The Occurrence of 13,14-Dihydro and 13,14-Cis-Unsaturated Prostaglandins in the Coral, *Plexaura homomalla*. Synthesis of 13,14-*cis*-Prostaglandin E_2, 15-Acetate Methyl Ester, and the 13,14-Cis Analogues of Prostaglandin $F_{2}\alpha$ and Prostaglandin $F_{2}\beta$

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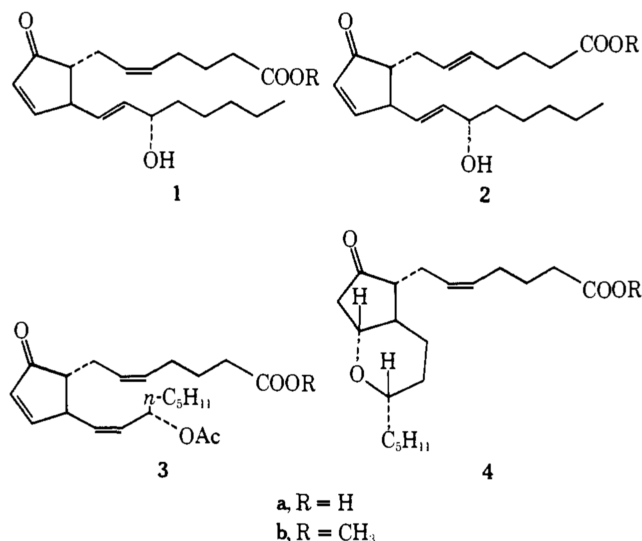
Abstract: Extracts from the coral, *Plexaura homomalla,* have been shown to contain derivatives of 13,14-dihydro- and 13,14-*cis*-PGA_2, and from the latter, 13,14-*cis*-$PGF_{2}\alpha$, 13,14-*cis*-$PGF_{2}\beta$, and 13,14-*cis*-PGE_2, 15-acetate methyl ester have been synthesized. The 13,14-dihydro-PGA_2 undergoes internal Michael addition of the 15-hydroxyl to the enone system as does 13,14-dihydro-PGA_1. In the latter case, the two epimeric (at C-11) Michael products (**11** and **12**) were separated, and structures assigned by CD and NMR spectra and compared with the corresponding data from the 5,6-dehydro compound **4** isolated from coral extracts.

Enzymatic hydrolysis of prostaglandin esters in the coral, *Plexaura homomalla,* obtained from the Cayman Islands, followed by extraction gives a mixture from which PGA_2 (**1a**), 5,6-*trans*-PGA_2 (**2a**), PGE_2, and traces of $PGF_{2}\alpha$ have been isolated by chromatography.[1] Further separation by silica gel chromatography of fractions less polar than PGA_2 have disclosed the presence of other prostaglandins of which 13,14-*cis*-PGA_2, 15-acetate (**3a**), 13,14-dihydro-PGA_2 acetate

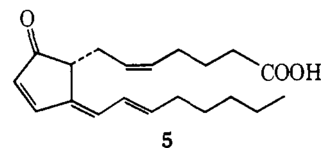


methyl ester (**13**), and 13,14-dihydro-PGA_2 internal Michael adduct **4a** have now been identified. The origin of the "unnatural" double bond isomers **2** and **3** presents an interesting biochemical problem, while the discovery of the presence of 13,14-dihydro prostaglandins may indicate that the early metabolic steps in the coral may be similar to those in mammals.[2]

The 13,14-*cis*-prostaglandin **3a** was obtained as an oil having a thin layer chromatographic mobility (A IX system[3]) near that of PGA_2 methyl ester **1b.** The proton NMR spectrum showed, however, that it was an acetate rather than a methyl ester of a PGA_2-like material, the acetate group evidently escaping the enzymatic hydrolysis step. The material was converted to its methyl ester with diazomethane and further purified by chromatography. The resulting acetate methyl ester **3b** was not identical with PGA_2 acetate methyl ester, its 15-epimer, or to 5,6-*trans*-PGA_2 acetate methyl ester,[1] 8-*iso*-PGA_2 acetate methyl ester,[4] or the allylic rearrangement product, methyl 9-keto-13-acetoxy-5-*cis*-10,14-prostatrienoate,[5] as shown by differences in their proton NMR spectra. The NMR spectrum of new material showed upfield shifts of the C-11 and C-13(14) protons and a downfield shift of the C-12 proton compound with that of PGA_2 acetate methyl ester (**1b,** acetate). A 100-MHz spectrum failed to resolve the olefinic protons sufficiently to get coupling constants. The mass spectrum of **3b** was similar to that of **1b** acetate, indicating identical empirical formulas and probable isomeric relationship.

Attempts to hydrolyze the acetate group of **3b** with methanol-$HClO_4$ or coral enzymes[1] failed, the latter giving back the acetate acid **3a** after 3.5 days incubation. The configuration at C-15 in the new prostaglandin was shown to be 15*S* by ozonization with oxidative workup and isolation of the α-acetoxyheptanoic acid from the lower side chain.[6] The ORD curve of this fragment showed the same sign and shape as that shown by the (*S*)-α-acetoxyheptanoic acid isolated after a similar ozonization of (15*S*)-PGA_2 acetate methyl ester (**1b** acetate). The CD curve of **3a** is positive and very similar to those of PGA_2 and 8-*iso*-PGA_2, showing that **3b** cannot be the acetate of 12-*iso*- or 8-*iso*-12-*iso*-PGA_2, which would be expected to show negative CD curves.

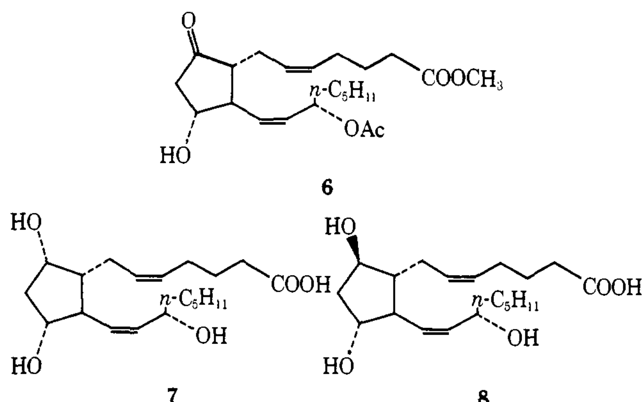
The ultraviolet spectrum of **3b** showed typical PGA type absorption at 217 nm, changing to 325 nm on addition of base. This latter behavior is also shown by **1** acetate due to elimination of acetic acid. The major product of this basic elimination of **3b** was identical in the NMR spectrum with the product **5** formed from PGA_2 acetate methyl ester.[7]



The infrared spectrum of **3b** resembles that of PGA_2 acetate methyl ester except that there is no absorption at 970 cm^{-1} (trans double bond). Both **1b** acetate and **2b** acetate show absorption peaks at 970 cm^{-1}, the latter about twice as strong as the former. This evidence for the lack of a trans double bond in **3b** and the elimination of all other possible sites of isomer-

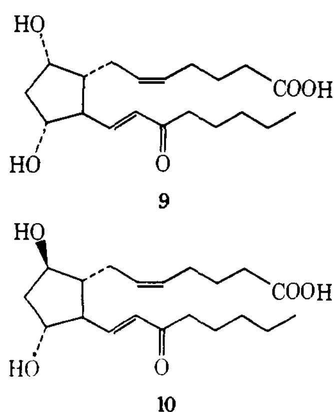
ization led to the assignment of structure **3** for the new coral prostaglandin.

Alkaline epoxidation of **3b** gave a mixture of 10,11-epoxides, which on aluminum amalgam reduction[1] led largely to one PGE_2 isomer assigned structure **6**.[8] Greater steric hindrance of the upper face of the ring by the 13,14-cis-olefinic side chain should decrease the extent of β-epoxidation. Sodium borohydride reduction of **6** followed by alkaline hydrolysis gave two isomeric PGF's, **7** and **8**, the more polar predominating (5:3).



6 **7** **8**

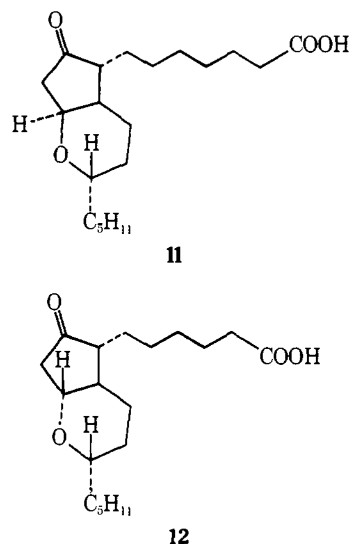
These were both considerably less polar on silica gel TLC plates than $PGF_{2}\alpha$ and $PGF_{2}\beta$, but by analogy with the relative polarities of $F_{2}\alpha$ and $F_{2}\beta$, coupled with the expected predominance of the 9β-alcohol due to increased steric hindrance to the β face of the ring, the less polar, minor isomer was assigned structure **7**, the more polar, **8**.[10] These assignments were substantiated by irradiation of **7** and **8** with 3500-Å light in the presence of diphenyl sulfide, a procedure known to produce cis–trans double bond isomerizations.[9] From **7**, a mixture of $PGF_{2}\alpha$ and 5,6-*trans*-$PGF_{2}\alpha$[1] was obtained, while from **10**, $PGF_{2}\beta$ and its 5,6-trans isomer were produced. Also, oxidation of the allylic alcohols at C-15 in **7** and **8** with dichlorodicyano-1,4-benzoquinone, while considerably slower than oxidation of $PGF_{2}\alpha$ and $PGF_{2}\beta$, did give materials identical by thin layer and gas chromatographic mobility with the 15-ketones **9** and **10**, produced by similar oxidations of $PGF_{2}\alpha$ and



9 **10**

$PGF_{2}\beta$, evidently arising by cis–trans isomerization of the resulting conjugated ketones.

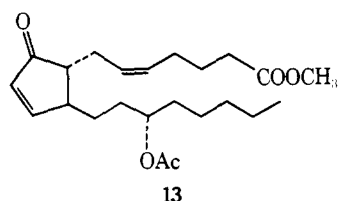
During the above chromatographic separation of **3a** from coral extracts, another prostaglandin, somewhat less polar than **3a**, was also isolated. This was esterified with diazomethane and rechromatographed to give a material which, on the basis of spectral comparisons with the known[11] mixture of isomers **11** and **12** obtained by Michael cyclization of 13,14-dihydro-PGA_1, was assigned structure **4**. This latter mixture of isomers at C-11 was evident from the NMR spectrum, which shows two sets of protons on carbon linked by ether oxygen, one set at δ 4.33 and 3.74, the other at δ 4.08 and 3.3. In the product **4** from coral, the upfield set was the major one seen; the downfield set was barely seen at higher spectrum amplitudes and must be due to a very minor constituent of the mixture. The isomeric mixture of **11** and **12**, made by cyclization of



11 **12**

13,14-dihydro-PGA_1, was separated on acid-washed silica gel to give two pure isomers **11** and **12**. The isomer with the upfield set of signals at δ 4.08 and 3.35, similar to the coral-derived **4**, was assigned the 11β-hydrogen isomer **12**, since in this structure both protons at C-11 and C-15 are diaxial to the six-membered ring. This assignment was supported by CD measurements,[12] which showed **12** had a CD curve similar to that of PGE_1 and considerably more negative than that of **11**. Compound **12** was obtained crystalline. Catalytic hydrogenation of the coral-derived **4b** gave a 5,6-dihydro methyl ester identical by NMR and TLC mobility with the methyl ester of **12**. The mass spectrum of **4a** was also consistent with the assigned structure.

We have also obtained evidence that the 15-acetate methyl ester (**13**) of 13,14-dihydro-PGA_2 is present in coral extracts.



13

A sample of (predominantly) PGA_2 acetate methyl ester chromatographic fractions from *P. homomalla* (Cayman Islands) was treated with 98% formic acid and the resulting mixture subjected to acid hydrolysis according to the method of Spraggins.[5] Chromatography of the products gave mostly a mixture of hydroxy methyl esters, but about 7% of the material was recovered as an acetoxy methyl ester which was assigned the 13,14-dihydro structure **13** on the basis of its IR, UV, NMR, and mass spectra. Evidently the nonallylic acetate at C-15 of compound **13** survived the formolysis conditions which convert allylic acetates to a mixture of allylic formates. The structure of **13** was confirmed by enzymatic hydrolysis of the ester groups and Michael cyclization of the resulting 13,14-dihydro-PGA_2 to a mixture containing **4**.

Experimental Section[13]

13,14-*cis*-PGA_2 Acetate (3a) and Its Methyl Ester (3b). A 78-g chromatographic fraction from a large scale silica gel chromatogram[14] of enzymatically hydrolyzed extracts[1] of Grand Cayman coral, *Plexaura homomalla*, was rechromatographed on 2 kg of silica gel, eluting with a gradient of 20–100% ethyl acetate–Skellysolve B.

Fractions of 600 mL were collected, from which portions 19–30 contained 11.24 g of material with TLC mobility similar to that of PGA_2 methyl ester. Rechromatography of a sample of this material on a 1/2 × 17 in. column of Brinkmann thin layer grade silica gel, eluting with 75% ethyl acetate-Skellysolve gave purified **3a** showing absorptions in the NMR spectrum at δ 7.35 (C-11, doublet of doublets, J = 5 and 2.5 Hz); δ; 6.22 (C-10 doublet of doublets, J = 5 and 2 Hz); five protons between δ 5.0 and 5.8 (C-5,6,13,14,15, unresolved); δ 3.7 (multiplet, one proton, C-12); and δ 2.02 (three protons, singlet, acetate (C-15)). The IR spectrum (neat) showed broad carboxyl absorption, 3600–2500 cm^{-1}, acetate at 1735 and 1240 cm^{-1}, carbonyls at 1700 cm^{-1}, and conjugated double bond at 1580 cm^{-1}.

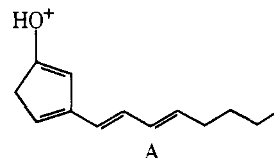
The remainder of the material in fractions 19–30 above was converted to methyl esters with excess ethereal diazomethane and rechromatographed on 1 kg of silica gel, eluting with 20–50% ethyl acetate-Skellysolve B. Fractions (350-mL each) 9–13 contained 3.99 g of homogeneous (by TLC) oil moving slightly faster on silica gel plates (A IX[3] or 50% EtOAc-cyclohexane) than PGA_2 acetate methyl ester. The NMR spectrum was like that of **3a** above except for an additional three-proton singlet at δ3.68 (OCH_3). The UV spectrum in EtOH showed λ_{max} 217 nm (9400) changing to 325 nm on addition of a drop of 50% aqueous KOH. The mass spectrum showed a small M^+ at m/e 390 and fragments at m/e 359 (M − 31), 350, 348, 346, 330 (M − 60), 299 (M − 60 − 31), 250 (M − $C_8H_{12}O_2$), 243, 230, 227, and 203. The IR spectrum showed absorptions at 1735, 1700, 1580, 1240, and 1025 cm^{-1} with essentially no absorption at 970 cm^{-1}, where PGA_2 and 5,6-trans-PGA_2 show substantial peaks. The CD curve showed a positive peak at 232 nm, with a molar rotation of 3.75×10^4.

Enzymatic Hydrolysis of 3b. A solution of 120 mg of **3b** in 0.4 mL of 95% ethanol was added to 10 mL of stirred deionized water. To this was added 1.1 g of coral acetone-powder enzyme[1] and the mixture was stirred at 25 °C for 1 day. Then 20 mL of acetone was added, stirred for 30 min, and filtered. The filtrate was concentrated, acidified with citric acid, and extracted with ethyl acetate. The extracts were washed, dried, and evaporated to leave a residue which by NMR still retained the acetate group, by TLC (A IX system[3]) was the same as **3a**, and on diazomethane treatment gave back **3b**. Repeating the hydrolysis for 3.5 days gave the same result except that an additional less polar TLC spot formed and the infrared spectrum showed an additional absorption at 1540 cm^{-1}, characteristic of the conjugated trienone **5**.

Treatment of 3b with Base. To a solution of 125 mg of **3b** in 0.8 mL of methanol was added 0.4 mL of 1 M $NaOCH_3$ in methanol. The mixture stood under N_2 for 1 h, then was acidified with 0.5 mL of 2 N H_2SO_4. The product was extracted with ethyl acetate, washed and dried, and the residue chromatographed on 25 g of thin-layer grade silica gel eluting with 25% ethyl acetate-Skellysolve B. Fractions 5–7 (10-mL fractions) contained the major product showing TLC mobility, λ_{max}^{EtOH} 326 nm, and NMR peaks at δ 7.75, 7.67, 6.46, 6.36, 6.28, 6.20, 6.10, 5.32, 5.25, and 3.69, characteristic of the methyl ester of **5**.[1,7] A small amount of a product eluted earlier than this showed shoulders at 280 and 225 nm on general UV end absorption, NMR peaks at δ 5.93, 5.45, 4.0–4.4, and 3.8. The UV absorption did not change on addition of base. The mass spectrum showed ions at m/e 348, 330, 317, 299, 277, 245, and 151 mass units.

Ozonization of 3b. The method of Nugteren et al.[15] was used. Samples of PGA_2 acetate methyl ester and **3b**, 200 mg each, were separately dissolved in 25 mL of chloroform, were cooled to −20 °C, and ozonized oxygen was passed through for 6 min (about 3.5 mM O_3/mM compound). The blue solutions stood at −20 °C for 10 min and then were evaporated in vacuo. To the residues were added 15 mL of acetic acid and 3 mL of 30% H_2O_2 and the solutions stood overnight. They were then concentrated in vacuo to a small volume, extracted with ethyl acetate, and the extracts washed with dilute HCl, saturated salt, dried with sodium sulfate, and evaporated. A small portion of each residue was treated with diazomethane and the methyl ester mixture injected on a 6 ft 3% OV 17 GLC column at 75 °C. Both samples had the same series of peaks, but of somewhat different relative intensities. The two most prominent peaks were due to dimethyl glutarate (7 min) and α-acetoxymethyl heptanoate (18.5 min). An attempt to collect the latter peak from a preparative GC column failed. The remainder of the acidic ozonization product samples were chromatographed on 25 g of Brinkmann TLC-grade silica gel using 10% acetone-CH_2Cl_2 for elution and 60–70 p.s.i. pressure. The fractions collected were assayed by GLC and the ones consisting largely of α-acetoxyheptanoic acid were combined. These still contained some monomethyl glutarate, which was confirmed by treatment of a portion with diazomethane, when the GLC peak for the impurity was found to coincide with authentic dimethyl glutarate. They were rechromatographed using 5% acetone-CH_2Cl_2 and the resulting fractions containing largely α-acetoxyheptanoic acid were submitted for ORD measurements. Both samples in methanol showed positive ORD curves of the same shape, but of somewhat different intensities due to differences in amount of impurity monomethyl glutarate present. The purest sample had $[\theta]_{229nm}$ = 1694 ± 27° (CH_3OH). The rotation of (S)-2-hydroxyheptanoic acid is reported[5] to be $[\alpha]^{20}_D$ +6° ($CHCl_3$). Pure methyl (S)-2-acetoxyheptanoate has been found by Ayer of these laboratories to have θ_{227}^{max} +2650°.

13,14-*cis*-PGE_2, 15-Acetate Methyl Ester (6) and Its 11β-Isomer. A solution of 600 mg of **3b** in 12 mL of methanol was cooled to −20 °C and 0.78 mL of 30% H_2O_2 was added followed by 0.12 mL of 1 N NaOH added dropwise over 1 h. After an additional 1 h at −20 °C, 1.8 mL of 1 N HCl was added, the mixture concentrated in vacuo, and extracted with ethyl acetate. This was washed, dried, evaporated, and the residue dissolved in 6 mL of diethyl ether, 0.6 mL of methanol, and 1 drop of water and treated with aluminum amalgam made[1] from 900 mg of granular aluminum. After stirring at 25 °C for 4 h the mixture was filtered through Celite and the filtrate evaporated. The crude product was chromatographed on 50 g of silica gel and eluted with 1 L of 20–50% ethyl acetate-Skellysolve B. Fraction 8 (50 mL) contained 42 mg of the less polar (11β) isomer of **6**, showing in the NMR spectrum a five-proton multiplet at δ 5.2–5.8 (C-5,6,13,14,15), one proton (C-11) at δ 4.2–4.5, three-proton singlet at δ 3.68 (OCH_3), and also at δ 2.03 (acetate). The major product, 146 mg, portions 9–13, consisting of **6** had an NMR spectrum very similar to that of the less polar isomer except that the C-11 proton was shifted upfield to δ 3.8–4.4; its mass spectrum showed peaks at m/e 348 (M − 60), 330, 317, 299, 277, 259, 250, 245, 208, and 190 (structure A).



A

The mass spectrum of the 11β-isomer alone was similar.

13,14-*cis*-$PGF_{2\alpha}$ (7) and 13,14-*cis*-$PGF_{2\beta}$ (8). A solution of 135 mg of the more polar isomer **6** from the preceding experiment in 2 mL of tetrahydrofuran was treated with 0.65 mL of hexamethyldisilazane and 0.11 mL of trimethylchlorosilane. After 2 h at room temperature the mixture was concentrated at 0.5-mm pressure. Some xylene was added, and the cloudy suspension was filtered through Celite and again concentrated in vacuo. The residue was dissolved in 4 mL of absolute ethanol, cooled to 0 °C, and 25 mg $NaBH_4$ added. After 1 h at 0 °C the mixture was acidified with aqueous acetic acid and allowed to stand 2.5 days at 8 °C. After concentrating in vacuo the residue was extracted with ethyl acetate, washed, dried, and evaporated. The residue was dissolved in 2.5 mL of methanol and treated under N_2 with 2.5 mL of 1 N NaOH. After 4 h at 25 °C, methanol was largely removed at reduced pressure and the residue extracted with methylene chloride to remove neutral material. The aqueous layer was acidified with 1 N HCl, saturated with NaCl, and extracted with ethyl acetate. The residue on evaporation was chromatographed on 15 g of acid-washed silica gel, eluting with 50–100% ethyl acetate-Skellysolve B. Two isomeric products were obtained, the less polar (**7**), 29 mg, and a more polar, 72 mg. The NMR spectra of these were consistent with structures **7** and **8** and both showed virtually identical mass spectra, m/e 336 (M^+ − 18), 334, 318, 300, 264, and 137 mass units. The R_f's on silica gel plates (A IX system[3]) of **7** and **8** were 0.39 and 0.26, respectively, compared to $PGF_{2\alpha}$ (0.15) and $PGF_{2\beta}$ (0.097). The less polar isomer **7** crystallized on standing in the refrigerator, mp 74–78 °C. Samples of **7** and **8** were converted to their methyl esters with ethereal diazomethane and were then identical in thin-layer mobility (A IX system) to authentic samples prepared by total synthesis.[10]

Ultraviolet Isomerization of 7 and 8. Solutions containing 5 mg of **7** and **8** in 5 mL of benzene, 0.5 mL of methanol, and 2 drops of diphenyl disulfide were separately irradiated in Pyrex tubes in a Rayonet photochemical reactor with 3500-Å light under nitrogen. After about 6 h, thin-layer chromatograph showed a more polar material forming having the same mobility (A IX system[3]) and color (vanillin-H_3PO_4

spray) as $PGF_{2}\alpha$ from **7** and from **8** a spot like $PGF_{2}\beta$ was formed. On treatment with diazomethane, these thin layer samples now showed spots with mobility like that of $PGF_{2}\alpha$ and $PGF_{2}\beta$ methyl esters, respectively, but when run on silver nitrate impregnated silica gel plates, the $PGF_{2}\alpha$ methyl ester spot now showed it was a mixture of two materials, one moving with $PGF_{2}\alpha$ methyl ester, the other with 5,6-*trans*-$PGF_{2}\alpha$ methyl ester.[16]

The irradiation mixture from **7** was chromatographed on 6 g of Porasil T, 15-25 μm (Waters Assoc.) in a 0.6 × 46 cm column, eluting with 2.5:2.5:95 methanol/acetic acid/methylene chloride. The fractions containing material moving like $PGF_{2}\alpha$ on thin layer were combined, esterified with diazomethane, and then silylated with bis-(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane and injected on a 4 ft 1% SE 52 column at 175 °C, isothermal. The irradiation material showed a major peak at 13.2 min, a minor one at 11.2 min (75 mL/min helium), and same retention times as silylated 5,6-*trans*-$PGF_{2}\alpha$ methyl ester and $PGF_{2}\alpha$ methyl ester, respectively.

Oxidation of 7 and 8 by Dichlorodicyano-1,4-benzoquinone; Formation of 9 and 10. Samples, 5 mg, of **7** and $PGF_{2}\alpha$ were separately oxidized in 0.5 mL of dioxane with 5 mg of dichlorodicyano-1,4-benzoquinone at 25 °C overnight.[17] In each case a new product was formed of R_f 0.31 in the A IX system[3] on silica gel thin layer chromatography. The products were converted to methyl esters with diazomethane and then trimethylsilylated and subjected to gas chromatography on a 6 ft 3% OV17 column at 250 °C. In each case a major peak occurred at a retention time of 3.6 min, and on admixture this peak still appeared homogeneous.

In the same manner **8** and $PGF_{2}\beta$ were separately oxidized with DDQ. Both materials gave a new product, **10**, of R_f 0.27 under the same TLC conditions as above and on gas chromatography of their methyl ester-Me_4Si derivatives, as above, gave a major peak of 3.2 min retention time.

Isolation of 4b from Extracts of *P. homomalla*. From the same large-scale chromatogram described in the first experiment, fractions 15-18 contained 16.2 g of an oil containing largely material slightly less polar than **3a**. A sample, 1 g, of this material was esterified with diazomethane and rechromatographed on 100 g of silica gel, eluting with 2 L of 0-40% ethyl acetate in Skellysolve B. Fractions 44-48 (20 mL) were combined on the basis of homogeneity by TLC to give 564 mg of an oil showing IR absorptions at 1735, 1240, 1040, 915, 860, and 730 cm^{-1}. The NMR spectrum showed two olefinic protons (C-5,6) as a multiplet at δ 5.4, a one-proton multiplet centered at δ 4.08 and another at 3.3, and a three-proton methoxyl absorption at δ 3.68. The sample, on gas chromatography (5 ft 3% OV-17 at 250 °C) showed a major peak of 3.5 min retention time, a minor one at 4.3 min. Both peaks show the same mass spectrum, *m/e* 350 (M^+), 332, 319, 247, 210, 192, 139, 121, 109, 96, 81, 79, 87, and 55 mass units and represent the two isomers (at C-11) of **4b**.

Hydrogenation of 4b to 12. A sample, 100 mg, of **4b** above was hydrogenated at atmospheric pressure in 5 mL of ethyl acetate with 15 mg of 5% Pd-charcoal catalyst until about 8 mL of H_2 had been absorbed and no more uptake occurred. The mixture was filtered and evaporated to give an oil which was identical by NMR spectrum and thin layer mobility with the major isomer **12** obtained from the known[11] mixture of **11** and **12** as described below. The NMR spectrum showed multiplets centered at δ 4.08 and 3.3, one proton each, and a three-proton singlet at δ 3.67 (OCH_3). At increased spectrum amplitude there were barely discernible proton absorptions at δ 3.75 and 4.33 due to the presence of small amounts of **11**.

Separation of 11 and 12. A mixture, 1.5 g, of **11** and **12** prepared as described in ref 11c was rechromatographed on 250 mg of acid-washed silica gel, eluting with 6 L of 5-25% ethyl acetate-Skellysolve B. Fractions 16 and 17 (250 mL) crystallized on standing and were recrystallized from ether-Skellysolve B to give 123 mg of **12**, mp 47-51 °C. Anal. Calcd for $C_{20}H_{34}O_4$; C, 70.87; H, 10.13. Found: C, 71.05; H, 9.80. The NMR spectrum showed one-proton multiplets centered at δ 4.08 and 3.3, and CD absorptions at 297 and 304 nm with molar rotations of -10×10^3 and -10.3×10^3, respectively, with inflections at about 185 and 315 nm. The methyl ester of this material was prepared with diazomethane and gave a NMR spectrum and thin layer mobility (A IX system) identical with the hydrogenation product above.

The fractions at the end of the peak of eluted material contained the slightly more polar noncrystalline isomer **11**, showing one-proton multiplets in the NMR spectrum at δ 4.33 and 3.74. The mass spectrum showed ions at *m/e* 338 (M^+), 295, 249, 210, 192, 96, 55, and 43 mass units and molar rotations as follows: 294 (-2.2×10^3), 304 (-2.1×10^3), 316 (-8×10^2), and 325 nm (4×10^2).

Methyl (15*S*)-15-Acetoxy-9-keto-5-*cis*-10-prostadienoate (13) from Coral Extracts. A 1-g sample of chromatographed material, predominantly PGA_2 acetate methyl ester, from extracts of *P. homomalla* from Grand Cayman Island was stirred 4.5 days in 50 mL of 98% formic acid containing 500 mg of $KHCO_3$ under N_2 at 25 °C. It was poured into ice, extracted with ethyl acetate, and the extracts were washed, dried, and evaporated. The resulting mixture of largely formate esters was dissolved in 50 mL of methanol and treated with 5 mL of 1N HCl for 7 h at 25 °C, when water was added and methanol was removed in vacuo. The residue was extracted, washed, and dried as above to give a mixture of products containing **1b** and its 15*R* isomer as well as a number of less polar materials. This was chromatographed after treatment with diazomethane on 300 g of silica gel, eluting with 20-70% ethyl acetate-Skellysolve B. The least polar material eluted, 71 mg, was assigned structure **13** on the basis of the following data: NMR absorptions at δ 7.58 (d of d, J = 2, 6 Hz), 6.13 (d of d, J = 2, 6 Hz), one proton each, a two-proton multiplet at about δ 5.4 (C-5,6 protons), a one-proton multiplet at δ 4.88, two overlapping triplets (C-15 proton), a three-proton singlet at δ 3.67 (OCH_3), a three-proton singlet at δ 2.02 (acetate); mass spectral ions at *m/e* 392 (M^+), 361, 332, 330, 301, 252, and 192 mass units.

For confirmation of structure **13**, it was converted to **4b** as follows. The ester groups were removed by an enzymatic hydrolysis by suspending 620 mg of coral esterase enzyme acetone powder[1] in 5 mL of water and adding the above material, dissolved in 0.2 mL of 95% EtOH, to the stirred enzyme mixture. After stirring 20 h, 12 mL of acetone was added and filtered after an additional hour. The filtrates were concentrated to remove acetone and extracted with ethyl acetate, which was washed, dried, and evaporated. A NMR spectrum of the residue confirmed the absence of ester groups. This residue was then treated in 2.5 mL of tetrahydrofuran with 2.0 mL of 0.5 N HCl for 3 days at 25 °C. It was then diluted with ethyl acetate, which was washed with saturated salt, dried with Na_2SO_4, and evaporated. Treatment with excess ethereal diazomethane converted the residue to the methyl esters. Chromatography on silica gel eluting with 25-100% ethyl acetate-Skellysolve B gave 25 mg of material having the same mobility on silica gel thin layer plates (R_f 0.70 in 50% EtAc-hexane) as **4b**, and identical NMR and mass spectra. On gas chromatography, the same two peaks were seen for the two isomeric (at C-11) cyclic ethers **4b** as before, but in more nearly equal amounts than in the **4b** isolated directly from the coral extracts.

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(12) We are indebted to W. Krueger and L. Pschigoda of these laboratories for these data.
(13) IR spectra were recorded with a Perkin-Elmer Model 221 spectrophotometer in Nujol mulls or as neat liquids between salt plates. The NMR spectra were run on a Varian A-60A spectrophotometer using deuteriochloroform solution with tetramethylsilane as internal standard. Mass spectra were recorded on an Atlas CH-4 instrument with ionization voltage of 70 eV or on a LKB Model 9000 gas chromatograph–mass spectrograph. UV spectra were recorded in 95% ethanol using a Cary Model 14 spectrophotometer. We are grateful to Dr. A. A. Forist and his associates for much of the analytical and spectral data.
(14) Kindly provided by H. Karnes of our Chemical Research Preparations

Unit.
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The Total Synthesis of *dl*-Vernolepin and *dl*-Vernomenin

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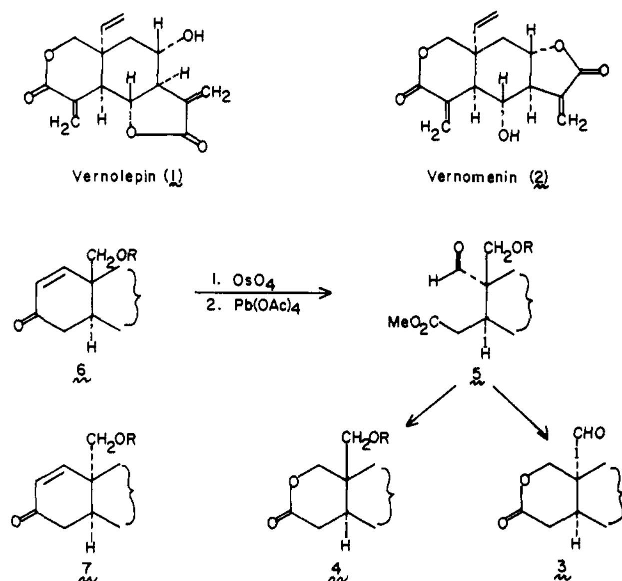
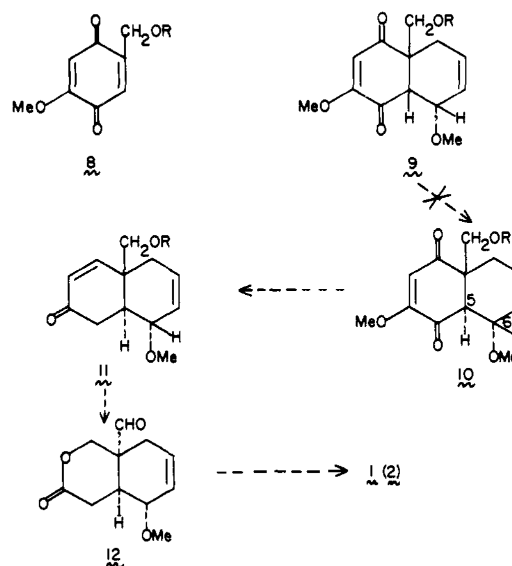
Abstract: The total syntheses of the racemates of the bis-α-methylenelactonic sesquiterpenes, vernolepin and vernomenin, have been achieved. These tumor inhibitors were synthesized in 17 steps starting with dienone ester **29**, itself the result of two Diels–Alder reactions. The key elements of the total syntheses were (i) the use of an angular carboxyl group to establish three centers of asymmetry in the B ring (**29** → **37**), (ii) the conversion of a cis-fused cyclohexenone to a cis δ-lactone (**37** → **41**), (iii) the protection of a δ-lactone as an ethylene glycol mixed orthoester (**41** → **42**), (iv) the opening of a very hindered epoxide to control the stereochemistry of the C ring (**52** → **54**), and (v) the bis-α-methylenation of the bisnor precursor (**55** → **1** and **56** → **2**) using dimethyl(methylene)ammonium iodide.

Background

In a previous paper,[1] we described an approach to the total synthesis of the tumor inhibitors vernolepin (**1**) and vernomenin (**2**).[2-4] Our route to the valerolactone segment involved oxidative degradation of a cyclohexenone using the indicated reactions. If one employed a precursor of the type **6**, model studies indicated that one could derive either the cis lactone **3** or a trans lactone **4** by suitable manipulations of the intermediate aldehydo ester **5**. An obvious corollary involved the supposition that one could derive the desired **3** from either a trans-fused enone **6** or a cis-fused enone **7**.



The synthetic scheme to bicyclic Δ^1-enones, which was available, was provided in characteristically elegant form by the Woodward school in the context of its classical total synthesis of steroids.[5] Translated to our needs, this involved recourse to a para quinone of the type **8** which was easily available in copious quantity from isovanillin, as previously described.[1] Cycloaddition of **8** with 1-methoxybutadiene gave, as expected from the maxim of cis-endo addition,[6] the adduct **9**. It was our intention to transform **9**, by equilibration,[5] to the trans epimer **10**. The further elaboration of **10** in the direction of vernolepin and vernomenin was to be achieved via intermediates **11** and **12**. Unfortunately, even after repeated experimentation, we were unable to effect the transformation of **9** → **10**.



At this juncture we faced a serious dilemma. If we were to use a cis-based Diels–Alder approach involving quinone **8**, we could not incorporate the oxygen functionality to establish the