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THE ISOLATION OF (−)-SARCOPHYTOL A AND (+)-MARASOL FROM THE CARIBBEAN GORGONIAN *PLEXAURA FLEXUOSA*[1]

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ABSTRACT.—The gorgonian *Plexaura flexuosa* from Puerto Rico was found to contain (1*E*, 3*E*,7*E*,11*E*)-(14*R*)-cembra-1,3,7,11-tetraen-14-ol [2], the (−)-antipode of the potent antitumor promoter (+)-sarcophytol A [5]. A new bicyclic oxa-bridged 2,5-dihydrofuran-containing cembranoid diterpene, (+)-marasol [4], was isolated as a minor constituent from samples of the same gorgonian. Their structures have been established through chemical and spectral studies.

RESULTS AND DISCUSSION

Diterpenes with the 14-carbon cyclic cembrane nucleus have been frequently found in soft corals of the order Alcyonacea as well as in many gorgonians. Their presence in soft corals is well documented, and cembranes continue to be the largest and most abundant group of compounds among the diterpenes isolated from soft corals (1). Among the numerous cembranoid diterpenes isolated from marine organisms is cembrene C [1], which was isolated in 1976 by Schmitz *et al.* (2) from a Pacific soft coral of the genus *Nephthea*. Many oxygenated diterpenoids having the cembrene C skeleton have been obtained from marine organisms, and many variations of substitution in the cembrane ring have been reported (1). We now report the isolation of two new cembranoid diterpenes related to cembrene C that have been isolated from the Caribbean gorgonian *Plexaura flexuosa* (Lamouroux) (phylum Cnidaria; class Anthozoa; subclass Alcyonaria; order Gorgonacea). The structure of the major secondary metabolite isolated was subsequently shown to be (1*E*,3*E*,7*E*,11*E*)-(14*R*)-cembra-1,3,7,11-tetraen-14-ol [2] on the basis of its combined spectral and chemical degradation data. This simple monohydroxycembratetraene was found to be the (−)-antipode of the potent antitumor promoter (+)-sarcophytol A [5] isolated from *Sarcophyton glaucum*, a common soft coral found in the coral reefs of Indo-Pacific coastal waters (3,4). In this paper we also describe the isolation and characterization of (+)-marasol [4], an oxa-bridged 2,5-dihydrofuran-containing cembranoid diterpene which was isolated as a minor constituent from samples of the same gorgonian. The structure of 4 was elucidated on the basis of chemical and spectral studies, particularly chemical shift correlation methods, homonuclear spin decoupling experiments, and nuclear Overhauser enhancement difference spectroscopy (nOeds).

RESULTS AND DISCUSSION

Freeze-dried material of *P. flexuosa*, collected in the coral reef of Desecheo Island, Puerto Rico in March 1989, was extracted with MeOH/CHCl$_3$, and the lipid extract obtained was subjected to repeated chromatography to give two cembrane-type diterpenes 2 and 4.

Compound 2, [α]D −190.39°, obtained as a uv-active colorless oil, corresponded to a molecular formula of C$_{20}$H$_{32}$O on the basis of its ^{13}C-nmr analysis and mass spectrum ([M]$^+$ *m/z* 288), and contained five unsaturations. Hrms of 2 gave major ions at *m/z* 288.2453 for C$_{20}$H$_{32}$O [M]$^+$ and *m/z* 273.2219 for C$_{19}$H$_{29}$O, *m/z* 270.2349 for C$_{20}$H$_{30}$, and *m/z* 245.1906 for C$_{17}$H$_{25}$O, which reflect fragmentations of [M − Me]$^+$,

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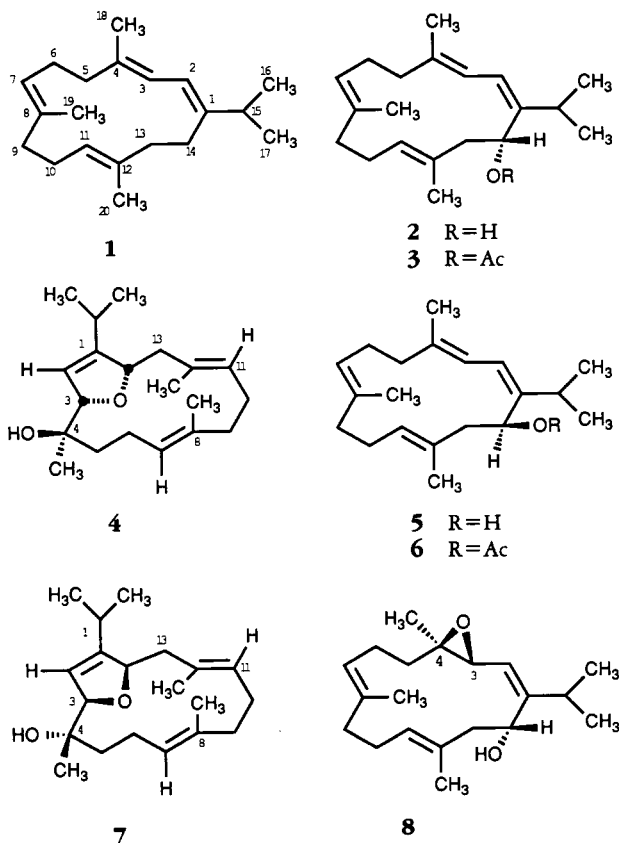
[M − H₂O]⁺, and [M − C₃H₇]⁺, respectively, from the actual molecular composition of $C_{20}H_{32}O$. This homogeneous component showed a hydroxyl absorption at 3380 cm⁻¹ in its ir spectrum, and its ¹³C-nmr spectrum in CDCl₃ (Table 1) indicated the presence of eight sp² carbons and only one carbon atom bearing oxygen atoms (δ 69.95). Accordingly, it appeared that **2** possessed four double bonds, a hydroxyl linkage, and one ring.

The ¹H-nmr spectrum of **2** (Table 1) in CDCl₃ indicated the presence of two secondary methyl groups due to an isopropyl group [δ 1.02, 1.08 (each 3H, d, J = 6.8 Hz), 2.55 (1H, sept, J = 6.8 Hz)], three vinylic methyl groups [δ 1.44, 1.57, 1.72 (each 3H, br s)], and four olefinic protons [δ 4.98 (2H, br m), 5.97 (1H, d, J = 11.4 Hz), 6.12 (1H, d, J = 11.4 Hz)]. A single oxygen-bearing-methine signal was observed at δ 4.98, which was enveloped by two olefinic protons, and a broad envelope of allylic proton signals, integrating as 10H, was found between δ 1.93 and 2.39.

The ¹³C-nmr spectrum of **2** exhibited twenty signals divided by APT (5) into four quaternary carbons (C-1, C-4, C-8, and C-12), six CH groups (C-2, C-3, C-7, C-11, C-14, and C-15), five methylenes (C-5, C-6, C-9, C-10, and C-13) and five Me groups (C-16, C-17, C-18, C-19, and C-20). The APT experiment indicated that four of the olefinic carbons (δ 146.99, 135.87, 134.53, 131.31) are nonprotonated vinyls and four are singly protonated (δ 125.38, 124.49, 121.21, 120.45). The same experiment also indicated that the only oxygenated carbon atom (δ 69.95) present in **2** was tertiary.

The isopropyl group was found to be linked to a quaternary sp² carbon on the basis of the multiplicity and chemical shift of the methine signal at δ 2.55 (H-15). Irradiation around δ 1.10 and at 2.55 changed the signals of the methine and methyl groups.

TABLE 1. ^{13}C- (75 MHz)^a and ^{1}H- (300 MHz)^b nmr Data for Cembrane Diterpenes **2** and **4**.

Carbon	2; δ_C ppm	2; δ_H ppm (J in Hz)	4; δ_C ppm	4; δ_H ppm (J in Hz)
1	146.99 (s)	—	150.85 (s)	—
2	120.45 (d)	6.12 (1H, d, 11.4)	119.26 (d)	5.41 (1H, d, 2)
3	121.21 (d)	5.97 (1H, d, 11.4)	87.07 (d)	4.67 (1H, complex m)
4	135.87 (s)^c	—	74.64 (s)	—
5	38.75 (t)^d	2.08 (2H, m)	38.39 (t)	1.88 (1H, m)
				1.57 (1H, m)
6	25.50 (t)^e	2.14 (2H, m)	25.29 (t)	2.27 (1H, m)
				2.05 (1H, m)
7	125.38 (d)^f	4.98 (1H, m)	128.36 (d)	5.25 (1H, br dd, 2.9, 10.3)
8	134.53 (s)^c	—	132.36 (s)^c	—
9	39.62 (t)^d	2.08 (2H, m)	39.72 (t)	2.15 (2H, m)
10	24.50 (t)^e	2.14 (2H, m)	22.08 (t)	2.38 (1H, m)
				1.92 (1H, m)
11	124.49 (d)^f	4.98 (1H, m)	131.13 (d)	5.12 (1H, br dd, 2.1, 9.1)
12	131.31 (s)^c	—	129.67 (s)^c	—
13	44.64 (t)	2.35 (1H, br d, 12.4)	42.92 (t)	2.45 (1H, dd, 5.9, 13.4)
		2.25 (1H, d, 9.1)		2.12 (1H, d, 13.4)
14	69.95 (d)	4.98 (1H, m)	84.48 (d)	4.84 (1H, complex m)
15	27.09 (d)	2.55 (1H, sept, 6.8)	26.47 (d)	2.33 (1H, sept, 6.8)
16	24.33 (q)^g	1.02 (3H, d, 6.8)^c	20.85 (q)^d	1.02 (3H, d, 6.8)^c
17	25.32 (q)^g	1.08 (3H, d, 6.8)^c	22.02 (q)^d	1.14 (3H, d, 6.8)^c
18	16.29 (q)	1.72 (3H, br s)	23.77 (q)	0.91 (3H, br s)
19	15.51 (q)^h	1.44 (3H, s)^d	15.59 (q)	1.55 (3H, br s)
20	18.12 (q)^h	1.57 (3H, s)^d	17.65 (q)	1.53 (3H, br s)

^{a}^{13}C-nmr spectra were recorded in CDCl$_3$. Multiplicities were obtained by an Attached Proton Test (APT) experiment. Assignments were made on the basis of heteronuclear chemical shift correlation methods, carbon atom multiplicities and chemical shift values. The δ values are in ppm and are referenced to the residual CHCl$_3$ signal (77.0 ppm).
^{b}^{1}H-nmr spectra were recorded in CDCl$_3$. Assignments were aided by ^{1}H-^{1}H and ^{1}H-^{13}C COSY and homonuclear spin-decoupling experiments. J values are reported in Hz, and the chemical shifts are given in δ units (ppm downfield from TMS).
$^{c-h}$Values with identical superscripts in each column may be interchanged.

respectively, into singlets. The ir absorptions at 1610 and 1670 cm^{-1} and the uv absorption at 256 nm (ε 19,300) of compound **2** indicated the presence of a conjugated diene. Specifically, the presence of a 1,1,4,4-tetrasubstituted conjugated diene in **2** was implied by the ^{1}H-nmr signals at δ 5.97 and 6.12 as an AB quartet (J = 11.4 Hz). The conformation of the diene was deduced as s-trans on the basis of the similarities of the ^{1}H-nmr, ir, and uv data with known models (2). The somewhat deshielded vinylic methyl signal at δ 1.72 is linked to the conjugated diene, and the slightly broadened nature of the signal at δ 5.97 (H-3) suggests allylic coupling with the methyl signal at δ 1.72. This was confirmed by autocorrelated proton 2D nmr, since cross peaks connecting the H-18 methyl protons and H-3 are observed in the contour plot of the ^{1}H-^{1}H COSY spectrum of alcohol **2**.

The structure of the C-1 to C-11 segment in **2** was confirmed by the ^{13}C-nmr data (Table 1) and the detection of two moles of levulinic acid on ozonolysis. The co-occurrence of isobutyric acid during the ozonolysis of compound **2** along with the ^{1}H-nmr data (Table 1) showed that the hydroxyl group is located at C-14. This was also deduced on the ground that the ^{13}C-nmr signals at δ 135.87 (s), 134.53 (s), and 131.31 (s) in **2**, ascribable to either C-4, C-8, or C-12, were all devoid of the β effects of a hydroxyl group (6). The methylene carbon signal at δ 44.64 (t) in its ^{13}C-nmr spectrum was as-

cribed to C-13, which is located between a carbinyl carbon and an sp^2 quaternary carbon. These results led us to propose a cembrane skeleton for compound **2**.

The geometry of each of the three trisubstituted double bonds was assigned as trans from their signals in the ^{13}C-nmr spectrum, which showed a significant shielding of methyl groups caused by vicinal carbons in the same way as in *trans*-polyisoprene (7). A careful literature survey revealed that the nmr (^{1}H and ^{13}C), ir, uv, and mass spectra of diterpenoid cembrane **2**, as well as the magnitude of its specific rotation, appeared identical with those reported for (+)-sarcophytol A [**5**] (3,4). However, solutions of the two cembrane alcohols in CHCl$_3$ showed opposite signs of rotation. Therefore, (+)-sarcophytol A [**5**] and **2** are clearly enantiomeric. Since a Horeau determination of the absolute configuration of (+)-sarcophytol A indicated the 14S configuration, **2** must possess the 14R configuration. A further chemical correlation study to confirm the absolute configuration of new diterpene **2** indicated again the 14R configuration. Acetylation of **2** with a mixture of Ac$_2$O and pyridine at 25° gave the tetraene monoacetate **3** identical as regards ir, ^{1}H-nmr, ms, and magnitude of its specific rotation with natural product (+)-sarcophytol A acetate [**6**] {which has the 14S configuration} but with opposite sign of rotation (3,4). Thus, tetraene acetates **3** and **6** must be enantiomers.

(+)-Marasol [**4**], [α]D +173.91°, was obtained as a uv-inactive colorless oil that corresponded to a molecular formula of C$_{20}$H$_{32}$O$_2$ on the basis of its ^{13}C nmr and hreims ([M]$^+$ *m/z* 304). Its ir spectrum displayed a strong band at 3450 cm^{-1} (broad, hydroxyl group) and the ^{13}C-nmr spectrum (Table 1) confirmed the presence of three trisubstituted double bonds [150.85 (s), 132.36 (s), 131.13 (d), 129.67 (s), 128.36 (d), and 119.26 (d)]. Hence **4**, having five degrees of unsaturation, must possess a bicyclic skeleton. The ^{13}C-nmr spectrum of **4** exhibited twenty signals divided by APT into four quaternary carbons (C-1, C-4, C-8, and C-12), six methines (C-2, C-3, C-7, C-11, C-14, and C-15), five methylenes (C-5, C-6, C-9, C-10, and C-13), and five methyl groups (C-16, C-17, C-18, C-19, and C-20). The APT experiment also indicated that two of the three oxygenated carbon atoms (δ 84.48, 87.07) are tertiary, while the third (δ 74.64) belonged to a quaternary carbon. The ir and uv spectra of (+)-marasol [**4**] showed the absence of a conjugated diene and suggested instead the presence of an ethereal linkage. This was also suggested by the signals at δ 84.48 (d) and 87.07 (d) in its ^{13}C-nmr spectrum. However, the downfield shift of these signals coupled with the absence of the corresponding methine or methyl signals around δ 3.0 and 1.3, respectively, excluded the possibility of a trisubstituted epoxide ring which has frequently been found in the cembrane-type diterpenes from soft corals.

The ^{1}H-nmr spectrum of **4** (Table 1) indicated the presence of two vinylic methyl groups on quaternary carbons [δ 1.53 (3H, s), 1.55 (3H, s)], an isopropyl group [δ 1.14 (3H, d, *J* = 6.8 Hz), 1.02 (3H, d, *J* = 6.8 Hz)], and a methyl group on a quaternary carbon atom bearing oxygen [δ 0.91 (3H, s)]. The isopropyl group was also found to be linked to a quaternary sp^2 carbon on the basis of the multiplicity and chemical shift of its methine signal at δ 2.33 (1H, sept, *J* = 6.8 Hz). Irradiation around δ 1.1 and at δ 2.33 changed the signals of the methine and methyl groups into singlets. Allylic proton signals were observed between δ 2.01 and 2.50 as a broad envelope integrating as 8H, including a doublet of doublets at δ 2.45 (1H, dd, *J* = 5.9, 13.4 Hz) and an overlapped doublet at δ 2.12 (1H, d, *J* = 13.4 Hz), while the remaining signals were at δ 4.67 (1H, complex m), 4.84 (1H, complex m), 5.12 (1H, br dd, *J* = 2.1, 9.1 Hz), 5.25 (1H, br dd, *J* = 2.9, 10.3 Hz), and 5.41 (1H, d, *J* = 2 Hz).

The absence of a conjugated diene system and the combined ^{1}H- and ^{13}C-nmr data of (+)-marasol suggest that C-14 participates in the formation of an ethereal linkage. A heteronuclear chemical shift correlation with broad band decoupling (CSCMBB) experiment (8,9) correlated the proton resonances at δ 4.67 and 4.84 with their correspond-

ing carbon resonances at δ 87.07 and 84.48, respectively. The nature of these low-field signals was reminiscent of a 2,5-dihydrofuran ring system (10–12). Irradiation at δ 4.67 (H-3) collapsed the doublet at δ 5.41 (H-2) into a broad singlet. The noticeably small vicinal coupling constant observed between H-2 and H-3 is in agreement with the proposed structure **4**. An examination of molecular models revealed that the dihedral angle (φ) between H-2 and H-3 approaches 60°; i.e., for equatorial-equatorial (J_{ee}) or equatorial-axial (J_{ea}) the predicted coupling is ca. 2.5 Hz. This value very closely resembles the actual vicinal coupling observed for the two protons ($^{H-H}J_{2,3} = 2$ Hz). During irradiation of the proton at δ 4.67 (H-3), modified absorption patterns were also observed distinctively at δ 4.84 (H-14), thereby confirming the presence of a characteristic α,α′ long-range dihydrofuran ring coupling in **4**, which has also been observed in structurally related model systems containing the 2,5-dihydrofuran ring moiety (10–12). Certain five-membered ring systems, such as 2,5-dihydrofuran, show remarkably large coupling constants between the formally homoallylic H-2 and H-5, which appear to vary with the cis-trans stereochemistry (13–15). It is very likely that the interaction observed between H-3 and H-14 is in fact a sum of interactions across the homoallylic system and across the four-sigma-bond pathway via the heteroatom (13–15). Similarly, irradiation at δ 4.84 (H-14) changed the signal at δ 4.67 (H-3) into a broad doublet ($J = 2$ Hz) and collapsed the doublet of doublets at δ 2.45 (H-13α) into a broad doublet ($J = 13.6$ Hz), thereby confirming the location of one of the methylene protons in position C-13. No modified absorption patterns were observed near the doublet at δ 2.12, suggesting lack of coupling between H-14 and the remaining diastereotopic proton in position C-13 (H-13β). A nearly perpendicular spatial relationship (φ ≈ 90°) between H-14 and H-13β can be observed upon examination of molecular models. Irradiation of the doublet of doublets at δ 2.45 (H-13α) collapsed the doublet at δ 2.12 (H-13β) into a broad singlet, confirming the location of both methylene protons in position C-13. The strong geminal coupling between the C-13 methylene protons was also evident from the intense cross peaks observed in the ^{1}H-^{1}H COSY spectrum of **4**. These findings revealed that the ethereal methine proton (H-14) at δ 4.84 is coupled only to one of the methylene protons at position C-13 (δ 2.45), and since the connectivity of the C-13 protons stopped there, C-12 (δ 129.67 or 132.36) must be a quaternary carbon. These results confirm the homoallylic nature and ring size of the oxa-bridged ring system. Irradiation at the vinylic methyl region sharpened the low-field signals at δ 5.12 and 5.25, indicating allylic coupling with the vinylic methyl groups. This was confirmed by autocorrelated proton 2D nmr, since cross peaks connecting the C-8 and C-12 methyl groups with their corresponding vinyl protons at C-7 and C-11 are observed in the contour plot of the ^{1}H-^{1}H COSY spectrum of **4**. Micro-ozonolysis afforded one mole of levulinic acid; therefore, the unsaturation pattern shown in structure **4** was the only one that could accommodate these structural features in a cembrane ring. The ^{13}C-nmr spectrum showed that both vinyl methyl signals resonated at less than 20 ppm, which suggested that the double bonds had the usual *E* configuration (7).

The relative spatial arrangement between the substituents at chiral centers C-3, C-4, and C-14 was established by nOeds (16) involving the irradiation of the C-3 substituted proton (δ 4.67) (17). This resulted in significant enhancement of the methine proton at C-14 (δ 4.84) and therefore established the cis geometry between H-3 and H-14 of the 2,5-dihydrofuran ring. Moreover, since no enhancement of the C-4 substituted methyl group (δ 0.91) was observed during irradiation of the bridgehead methine (δ 4.67), the quaternary methyl and the H-3 proton should not be within nOe proximity. Therefore a trans spatial arrangement can be assumed.

During an examination of the complex autoxidation products of (+)-sarcophytol A

[5], Kobayashi and co-workers (18, 19) showed that the dihydrofuran 7 was one of the major components. Therefore, (+)-marasol [4] is the likely enantiomer of compound 7 and likewise is an artifact derived from the labile (−)-sarcophytol A [2]. We suggest that 2 and 4 are biogenetically related via transannular cyclization through the interaction of an initial intermediate (3S),(4R)-epoxide 8 with its 14-hydroxyl group. Since the absolute configuration of compound 2 has been established and both compounds 2 and 4 have identical relative configuration at C-14, one can safely assume that they also have the same absolute configuration. Therefore, the absolute configuration of all three chiral centers must be as shown in structure 4. Finally, cembrane diterpenes 2 and 4 appear to be present in the crude extract of *P. flexuosa* and are not just artifacts derived from autoxidation of cembrene C [1]. Supporting this contention, no traces of hydrocarbon 1 were detected throughout the present work. Furthermore, 2 survives chromatographies over Si gel as does dihydrofuran 4, and no isomerization of 2 to 4 appeared to occur on the latter adsorbent, as judged by hplc analysis of eluted material. However, (−)-sarcophytol A [2] was found to be markedly reactive when kept in CHCl₃ at room temperature, and within several days most of the starting material was consumed. ¹H-nmr, glc-ms, and tlc analyses of the complex mixture showed that compound 4 was one of the major components.

In the present work we have demonstrated the presence of new cembranoid diterpenes 2 and 4 in the Caribbean gorgonian *P. flexuosa*. While it is well known that a number of terrestrial plants produce both antipodes for a given metabolite (usually sesquiterpenes) (20), similar antipodal relationships appear to be much rarer among marine organisms. The clear-cut antipodal relationship that exists between cembranoid diterpenes 2 and 5 (and 4 and 7), isolated from the Caribbean and the Pacific, respectively, represents an obvious exception. Since the cembranoid diterpene 2, the principal metabolite from *P. flexuosa*, has the proper stereochemistry to be the logical biosynthetic precursor to 4, transannular back-side attack of the C-14 hydroxyl group at C-3 of a 3,4-epoxy intermediate 8 should lead exclusively to compound 4. The optical antipode of the form found in the Caribbean could a priori yield dihydrofuran diterpene 7.

Inasmuch as (+)-sarcophytol A [5] has recently been shown to be active as a potent antitumor-promoter in a two-stage mouse skin carcinogenesis model, as well as having an inhibitory effect on the hyperplasia of mouse skin (21), it is very likely that (−)-sarcophytol A [2] also exhibits antitumor-promoting activity. However, owing to the limited amounts available and since both 2 and 4 are quite labile to autoxidation, we were unable to examine these new cembrene diterpenes for any kind of biological activity. Takayanagi *et al.* (22) reported recently the first total synthesis of (+)-sarcophytol A in a highly stereo- and enantioselective manner, which could in principle be modified to obtain also (−)-sarcophytol A [2].

EXPERIMENTAL

GENERAL PROCEDURES AND ISOLATION.—¹H and ¹³C nmr were measured at 300 and 75 MHz, respectively, with a General Electric QE-300 spectrometer. ¹H-nmr chemical shifts are reported as δ values in ppm relative to either TMS (0.0 ppm) or CHCl₃ (7.26 ppm). Data is reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), and coupling constant (Hz). ¹³C chemical shifts are reported in ppm relative to CDCl₃ (77.0 ppm). Ir spectra were recorded on a Nicolet 600 FT-IR spectrophotometer, and uv spectra were recorded on a Hewlett-Packard Chem Station 8452A spectrometer. All hplc was performed on a Whatman Magnum 9 10/50 Partisil semipreparative column and a Beckman Ultrasphere-ODS semipreparative column. Separations were monitored simultaneously by refractive index and uv absorption. Low resolution mass spectra (lrms), electron impact (ei) were recorded on a Hewlett-Packard 5995A spectrometer. Glc-ms analyses were performed on a Hewlett-Packard 59970 MS Chem Station using a capillary HP-1 (cross-linked methyl silicone gum) column (12 m, 0.20 mm), and the temperature was programmed from 35 to 250° at 10°/

min. Analytical tlc was performed using 0.25 mm Analtech Uniplate precoated Si gel plates. Optical rotations were determined on a Perkin-Elmer Polarimeter Model 243B. Ozone was generated with a Polymerics, Inc. Ozonator Model T-408. All solvents used were either spectral grade or were distilled from glass prior to use. Minced and freeze-dried *P. flexuosa* (165 g), collected at Desecheo Island, Puerto Rico in March 1989, were extracted exhaustively with $CHCl_3$-MeOH (1:1). A voucher specimen is stored at the Chemistry Department of the University of Puerto Rico. The dried residue after concentration amounted to ca. 3 g and was partitioned against hexane and H_2O, giving after subsequent rotaevaporation 1.5 g of lipids as a viscous dark green oil. The hexane extract was dissolved in hexane-EtOAc (90:10) and passed through a column containing 70 g of Si gel (35–75 mesh, Analtech). The less polar portion of the lipids was fractionated roughly into fractions A through G on the basis of tlc analyses. Repeated cc of fraction C (ca. 50 mg) by hplc {Ultrasphere-ODS Si gel with MeOH-H_2O (9:1)} gave a pure sample of **2** (ca. 34 mg). Fraction D (61.5 mg), which consisted by tlc analysis of a mixture of **2** and **4**, was dissolved in hexane-EtOAc (90:10) and purified successively through normal phase hplc [Partisil Si gel with hexane-EtOAc (90:10)] and reversed-phase hplc [Ultrasphere-ODS Si gel with MeOH-H_2O (95:5)] to give another ca. 24 mg of pure **2** and 13 mg of analytically pure **4**.

(1E,3E,7E,11E)-(14R)-CEMBRA-1,3,7,11-TETRAEN-14-OL [**2**].—(−)-*Sarcophytol A* [**2**]: colorless oil; $[\alpha]^{29}D$ − 190.39° (c = 1.06 g/100 ml, $CHCl_3$); uv λ max ($CHCl_3$) 256 nm (ε 19,300); ir (neat) 3380, 2955, 2922, 2853, 1670, 1610, 1457, 1445, 1380, 1009 cm^{-1}; hreims *m/z* $[M]^+$ 288.2453 (2.7%) ($C_{20}H_{32}O$ requires 288.2454), 273 (4), 270 (0.5), 245 (2.8), 205 (2.3), 175 (1.4), 149 (2.3), 138 (13.8), 137 (100), 121 (5.1), 119 (4.5), 109 (68.8); ^1H and ^{13}C nmr see Table 1.

(+)-MARASOL [**4**].—Colorless oil; $[\alpha]^{29}D$ + 173.91° (c = 0.69 g/100 ml, $CHCl_3$); ir (neat) 3450, 2862, 2927, 2909, 2872, 2852, 1457, 1438, 1393, 1369, 1253, 1188, 1104, 1085, 1030, 978, 949 cm^{-1}; hreims *m/z* $[M]^+$ 304.2415 (5%) ($C_{20}H_{32}O_2$ requires 304.2404) 289 (0.2), 286 (3), 261 (1.5), 246 (0.1), 243 (1), 236 (2), 221 (6), 194 (3), 193 (2), 176 (3), 163 (8), 161 (8), 154 (10), 153 (100); ^1H and ^{13}C nmr see Table 1.

(1E,3E,7E,11E)-(14R)-ACETOXYCEMBRA-1,3,7,11-TETRAENE [**3**].—Treatment of the alcohol **2** (32 mg, 0.11 mmol) with a mixture of Ac_2O (2 ml) and pyridine (1 ml) afforded, after stirring at 25° for 18 h followed by usual workup and rapid chromatography on hplc [Ultrasphere-ODS Si gel with MeOH-H_2O (95:5)], the acetate **3**: colorless oil, $[\alpha]^{29}D$ − 214.93° (c = 0.67 g/100 ml, $CHCl_3$); uv λ max ($CHCl_3$) 256 nm; ir (neat) 2958, 2922, 2859, 1740, 1239 cm^{-1}; lreims $[M]^+$ 330 (2.6), 273 (1.2), 270 (1.6), 255 (2), 227 (3.5), 205 (4), 187 (6), 177 (4), 159 (20), 152 (19), 145 (10), 137 (71), 119 (34), 109 (100), 105 (25); ^1H nmr (300 MHz, $CDCl_3$) δ 1.03 (6H, br t, J = 6.8 Hz, isopropyl methyls), 1.44, 1.55, 1.70 (each 3H, br s, vinyl methyls), 1.98 (3H, s, acetate methyl), 2.03–2.45 (10H, br m, allylic protons), 2.55 (1H, sept, J = 6.8 Hz, H-15), 4.98 (1H, br t, vinyl H), 5.05 (1H, br t, vinyl H), 6.02 (1H, dd, J = 4.3, 9.6 Hz, CHOAc), 6.07 (1H, d, J = 11.6 Hz) and 6.18 (1H, d, J = 11.6 Hz) (diene protons), ^{13}C nmr (75 MHz, $CDCl_3$) δ 170.01 (s), 142.25 (s), 136.79 (s), 134.37 (s), 130.20 (s), 126.32 (d), 124.45 (d), 122.24 (d), 121.58 (d), 72.69 (d), 41.96 (t), 39.77 (t), 38.65 (t), 27.92 (d), 25.56 (t), 24.91 (q), 24.61 (t), 24.00 (q), 21.31 (q), 18.16 (q), 16.27 (q), 15.56 (q).

MICRO-OZONOLYSIS OF (+)-MARASOL.—Dihydrofuran **4** (3 mg, 0.0098 mmol) was dissolved in EtOAc (3 ml) and cooled to −78° in a dry ice/Me_2CO bath. Ozone in O_2 (2% v/v) was bubbled through the solution for 4 min. After removal of the solvent at 35° in vacuo, the ozonide was decomposed upon addition of H_2O (3 drops) followed by heating to 90° for 1 h in the presence of H_2O_2 (3 drops). The resulting residue was analyzed by glc-ms. The retention time of one of the major components was 9.8 min. Under identical conditions an authentic sample of levulinic acid showed identical retention time and mass spectral fragmentation patterns.

MICRO-OZONOLYSIS OF (−)-SARCOPHYTOL A [**2**].—A stirred solution of **2** (6 mg, 0.021 mmol) in EtOAc (3 ml) at −78° was treated with a stream of O_2/O_3 until the reaction mixture attained a pale blue coloration (3 min). After removal of the solvent under reduced pressure at 35°, H_2O was added to the residue and the mixture was kept at 100° for 1 h in the presence of a few drops of H_2O_2. Injection of the residue in the glc-ms system followed. The identification of the fragments was established by comparison of the retention times and mass spectra recorded with those of authentic samples of levulinic acid (Rt 9.8 min) and isobutyric acid (Rt 11.5 min).

NOEDS EXPERIMENTS.—The nOe difference spectroscopy experiments were performed as outlined by Hall and Sanders (17). All samples prepared for nOeds were degassed by bubbling argon through the solution while being kept at 0° for 1 h and then sealed around the cap with parafilm. Solutions were made up in $CDCl_3$ such that, after degassing and a loss of a significant volume of $CDCl_3$, the final concentration was 0.05–0.07 M.

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