8" column of 20% FFAP stationary phase, flow rate = 65 ml./min., and a column temperature of 125°C). There were only two major components in the sample of methyl esters in a ratio of approximately 40:60. The smaller of these two peaks had a retention time of 7.7 minutes, while the larger one's retention time was 9.1 minutes. The following standards were run for their retention time.

TABLE X

RETENTION TIMES FOR STANDARD CARBOXYLIC ACID METHYL ESTERS ON 5', 20% FFAP G.C. COLUMN

Compounds	Retention Time
Methyl butyrate	0.95
Methyl heptanoate	1.55
Dimethyl succinate	4.90
Dimethyl glutarate	7.70
Methyl adipate	12.40

The above retention times as well as the sample history strongly suggest the second largest peak to be that of dimethyl glutarate.

Methyl 15(R)-Acetoxy-9-Oxo-5-Cis,10,13-Trans-Prostatienoate (II) From 15-Epi-PGA_2 (I): The methyl ester of I was made by the reaction of I with excess diazomethane in an ether solution. The resulting methyl ester (0.0913 grams) showed a characteristic methoxy signal at δ3.60 and a TLC mobility of R_{dye} = 0.51 on silica gel H plates and 40:60 ethyl acetate: benzene as solvent. The methyl ester was dissolved in 50 ml. benzene along with 0.3 ml. of a 1 N solution of actic anhydride-pyridine in benzene. After 24 hours, the TLC showed no reaction and an additional 0.2 ml. of standard reagent was added. After an additional 24 hours, still no reaction had occurred by TLC, so 5.0 ml. more standard reagent was added. After 24 hours, a small amount of product was formed, R_{dye} = 0.87. An additional 5 ml. of standard reagent was added and after 24 hours the reaction mixture by TLC appeared to be about a 50:50 mixture of product and reactant. Five ml. more standard reagent was added and after 3 additional days reaction time no remaining starting material

could be seen by TLC (product:R_{dye} = 0.87). The volume of the reaction mixture was reduced to approximately 10 ml. on a rotary evaporator. The reacion product was chromatographed on 70 grams silicic acid (CC-4) in a 1.25" diameter column using 8% ethyl acetate in benzene as solvent. A flow rate of 3 ml./min. was used and 100 ml. fractions taken. Compound II was recovered in fractions 3 through 5 (0.0061, 0.0487, and 0.0182 grams). Its mass spectrum as well as NMR, and IR spectra were identical with that of natural II from the gorgonian.

Partial Hydrogenation of 15-Epi-PGA_2 (I): A sample of I (0.3626 grams) was hydrogenated in 50 ml. ethyl acetate using 0.10 grams of 5% rhodium on carbon as catalyst at 50 psig hydrogen in a Parr hydrogenator. The reaction was interrupted at intervals of 1 hour and 1.67 hours and then hydrogenated for an additional 2 hours. At these intervals, the catalyst was filtered from the reaction mixture and the solvent removed. The reaction product was then analyzed by nuclear magnetic resonance and infrared spectroscopy. The extent of hydrogenation of the delta-five and thirteen double bonds of I were determined from the NMR vinyl integral after hydrogenation divided by the vinyl integral in the starting material multiplied by 100. The integrals were normalized against the methyl absorption of I (δ0.90) that remained unchanged in the hydrogenation product.

TABLE XI

HYDROGENATION DATA FOR 15-EPI-PGA_2 USING 5% RHODIUM ON CARBON AS CATALYST

Reaction Time	% Hydrogenation By NMR Absorption For	
	Delta-Thirteen Double Bond	Delta-Five Double Bond
1 hour	47%	67%
1.67 hours	88%	97%

The infrared spectrum of I contains an absorption (970 cm^{-1}) similar to the absorption in PGA_2 for the delta-thirteen double bond. This absorption disappeared on hydrogneation of I. This disappearance was followed by measuring an extinction coefficient at the various stages of hydrogenation. The extinction coefficient was defined as follows:

$$\text{Time} = \frac{\log T_{925\ cm^{-1}} - \log T_{970\ cm^{-1}}}{(\text{cell length})(\text{Conc. moles/liter})}$$

The transmission at 925 cm^{-1} is the base line. By dividing the extinction coefficient at T_t by T_o and multiplying by 100, one obtains a percent hydrogenation as reflected by this trans absorption (970 cm^{-1}). These data are shown in Table XII.

Another infrared absorption bond of I (1350 cm^{-1}) was found to disappear on hydrogenation (see Table XIII). The base line of the spectrum near this absorption (1333 cm^{-1}) was used to calculate an apparent extinction coefficient.

TABLE XII

DISAPPEARANCE OF IR ABSORPTION (970 cm^{-1}) OF 15-EPI-PGA_2 ON HYDROGENATION

Reaction Time	% Transmission 970 cm^{-1}	% Transmission 925 cm^{-1}	Concentration Moles/Liters	Calculated Extinction Coefficient	% Hydrogenation
0	39	72	.236	9.78	0
1 hour	63	81.5	.165	5.92	40
1.67 hours	72	66	.297	1.08	89

Cell length: 0.155 mm

TABLE XIII

DISAPPEARANCE OF IR ABSORPTION (1350 cm^{-1}) OF 15-EPI-PGA_2 ON HYDROGENATION

Hydrogenation Time	% Transmission 1333	% Transmission 1350	Concentration Moles/Liters	Apparent Extinction Coefficient	% Hydrogenation By IR
0	60	54	0.236	1.69	0
1 hour	75	72.5	0.165	.78	70
1.67 hours	60.5	58.7	0.297	.39	99
3.67 hours	71	70	0.138	.38	100

Cell length: 0.115 mm

$$\text{Time} = \frac{\log {}^{T}1333 - \log {}^{T}1350}{(\text{cell length})(\text{Conc. moles/liter})}$$

The above equation was used to calculate the values found in Table XIII for the various stages of hydrogenation of I.

15(R)-Hydroxy-9-Oxo-5-Cis-$\Delta^{8,12}$-13-Trans-Prostatrienoic Acid (15-Epi-PGB2, VI): A sample of 15-epi-PGA_2 (0.1242 grams) was dissolved in 5 ml. MeOH and cooled to 0°C in an ice bath. Five ml. of 1.0N NaOH (50:50, MeOH:H_2O) was added dropwise to the 15-epi-PGA_2 solution and the resulting solution was allowed to warm to room temperature and was stirred at room temperature for 8 hours. The reaction solution was transferred to a separatory funnel with 50 ml. water. It was extracted with two 50 ml. portions of chloroform. After acidification to a pH of approximately 2, the aqueous mixture was again extracted with three 50 ml. portions chloroform. The extracts were combined, dried over Na_2SO_4, and yielded 0.1236 grams of an oil (R_f = 0.52, silica gel H plates and 1% HOAc/ethyl acetate as solvent).

This product was chromatographed on 70 grams silicic acid (CC-4) using 20% ethyl acetate in benzene as solvent. A flow rate of 2 ml./min. was used and 100 ml. fractions were taken. After 17 fractions were taken, the solvent was changed to 30% ethyl acetate with 15-epi-PGB_2 (VI) being eluted in fraction 19) 0.0138 and 20) 0.0429 grams. $[\alpha]_D^{25}$ = -42.3 (C = 0.567, $CHCl_3$); CD Cotton effect (CH_3OH) at 280 mμ = -2880°.

The IR spectrum ($CHCl_3$) was consistent with the structure with peaks at 3600, 3400, 1710, 1690, 1645, 1600, 1440, 1410, 1370, 1300, 1050, and 970 cm^{-1}.

The NMR spectrum ($CDCl_3$) showed resolved peaks at δ6.90 (1,d,J=16),

6.27 (1, dd, J=16, 5 Hz), 5.36 (2, perturbed triplet), 4.19 (1,m), 3.01 (2, m) and 0.90 (3, perturbed triplet).

15(S)-Hydroxy-9-Oxo-5-Cis-$\Delta^{8,12}$-13-Trans-Prostatrienoic Acid (PGB_2, VII): Fourteen milligrams PGE_2 was dissolved in 2 ml. MeOH and the solution was purged with nitrogen. Two ml. of 0.5N NaOH in 50:50, MeOH:H_2O was added dropwise to the PGE_2 solution that had been cooled to 0°C in an ice bath. The solution was allowed to warm to room temperature and was stirred for 8 hours. The solution was diluted to 25 ml. with water and acidified with HCl to a pH of approximately 2. The aqueous mixture was extracted with five 50 ml. quantities of $CHCl_3$. After drying over $MgSO_4$, and removing solvent, 0.0128 gms. of VII was recovered. This was chromatographed on 70 grams silicic acid (CC-4) using 20% ethyl acetate in benzene as solvent. After seventeen 100 ml. fractions were taken, the solvent was changed to 30% ethyl acetate. A 500 ml. fraction was taken. This fraction contained 11.2 m gms. PGB_2, R_f = 0.52 on silica gel H plates and using 1% HOAc in ethyl acetate as solvent. $[\alpha]_D^{25}$ = 36° (c = 0.11, $CHCl_3$), CD Cotton effect (CH_3OH) at 295 mμ = 2295°.

Ozonolysis of Methyl 15(R)-Acetoxy-9-Oxo-5-Cis,10,13-Trans-Prostatrienoate (II): The diester (II, 0.8555 grams) was dissolved in 60 ml. chloroform in a 100 ml. ozonolysis flask for ozonolysis on an Orec Ozonator (model 0366). The reaction was carried out at -15°C with mechanical stirring. The following conditions were set on the ozonator:

air flow = 7.5 liters/min.

current = 2 amperes

cooling water = 1 liter/min.

After four minutes reaction time, the ozone generator and air flow were turned off. A blue color had developed in the $CHCl_3$ solution. The reaction solution was stirred for four more minutes and then the air flow was turned on for two minutes to remove the excess ozone. The chloroform solvent was removed at 25°C on a rotary evaporator. The resulting oil was dissolved in 25 ml. acetone (distilled over $KMnO_4$) and cooled to 0°C while being stirred magnetically. Jones reagent (3 ml.) was added dropwise with a pressure equalized addition funnel. This mixture was allowed to warm to room temperature for three minutes and 100 ml. water were added. An additional 3 ml. of Jones reagent was added and the aqueous mixture was transferred to a separatory funnel with 100 ml. chloroform. The aqueous layer was extracted with five 100 ml. quantities of $CHCl_3$. After drying the combined $CHCl_3$ extracts, and removing the solvent, an oil (1.0069 grams) was recovered. This oil was chromatographed on 70 grams silicic acid (CC-7) in a 1" diameter column using 15% ethyl acetate in benzene as solvent. A flow rate of 2 ml./min. was used and 100 ml. fractions were used.

Fraction	Weight of Fraction	Fraction	Weight of Fraction
1	0.0000	6	0.0190
2	0.0878	7	0.1040
3	0.2827	8	0.0842
4	0.0462	9	0.0312
5	0.0100	10	0.0211
		11	0.0090

Fractions 7 - 10 contained monomethyl glutarate (VIII). Its NMR spectrum ($CDCl_3$) is identical with that of authentic material with absorptions at 10.93 (1, exchangeable), 3.70 (3,S), and 2.7 to 1.8 (6 proton absorptions). The IR spectrum ($CHCl_3$) shows peaks at 1740 and 1715 (unresolved), 1445, 1430, 1380, 1300, 1220, and 1175 cm^{-1}. On esterification in methyl alcohol with trace of H_2SO_4 the dimethyl ester was formed and showed the same retention time (9.8 minutes) on a 3% SE-30, 5' x 1/8" column at 90°C as did an authentic preparation from glutaric acid.

The fractions 2 - 4 (α-acetoxy heptanoic acid, IX) were rechromatographed using 13% ethyl acetate in benzene as solvent using the same type column described above. Fractions 4) 0.1391 and 5) 0.0579 grams were combined and showed characteristic NMR peaks at 8.46 (1, exchangeable), 5.05 (1,T, J=6.5 Hz), 2.14 (3,S), and 0.90 (3, perturbed triplet): $[\alpha]_D^{25}$ = 21.4 (C = 1.96, $CHCl_3$).

Compound IX (0.1960 grams) was hydrolyzed in 75 ml. of 0.1N NaOH at 25°C for 2.5 hours. At this time the aqueous reaction solution was carefully acidified with HCl and extracted with five 100 ml. portions of $CHCl_3$. After drying over Na_2SO_4, the combined chloroform extracts yielded, on removing the solvent, 0.1612 grams of α-hydroxy heptanoic acid (X), mp = 51 - 56.5°C $[\alpha]_D^{25}$ = -16.7°C (C = 1.612, $CHCl_3$). On recrystallization from benzene pentane 0.0692 grams of X, m.p. 63.5 - 65°C, was recovered.

Anal. Calcd for $C_7H_{14}C_3$: C, 57.51%; H, 9.65%; found: C, 57.8%; H, 9.55%.

The mass spectrum supports the structure for X with peaks at 128 (m-18), 101 (m-45), and 83 (m-18-45). $[\alpha]_D^{25} = -13$ (c = 0.692, $CHCl_3$). The ORD spectrum (CH_3OH) shows peaks at $[\alpha]_{265} = -910°$, $[\alpha]_{250} = 780°$, $[\alpha]_{216} = -16{,}500°$.

The NMR spectrum showed resolved peaks at δ7.19 (2, exchangeable), 4.28 (1,m), and 0.92 (3, perturbed triplet).

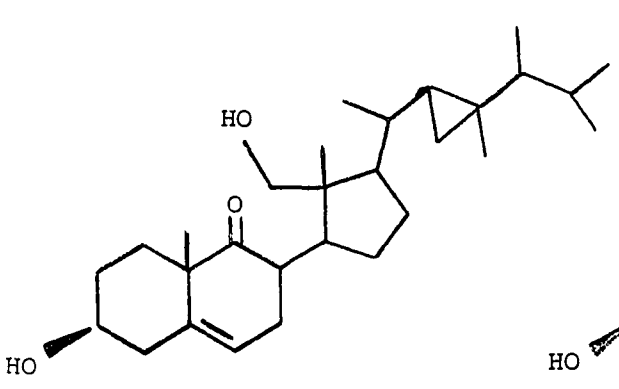
II. TWO NOVEL MARINE STEROIDS: Δ^5-3,11,DIHYDROXY-9,11-SECO-GORGOSTEN-9-ONE (I) AND ITS 5α,6α-EPOXIDE (II) FROM THE GORGONIAN PSEUDOPTEROGORGIA AMERICANA (GMELIN)

INTRODUCTION

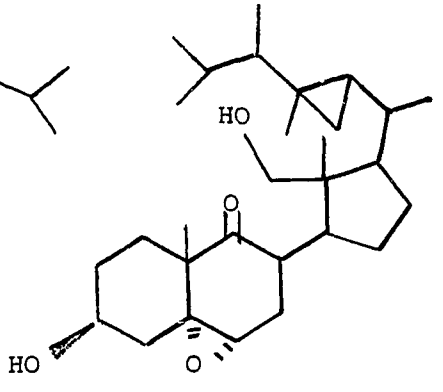
The gorgonian, Pseudopterogorgia americana (Gmelin) has been reported by laboratories[21] to contain a solid with interesting functionality. The initial isolation was done by Dr. L. S. Ciereszko on material collected near Bermuda. The present work was done with Pseudopterogorgia americana collected near Miami. As before, a crystalline precipitate was observed on hot hexane extraction of this gorgonian; however, this precipitate differed from the material from Bermuda in that the Miami gorgonian contained a second component which proved to be the 5α,6α-epoxide of the first. These compounds were separated as their diacetates on silicic acid chromatography. Preliminary spectral data on these compounds showed them to be novel compounds, each containing a cyclopropane ring, and thirty carbon atoms. Further examination of these compounds led to their identification as 9,11-Seco-gorgosterol derivative, a new class of marine steroids.

RESULTS AND DISCUSSION

The air dried cortex of Pseudopterogorgia americana was found to contain 0.03% of the novel steroid (I), figure 10, and 0.02% of its 5α, 6α-epoxide (II), figure 11. In addition to an open C-ring, these steroids possess an eleven carbon side chain containing a cyclopropane group. This side chain appears to be identical to that of gorgosterol, a marine sterol whose occurrence in the various gorgonians has been reported in our laboratories [22] and whose structure proof paralleled this work.[23]



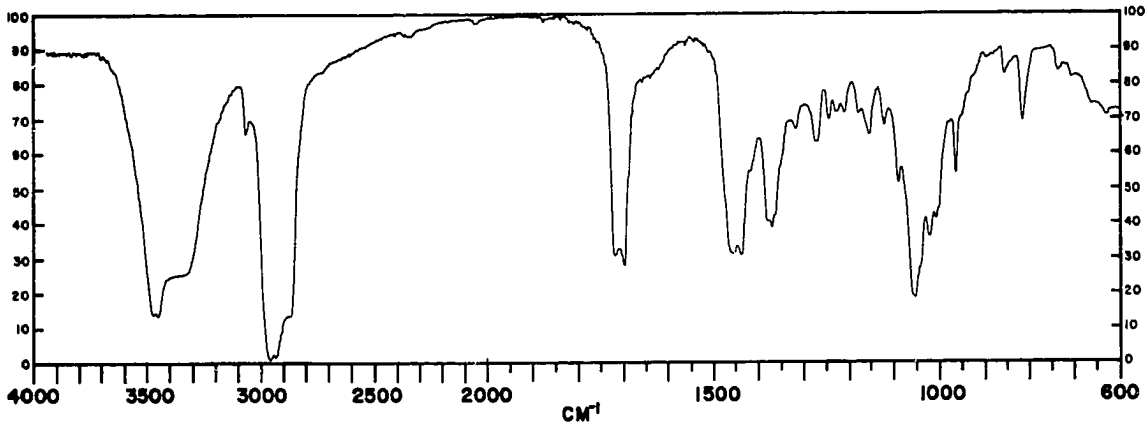
I



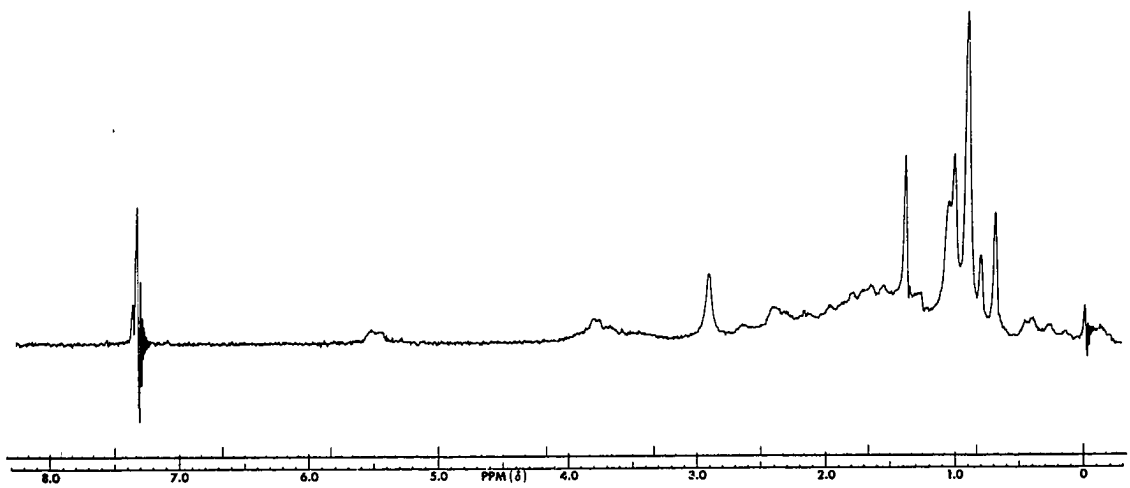
II

Figure 10
Spectra of Δ^5-9,11-Seco-Gorgosten-3,11-Diol-9-One (I)

IR Spectrum (KBr) of I



NMR Spectrum ($CDCl_3$) of I



Mass Spectrum of I

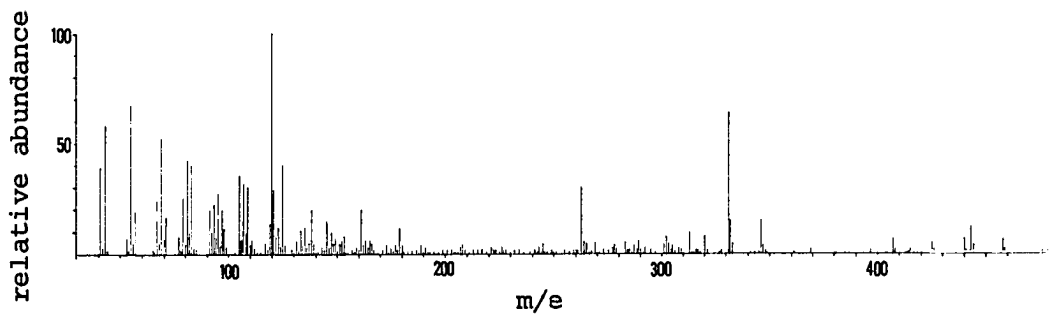
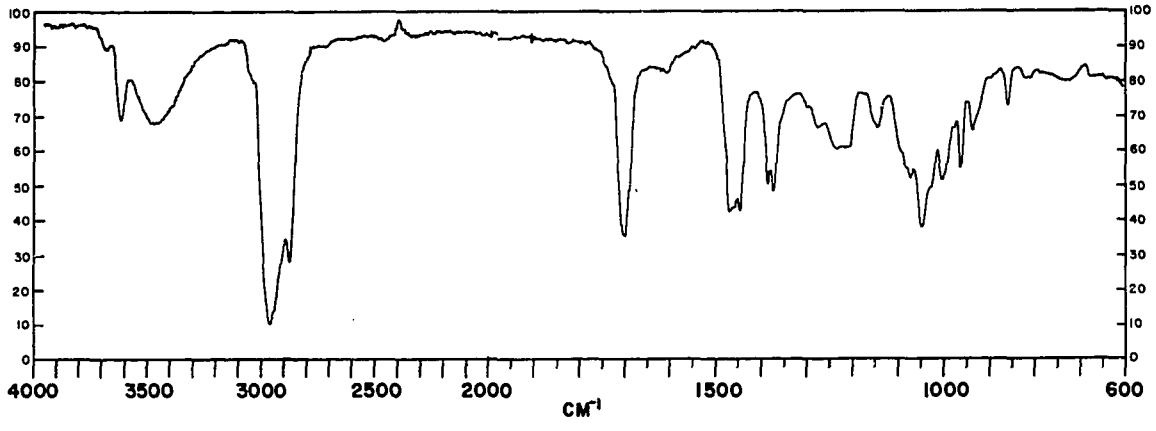
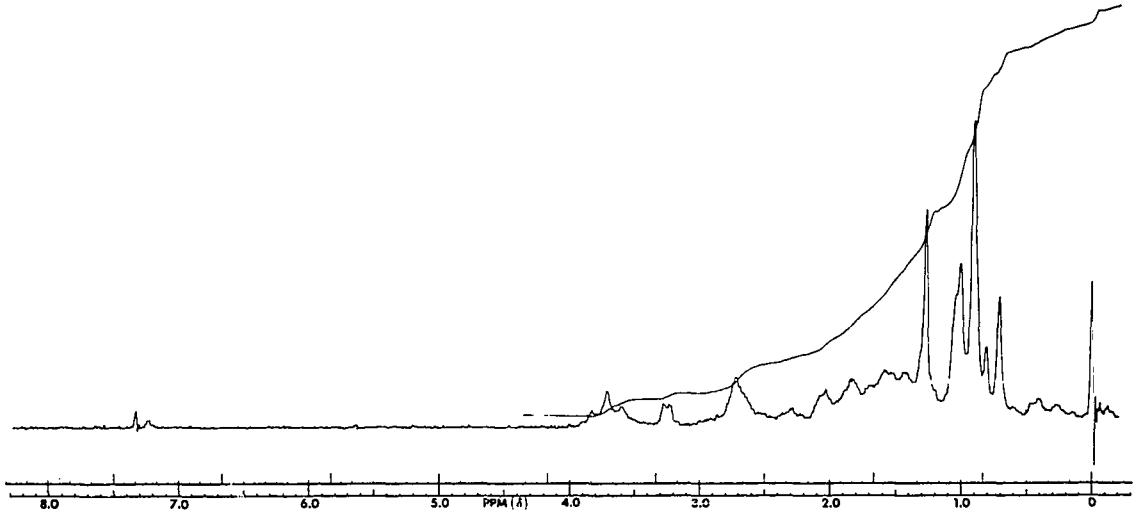


Figure 11
Spectra of 5α,6α-Epoxy-9,11-Seco-Gorgostan-3,11-Diol-9-One (II)

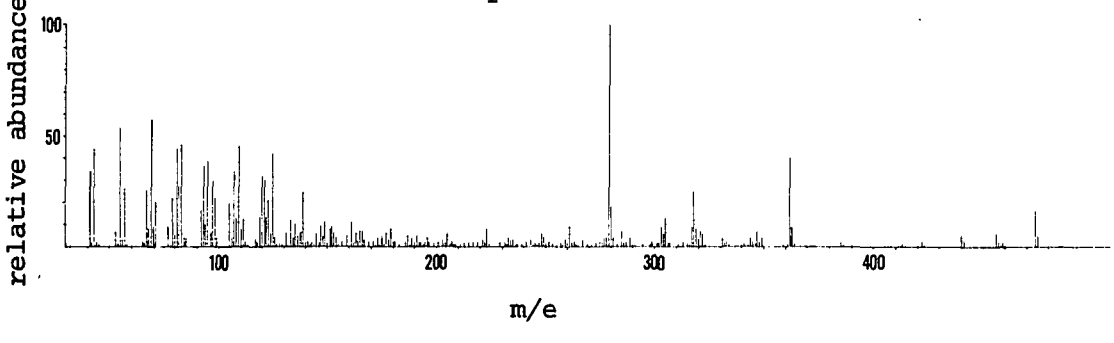
IR Spectrum ($CHCl_3$) of II



NMR Spectrum ($CDCl_3$) of II



Mass Spectrum of II

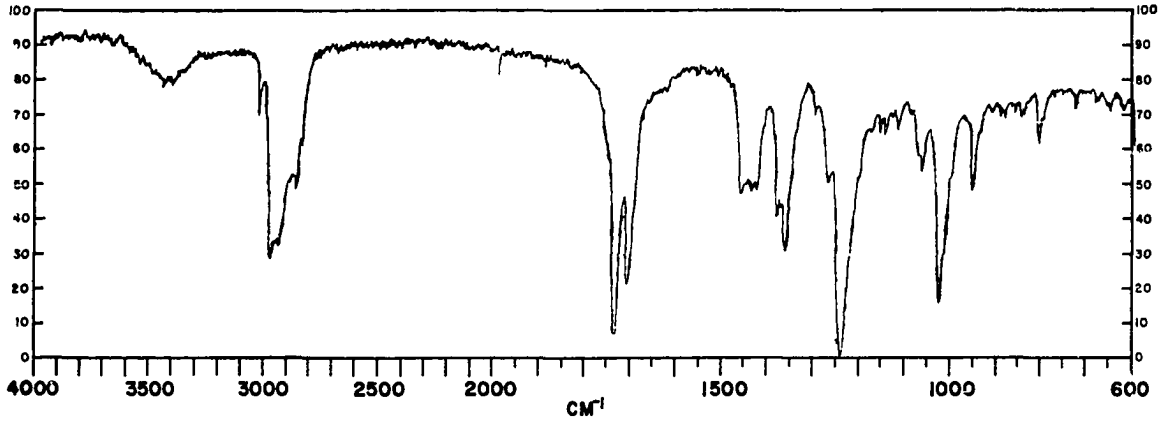


Previously air dried, ground, and cold hexane extracted cortex was again extracted with hot hexane. The highly oxygenated steroids (I) and (II) precipitated from the resulting hot solution under these conditions and were purified as their diacetates by silicic acid chromatography (Silicar CC-7) using 3% ethyl acetate in benzene as the eluting solvent.

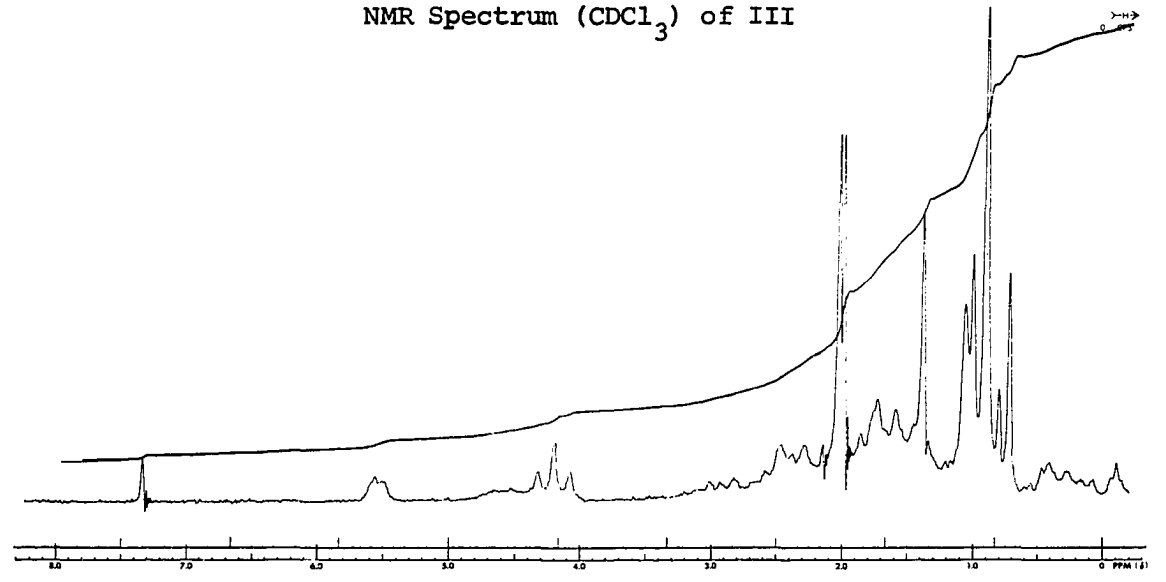
The mass spectrum (M^+458) as well as combustion data for I shows its molecular composition is $C_{30}H_{50}O_3$. Its IR spectrum (KBr) shows a split carbonyl absorption occurring at 1720 and 1700 cm^{-1} indicating strong hydrogen bonding of the ketone function. Compound I forms diacetate III on treatment with acetic anhydride in pyridine whose combustion data and mass spectrum (M^+542) shows its composition to be $C_{34}H_{54}O_5$, figure 12. The diacetate III exhibits normal ketone absorption in the UV (292 mμ,ε=40) and a negative Cotton effect in its circular dichroism spectrum (-10,000°). Its IR spectrum ($CHCl_3$) shows normal ketone absorption at 1710 cm^{-1} and ester absorption at 1740 cm^{-1} for the two acetates. The NMR spectrum (60 MHz, $CDCl_3$) of III shows a single vinyl proton (C-6) at δ5.50(m), a methine proton at δ4.51(m) for the secondary acetate hydrogen (C-3) and a methylene signal δ4.19 (t,J=8 Hz) for the primary acetate hydrogens at C-11. The spectrum also shows resolved singlet methyl signals at δ1.36 and 0.70 (C-19 and C-18), acetate methyls at δ1.99 and 1.95 and four unresolved high field protons in the cyclopropane region.

In the 220 MHz NMR spectrum ($CDCl_3$) of III the primary acetate methylene appears as the XY of an ABXY. In addition to the acetate methyls, the spectrum shows seven resolved methyl signals, four secondary δ1.05 (3,d,1Hz), 0.95 (3,d,J=6.5Hz), 0.93 (3,d,J=6.5Hz), and 0.85 (3,d,

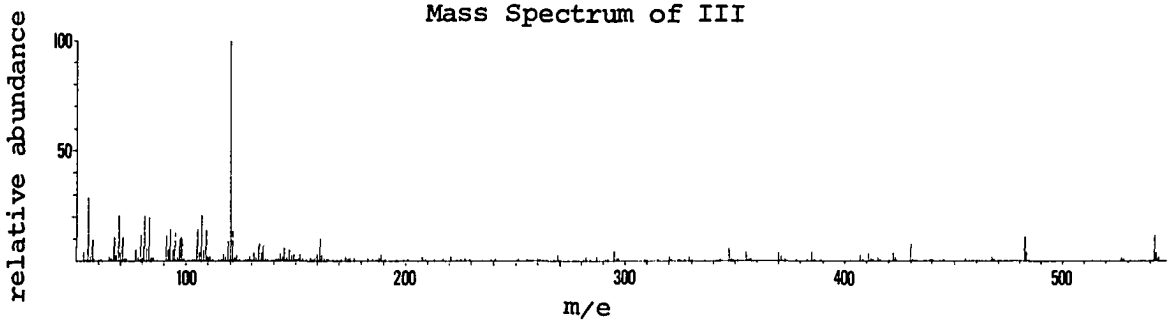
Figure 12
Spectra of Δ^5-3,11-Diacetoxy-9,11-Seco-Gorgosten-9-One (III)
IR Spectrum (KBr) of III



NMR Spectrum ($CDCl_3$) of III



Mass Spectrum of III



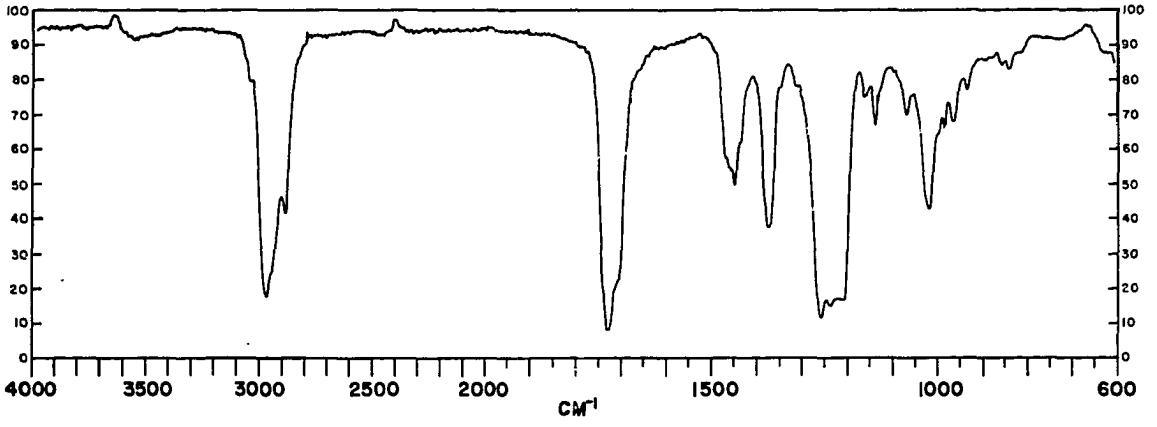
J=7Hz) and three tertiary methyl groups δ1.137 (3,S), 0.88 (3,S) and 0.70 (3,S). A large upfield shift for the low field singlet methyl group was observed on solvent change to benzene ($\delta 1.37_{(CDCl_3)} - \delta 1.18_{(Bz)} = \Delta\delta 0.19$) which is characteristic for the methyl of an axial 2-methyl cyclohexanone system.[24] The cyclopropane region shows three distinct areas of absorption. Double doublet absorptions at δ~0.12 (1,dd,J=4,4.5Hz) and 0.48 (1,dd,J=9,4Hz) are consistent for a methylene of a cyclopropane with the high field hydrogen being shielded by two cis substituted alkyl chains.[25] These methylene hydrogens are coupled to a third cyclopropane hydrogen,δ0.25, which is cis to the lower field methylene hydrogen (J=9Hz) and trans to the higher field methylene hydrogen. On solvent change to benzene the signals of the methylene of the cyclopropane are shifted to -δ0.15 (1,dd,J=4,4.5Hz) and 0.46(1,dd,J=9,4Hz). The third cyclopropane hydrogen is still unresolved from the non-cyclopropane-hydrogen, δ0.25.

On hydrogenation of III over PtO_2 at 45 psig its dihydro derivative (IV, M^+544) is formed, figure 13. The hydrogenation occurred exclusively from the beta side, forming a cis fused A/B ring system as indicated by the large downfield shift (δ0.48) of the secondary acetate proton at C-3 which has gone from an axial orientation in III to equatorial in IV. On extended hydrogenation in acetic acid no additional carbon-carbon double bonds were reduced. This suggests only one carbon-carbon double bond and requires four rings in III to account for its eight degress of unsaturation.

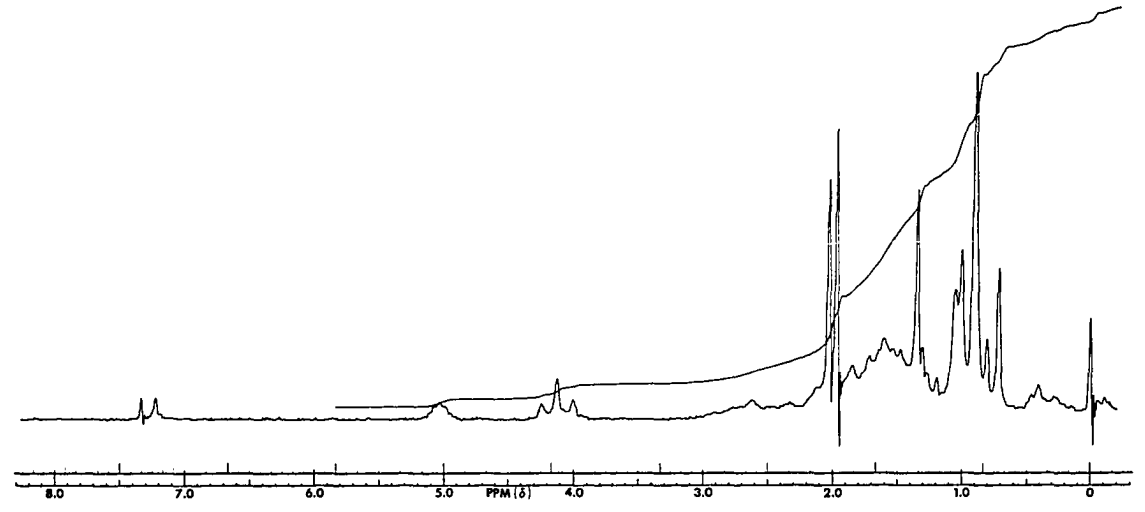
Epoxidation of III with the nonselective trifluoroperacetic acid gave a mixture consisting of an oily epoxide (V, M^+558), figure 14, and a solid epoxide (VI, M^+558), figure 15. The shifts to higher field of

Figure 13
Spectra of 3,11-Diacetoxy-5β-Dihydro-9,11-Seco-Gorgostan-9-One (IV)

IR Spectrum ($CHCl_3$) of IV



NMR Spectrum ($CDCl_3$) of IV



Mass Spectrum of IV

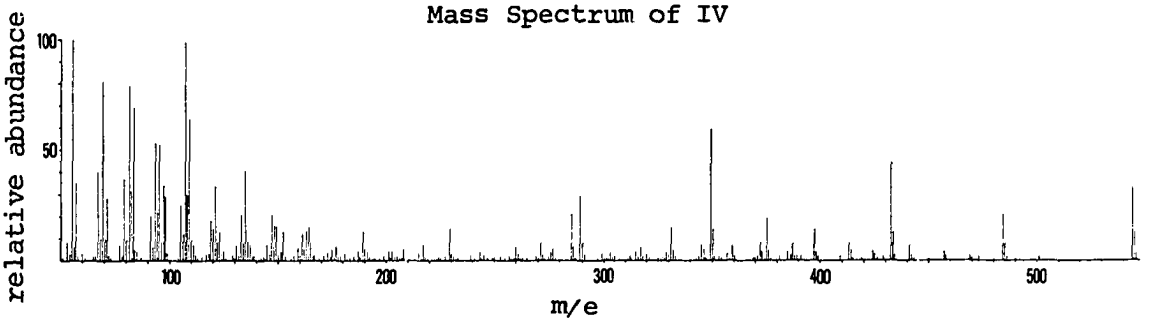
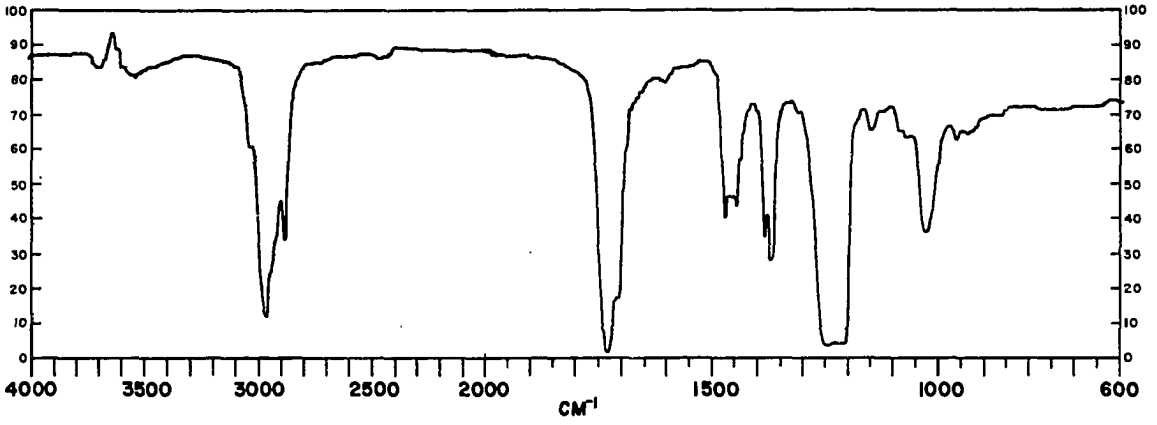
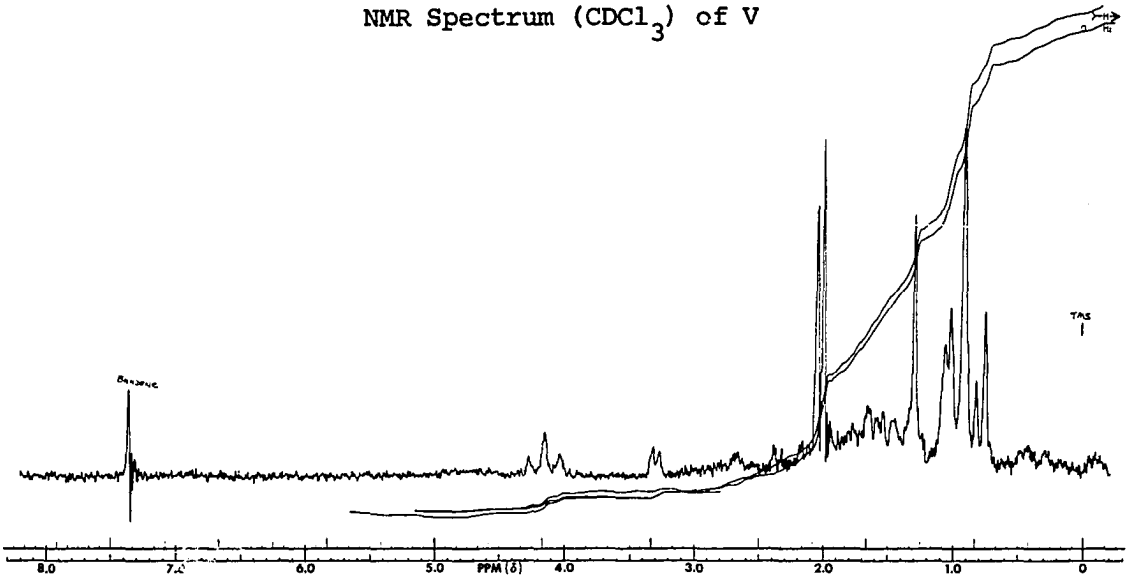


Figure 14
Spectra of 5α,6α-Epoxy-3,11-Diacetoxy-9,11-Seco-Gorgostan-9-One (V)

IR Spectrum ($CHCl_3$) of V



NMR Spectrum ($CDCl_3$) of V



Mass Spectrum of V

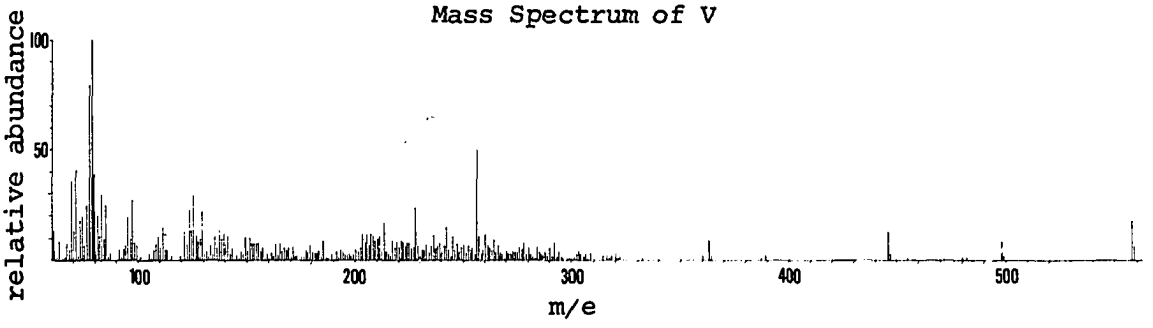
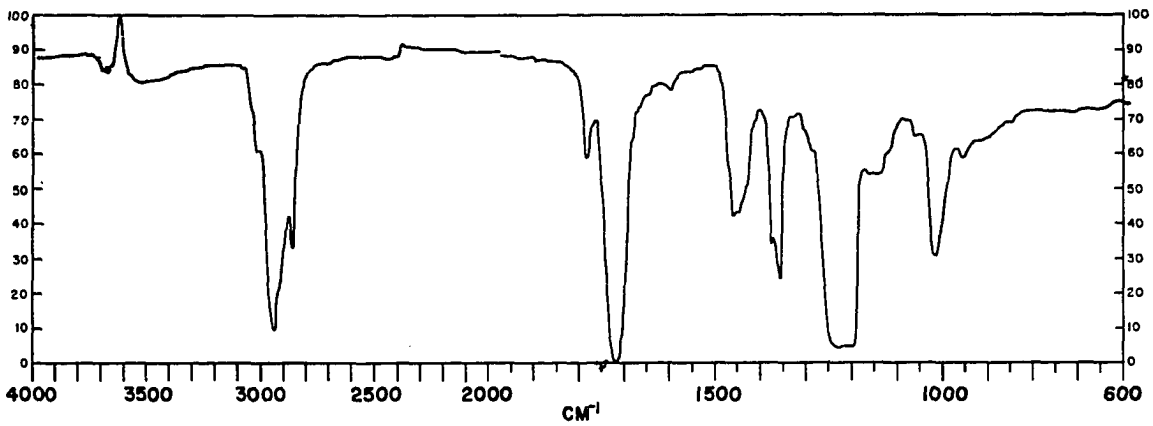
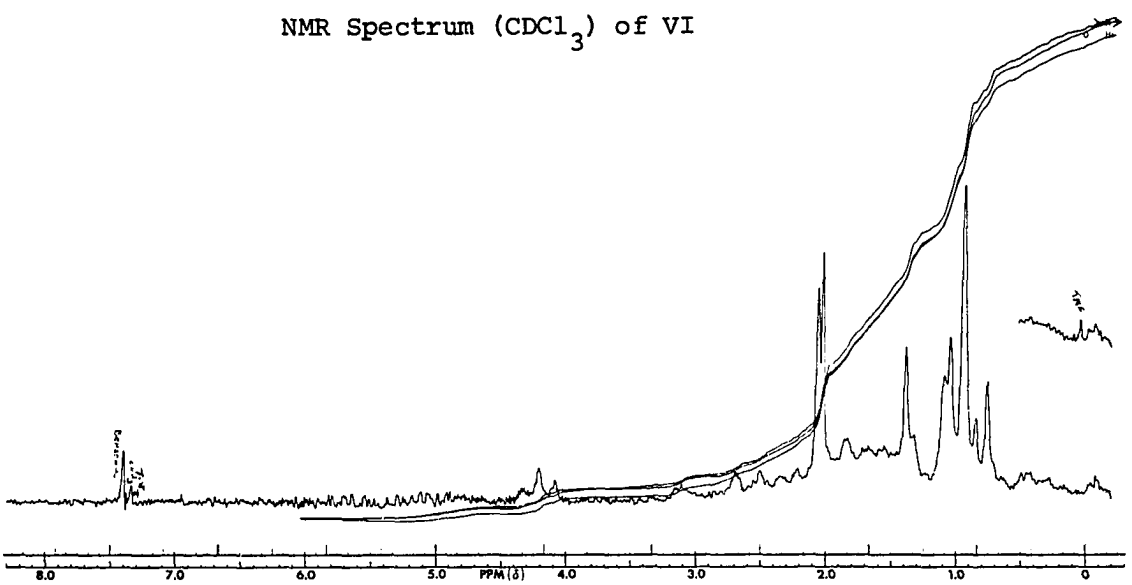


Figure 15
Spectra of 5β,6β-Epoxy-3,11-Diacetoxy-9,11-Seco-Gorgostan-9-One (VI)

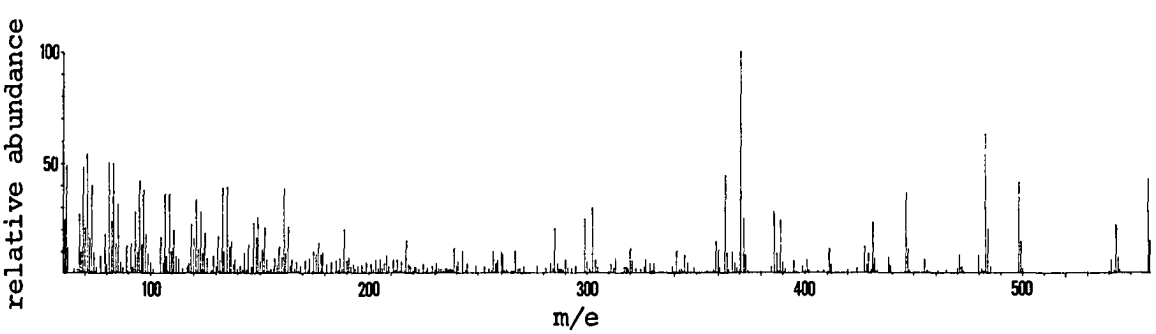
IR Spectrum ($CHCl_3$) of VI



NMR Spectrum ($CDCl_3$) of VI



Mass Spectrum of VI



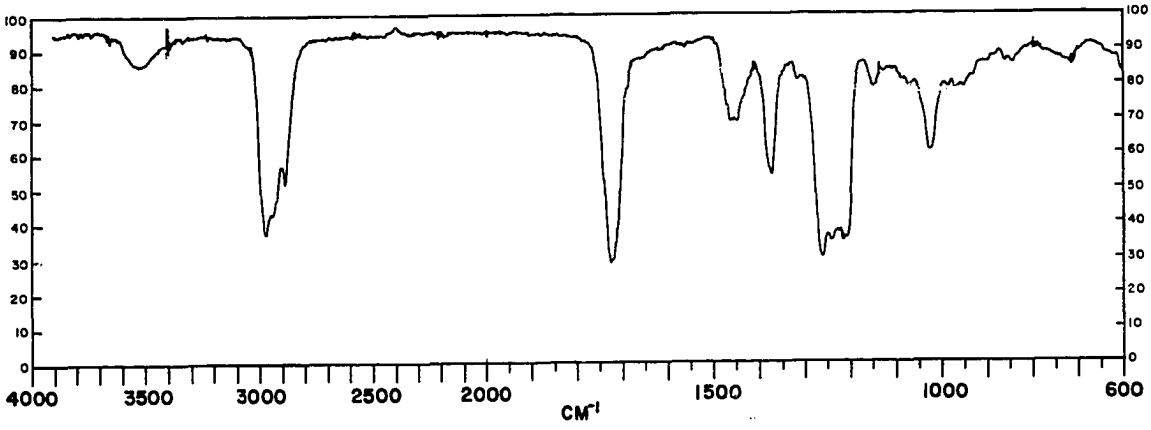
of the C-19 methyl in the NMR spectra of IV, V, and VI compared to III are completely consistent with functional group changes at the 5,6-double bond. The epoxide function in VI is assigned the beta orientation due to its greater deshilding effect on the C-19 methyl (8 Hz) compared to that in the alpha epoxide, V.[26]

The alpha epoxide (V) had the same chromatographic mobility, specific rotation, NMR (60 Hz, $CDCl_3$) and IR ($CHCl_3$) spectra as the diacetate of the natural occurring diol (II). On very mild hydrolysis of V using 1.5 equivalents KOH methanol, II was formed along with a slower moving minor component detected by thin layer chromatography. After chromatography, the II obtained was shown by melting point and mixed melting point to be identical with the natural diol. On storage for several months, the melting range of II changed from 132-132.5°C to 124-129°C.

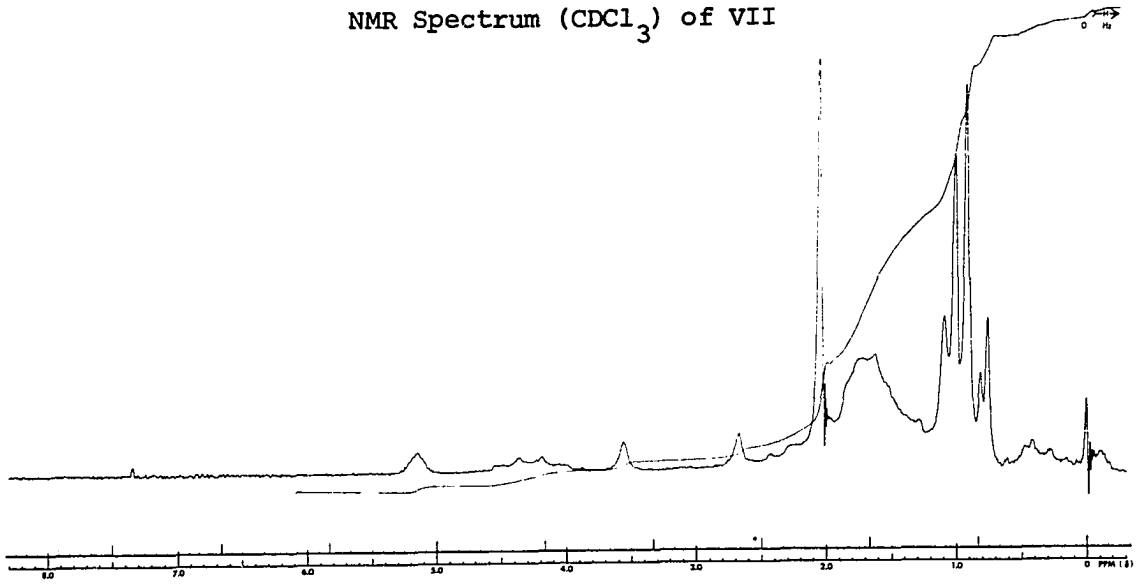
On hydrogenation of III with PtO_2 in glacial acetic acid containing $HClO_4$ the ketone carbonyl was reduced forming VII (M^+546), figure 16. The hydrogen again added from the beta side forming an axial alcohol at C-9 (δ3.56, broad singlet). In the 220 MHz spectrum, H-9 appeared as a broad singlet, $W_{.5}$ = 5 Hz. On treatment of III with $NaBH_4$ in absolute ethanol the diol VIII (M^+504) was obtained, figure 17. Its mass spectrum and combustion data show its composition to be $C_{32}H_{56}O_4$. In addition to reduction of the carbonyl group, the primary acetate C-11 was hydrolyzed with probable assistance by the C-9 alcohol. On selective acetylation of VIII with acetic anhydride and pyridine, the 11-acetate (VII) was formed. The product showed the same spectra and chromatographic properties as VII. Strong hydrogen bonding between the

Figure 16
Spectra of 3,11-Diacetoxy-5β-Dihydro-9,11-Seco-Gorgostan-9-Ol (VII)

IR Spectrum ($CHCl_3$) of VII



NMR Spectrum ($CDCl_3$) of VII



Mass Spectrum of VII

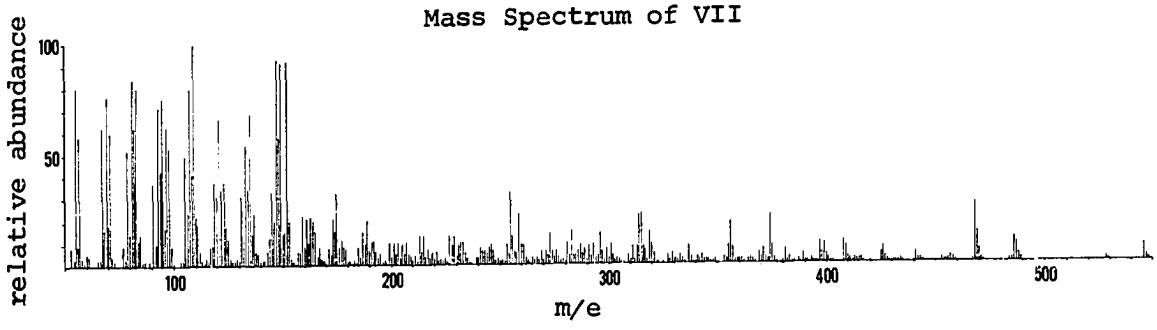
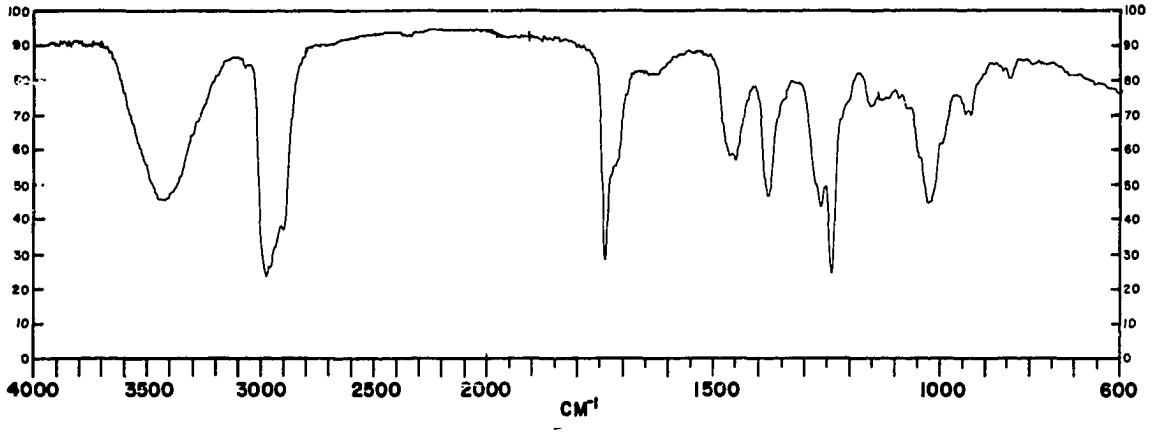
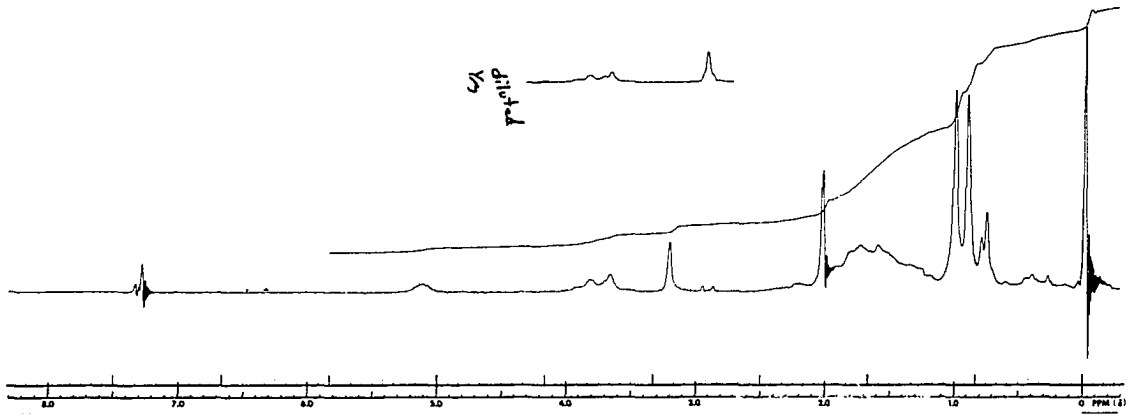


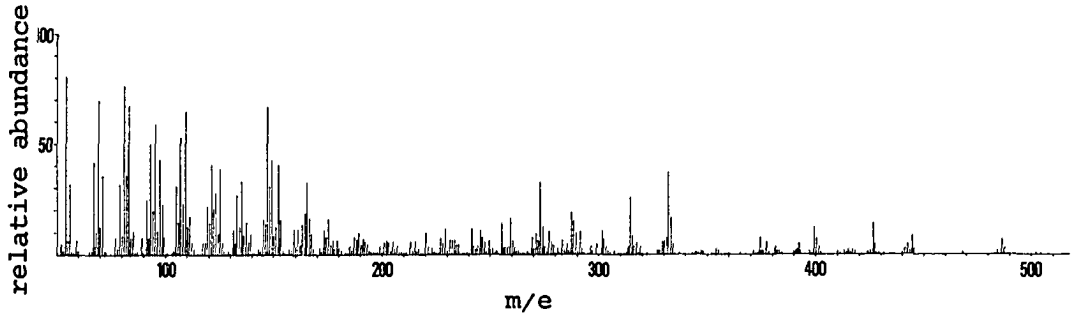
Figure 17
Spectra of 3-Acetoxy-5β-Dihydro-9,11-Seco-Gorgostan-9,11-Diol (VIII)
IR Spectrum (KBr) of VIII



NMR Spectrum ($CDCl_3$) of VIII



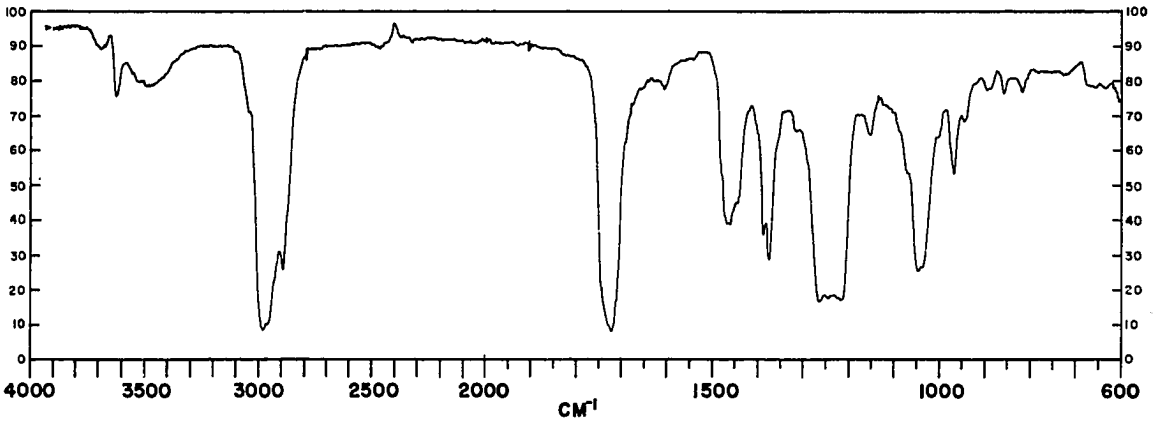
Mass Spectrum of VIII



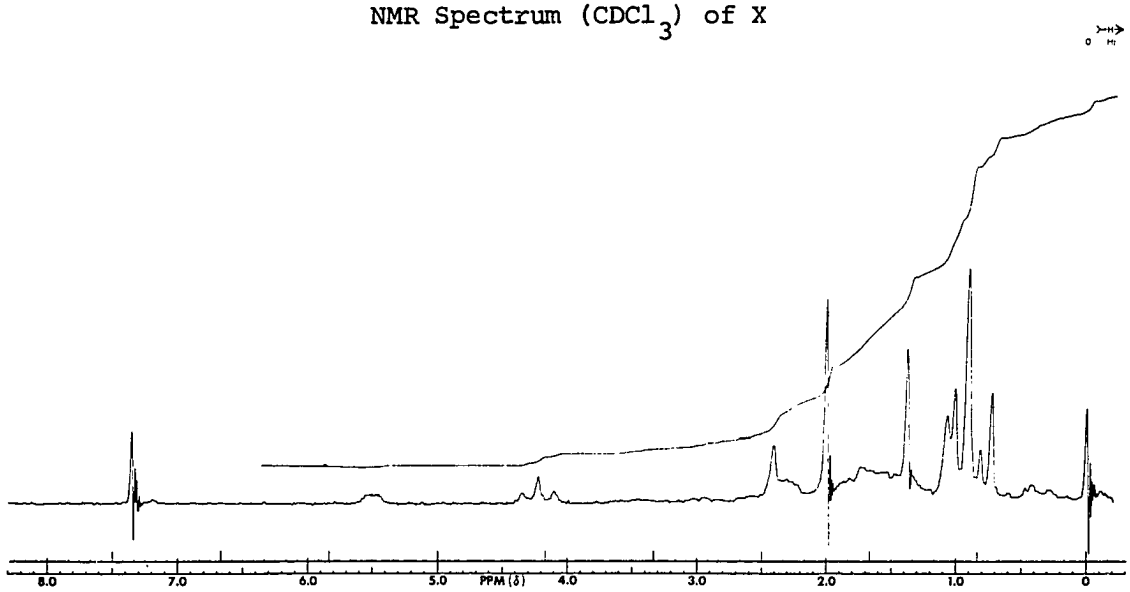
C-9 and C-11 alcohols of VIII was indicated by the NMR spectrum ($CDCl_3$) chemical shift of the C-9 proton (δ3.70, broad singlet). The downfield shift ($\Delta\delta$ = 0.14) of the C-9 proton in VIII, compared to that in VII, can be explained by polarization of the C-9 carbon oxygen bond due to hydrogen bonding with the C-11 alcohol. Treatment of III with $NaBD_4$ in absolute ethyl alcohol yielded IX (M^+505) which provided a convenient deuterium tag for studying the fragmentation of the diol VIII.

Selective hydrolysis of III with KOH in methanol gave good yields of the 3-ol-11-acetate X(M^+500, figure 18) along with I. The NMR spectrum of X (60 MHz, $CDCl_3$) shows resolved signals for the single vinyl proton at δ5.51 (m), the methine of the secondary alcohol at 3.55 (m), the methylene of the primary acetate at 4.23 (t,J=7Hz) and singlet methyl signals at δ2.00, (acetate), 1.38, (C-19), and 0.73, (C-18). On oxidation of X with Jones reagent, the unstable Δ^5-3,9-diketone (XI) was formed. The IR spectrum of the crude reaction product showed absorptions at 1740 cm^{-1} (11-acetate) and 1710 cm^{-1} for the non-conjugated ketones, C-3 and C-9. The oxidation product was chromatographed on silicic acid (Silicar CC-7) and found to isomerize on the column to the Δ^4-3,9-diketone (XII) that showed only one spot by TLC. Its IR spectrum ($CHCl_3$) showed strong carbonyl absorption at 1680 cm^{-1} (conjugated ketone) as well as at 1740 cm^{-1} (acetate) and 1710 cm^{-1} (unconjugated ketone, C-9). The mild conditions required for shifting the 5,6 double bond into conjugation with the ketone at C-3 verifies the homoallylic relationships between the 3-hydroxyl and the 5,6 double bond of I. The absence of an absorption at 1620 cm^{-1} in XII suggested the transoid conjugated ketone.[27] Its NMR spectrum showed a downfield shift for the

Figure 18
Spectra of Δ^5-11-Acetoxy-9,11-Seco-Gorgosten-3-Ol-9-One (X)
IR Spectrum ($CHCl_3$) of X



NMR Spectrum ($CDCl_3$) of X



Mass Spectrum of X

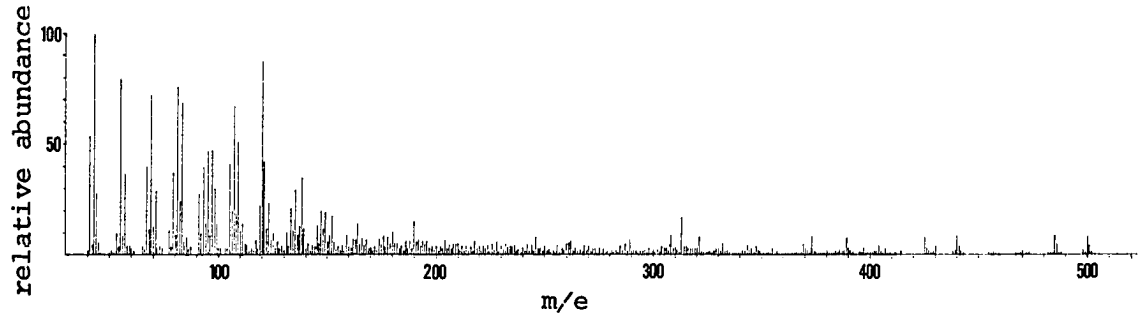
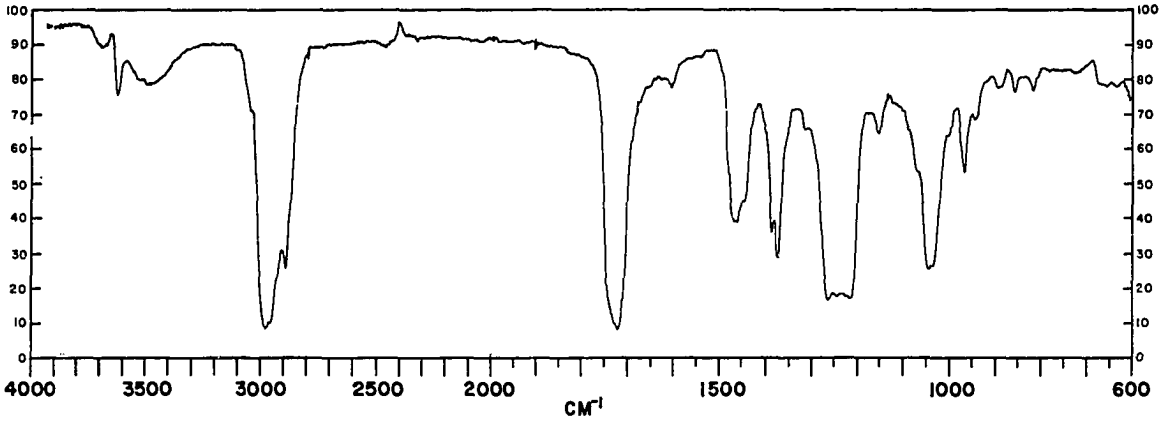
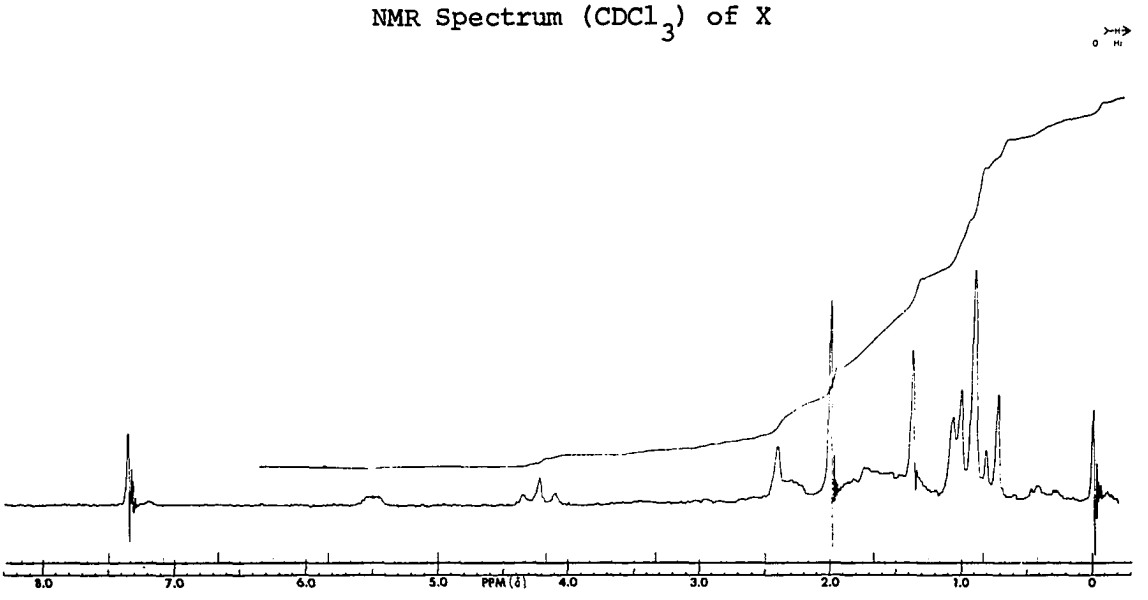


Figure 18
Spectra of Δ^5-11-Acetoxy-9,11-Seco-Gorgosten-3-Ol-9-One (X)

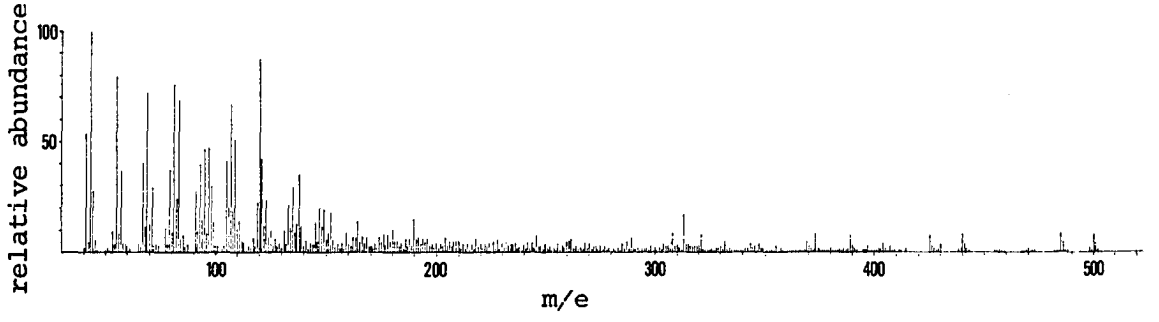
IR Spectrum ($CHCl_3$) of X



NMR Spectrum ($CDCl_3$) of X



Mass Spectrum of X



C-19 methyl to δ1.50 compared to 1.38 for compound X. The instability of XII prevented complete characterization. Oxidation of I with Jones reagent yielded again a reaction product containing little or no conjugated ketone with expected carbonyl absorption in the IR spectra at 1710 cm^{-1}. On Silicar CC-7 partial isomerization gave the conjugated Δ^4-3,9-diketone-11-carboxylic acid (XIII) which without purification showed the following spectral data: IR (film) 1700 and 1680 cm^{-1}; UV (MeOH) 239 mμ (ε = 7,000). The ultra-violet absorption (239 mμ) of XIII is compatible with the expected unsubstituted C-4 position of the conjugated ketone. The absence of any 1620 cm^{-1} in the IR spectrum again points to a transoid conjugated ketone.[27]

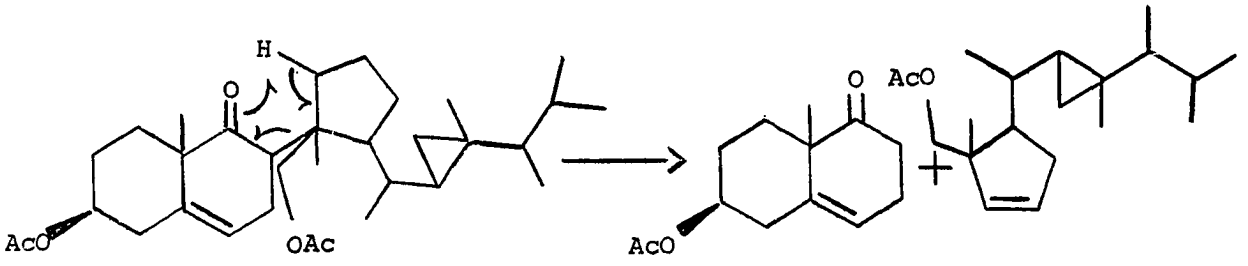
On treatment of I with sodium methoxide in deuteromethanol (DOMe) at room temperature for a total of eight days, a product was obtained that showed no appreciable change by NMR (60 MHz, $CDCl_3$). The product was analyzed by mass spectroscopy for increase in molecular weight of the parent ion (M^+458) to determine the number of hydrogens alpha to the ketone group. The mass spectrum (75 ev) of the product showed peaks at 458, 1.3%; 459, 1.9%; 460, 1.6%; 461, 0.9%; 462, 0.6%; 463, 0.3%, suggesting partial incorporation of three deuterium atoms. This product was then dissolved in deuteromethanol with sodium methoxide and refluxed for two days. The product was again analyzed by mass spectroscopy (25 ev) for deuterium incorporation and showed peaks at 458, 1.4%; 459, 4.8%; 460, 2.9%; 461, 2.4%; 462, 1.4% 463, 0.5%; and showed no further increase in the deuterium incorporation in I. The product from the second attempt at deuterium incorporation was acetylated with acetic anhydride and pyridine in benzene and chromato-

graphed on Silicar CC-7 to obtain deuterium enriched diacetate III. The mass spectrum (normal M^+542) showed again partial incorporation of three deuterium atoms; 542, 0.7%; 543, 6.7%; 544, 2.8%; 545, 3.0%; 546, 1.0%; 547, 0.4%. The NMR (100 MHz, $CDCl_3$), however, showed the complete loss of the one proton signal at δ2.99 assigned to the C-8 position. This signal in the 220 MHz NMR spectrum is clearly coupled to three near neighbors (dt, J=12,4Hz). The axially oriented C-8 hydrogen shows large diaxial coupling with the axial hydrogen of the methylene at C-7 and two smaller couplings with the equatorial hydrogen at C-7 and the single hydrogen at C-14. This demonstrates the absence of a methyl substitution at C-14 which is a common site of substitution in thirty carbon steroids.

The 220 MHz spectrum of the diacetate showed that deuterium incorporation had occurred exclusively at the alpha position, C-8, and further, that incorporation at this center was complete. Therefore, the mass spectral behavior of the 8-deutero compound, which suggests the partial incorporation of three or more deuterium atoms, must be considered anomolous. The anomaly is further compounded by the fact that the total relative intensities of the group of M^+ ions is only about one third of the relative intensity of the normal molecular ions in the mass spectra of I and III. Although no rationalization of these observations can be offerred at this time, it appears that an unusual exchange of the 8-H (D) occurs as the molecule enters the mass spectrometer, resulting in a thorough distribution of the single deuterium atom amongst several molecules.

The high resolution mass spectrum of III shows peaks at 320

($C_{21}H_{36}O_2$) and 222 ($C_{13}H_{18}O_3$) for the McLafferty cleavage of its 8,14-bond. Strong peaks are also present at 260 ($C_{19}H_{32}$) and 162 ($C_{11}H_{14}O$) that represent loss of acetic acid from these fragments. These data, along with the mass spectra data for compounds VIII and IX, point to a decalone system for III substituted with an alkyl substitution ($C_{21}H_{35}O_2$) alpha to the ketone.



The comparison of the mass spectrum of VIII to that of IX allows one to determine the molecular fragments that contain the reduced ketone function by the deuterium tag in IX at C-9. A high resolution mass spectrum of VIII was also obtained which gave the chemical composition of each fragment. These data show that VIII fragments with losses of one or two moles of water, loss of acetic acid, and loss of the fragments $-C_8H_{16}$ and $-C_{11}H_{21}$. Combined losses of these elements were observed in the spectrum of VIII with the corresponding peak in the spectrum of IX occurring at one greater mass unit. Table XIV shows some of these fragmentations.

TABLE XIV

SOME MASS SPECTRUM FRAGMENTATIONS OF VIII
THAT RETAIN DEUTERIUM IN THE SPECTRUM OF IX

			Loss of Fragments				
Mass Weight	Fragment Composition	% of Base Peak	H_2O	H_2O	HOAc	$-C_8H_{16}$	$-C_{11}H_{21}$
504	$C_{32}H_{56}O_4$	1					
486	$C_{32}H_{54}O_3$	7	X				
468	$C_{32}H_{52}O$	1	X	X			
444	$C_{30}H_{52}O_2$	9			X		
426	$C_{30}H_{50}O$	14	X		X		
392	$C_{24}H_{40}O_4$	5				X	
374	$C_{24}H_{38}O_2$	8	X			X	
332	$C_{22}H_{36}O_2$	37			X	X	
314	$C_{22}H_{34}O$	26	X		X	X	
296	$C_{22}H_{32}$	4	X	X	X	X	
309	$C_{19}H_{33}O_3$	1	X				X
291	$C_{19}H_{31}O_2$	11	X	X			X
273	$C_{19}H_{29}O$	33	X		X		
255	$C_{19}H_{27}$	14	X	X	X		X

In addition, VIII easily looses H_2 from its molecular ion and then fragments in the already described manner. The loss of C_2H_5O ($-CH_2CH_2OH$) and HOAc was observed (399, 13%), while the fragment $C_{19}H_{33}O$ (227, 11%) was present in the spectra of both VIII and IX. This represents the loss of the eleven carbon A/B ring system along with H_2.

The data presented are clearly consistent with a six on six A/B ring system with a ketone in the C-9 position of the steroid system. The formation of an axial oriented secondary acetate in IV on hydrogenation of III confirms the ring size containing this function. Its homoallylic position relative to the trisubstituted double bond was demonstrated by the oxidation products of the C-3 hydroxyl in I and X.

The lowfield chemical shift (δ1.36) of the C-19 singlet methyl group of III and the large solvent shift for this signal ($\delta_{chloroform} - \delta_{benzene}$) demonstrates the relationship of the ketone and this methyl group as part of an axial α-methyl cyclohexanone system. Hydrogenation or epoxidation of the double bond of III, oxidation of the C-3 hydroxyl groups in I or X, and reduction of the ketone function have their greatest effects on the chemical shift of the C-19 methyl group. This evidence locates the double bond, the secondary hydroxyl and the ketone of I all in the A/B ring system and is consistent with the mass spectral evidence. The steroid position C-1 is eliminated for the ketone because of a necessary beta relationship with the hydroxyl group at C-3 in I. Since an α,βunsaturated ketone is not formed from I under acid or basic conditions, the C-1 position for the ketone is not possible. Furthermore, since I has only one exchangeable hydrogen alpha to the ketone, only the C-9 position is consistent with all the data. The

negative Cotton effect of III is also completely consistent with this position.

The multiplicity in the NMR spectrum (100 MHz, $CDCl_3$) of the C-6 proton in VI clearly shows an adjacent methylene group. The NMR multiplicity of the single proton alpha to the ketone (dt) of III again suggests a methylene at C-7. The C-8 proton of III appears to be virtually coupled to the vinyl proton C-6 as shown by a sharpening of the latter signal when C-8 is substituted with deuterium. The NMR spectra of the ketone reduction products VII and VIII indicate axial alcohols at C-9. These data together confirm the B-ring as well as the A/B ring system of I with the ketone at C-9.

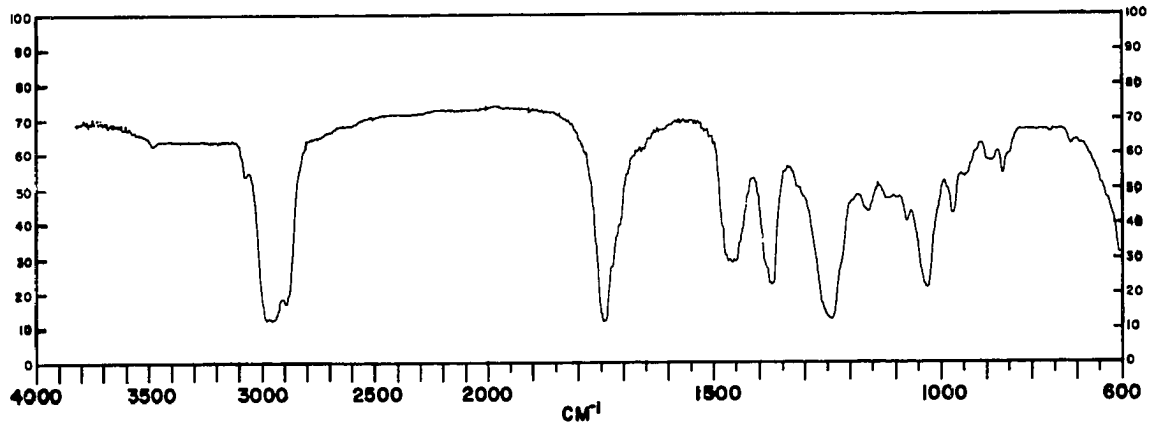
For a steroid system with a C-9 ketone, an open C-ring is necessary. The remaining part of this open C-ring (methylenes C-11 and C-12) is present in I as a beta substituted ethyl alcohol. The methylene of the primary alcohol of I is shown by NMR (220 MHz) to be the XY of an ABXY system. The magnetic and chemical difference in the hydrogens of each methylene (C-11 and C-12) is partly due to the asymmetric center at C-13 and probably partly due to the hydrogen bond ring formation between the ketone and the primary hydroxyl at C-11.

An attempt was made to work chemically into the D-ring of I. Compound VII was dehydrated with thionyl chloride in pyridine. By mixing the reagents at 0°C and allowing the mixture to warm to room temperature, products were obtained that were separated by silicic acid (Silicar CC-7) chromatography using benzene as solvent. The faster moving fractions appear to be an unseparated mixture of compounds with tetra- and tri-substituted bonds as shown by comparing the

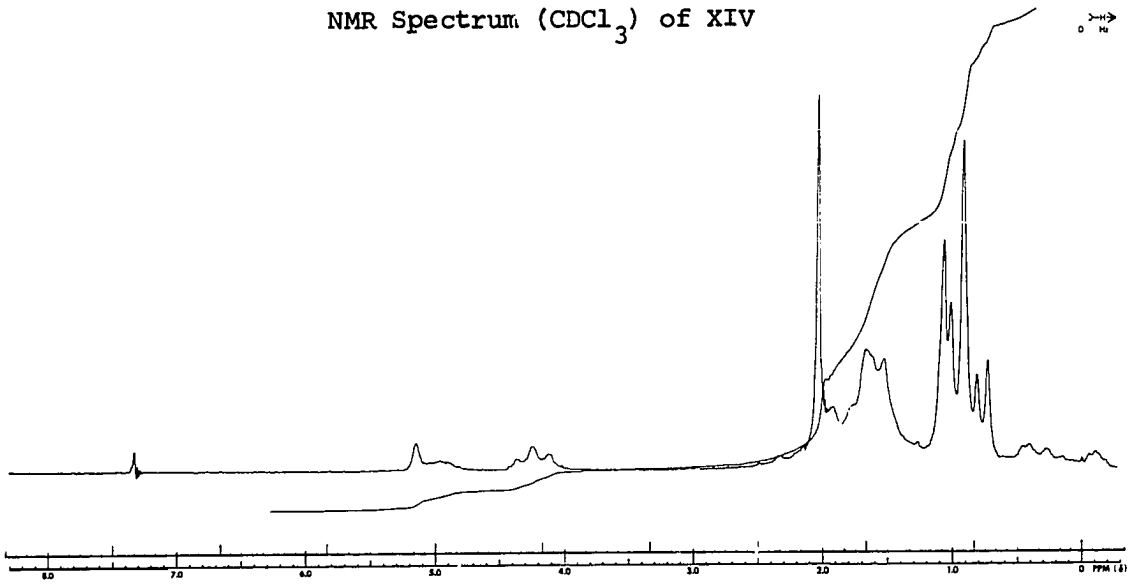
NMR spectrum integrals ($CDCl_3$) for the vinyl and secondary acetate signals. It was decided that the products containing a trisubstituted double bond were rearranged because of the appearance of its vinyl hydrogen absorption. The signal showed large couplings in contrast to the small allylic couplings anticipated for the expected product. A slower moving cleanly separated component XIV, figure 19, found to make up about half the dehydration mixture, appeared on the basis of its NMR spectrum to be the desired dehydration product. Its vinyl proton appeared in the NMR spectrum as a broad singlet and the entire spectrum integrated perfectly for the various resolved signals. On ozonolysis of XIV, a mixture of two products was obtained. The slower component XV was separated from the faster moving component by silicic acid (Silicar CC-7) chromatography using a maximum flow rate. When the acid XV was allowed to spend longer times on the silicic acid column it was partially converted to the faster moving reaction product. This suggests that the free acid (XV) is partially lactonized under these acidic reaction and chromatographic conditions.

The ketoacid (XV) was treated with trifluoroperacetic acid and converted to the corresponding Baeyer-Villiger ester products. The conversion was confirmed by the difference in R_f values of product and reactant. Loss of ketone carbonyl absorption (1710 cm^{-1}) and appearance of additional ester carbonyl absorption (1740 cm^{-1}) in the IR spectrum of the product compared to the IR spectrum of XV also confirmed the conversion to the Baeyer-Villiger ester products. This product was saponified with NaOH in aqueous methanol and the saponification mixture was repeatedly extracted with chloroform to obtain the neutral

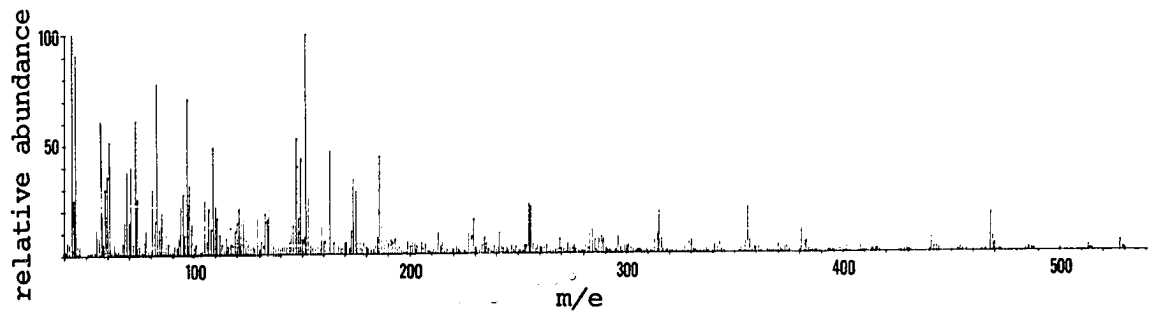
Figure 19
Spectra of A Dehydration Product of VII (XIV)
IR Spectrum (film) of XIV



NMR Spectrum ($CDCl_3$) of XIV



Mass Spectrum of XIV



materials. After careful acidification with concentrated HCl the reaction mixture was again extracted with chloroform. Only the acid fraction contained product. It crystallized to a solid m.p. 110-111.5°C, but contained a minor impurity by TLC. This product showed a molecular ion at m/e 474, which corresponds to the full molecule, and is incompatible with the anticipated Baeyer-Villiger oxidation at the 8,14-bond. It must be assumed, therefore, that the starting olefin (XIV) in this sequence is also a rearranged product of dehydration. On mixing the reagents for dehydration of VII at -70°C a sulfate ester was formed that failed to eliminate at room temperature but reverted to VII on treatment with water. On mixing the reagents at room temperature (25°C) the dehydration of VII went almost exclusively to XIV as shown by the NMR spectrum of the crude reaction product.

Spectral evidence is available for the D-ring of this novel steroid system. The high field C-18 singlet methyl in the NMR spectrum of I (δ0.70, $CDCl_3$) and its corresponding absorption in each of the described derivatives of I are characteristic of the C-18 angular methyl of 14α-androstane derivatives. The C-18 methyl of both 5α,14α-androstane and 5β,14α-androstane absorb at δ0.69 (NMR, $CDCl_3$) but at 0.99 in the corresponding 14β- derivatives.[28] This demonstrates the shielding effect of a cis 8,14-bond on the 18-methyl group compared to a cis substituted carbon-hydrogen bond to the 18-methyl group. Therefore, I and its derivatives appear by their NMR spectra to have a cyclopentane D-ring with its C-14 position substituted with hydrogen on the alpha side.

Additional evidence for the D-ring of I was obtained from mass spectrometry. Steroids are known to fragment with loss of side chain

and also loss of D-ring carbons C-15, C-16, and C-17 along with their substituents.[29] The latter fragmentation is sometimes accompanied by a transfer of hydrogen from the C-8 position. Fragmentations occur in I and its derivatives with losses of $-C_{11}H_{21}$ and $-C_{14}H_{27}$ along with losses of H_2O and/or acetic acid. These precise masses were verified in the high resolution mass spectra of I, III and VIII. These data support for I an eleven carbon side chain containing one unsaturation equivalent ($-C_{11}H_{21}$ fragment) and a cyclopentane D-ring with unsubstituted methylenes at carbons C-15 and C-16. The "unsaturation" in this novel side chain is necessarily the cyclopropane ring.

The mass spectra of I and its derivatives show losses of $-C_3H_7$ (isopropyl) and $-C_5H_{11}$ (3-methyl-2-butyl) alone and in combination with losses of H_2O and/or acetic acid. These mass losses were verified in the high resolution mass spectra of I, III and VIII and required a C-24 methyl substitution. These fragments account for three of the four secondary methyl groups observed in all the available 220 MHz NMR spectra of derivatives in this series. The chemical shifts for the methyl groups in compounds III and V appear in Tables XV (for chloroform solvent) and Table XVI (for benzene solvent). The isopropyl methyls were easily recognized as they appear close together as overlapping doublets or as an apparent triplet. The absorption of the 21-methyl was assigned on the basis of a long effect of the C-9 ketone on the methine hydrogen at C-20, and an acetate effect at C-11 on the chemical shift of the C-21 methyl group. The remaining secondary methyl (24) was assigned by difference.

TABLE XV

CHEMICAL SHIFTS OF THE NON-ACETATE METHYL GROUPS IN THE DIACETATE (III) AND ITS ALPHA-EPOXIDE (V) AT 220 MHZ ($CDCl_3$)

Methyl Assignment	Diacetate (III)		Diacetate-Alpha Epoxide (V)	
	Chemical Shift (δ)	Coupling Constant	Chemical Shift (δ)	Coupling Constant
C-18	0.70	S	0.73	S
C-24	0.85	d,J=7	0.86	d,J=7
Cyclopropyl	0.88	S	0.89	S
Isopropyl	0.93, 0.95	d,J=6.5	0.92, 0.95	d,J=6.5
C-21	1.05	d,1*	1.04	d,1*
C-19	1.37	S	1.27	S

*Absorption spacing, but not coupling constant.

TABLE XVI

CHEMICAL SHIFTS OF THE NON-ACETATE METHYL GROUPS IN THE DIACETATE (III) AND ITS ALPHA-EPOXIDE (V) AT 220 MHZ IN BENZENE

Methyl Assignment	Diacetate (III)		Diacetate-Alpha-Epoxide (V)	
	Chemical Shift (δ)	Coupling Constant	Chemical Shift (δ)	Coupling Constant
C-18	0.57	S	0.61	S
Cyclopropyl	0.89	S	0.91	S
C-24	0.90	d,J=7	0.91	d,J=6.5
Isopropyl	0.98, 1.00	d,J=6.5	0.99 1.02	d,J=6.5
C-21	1.11	d,J=7	1.08	d,J=6.5
C-19	1.18	S	1.27	S

Table XV shows the 21-methyl occurs as finely spaced doublet in the NMR spectra ($CDCl_3$) of III or V, due to the close chemical shift of the methine proton at C-20. In benzene the chemical shifts of these vicinal hydrogens (21-methyl and 20 carbinyl) are separated in part as a result of a small downfield shift of the methyl signal, resulting in the 21-methyl absorption appearing as a normal doublet (J=7 Hz, see Table XVI) with an upfield tilt. It was concluded that the spreading of these chemical shifts in benzene was due to long range solvent effect of the 9-ketone, since the finely spaced doublets for the 21-methyl in

the 9-ols VII and VIII did not change appearance in benzene. The requisite proximity for the operation of these effects leads to the assignment of this secondary methyl group to the side chain position nearest to the 9-keton; i.e., 21-methl.

TABLE XVII

CHEMICAL SHIFT OF THE NON-ACETATE METHYL GROUPS IN THE TETRAHYDRODIACETATE (VII) AND ITS C-3 MONOACETATE (VIII) IN BENZENE AT 100 MHZ

Methyl Assignment	11-Acetate (VII)		11-Hydroxyl (VIII)	
	Chemical Shift (δ)	Coupling Constant	Chemical Shift (δ)	Coupling Constant
C-18	0.70	S	0.73	S
C-24	0.92	d,J=6.5	0.90	d,J=6.5
Cyclopropyl	0.91	S	0.92	S
C-19	1.01	S	1.01	S
Isopropyl	1.02*	d,J=6.5	1.01*	d,J=6.5
C-21	1.12	d,1**	1.00***	d,1***

* Both isopropyl methyls have same chemical shift.

** Absorption spacing, but not coupling constant.

*** Absorption not resolved.

TABLE XVIII

CHEMICAL SHIFTS OF THE C-24 AND C-21
SECONDARY METHYLS IN VII AND VIII
IN CHLOROFORM AT 100 MHZ

Methyl Assignment	Chemical Shifts (δ)	
	For VII (11-Acetate)	For VIII (11-Hydroxyl)
C-24	0.86	0.87
C-24	1.07	0.98

A large downfield shift for the 21-methyl is observed on introduction of the 11-acetate (VII) into VIII (see Table XVIII, $\Delta\delta$ = 0.09, $CDCl_3$). In benzene this shift is even larger ($\Delta\delta$= 0.12, Table XVII) while the remaining methyl groups show little or no shift. In both solvents the shift is attributed to the deshielding effect of C-11 acetate of VII on the 21-methyl relative to the C-11 hydroxyl of VIII. This acetate effect as well as the ketone effect supports the presence of the 21-methyl group and its assignment as the highest field secondary methyl in the NMR spectra of each of the described derivatives of I.

The presence of the C-21 secondary methyl group fixed the cyclopropane at carbons C-22 and C-23 when taken together with the mass losses of the isopropyl and 3-methyl-2-butyl groups from the derivatives of this steroid series. The remaining tertiary methyl group of I must then be substituted on the cyclopropane at either C-22 or C-23 which then accounts for three cyclopropane hydrogens in I.

The 200 MHz spectrum (benzene) of the epoxide V, see Table XVI, shows the seven methyl groups to be well separated and the overlapping

hydrogens in the cyclopropane region separated. The 100 MHz NMR spectrum (benzene) of V shows the secondary methyls and the cyclopropany methyl to be highly overlapped. However, the high field half and low field half, respectively, of the secondary methyls (C-24 and C-21) doublets are visible and were verified by their chemical shifts. In the same manner, the low field member of the higher field isopropyl doublet and the high field member of the lower field isopropyl doublet were identified from the spectrum. On decoupling this spectrum at 100 MHz, the three cyclopropane hydrogens were found to be coupled to one another (cis, J=7 Hz; trans, J=4.5 Hz; geminal, J=5.5 Hz). On irradiation of the signal at δ0.46 (dd, J=4.5, 7 Hz), the signal at δ-0.16 (dd, J=4.5, 5.5 Hz) was decoupled to a perturbed doublet (J=5.5Hz). Irradiation of the non-cyclopropane signal at δ0.15 caused the doublet methyl at δ0.95 to collapse to a singlet and irradiation of this doublet methyl group caused perturbation of the unresolved non-cyclopropane signal at δ0.15. The chemical shift of the non-cyclopropane proton (δ.15) was determined by virtue of this decoupling experiment. In addition to the new absorption at 0.95 in the decoupled spectrum that represented partial decoupling of a secondary methyl, the spectrum also shows the outer members of the secondary methyls C-24 and C-21. These signals are not well resolved and therefore are difficult to interpret; however, it strongly suggests that the non-cyclopropane proton is a methine proton coupled to one methyl group with a chemical shift of 0.95; i.e., one of the isopropyl methyls.

The tertiary cyclopropane methyl group must be placed at either C-22 or C-23. Intuitively, one would not expect the common large loss

of fragment C_8H_{16} from each of these steroid derivatives from a C-22 methyl substitution. With the methyl group at C-23 and with cleavage of two carbon-carbon cyclopropane bonds, the loss of C_8H_{16} is obtained directly. Therefore, C-23 is the position of choice for the cyclopropane methyl group. The cyclopropane methyl position as well as the stereochemistry of the side chain will be verified by the X-ray study now underway on Δ^5-3-p-iodobenzoxy-11-acetoxy-9,11-seco-gorgosten-9-one (XVI). This heavy atom compound was made from X by reaction with p-iodobenzoyl chloride in pyridine. The reaction mixture was directly chromatographed yielding the oil (XVI) that was difficult to crystallize. It crystallized in small clusters on evaporation from methanol-benzene-pentane. On evaporation of a solution of chloroform-methanol (approximately 10:1) long retangular cryctalline bars were recovered for the X-ray study.

EXPERIMENTAL

All melting points were corrected by adding 1.5°C and were taken on a Thomas Hoover, Capillary Melting Point Apparatus. All solvents were distilled before using.

NMR spectra were taken on Varian A-60 as well as 100 and 220 MHz Varian spectrometers using tetramethylsilane (TMS) as an internal reference. Chemical shifts were reported in δ-valves (ppm from TMS). The multiplicity of the signals are denoted by the symbols: s, singlet; d, doublet; dd, doubled doublet; t, triplet; q, quartet; m, multiplet. Coupling constants are reported in Hz.

Carbon hydrogen analyses were carried out by the Alfred Bernhardt Laboratories, Mulheim, West Germany and Chemalytics Incorporated, Tempe, Arizona.

Collection of Pseudopterogorgia americana (Gmelin): The gorgonian, Pseudopterogorgia americana, was collected on the coral reefs off the Florida Keys. This gorgonian is very slimy to the touch and grows firmly attached to the bottom. It was cut from the reef with pruning shears.

Collection Work-Up: The gorgonian was dried in air until it became brittle. It was then cut into pieces about an inch long and ground in a Waring blender. The ground cortex was separated from the protein skeleton by the use of a sieve with a mesh size of 10.

Extraction of Pseudopterogorgia americana: The dried cortex (16,750 grams) was extracted with cold hexane until the extract was colorless. The cortex was dried and placed in a continuous extractor. On extraction of the cortex with hot hexane, 7.8 grams of crude crystals were precipitated during the second day. The red colored crystals were triturated with cold benzene to remove the red pigment and other soluble materials. A total of 4.29 grams of colorless crystals were recovered by repeated concentration of the benzene extracts and filtration of the crystals.

The hot hexane extraction of the cortex was continued for several days and 24.7 grams of a red viscous oil precipitated. Both the precipitated crystals and red colored oil were found to contain Δ^5-9,11-seco-gorgosten-3,11-ol-9-one (I) and its 5α,6α-epoxide (II).

Isolation of Δ^5-9,11-Seco-Gorgosten-3,11-Diol-9-One (I): A quantity (1.85 grams) of the crude Pseudopterogorgia americana crystals was stirred with 6.0 ml. acetic anhydride and 6.0 ml. pyridine in 20 ml. benzene for 15 hours in a stopped 100 ml. flask at room temperature. The solution was diluted to 50 ml. with benzene while transferring to a spearating funnel. The benzene phase was extracted with three portions of 5% HCl and one portion of water. After drying over Na_2SO_4, the benzene solutions yielded 2.31 grams of oil. This oil was chromatographed on 156 grams of silicic acid (Silicar CC-7) using 3% EtOAc/Bz as solvent, flow rate of 110 ml./hour and 100 ml. fractions were taken. The following fractions were taken containing the diacetates of I (R_{dye} = 1.0) and II (R_{dye} = 0.96 using silica gel H plates and 40% ethyl acetate in benzene as solvent).

Fractions	Weight	Fractions	Weight
5	0.0000	12	0.0000
6	0.1886	13	0.0448
7	0.8030	14	0.0654
8	0.4857	15	0.0474
9	0.1406	16	0.0265
10	0.0900	17	0.0220
11	0.0188	18	0.0120
Total	1.7267 g.	Total	0.2181 g.

Fractions 6-11 contain the solid, Δ^5-3,11-diacetoxy-9,11-seco-gorgosten-9-one (III) which, after recrystallization from benzene-pentane, had a melting point of 102.5-103°C.

Anal. Calcd for $C_{34}H_{54}O_5$: C, 75.28%; H, 9.96%; O, 14.76%; found: C, 75.63%; H, 10.09%; O, 14.25%. $[\alpha]_D^{25} = -33°$ (C = 1.45, $CHCl_3$).

The mass spectrum is consistent for the structure with peaks at m/e 542, 482, 430, 347, 161, 145, 135, 133, 121, and 120. The UV spectrum (hexane) at 292 mμ, ε= 40; the (CH_3OH) Cotton effect at 291 mμ (-10,000).

The IR spectrum (KBr) shows peaks at 1735, 1710, 1465, 1435, 1385, 1365, 1240, 1070, 1030, 960 and 815 cm^{-1}.

The NMR spectrum ($CDCl_3$) shows resolved peaks at δ5.50 (1,m), 4.19 (1,m), 4.51 (2, T, J=8), 1.99 (3,S), 1.95 (3,S), 1.36 (3,S), and 0.70 (3,S).

The diacetate III (0.3980 grams) was hydrolyzed in 20 ml. methanol containing 0.530 grams KOH in a stoppered round bottom flask. The mixture was stirred overnight (16 hours) at room temperature. After this

time the volume was reduced on a rotary evaporator to approximately 5 ml. Benzene (50 ml.) was added to transfer the solution to a separatory funnel, and the solution was extracted with three portions of water. After drying the benzene solution over Na_2SO_4 the solvent was removed yielding 0.3504 grams of I, m.p.=170-172.5°C. Recrystallization from chloroform yielded long needles, m.p.=172.5-173.5°C. Pseudopterogorgia americana collected in Bermuda yielded insoluble crystals not contaminated with II. On recrystallization of the crystals from benzene-pentane I is obtained directly, m.p.=170-172.5°C.

Anal. Calcd for $C_{30}H_{50}O_3$: C, 78.60%; H, 10.91%; found: C, 78.30%; H, 11.35%. $[\alpha]_D^{25} = -33$ (C = 2.15, $CHCl_3$).

The mass spectrum is consistent with the structure with peaks at m/e 458, 443, 440, 407, 369, 346, 331, 320, 302, 263, 180, and 161.

The IR spectrum (KBr) shows peaks at 3470, 3360, 3060, 2970, 2880, 1720, 1700, 1465, 1445, 1375, 1060, 965, and 820 cm^{-1}.

The NMR spectrum ($CDCl_3$) shows resolved peaks at δ5.49 (1,m), 3.45 (1,m), 3.80 (2,m), 1.39 (3,S) and 0.70 (3,S).

Isolation of 5α,6αEpoxy-9,11-Seco-Gorgostan-3,11-Diol-9-One (II): The alpha epoxide II is the only detectable contaminant of I in the crude Pseudopterogorgia americana crystals obtained as a precipitate in the hot hexane extraction of dried cortex. The diols I and II were separated as their diacetates as described before. Fractions 13-18 contained the diacetate V, 5α,6α-epoxy-3,11-diacetoxy-9,11-seco-gorgostan-9-one. $[\alpha]_D^{25} = -15.7°$ (C = 3.88, $CHCl_3$); UV spectrum (CH_3OH) at 280 mμ (ε = 61).

The mass spectrum is consistent with the structure with peaks at m/e 558, 498, 446, 364, 256, 228, 213, 185 and 125.

The IR spectrum ($CHCl_3$) shows peaks at 1730 and 1710 (not resolved), 1470, 1455, 1445, 1385, 1365, (1240 and 1220 unresolved) 1145 and 1025 cm^{-1}.

The NMR spectrum ($CDCl_3$, 60 Hz) shows resolved peaks at δ4.70 (1,m), 4.14 (2, T, J=7), 3.29 (1,d,J=3), 2.03 (3,S), 1.99 (3,S), 1.28 (3,S), and 0.75 (3,S).

The diacetate V(0.3883 grams), 0.7 m moles) was hydrolyzed in 10 ml. MeOH containing 0.0840 grams KOH (1.5 m moles) for 18 hours at room temperature. Twenty-five ml. of water saturated with NaCl was added and the aqueous phase was extracted with four 50 ml. quantities of chloroform. After drying and removing the solvent, the combined chloroform extractions yielded 0.2346 grams of II, m.p. 119-126°C, (R_f = 0.43 and trace impurity R_f = 0.19 using 1% HOAc/EtOAc as solvent and silica gel H plates). On recrystallization from chloroform-pentane the melting point was raised to 124-129°C (R_f = 0.43 one spot). Another preparation involving a silicic acid CC-7 chromatography of II and then recrystallization from benzene-pentane yielded II, m.p. = 132-132.5°C. On standing in a stoppered sample bottle for 14 months the melting point was reduced to 124-129°C. Recrystallization from benzene-pentane failed to improve the melting point. $[\alpha]_D^{25}$ = -1.5 (C = 1.38, $CHCl_3$).

The mass spectrum of the diacetate V is consistent with the structure with peaks at m/e 474, 456, 362, 347, 318, 305, 279, 261, 223, 205, 179, 161, and 125.

The IR spectra shows peaks at IR ($CHCl_3$) 3620, 3470, 1700, 1470, 1445, 1385, 1370, 1145, 1050, 1000, 960, 935, and 855 cm^{-1} and IR (KBr) 3400, 3060, 1705, 1465, 1450, 1385, 1375, 1270, 1210, 1150, 1055, 1030,

1010, 965, 940, 860, and 810 cm^{-1}.

The NMR spectrum ($CHCl_3$) shows resolved peaks at δ3.71 (2,m), 3.60 (1,m), 3.26 (1,d,J=3Hz), 1.27 (3, S) and 0.71 (3,S).

Percentage of Δ^5-9,11-Seco-Gorgosten-3,11-Ol-9-One (I) and Its 5α,6α Epoxide (II) In The Cortex of Pseudopterogorgia americana: The insoluble crystals (4.29 grams) from the hot hexane extraction of 16,750 grams of Pseudopterogorgia americana cortex corresponds to 0.021% of I and 0.0031% of II in the dried cortex. The diols were also isolated from the insoluble red oil (24.2 grams) recovered from the hot hexane extraction.

The red oil (24.2 grams) was acetylated with 0.5 moles of acetic anhydride and pyridine in 25 ml. chloroform overnight. After work-up the product (25.0 grams) was chromatographed in a 3" diameter column using 5% ethyl acetate in benzene as solvent. A flow rate of 3 ml./min. was used and 100 ml. fractions were taken. Fractions 13 through 17 (5.66 grams) and 18 through 27 (3.0108 grams) contained the diacetates III and V. The combined fractions 13 - 17 were rechromatographed and were found to contain 32% of the diacetate III and 15% of the diacetate V. The fractions 18 - 27 contained no diacetate III and approximately 58% of V. Based on the weight of their corresponding diols, these results increased the amount of I and II in the dried cortex to 0.030% of I and 0.02% of its alpha epoxide (II). No detectable amounts of I and II were found in the soluble extract fractions.

3,11-Diacetoxy-5β-Dihydro-9,11-Seco-Gorgostan-9-One (IV): The diacetate (III), 0.5363 grams, was hydrogenated in 25 ml. ethyl acetate containing 0.10 grams PtO_2. The reaction was carried out in a Parr hydrogenator

for three days. The catalyst was filtered and the solvent removed on a rotary evaporator. The resulting oil was chromatographed on 70 grams silicic acid (CC-7) using 3% ethyl acetate in benzene as solvent. A flow rate of 1 ml./min. was used and 50 ml. fractions were taken. The dihydrodiacetate (IV) was recovered (0.5208 grams) in fractions 6 through 10; UV max. (hexane) at 295 mμ (ε = 37).

The mass spectrum is consistent with the structure with peaks at m/e 544, 501, 484, 473, 469, 457, 441, 432, 413, 397, 387, 375, 359, 349, 331 and 289.

The IR spectrum ($CHCl_3$) shows peaks at 1730 and 1710 (unresolved), 1445, 1375, 1255, 1235, 1215, 1160, 1140, 1070, 1020, 985, 965, and 935 cm^{-1}.

The NMR spectrum ($CDCl_3$, 60 Hz) shows resolved peaks at δ5.03 (1,m), 4.12 (2, T, J=7 Hz), 2.03 (3, S), 1.99 (3,S), 1.33 (3,S) and 0.71 (3,S).

Epoxidation of the Diacetate (III): A solution of trifluoroperacetic acid was prepared from trifluoroacetic anhydride and 90% H_2O_2. Five ml. of methylene chloride containing 0.66 ml. of trifluoroacetic anhydride (4.7 m moles) was added dropwise to 5 ml. methylene chloride containing 0.11 ml. 90% H_2O_2 (3.9 m moles) that was cooled to 0°C in an ice bath. One ml. of the resulting trifluoroperacetic acid solution (0.39 m moles) was added dropwise to 5 ml. solution of III (0.1398 grams) in methylene chloride containing 0.11 grams of solid Na_2HPO_4. After the addition was completed the resulting mixture was refluxed for 30 minutes.

The reaction mixture was filtered at room temperature. The solid was washed with three 5 ml. quantities of methylene chloride which were added to the filtrate. The methylene chloride solution was diluted to 25 ml. with transfer to a separatory funnel. The resulting solution was extracted with two 25 ml. portions of 5% $NaHSO_3$, two 25 ml. portions Na_2CO_3 and a 25 ml. portion of water. The methylene chloride solution was dried over $MgSO_4$ and on removing the solvent yielded an oil, 0.1495 grams.

Thin layer chromatography (80:20, Benzene:ethyl acetate) on silica gel H plates showed the product to be a mixture of two components, R_{dye} = 0.78, 0.64.

This mixture was separated on a column of 175 grams of silicic acid (Silicar CC-7) using 3% ethyl acetate in benzene as solvent. A flow rate of 2 ml./min. was used and 50 ml. fractions were taken. Fractions 20 through 24 contained IV (0.0689 grams) identical with the diacetate of the naturally occurring α-epoxide (V) by TLC as well as by NMR and IR spectra.

Fractions 25 through 30 contained no material and were discarded. The eluting solvent was changed to 10% ethyl acetate in benzene. The second component of this mixture (VI) was washed from the column (0.0430 grams) in a 1000 ml. fraction. M.p. = 140-141.5°C. UV max. (hexane) at 276 mμ (ε = 64).

The mass spectrum is consistent with the structure with peaks at m/e 558, 543, 498, 483, 471, 455, 446, 436, 389, 386, 371, 363, 303, 299, 285, 267, 217, 189 and 161.

The IR spectrum ($CHCl_3$) 1725 (broad), 1465, 1380, 1365, (1255,

1235, 1210 not resolved), 1065, 1025 and 960 cm^{-1}.

The NMR spectrum ($CDCl_3$) δ4.89 (1,m), 4.17 (2, T, J=8 Hz), 3.09 (1,m), 2.01 (3,S), 1.98 (3,S), 1.35 (3,S), and 0.73 (3,S).

3,11-Diacetoxy-5β-Dihydro-9,11-Seco-Gorgostan-9-Ol (VII): Dihydrodiacetate (IV) was dissolved in 15 ml. glacial acetic acid containing two drops $HClO_4$. This solution was hydrogenated using 0.10 grams Rh/C as catalyst at 46 psig for three days. The reaction solution was filtered from its catalyst into a 125 ml. Erlenmeyer flask with washes totaling 50 ml. of chloroform. Water (50 ml.) was added and sodium bicarbonate was carefully added with mixing to achieve neutralization. The two phases were transferred to a separatory funnel with washings. The chloroform layer was removed and the aqueous phase extracted with two additional 50 ml. portions chloroform. After drying, the combined chloroform extractions yeilded an oil (VII, 0.1043 grams). This oil was chromatographed on 70 grams silicic acid (CC-7) in a 1.25" diameter column using 3% ethyl acetate in benzene as solvent. A flow rate of 1 ml./min. was used and 50 ml. fractions were taken. Fractions 11 - 15 yielded 0.0863 grams of VII (R_{dye} = 1.0 on silica gel H plates using 40% ethyl acetate in benzene as solvent).

Compound VII was also made by selective acetylation of the diol VIII. Compound VIII (0.3300 grams) was dissolved in 5 mls. of benzene that was 1.0 M in acetic anhydride and pyridine. The reaction mixture was allowed to stand in a stoppered round bottom flask for 24 hours at room temperature. At this time TLC (R_{dye} = 1.0 using 40% ethyl acetate/benzene as solvent) showed the reaction was complete. The reaction mixture was chromatographed on 70 grams silicic acid (CC-7) in a 1.25"

diameter column using 3% ethyl acetate in benzene as solvent. A flow rate of 1 ml./min. was used and 50 ml. fractions were taken. Fractions 10 through 16 contained the diacetate VII (0.2660).

The mass spectrum is consistent with the structure with peaks at m/e 546, 528, 503, 486, 475, 458, 434, 374, 366, 315, 255, and 175.

The IR spectrum ($CHCl_3$) shows peaks at 3530, 1730, 1455, 1375, 1265, 1245, 1220, 1150, and 1025 cm^{-1}.

The NMR spectrum ($CDCl_3$) shows resolved peaks at δ5.15 (1,m), 4.27 (2,m) 3.56 (1,m), 2.03 (3,S), and 0.75 (3,S).

3-Acetoxy-5β-Dihydro-9,11-Seco-Gorgostan-9,11-Diol (VIII): The dihydrodiacetate (IV, 0.4816 grams) was dissolved in 18 ml. absolute ethyl alcohol along with 0.160 grams sodium borohydride at 0°C in a stoppered Erlenmeyer flask. The reaction solution was maintained at 5°C and followed by silica gel H TLC using 40% ethyl acetate in benzene as solvent. After 144 hours the reaction was stopped. The reaction product showed primarily two spots, R_{dye} = 0.0, 0.73.

The reaction mixture was taken up in 100 ml. of $CHCl_3$ with transferring to a separating funnel. Water (100 ml.) was added and extracted against the $CHCl_3$ layer. The aqueous layer was extracted with two additional portions of chloroform. After drying, the chloroform was removed yielding a solid that was chromatographed on a 1.25" diameter column packed with 70 grams silicic acid (CC-7) using 10% ethyl acetate in benzene as solvent. A flow rate of 0.5 ml./min. was used and 50 ml. fractions were taken. Fractions 11 through 17 contained VIII (0.3494 grams). An additional 0.0370 grams, R_{dye} = 0.0, presumably the corresponding triol, was washed from the column with 500 ml. of 30% acetate in benzene.

On recrystallization from benzene-pentane, the melting point of VIII was 191.5-192°C.

Anal. Calcd for $C_{32}H_{56}O_4$: C, 76.19; H, 11.11; found: C, 76.34; H, 11.34.

The mass spectrum is consistent with the structure with peaks at m/e 504, 486, 444, 426, 392, 374, 332, 314, 296, 309, 291, 273, and 255.

The IR spectrum (KBr) shows peaks at 1735, 1455, 1375, 1260, 1235, 1150, 1025, 945, 930 and 845 cm^{-1}.

The NMR spectrum ($CDCl_3$) shows resolved peaks at δ5.15 (1,m), 3.81 (1,m), 3.70 (1,m), 2.04 (3,S) and 0.76 (3,S).

3-Acetoxy-9-Deutero-5β-Dihydro-9,11-Seco-Gorgosten-9,11-Diol (IX): The dihydrodiacetate (IV, 0.0606 grams) was dissolved in 5 ml. absolute ethanol along with 0.0280 grams sodium borodeuteride at 0°C in an Erlenmeyer flask. The reaction solution was maintained at 5°C. After 144 hours the reaction was stopped. TLC of this product using 40% ethyl acetate in benzene as solvent showed two spots R_{dye} = 0.0, 0.73.

The reaction product was taken up in 50 ml. $CHCl_3$ and transferred to a separatory funnel. Water (50 ml.) was added and saturated with NaCl. The aqueous layer was extracted against three 50 ml. portions of chloroform. After drying, the combined chloroform solution yielded an oil, 0.0520 grams. After chromatography using the same conditions as for VIII, 0.0390 grams of the 9-deutero derivative IX was recovered.

The mass spectrum is consistent with the structure with peaks at m/e 505, 487, 469, 445, 427, 393, 375, 333, 315, 297, 310, 292, 274, and 256.

Δ^5-11-Acetoxy-9,11-Seco-Gorgosten-3-Ol-9-One (X): The diacetate (III) (0.3103 grams) was dissolved in 25 ml. MeOH and 2 ml. benzene. A solution (0.7 ml.) of 1N KOH in 50% MeOH - 50% H_2O was added. After 45 minutes the reaction was stopped. Chloroform (50 ml.) was added while transferring the reaction mixture to a separatory funnel. Then 50 ml. water was added. The aqueous layer was just acidified with acetic acid and extracted three times with 50 ml. portions $CHCl_3$. After drying over anhydrous Na_2SO_4, the chloroform solution yielded an oil, 0.2975 grams. Thin layer chromatography on silica gel H plates using 40% ethyl acetate in benzene as solvent showed only two major components, R_{dye} = 0.17, 0.48.

This mixture was chromatographed on 70 grams of silicic acid (CC-7) using 20% ehtyl acetate in benzene as solvent. A flow rate of 1 ml./min. was used and 50 ml. fractions were taken.

Fractions 8 through 11 contained the faster moving monoacetate (X) (0.1492 grams). After fraction 15 was taken, the column was washed with 300 ml. ethyl acetate that yielded the diol I (0.0900 grams), m.p. 170-172.5°C. An additional wash of 500 ml. yielded no additional material. Compound X shows the following spectral data.

The mass spectrum is cor · tent with the structure with peaks at m/e 500, 485, 470, 457, 440, 425, 389, 373, 332, 321, 313, 246, 190 and 164.

The IR spectrum ($CHCl_3$) shows peaks at 3520, 3480, 1720 (broad), 1460, 1385, 1370, 1260, 1240, 1210, 1155, 1040, 965, 945, 895, 860, and 820 cm^{-1}.

The NMR spectrum ($CDCl_3$, 60 MHz) showed resolved peaks at δ5.51

(1,m), 4.23 (1,m), 3.55 (1,m), 2.00 (3,S), 1.38 (3,S) and 0.73 (3,S).

Δ^4-11-Acetoxy-9,11-Seco-Gorgosten-3,9-Dione (XI): The monoacetate (X) 0.0599 grams, was dissolved in 5 ml. acetone (distilled over $KMNO_4$) and cooled to 0°C. Jones reagent (0.2 ml.) was added dropwise. The reaction mixture was allowed to warm up to room temperature and stand for 20 minutes. Chloroform (50 ml.) was added to the reaction mixture with transferring to a separatory funnel. Water (50 ml.) was added and the aqueous layer was extracted with four portions of chloroform. The combined chloroform extracts were dried over $MgSO_4$ and on removing the solvent yielded an oil. Its IR spectrum ($CHCl_3$) showed unconjugated ketone absorption at 1715 cm^{-1}. The NMR spectrum ($CDCl_3$) showed a singlet at $\delta 1.5$ for the C-19 methyl group. This oil was chromatographed on 70 grams silicic acid (CC-7) in a 1.25" diameter column. A flow rate of 2 ml./min. was used and 50 ml. fractions were taken. The first eleven fractions were taken using 2% ethyl acetate in benzene as solvent. Two additional fractions were taken at 5%, 10% and 20% ethyl acetate in benzene. Finally XI (0.0449 grams) was washed from the column with 200 ml. ethyl acetate. TLC showed only one spot (R_{dye} = 0.78 using silica gel H plates and 40% ethyl acetate in benzene as solvent). IR spectrum ($CHCl_3$) showed carbonyl absorptions at 1740 cm^{-1} (acetate) 1710 (unconjugated ketone at C-9) and 1680 (conjugated ketone at C-3).

Deuteration of Δ^5-9,11-Seco-Gorgosten-3,11-Diol-9-One (I): The diol I (0.2317 grams) was dissolved in 2 ml. of MeOD along with approximately 0.01 grams sodium metal. This solution was stirred in a stoppered round bottom flask for two days. Saturated NaCl (25 ml.) was added along with 50 ml. chloroform. The aqueous phase was extracted with a

total of five 50 ml. portions of chloroform. After drying over $MgSO_4$, the combined chloroform extractions yielded 0.2107 grams. The NMR spectrum (60 MHz) of the product showed no significant change from the starting material. After an additional two days reaction time 0.1676 grams of product was recovered as before. Since no significant change was seen in the NMR spectrum (60 MHz), this product was subjected to four more days of deuteration and 0.1267 grams of product was recovered. The product's NMR spectrum (60 MHz) still showed little or no change. The mass spectrum shows peaks at 458, 1.3%; 459, 1.9%; 460, 1.6%; 461, 0.9%; 462, 0.6%; 463, 0.3%.

The procedure was repeated at reflux temperature for three days. No significant change in the amount of deuterium incorporated was shown by the mass spectrum of the product: 458, 1.4%; 459, 4.8%; 460, 2.9%; 461, 2.4%; 462, 1.4%; 463, 0.5%.

The product was acetylated with 20 ml. of 1.0 M acetic anhydride and pyridine in benzene for 16 hours. Five ml. of the reaction was worked up by dilution to 50 ml. and extraction with three 50 ml. portions of 5% HCl. On drying and removing the benzene solvent, 0.0183 grams of diacetate III was recovered. The remaining 15 ml. was chromatographed on 70 grams of silicic acid (CC-7) using 3% ethyl acetate in benzene as solvent. Fractions of 50 ml. were taken. Fractions 6 - 10 contained 0.0492 grams of III, m.p. 97 - 99.5°C. Its mass spectrum showed peaks at 542, 0.7%; 543, 6.7%; 544, 2.8%; 555, 3.0%; 556, 1.0%; 557, 0.4%. The 100 MHz NMR spectrum ($CDCl_3$) showed the complete absence of a single proton at δ2.99 (1,dt, J=4,4,12 Hz). The mass spectral data then indicates intermolecular exchange of hydrogen or deu-

terium between several carbon atoms of I or III in the inlet of the mass spectrometer.

Dehydration of 3,11-Diacetoxy-5β-Dihydro-9,11-Seco-Gorgostan-9-Ol (VII): Compound VII was dissolved in 5 ml. pyridine and cooled in an ice bath. Thionyl chloride (0.2 ml.) was added and the reaction mixture allowed to warm to room temperature with stirring. After one hour, 100 grams of ice was added to the reaction mixture. The hydrolyzed reaction mixture was transferred to a separatory funnel with 50 ml. chloroform. The aqueous phase was separated and the chloroform layer extracted with three 50 ml. portions of 10% HCl. After drying over Na_2SO_4 and removing the chloroform, an oil was recovered which was chromatographed on 70 grams of silicic acid (CC-7) in a 1.25" diameter column. Fractions of 50 ml. were taken and 1 ml./min. flow rate was used. Fractions 10 - 20 contained 0.1466 grams of a mixture of tri-and tetra-substituted olefins. Fractions 21 - 26 contained the olefin XIV that was carried through a reaction series of ozonolysis, Baeyer-Villiger oxidation and saponification only to find the olefin was rearranged product of dehydration and not the expected Δ^8-3,11-diacetoxy-5β-dihydro-9,11-seco-gorgostene. The unknown olefin XIV is characterized by the following spectra.

The mass spectrum showed peaks at m/e 528, 513, 468, 441, 381, 356, 315, 256, 255, 241, 229, 186, 174, and 152.

The IR spectrum (film) showed peaks at 3063, 1740, 1460, 1370, 1240, 1160, 1120, 1075, 1025, 970, and 860 cm^{-1}.

The NMR spectrum ($CDCl_3$ showed resolved absorptions at δ5.15 (1,m), 4.96 (1,m), 4.24 (2, t, J=7.5 Hz), 2.63 (3,S), 2.03 (3,S), and 0.73 (3,S).

Δ^5-3-P-Iodobenzoyl-11-Acetoxy-9,11-Seco-Gorgosten-9-One (XV): The monoacetate X (0.0851 grams) was dissolved in 5 ml. benzene and 2 ml. pyridine containing 0.53 grams crystalline p-iodobenzoic and 1.0 ml. thionyl chloride on a steam bath for six hours. The p-iodobenzoyl chloride was recovered in crystalline form after removing the last traces of gaseous by-products under vacuum. The acid chloride was used without further purification.

The reaction solution was allowed to stand for 17 hours at room temperature. At this time, TLC showed no remaining starting monoacetate X. The product mixture was directly chromatographed on 70 grams silicic acid (CC-7) using 1% ethyl acetate in benzene for the first sixteen 50 ml. fractions. The solvent was changed to 5% ethyl acetate in benzene and 100 ml. fractions were taken. The p-iodobenzoate XV was recovered in the first three fractions (0.0368, 0.0480, and 0.0121 grams respectively). These oily fractions resisted crystallization. Finally the combined neat fractions were dissolved in pentane and the solution was allowed to evaporate very slowly. One crystal was recovered from the side of the flask. The oil XV was redissolved and the single crystal was used to seed the solution. After slow evaporation (24 hours) the oil crystallized to clusters of small needles. These crystals were dissolved in approximately 10:1 methanol:chloroform and on slow evaporation of the solvent long needles were recovered, m.p. 104.5-106.5°C. These crystals were used for an X-ray study to be performed by Dr. Dick Van der Helm and Dr. Eric Enwall.

Anal. Calcd for $C_{39}H_{53}O_5$ I: C, 64.25%; H, 7.54%; found: C, 64.44%; O, 8.14%.

The mass spectrometer parent ion is 830.

The IR spectrum (KBr) shows peaks at 3070, 1720 (broad), 1590, 1470, 1390, 1370, 1315, 1305, 1270, 1255, 1240, 1180, 1115, 1105, 1080, 1025, 1010, 965, 840, and 755 cm^{-1}.

The NMR spectrum ($CDCl_3$) δ7.27 (4,m), 5.58 (1,m), 4.77 (1,m), 4.23 (2, T, J=7.5 Hz), 2.01 (3,S), 1.42 (3,S) and 0.73 (3,S).

SUMMARY

Plexaura homomalla (Esper) was found to be a rich natural source of two new 15-epi-PGA_2 prostaglandins derivatives. Their isolation from a marine organism is the first from natural non-mammalian source for this class of compounds and the richest known natural source. Since prostaglandins are becoming important as drugs and their total synthesis difficult, this sea organism appears to be an important source for the 15-epi-prostaglandins which may be used as intermediates for PGE_2, $PGF_{2\alpha}$, as well as for the other biologically useful prostaglandin derivatives.

The gorgonian, Pseudopterogorgia americana (Gmelin) was found to contain Δ^5-9,11-Seco-gorgosten-3,11-diol-9-one and its 5α,6α-epoxide. These novel C_{30}-seco-steroid diols contain an eleven carbon atom side chain containing cyclopropane and are derivatives of the marine sterol gorgosterol. All three of these steroids contain the same previously unknown, biologically unprecedented, eleven carbon side chain. In addition, these Pseudopterogorgia americana steroids have an open C-ring at the C-9,11 position. The mixed ester, Δ^5-11,acetoxy-9,11-seco-gorgosten-9-one-3-p-iodobenzoate has been prepared for an X-ray diffraction study.

BIBLIOGRAPHY

1. A. J. Weinheimer and R. L. Spraggins, "Tetrahedron Letters", 5185 (1969).

2. A. J. Weinheimer and R. L. Spraggins, "Food-Drugs From The Sea Conference" and Proceedings (1969).

3. A. J. Weinheimer and R. L. Spraggins, "Abstracts", 158th National Meeting of the American Chemical Society, New York (1969).

4. Proceedings of the Second Nobel Symposium, p. 24 (1966), edited by S. Bergstrom and B. Samuelsson, Interscience Publishers, A Division of John Wiley and Son.

5. M. W. Goldblatt, Chem. Ind. (London), 52, 1056 (1933).

6. V. S. von Euler, U.S. Arch. Exptl. Pathol. Pharmakol, 175,78.

7. S. Bergstrom and J. Sjovall, Acta. Chem. Scand., 14, 1693, 1701, 1706 (1960).

8. S. Bergstrom, Science, 157, 382 (1967).

9. W. B. Stavinoha, Private communication on testing of the methyl, acetate diester (15-epi-PGA_2) on smooth muscle.

10. J. Nakano, J. Pharm. and Pharmacol., 782 (1969).

11. Authentic sample of the prostaglandin PGA_2 and its NMR spectrum was supplied by Dr. John Pike of the Upjohn Company.

12. P. W. Ramwell, J. E. Shaw, G. B. Clark, M. F. Grostic, D. G. Kaiser, and J. E. Pike, Progress in the Chemistry of Fats And Other Lipids, IX, p. 256 (1968).

13. P. W. Ramwell, J. E. Shaw, G. B. Clark, M. F. Grostic, D. G. Kaiser, and J. E. Pike, Progress in the Chemistry of Fats And Other Lipids, IX, p. 252 (1968).

14. E. G. Daniels, W. C. Krueger, F. P. Kupiecki, J. E. Pike, and W. P. Schneider, J. Am. Chem. Soc., 90, 5894 (1968).

15. M. Hamberg and B.Samuelsson, J. Biol. Chem., 241, 257-263 (1966).

16. A. S. Narvla and S. Dev, "Tetrahedron Letters", 1733 (1969).

17. The Sadtler Standard Spectra NMR No. 336, Published by Sadtler Research Laboratories, Philadelphia, Pa.

18. D. H. Nugteren, D. A. Van Dorp, S. Bergstrom, M. Hamberg, and B. Samuelsson, Nature, 212, 38 (1966).

19. C. G. Baker and A. Meister, J. Amer. Chem. Soc., 73, 1336 (1951).

20. S. Bergstrom, R. Ryhage, B. Samuelsson and J. Sjovall, J. Biol. Chem., 238, 3555 (1963).

21. L. S. Ciereszko, D. H. Sifford, and A. J. Weinheimer, Annals of the New York Academy of Sciences, 90, 917 (1960).

22. L. S. Ciereszko, M. A. Johnson, R. W. Schmidt and C. B. Koons, Comp. Biochem. Physiol., 24, 899 (1968).

23. R. L. Hale, J. Leclercq, B. Tursch, C. Djerassi, R. A. Gross, Jr., A. J. Weinheimer, K. Gupta and P. J. Schever, J. Am. Chem. Soc., 92, 2179 (1970).

24. N. S. Bhacca and D. H. Williams, Applications of NMR Spectroscopy In Organic Chemistry, p. 166, Holden-Day Inc., San Francisco, London, Amsterdam (1964).

25. D. T. Longone and A. H. Miller, Chemical Communications, 447 (1967).

26. K. Tori, T. Komeno, and T. Nakagawa, J. Organic Chem, 29, p. 1136 (1964).

27. K. Nakanishi, Infrared Absorption Spectroscopy, p. 165, Publisher: Hold-Day, Inc., San Francisco and Nankodo Company Limited, Tokoyo (1962).

28. N. S. Bhacca and D. W. Williams, Applications of NMR Spectroscopy In Organic Chemistry, p. 19 and 23, Holden Day, Inc., San Francisco, London, Amsterdam (1964).

29. L. Tokes, G. Jones, and C. Djerassi, J. Am. Chem. Soc., 5465 (1968).