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FURTHER BUTENOLIDES FROM THE CARIBBEAN OCTOCORAL *PTEROGORGIA CITRINA*[1]

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ABSTRACT.—Seven fatty acid derivatives containing one or two butenolide moieties along with ancepsenolide [**1**], a known bis-butenolide of marine origin, were isolated from the Caribbean gorgonian *Pterogorgia citrina*. The structures of the new metabolites were elucidated on the basis of spectroscopic data and were confirmed upon chemical conversion to either ancepsenolide or to its homolog, homoancepsenolide [**4**].

The presence of endogenous secondary metabolites is believed to endow marine organisms with a chemical means of defense. Crude extracts of many gorgonians (sea fans and sea whips) have been shown to contain antimicrobial and cytotoxic materials, and subsequent chemical investigations have resulted in the isolation and identification of a host of new terpenes, sterols, and prostaglandins (1). In the course of our investigations on the chemical constituents of Caribbean coelenterates, we have isolated seven new lipids, each containing one or two butenolide moieties, from the gorgonian octocoral, *Pterogorgia citrina* Esper, collected off the west coast of Puerto Rico. We report herein the isolation and structure elucidation of these butenolides on the basis of their spectral properties and chemical reactions.

RESULTS AND DISCUSSION

Specimens of *P. citrina* were collected at Mona Island, 80 km from the Puerto Rican coast. The sample was freeze-dried and then extracted exhaustively with a 1:1 mixture of MeOH-$CHCl_3$. The 14% extract, based on dry weight, was defatted with hexane and then partitioned against $CHCl_3$ and H_2O. After filtration and concentration, the $CHCl_3$ extract (8.1%) was separated after successive chromatography on Bio Beads SX-2 and Si gel, followed by subsequent purification by hplc, into the known ancepsenolide [**1**] and seven new butenolide derivatives.

The main fraction isolated from hplc consisted of a white solid, mp 96.0–97.9°, which was identified as ancepsenolide [**1**] by comparison of its physical and spectral properties to those of material isolated previously by Schmitz and coworkers from the Caribbean gorgonian *Pterogorgia anceps* (2). Although no evidence concerning the relative or absolute stereochemistry of ancepsenolide at C-5 and C-5′ has been obtained, the fact that **1** exhibits optical activity despite having a symmetrical structure, suggests that it must have the same absolute stereochemistry at both chiral centers. The absolute stereochemistry shown in structure **1** [5*S*, 5′*S*] and that depicted in structures **2–8** (also optically active compounds) has been chosen arbitrarily.

Ancepsenolide acetate [**2**] is an amorphous optically active powder ($[\alpha]^{25}D$ −11.2°, mp 53.9–54.9°) with elemental composition $C_{24}H_{38}O_6$, determined by hrfabms measurements of the molecular ion at *m/z* 429.2824 $[M+Li]^+$ and from ^1H- and ^{13}C-nmr data (Table 1). The ir spectrum of **2** exhibited a strong broad absorption at 1748 cm^{-1} with a shoulder at 1777 cm^{-1}, consistent with the presence of butenolide and acetate

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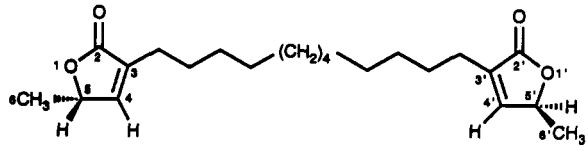
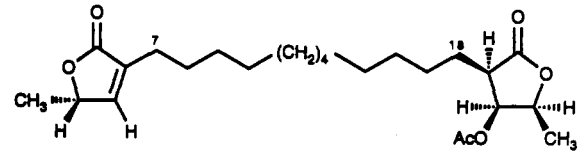


1



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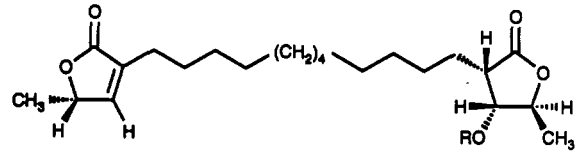
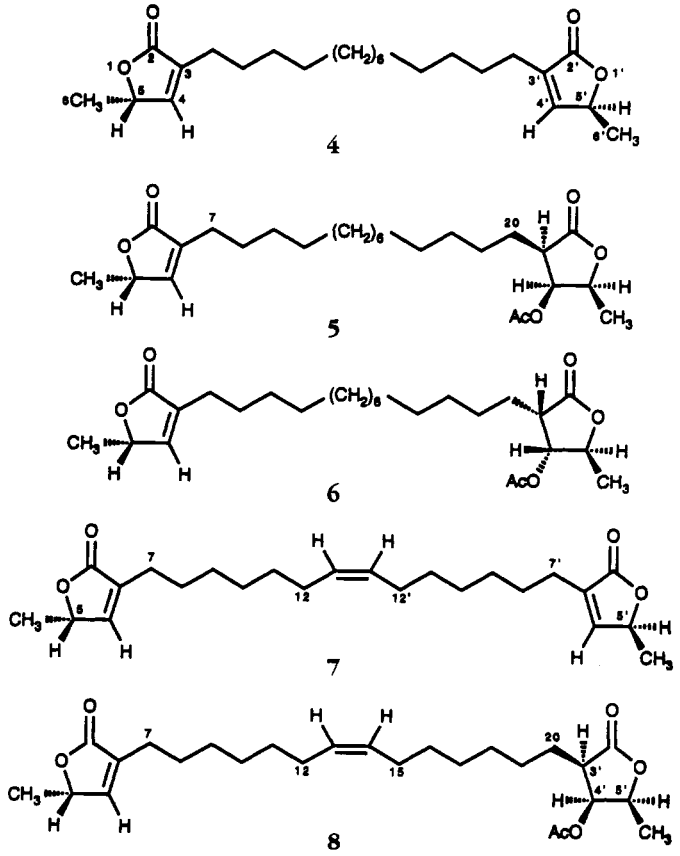


3

a R=Ac
b R=H

functionalities, respectively (2). The presence of a butenolide moiety was confirmed by the uv absorption (λ max 212 nm, ε 8,300) and one-proton multiplets in the ^{1}H-nmr spectrum at δ 4.97 and 6.96, identical with those present in ancepsenolide [**1**]. The position of the acetate moiety in **2** was suggested by the presence of two distinct one-proton multiplets at δ 5.58 (dd, J=3.6 and 5.1 Hz, H-4′) and 4.56 (dq, J=3.3 and 6.3 Hz, H-5′), and a third well-resolved absorption at δ 2.70 (m, H-3′). Confirmation of structure **2**, including relative stereochemistry, was achieved by conversion of ancepsenolide acetate [**2**] to ancepsenolide [**1**] upon treatment with DBU in THF at 35°. This conversion confirmed the anti stereochemistry between H-3′ and the acetate moiety at C-4′ as well as the overall relative stereochemistry of ancepsenolide acetate [**2**]. Examination of the literature revealed that **2**, although a new natural product, has been prepared earlier by Schmitz and Lorance by partial synthesis (3). The physical and chemical data reported for the synthetic material match closely those reported here for naturally occurring ancepsenolide acetate [**2**].

^{1}H- and ^{13}C-nmr spectra of butenolide **3a** displayed signals similar to those of ancepsenolide acetate [**2**]. Hrfabms and ^{13}C-nmr measurements of **3a** suggested a formula of $C_{24}H_{38}O_6$ with six degrees of unsaturation, which established that this material was isomeric with **2**. Ir signals at 1769 and 1744 cm^{-1}, coupled with ^{13}C-nmr signals at 176.31, 173.86, and 170.26 ppm (Table 1), indicated carbonyl and acetoxy functions for this metabolite. The relative stereochemical assignments indicated in structure **3a** for the substituents in the acetylated lactone ring are based on coupling-constant data (Table 2), and ^{1}H-^{1}H COSY and NOESY nmr experiments. Also, the noticeable lower-field shift value for the Me-6′ resonance in **3a** (δ 1.37, 18.01) in comparison to **2** (δ 1.29, 13.94) argues for a trans- H-4′, 5′ arrangement in **3a** as opposed to the cis- H-4′, 5′ assignment in **2**. As expected, a vicinal axial -OAc group in a lactone such as **3a** causes the resonance of an equatorial proton (H-5′) to move upfield by 0.1 ppm and the resonance of an axial -CH_3 group (Me-6′) to move downfield by approximately 0.1 ppm (Table 2). Loss of AcOH from this material on treatment with DBU/THF at 35° gave ancepsenolide [**1**] in good yield, a fact which requires that the acetoxy group of **3a** must be attached to the β-carbon of the second five-membered



lactone ring and that the relative stereochemistry of all four chiral centers be as shown in structure **3a**. A literature search revealed that compound **3a**, herein reported as a new natural product, was identical with hydroxyancepsenolide acetate, a synthetic compound prepared by Schmitz *et al.* upon acetylation of hydroxyancepsenolide [**3b**], a natural product isolated from the gorgonian *P. anceps* (4).

Homoancepsenolide [**4**] was obtained as a white optically active ($[\alpha]^{25}D$ +16.7°) solid, mp 99.6–101.6°, after repeated reversed-phase hplc. Hrfabms and ^{13}C-nmr spectral analyses established the molecular formula of homoancepsenolide as $C_{24}H_{38}O_4$ (*m/z* 397.2914 $[M+Li]^+$). A maximum in the uv spectrum was observed at 214 nm (ε 11,800) and the ir of **4** exhibited a single intense absorption at 1750 cm^{-1}. These facts along with resonances in the ^1H-nmr spectrum at δ 6.96 (br s, 2H) and 4.97 (m, 2H) are indicative of a bis-butenolide grouping in **4**. The ^1H- and ^{13}C-nmr spectra of homoancepsenolide displayed signals almost identical to those recorded for ancepsenolide [**1**] including a strong, sharp absorption at δ 1.23 confirming the presence of a long methylene chain. In view of this and with their molecular ions differing by 28 mass units, we propose that homoancepsenolide [**4**] and ancepsenolide [**1**] differ from each other only by two methylene groups in the alkyl chain. Since homoancepsenolide, with a symmetrical structure, is an optically active compound, it must possess identical absolute stereochemistry at C-5 and C-5′.

Homoancepsenolide acetate [**5**] was isolated and purified by reversed-phase hplc to give a colorless semisolid. Hrfabms and ^{13}C-nmr analyses established the molecular formula as $C_{26}H_{42}O_6$ (*m/z* 451.3047 $[M+H]^+$). The prominent features of the ir spectrum were three strong, broad carbonyl absorptions having maxima at 1777, 1755,

TABLE 1. ^{13}C-Nmr ($CDCl_3$, 75-MHz) Data for Butenolides **1–8**.[a]

1 (δ c)	**2** (δ c)	**3a** (δ c)	**4** (δ c)	**5** (δ c)	**6** (δ c)	**7** (δ c)	**8** (δ c)
173.65 (s, C-2, 2′)	176.12 (s, C-2′)	176.31 (s, C-2′)	173.69 (s, C-2, 2′)	176.33 (s, C-2′)	176.16 (s, C-2′)	173.66 (s, C-2, 2′)	176.19 (s, C-2′)
148.81 (d, C-4, 4′)	173.61 (s, C-2)	173.86 (s, C-2)	148.77 (d, C-4, 4′)	173.83 (s, C-2)	173.73 (s, C-2)	148.82 (d, C-4, 4′)	173.68 (s, C-2)
134.17 (s, C-3, 3′)	169.81 (s, $OCOCH_3$)	170.26 (s, $OCOCH_3$)	134.30 (s, C-3, 3′)	169.94 (s, $OCOCH_3$)	170.17 (s, $OCOCH_3$)	134.21 (s, C-3, 3′)	169.83 (s, $OCOCH_3$)
77.23 (d, C-5, 5′)	148.70 (d, C-4)	148.83 (d, C-4)	77.26 (d, C-5, 5′)	148.81 (d, C-4)	148.72 (d, C-4)	129.75 (d, C-13, 13′)	148.79 (d, C-4)
29.41 (t)	134.39 (s, C-3)	134.33 (s, C-3)	29.53 (t)	134.33 (s, C-3)	134.43 (s, C-3)	77.26 (d, C-5, 5′)	134.23 (s, C-3)
29.35 (t)	77.22 (d, C-5)	80.26 (d, C-5′)	29.43 (t)	77.26 (d, C-5)	80.17 (d, C-5′)	29.51 (t)	129.76 (d, C-13)[b]
29.16 (t)	77.18 (d, C-5′)	77.28 (d, C-5)	29.36 (t)	77.23 (d, C-5′)	77.30 (d, C-5)	29.01 (t)	129.74 (d, C-14)[b]
29.05 (t)	72.47 (d, C-4′)	75.19 (d, C-4′)	29.22 (t)	72.36 (d, C-4′)	75.29 (d, C-4′)	28.85 (t)	77.26 (d, C-5)
27.33 (t, C-8, 8′)	45.68 (d, C-3′)	41.49 (d, C-3′)	29.12 (t)	45.65 (d, C-3′)	41.55 (d, C-3′)	27.35 (t, C-8, 8′)	77.23 (d, C-5′)
25.07 (t, C-7, 7′)	29.48-29.12 (t, 8×C)	29.68-29.17 (t, 8×C)	27.40 (t, C-8, 8′)	29.58-29.18 (t, 10×C)	29.59-29.18 (t, 10×C)	27.08 (t, C-12, 12′)	72.39 (d, C-4′)
19.09 (q, Me-6, 6′)	27.44 (t)	27.43 (t)	25.13 (t, C-7, 7′)	27.40 (t)	27.45 (t)	25.11 (t, C-7, 7′)	45.60 (d, C-3′)
—	27.31 (t)	27.41 (t)	19.15 (q, Me-6, 6′)	27.31 (t)	27.45 (t)	19.13 (q, Me-6, 6′)	29.52 (t, 2×C)
—	25.16 (t, C-7)	25.17 (t, C-7)	—	25.16 (t, C-7)	25.19 (t, C-7)	—	29.21 (t)
—	23.73 (t, C-18)	23.67 (t, C-18)	—	23.66 (t, C-20)	23.75 (t, C-20)	—	29.02 (t)
—	20.23 (q, $OCOCH_3$)	20.74 (q, $OCOCH_3$)	—	20.35 (q, $OCOCH_3$)	20.67 (q,$OCOCH_3$)	—	28.85 (t, 2×C)
—	19.16 (q, Me-6)	19.20 (q, Me-6)	—	19.20 (q, Me-6)	19.21 (q, Me-6)	—	27.35 (t)
—	13.94 (q, Me-6′)	18.01 (q, Me-6′)	—	13.98 (q, Me-6′)	18.03 (q, Me-6′)	—	27.25 (t)
				—	—	—	27.08 (t, 2×C)
				—	—	—	25.12 (t, C-7)
				—	—	—	23.66 (t, C-20)
				—	—	—	20.26 (q, $OCOCH_3$)
				—	—	—	19.14 (q, Me-6)
				—	—	—	13.92 (q, Me-6′)

[a]^{13}C-Nmr multiplicities were obtained by Attached Proton Test (APT) sequences. Assignments were made on the basis of homonuclear and heteronuclear chemical shift correlation methods and comparison to known models. The δ values are in parts per million downfield from TMS.

[b]Values with identical superscripts in each column may be interchanged.

TABLE 2. Selected ^{1}H-Nmr Absorptions and Coupling Constants for Lactones **2**, **3a**, **5**, and **6**.[a]

Proton	Compound **2**[b]			Compound **3a**[b,c]			Compound **5**[b]			Compound **6**[b,c]		
	δ	mult.	*J*	δ	mult.	*J*	δ	mult.	*J*	δ	mult.	*J*
3′	2.70	m	5.1 Hz ($J_{H3'-H4'}$)	2.70	m	6.0 Hz ($J_{H3'-H4'}$)	2.71	m	5.1 Hz ($J_{H3'-H4'}$)	2.70	m	6.1 Hz ($J_{H3'-H4'}$)
4′	5.58	dd	3.3 Hz ($J_{H4'-H5'}$)	5.10	d	<0.5 Hz ($J_{H4'-H5'}$)	5.59	dd	3.3 Hz ($J_{H4'-H5'}$)	5.10	d	<0.5 Hz ($J_{H4'-H5'}$)
5′	4.56	dq	6.3 Hz ($J_{H5'-Me6'}$)	4.47	q	6.9 Hz ($J_{H5'-Me6'}$)	4.56	dq	6.3 Hz ($J_{H5'-Me6'}$)	4.47	q	6.9 Hz ($J_{H5'-Me6'}$)
Me-6′	1.29	d	6.3 Hz ($J_{H5'-Me6'}$)	1.37	d	6.9 Hz ($J_{H5'-Me6'}$)	1.30	d	6.3 Hz ($J_{H5'-Me6'}$)	1.37	d	6.9 Hz ($J_{H5'-Me6'}$)

[a]Values measured in $CDCl_3$ at 300 MHz.

[b]A dihedral angle close to 0° would be expected between protons H-3′, H-4′, and H-5′ if they are cis to one another. This is consistent with the larger coupling, $J_{H3'-H4'}$ and $J_{H4'-H-5'}$>3.0 Hz, assigned to these protons.

[c]A trans- orientation of protons H-4′ and H-5′ imposes a dihedral angle of 105–115° between these protons. This is consistent with the smaller coupling constant, $J_{H4'-H5'}$<0.5 Hz.

and 1748 cm^{-1}. An absorption maximum in the uv spectrum at 212 nm (ϵ 13,200) and absorptions in the ^{1}H-nmr spectrum at δ 6.97 (d, 1H, J=1.2 Hz) and 4.99 (dq, 1H, J=1.2, 6.6 Hz) confirmed the presence in **5** of a substituted butenolide ring identical with those present in homoancepsenolide [**4**]. The signals due to H-4, H-5, H-3′, H-4′, and H-5′ were almost identical to those of **2**, suggesting that these two compounds possess the same type of substituted γ-lactone rings. The mass spectral molecular ion of **5** was 28 mass units larger than that of **2**; this difference is consistent with the enlargement of the long methylene chain in **5** by two -CH_2- units. A NOESY nmr experiment revealed a positive nOe effect between H-3′ and protons H-4′ and H-5′. H-4′ in turn showed a strong nOe cross-peak to H-5′, supporting the all cis- orientation between these protons. Moreover, analysis of proton-proton coupling contants (Table 2), a COSY nmr experiment, and the chemical conversion of **5** to homoancepsenolide [**4**] with DBU/THF at 35°, all demonstrated that the relative stereochemistry of **5** was in line also with the identical stereochemistry of these groups in **2**.

The least abundant of the secondary metabolites isolated from *P. citrina*, hydroxy-homoancepsenolide acetate [**6**], had the molecular formula $C_{26}H_{42}O_6$ and therefore was isomeric with homoancepsenolide acetate [**5**]. The ^{1}H- and ^{13}C-nmr spectra of **6** were almost identical with those of **3a**. The ir spectrum contained ester bands at 1777, 1749, and 1738 cm^{-1} and the uv spectrum showed an absorption maximum at 212 nm (ϵ 7,300). That the stereochemistry for **6** at chiral centers 3′, 4′, 5, and 5′ was identical to that of **3a** could be shown by the similarity of the J values involving protons at these sites (Table 2), and by COSY and NOESY nmr experiments. For instance, a positive nOe effect between H-4′ and H-3′, supporting a cis- orientation, could be observed. On the other hand, the absence of nOe effects between H-5′ and protons H-3′ and H-4′ favors a structure where the former proton is trans- oriented with respect to H-3′ and H-4′ as in homolog **3a**. As before, we succeeded in converting butenolide acetate **6** into homoancepsenolide [**4**] upon treatment with DBU/THF at 35°.

13,13′-Dehydrohomoancepsenolide [**7**], a viscous oil with $[\alpha]^{25}D$ +29.7°, has the molecular formula $C_{24}H_{36}O_4$, as deduced from hrfabms. The single, broad ir band at 1747 cm^{-1} and the uv absorption at 214 nm (ϵ 14,800) were in agreement with a bis-butenolide functionality. The ^{1}H-nmr spectrum contained signals at δ 6.96 (br s, 2H, H-4, 4′), 5.31 (br t, 2H, J=4.8 Hz, H-13, 13′), 4.97 (dq, 2H, J=1.2 and 6.6 Hz, H-5, 5′), 2.23 (br t, 4H, J=7.8 Hz, H-7, 7′), 1.98 (m, 4H, H-12, 12′), 1.52 (m, 4H, H-8, 8′), 1.37 (d, 6H, J=6.6 Hz, Me-6, Me-6′), and a sharp absorption at δ 1.30 due to a methylene chain. The ^{13}C-nmr spectrum of **7** (Table 1) revealed only twelve carbon resonances, and together with the hrfabms spectrum, these data suggested that the molecule must possess a symmetrical structure. The fact that only half of the expected resonances could be detected in the ^{13}C-nmr spectrum implies the presence of two equivalent lactone carbonyls [δ 173.66 (s)], two identical trisubstituted double bonds [δ 148.82 (d), 134.21 (s)], and one symmetrically disubstituted double bond [δ 129.75 (d)]. These signals account for five of the seven degrees of unsaturation required by the molecular formula; therefore **7** must be bicyclic. A heteronuclear chemical shift correlation with broad-band decoupling (CSCMBB) experiment correlated the proton resonances at δ 1.98 (H-12, 12′) with their corresponding carbon resonance at δ 27.08 (C-12, 12′). The low-field nature of this allylic carbon resonance suggested *Z*- geometry for the Δ^{13} disubstituted double bond (5), a contention also supported by the absence in the ir spectrum of a strong, sharp band near 968 cm^{-1} (6). Hydrogenation of the natural product resulted in the uptake of 1 mole of hydrogen and gave the dihydro derivative homoancepsenolide [**4**], in good yield. Because 13,13′-dehydrohomo-ancepsenolide exhibits optical activity despite having a symmetrical structure, C-5 and

C-5′ must have identical absolute stereochemistry. Notwithstanding this, only relative stereochemistry is implied in structure **7**.

The formula of lactone **8** was confirmed by hrfabms to be $C_{26}H_{40}O_6$, *m/z* 449.2885 $[M+H]^+$, which implies seven degrees of unsaturation. The ^{13}C-nmr spectrum (Table 1) confirmed that there were only seven sp^2 carbons, namely, three carbonyl carbons and four olefinic carbons; hence, **8** must contain two rings. One of the rings was formulated as a γ-methyl-α-alkyl substituted butenolide moiety on the basis of a broad ir absorption at 1748 cm^{-1}, a uv absorption maximum at 214 nm (ε 8,400), a carbonyl carbon singlet peak at 173.68 ppm and a mutually coupled pair of multiplet ^{1}H-nmr signals at δ 6.96 (br s, 1H) and 4.96 (dq, 1H). Lactone **8**, named 13,14-dehydrohomoancepsenolide acetate, also showed strong ir absorptions at 1778 and 1754 cm^{-1} and was characterized as a homolog of ancepsenolide acetate [**2**] and also as an analog of homoancepsenolide acetate containing one disubstituted double bond in the linear alkyl chain by comparison of ^{1}H- and ^{13}C-nmr data with those of **5**. The ^{1}H-nmr spectrum provided further confirmation revealing the presence of two vinylic protons which appeared as a multiplet of closely spaced lines centered at δ 5.30. Elimination of one equivalent of AcOH from **8** with DBU/THF at 35° gave 13,13′-dehydrohomoancepsenolide [**7**] in good yield. This result requires the acetoxy group to be attached to the β carbon of the second γ-lactone ring, that the disubstituted double bond in the aliphatic chain, with a *Z*-configuration, be located as shown in structure **8**, and that the stereochemistry at C-5 be identical to that of **7**. The relative stereochemistry at C-3′, C-4′, and C-5′ was confirmed through a series of ^{1}H-^{1}H COSY and NOESY nmr experiments which showed identical results to those obtained for **2** and **5**.

Throughout this work we encountered samples of butenolides exhibiting variable small (both positive and negative) optical rotations. Although all the samples appeared to be homogeneous (as judged by tlc, hplc and ^{1}H and ^{13}C nmr), optical rotations alone appear insufficient to draw stereochemical conclusions rigorously. For example, compounds **2** and **5** and compounds **3a** and **6** should in theory provide identical molecular rotations, but they did not in the present work. One possibility is that **2**, for example, may exist as mostly the meso- compound with some excess of one enantiomer. This alone would be consistent with the variability observed, both here and during the original isolation of ancepsenolide (2,4), in molecular rotation. Another possibility which should not be discounted is that the isomers described here are epimeric only at C-5′ and not at C-3′ or C-4′, a contention once again consistent with a meso- mixture of bis-butenolides.

The new butenolides reported here were not active against *Escherichia coli* or *Staphylococcus aureus* at doses of 10, 5, and 1 μg of test compound per disc. Some of the new metabolites (compounds **2**, **4**, and **7**) were also screened for antitumor activity. However, they did not show significant cytotoxicity against a human colon tumor (HCT 116) cell line (IC_{50}>50 μg/ml).

EXPERIMENTAL

GENERAL EXPERIMENTAL DETAILS.—These have been described elsewhere (7). All hplc separations were performed on a DuPont Zorbax C8 semi-preparative column (9.4 mm×25 cm) using a flow-rate of 2 ml/min. All separations were monitored simultaneously by refractive index and uv absorption (λ max set to 220 nm).

COLLECTION AND EXTRACTION OF *PTEROGORGIA CITRINA*.—The Caribbean gorgonian *P. citrina* was collected by hand using Scuba at depths of 15-20 m in April 1992 from Mona Island, Puerto Rico. Upon arrival, the gorgonian was freeze-dried and kept frozen until extraction. A voucher specimen is stored at the Chemistry Department of the University of Puerto Rico. The dried animal (775 g) was blended with MeOH-$CHCl_3$ (1:1) (3×1 liter), and after filtration, the crude extract was evaporated under vacuum to yield a green residue (109 g). After partitioning the crude oil between hexane and H_2O, the aqueous suspension was

extracted with $CHCl_3$ (3×1 liter) and the extract subsequently filtered. The resulting filtrate was concentrated *in vacuo* to yield 63.1 g of a dark green oily residue. A portion of this residue (11.0 g) was dissolved in 10 ml of toluene and then was fractionated by size-exclusion chromatography on a Bio-Beads SX-2 column using toluene as eluent. The combined butenolide-rich fractions (tlc guided) were concentrated to a greenish oil (5.20 g) and a portion of this residue (1.90 g) was chromatographed over a Si gel column (80.0 g) with 25% EtOAc in hexane. The lipid mixture was fractionated roughly into fractions A–J on the basis of tlc analyses. Subsequent purification of fraction G (0.44 g) by hplc [Zorbax C-8 Si gel with MeOH-H_2O (75:25)] afforded the following butenolide derivatives: ancepsenolide acetate [**2**] (61.0 mg, 0.12%, *R*, 35.9 min), hydroxyancepsenolide acetate [**3a**] (5.3 mg, 0.01%, *R*, 38.9 min), ancepsenolide [**1**] (75.6 mg, 0.15%, *R*, 42.0 min), 13,14-dehydrohomoancepsenolide acetate [**8**] (45.3 mg, 0.09%, *R*, 49.0 min), 13,13′-dehydrohomoancepsenolide [**7**] (30.5 mg, 0.06%, *R*, 56.0 min), homoancepsenolide acetate [**5**] (32.0 mg, 0.06%, *R*, 72.2 min), hydroxyhomoancepsenolide acetate [**6**] (22.5 mg, 0.04%, *R*, 78.7 min), and homoancepsenolide [**4**] (29.7 mg, 0.06%, *R*, 85.7 min). Ancepsenolide [**1**] was identified by comparison of its mp, ir, specific rotation, ^{1}H-nmr, and uv spectral data with reported values (2).

Ancepsenolide [**1**].—White solid: mp 96.0–97.9°, lit. 90.5–92.0° (2), $[\alpha]^{25}D$ +27.1° (*c*=2.7, $CHCl_3$); ir ν max (neat) 3020, 2929, 2856, 1751, 1320, 1216, 1082, 1028, 758, 668 cm^{-1}; uv λ max (MeOH) 214 (ε 14,400) nm; ^{1}H nmr ($CDCl_3$, 300 MHz) δ 6.96 (2H, br s, H-4, 4′), 4.97 (2H, br q, *J*=6.6 Hz, H-5, 5′), 2.23 (4H, br t, *J*=7.8 Hz, H-7, 7′), 1.51 (4H, m, H-8, 8′), 1.38 (6H, d, *J*=6.6 Hz, Me-6, 6′), 1.23 (16H br s); ^{13}C nmr ($CDCl_3$, 75 MHz) see Table 1; lrfabms *m/z* $[M+H]^+$ 363.3 (100%) (calcd for $C_{22}H_{35}O_4$, 363.3).

Ancepsenolide acetate [**2**].—White solid: mp 53.9–54.9°, lit. 55.4–56.5° (3), $[\alpha]^{25}D$ −11.2° (*c*=1.1, $CHCl_3$); ir ν max (neat) 3020, 2963, 2929, 2856, 1777, 1748, 1262, 1216, 1096, 1021, 805, 758, 669 cm^{-1}; uv λ max (MeOH) 212 (ε 8,300) nm; ^{1}H nmr ($CDCl_3$, 300 MHz) δ 6.96 (1H, br s, H-4), 5.58 (1H, dd, *J*=3.3 and 5.1 Hz, H-4′), 4.97 (1H, dq, *J*=1.2 and 6.6 Hz, H-5), 4.56 (1H, dq, *J*=3.3 and 6.3 Hz, H-5′), 2.70 (1H, m, H-3′), 2.24 (2H, br t, *J*=7.8 Hz, H-7), 2.11 (3H, s, -OAc), 1.78 (1H, m, H-18), 1.52 (3H, m, H-8, 18′), 1.38 (3H, d, *J*=6.9 Hz, Me-6), 1.29 (3H, d, *J*=6.3 Hz, Me-6′), 1.23 (18H, br s); ^{13}C nmr ($CDCl_3$, 75 MHz) see Table 1; hrfabms *m/z* $[M+Li]^+$ 429.2824 (100%) (calcd for $C_{24}H_{38}O_6Li$, 429.2829).

Hydroxyancepsenolide acetate [**3a**].—Colorless semisolid: lit. mp 68.3–70.3° (4), $[\alpha]^{25}D$ +3.7° (*c*=2.2, $CHCl_3$); ir ν max (neat) 3081, 2963, 2924, 2853, 1769, 1744, 1653, 1261, 1096, 1023, 864, 799 cm^{-1}; uv λ max (MeOH) 212 (ε 7,300) nm; ^{1}H nmr ($CDCl_3$, 300 MHz) δ 6.96 (1H, br s, H-4), 5.10 (1H, d, *J*=6.0 Hz, H-4′), 4.97 (1H, dq, *J*=1.2 and 6.6 Hz, H-5), 4.47 (1H, q, *J*=6.9 Hz, H-5′), 2.70 (1H, m, H-3′), 2.24 (2H, br t, *J*=8.1 Hz, H-7), 2.08 (3H, s, -OAc), 1.78 (1H, m, H-18), 1.52 (3H, m, H-8, 18′), 1.38 (3H, d, *J*=6.6 Hz, Me-6), 1.37 (3H, d, *J*=6.9 Hz, Me-6′), 1.23 (18H, br s); ^{13}C nmr ($CDCl_3$, 75 MHz) see Table 1; hrfabms *m/z* $[M+H]^+$ 423.2737 (48%) (calcd for $C_{24}H_{39}O_6$, 423.2747).

Homoancepsenolide [**4**].—White solid: mp 99.6–101.6°, $[\alpha]^{25}D$ +16.7° (*c*=1.8, $CHCl_3$); ir ν max (neat) 3020, 2929, 2856, 1750, 1262, 1216, 1095, 1026, 758, 669 cm^{-1}; uv λ max (MeOH) 214 (ε 11,800) nm; ^{1}H nmr ($CDCl_3$, 300 MHz) δ 6.96 (2H, br s, H-4, 4′), 4.97 (2H, m, H-5, 5′), 2.24 (4H, br t, *J*=7.8 Hz, H-7, 7′), 1.51 (4H, m, H-8, 8′), 1.38 (6H, d, *J*=6.6 Hz, Me-6, 6′), 1.23 (20H, br s); ^{13}C nmr ($CDCl_3$, 75 MHz) see Table 1; hrfabms *m/z* $[M+Li]^+$ 397.2914 (100%) (calcd for $C_{24}H_{38}O_4Li$, 397.2932).

Homoancepsenolide acetate [**5**].—Colorless semisolid: $[\alpha]^{25}D$ −5.4° (*c*=3.2, $CHCl_3$); ir ν max (neat) 2927, 2854, 1777, 1755, 1748, 1460, 1260, 1081, 1026, 800 cm^{-1}; uv λ max (MeOH) 212 (ε 13,200) nm; ^{1}H nmr ($CDCl_3$, 300 MHz) δ 6.97 (1H, d, *J*=1.2 Hz, H-4), 5.59 (1H, dd, *J*=3.3 and 5.1 Hz, H-4′), 4.99 (1H, dq, *J*=1.2 and 6.6 Hz, H-5), 4.56 (1H, dq, *J*=3.3 and 6.3 Hz, H-5′), 2.71 (1H, m, H-3′), 2.25 (2H, br t, *J*=8.1 Hz, H-7), 2.12 (3H, s, -OAc), 1.80 (1H, br m, H-20), 1.54 (3H, m, H-8, 20′), 1.39 (3H, d, *J*=6.6 Hz, Me-6), 1.30 (3H, d, *J*=6.3 Hz, Me-6′), 1.24 (22H, br s); ^{13}C nmr ($CDCl_3$, 75 MHz) see Table 1; hrfabms *m/z* $[M+H]^+$ 451.3047 (100%) (calcd for $C_{26}H_{43}O_6$, 451.3061).

Hydroxyhomoancepsenolide acetate [**6**].—Colorless semisolid: $[\alpha]^{25}D$ +16.9° (*c*=1.7, $CHCl_3$); ir ν max (neat) 3093, 2963, 2917, 2848, 1777, 1749, 1738, 1653, 1260, 1092, 1023, 863, 799, 704 cm^{-1}; uv λ max (MeOH) 212 (ε 7,300) nm; ^{1}H nmr ($CDCl_3$, 300 MHz) δ 6.96 (1H, m, H-4), 5.10 (1H, d, *J*=6.1 Hz, H-4′), 4.97 (1H, dq, *J*=1.7 and 6.8 Hz, H-5), 4.47 (1H, q, *J*=6.9 Hz, H-5′), 2.70 (1H, m, H-3′), 2.23 (2H, dt, *J*=3.6 and 7.2 Hz, H-7), 2.08 (3H, s, -OAc), 1.75 (1H, m, H-20), 1.52 (3H, m, H-8, 20′), 1.38 (3H, d, *J*=6.6 Hz, Me-6), 1.37 (3H, d, *J*=6.9 Hz, Me-6′), 1.23 (22H, br s); ^{13}C nmr ($CDCl_3$, 75 MHz) see Table 1; hrfabms *m/z* $[M+H]^+$ 451.3032 (55%) (calcd for $C_{26}H_{43}O_6$, 451.3061).

13,13′-Dehydrohomoancepsenolide [**7**].—Colorless oil: $[\alpha]^{25}D$ +29.7° (*c*=2.1, $CHCl_3$); ir ν max (neat) 3079, 2980, 2928, 2855, 1747, 1653, 1454, 1319, 1201, 1084, 1027, 861 cm^{-1}; uv λ max (MeOH) 214 (ε 14,800) nm; ^{1}H nmr ($CDCl_3$, 300 MHz) δ 6.96 (2H, br s, H-4, 4′), 5.31 (2H, br t, *J*=4.8 Hz, H-13, 13′), 4.97 (2H, dq, *J*=1.2 and 6.6 Hz, H-5, 5′), 2.23 (4H, br t, *J*=7.8 Hz, H-7, 7′), 1.98 (4H, m, H-12, 12′), 1.52 (4H, m, H-8, 8′), 1.37 (6H, d, *J*=6.6 Hz, Me-6, 6′), 1.30 (12H, br s); ^{13}C nmr ($CDCl_3$, 75 MHz) see Table 1; hrfabms *m/z* $[M+Li]^+$ 395.2764 (45%) (calcd for $C_{24}H_{36}O_4Li$, 395.2775).

13,14-Dehydrohomoancepsenolide acetate [**8**].—Colorless oil: $[\alpha]^{25}D$ −12.9° (*c*=3.0, $CHCl_3$); ir ν max (neat) 3079, 2989, 2929, 2856, 1778, 1754, 1748, 1653, 1459, 1373, 1319, 1230, 1180, 1127, 1025, 871, 756 cm^{-1}; uv λ max (MeOH) 214 (ε 8,400) nm; ^1H nmr ($CDCl_3$, 300 MHz) δ 6.96 (1H, br s, H-4), 5.56 (1H, dd, *J*=3.3 and 5.1 Hz, H-4′), 5.30 (2H, br t, *J*=4.8 Hz, H-13, 14), 4.96 (1H, dq, *J*=1.5 and 6.9 Hz, H-5), 4.54 (1H, dq, *J*=3.3 and 6.3 Hz, H-5′), 2.68 (1H, m, H-3′), 2.22 (2H, br t, *J*=7.8 Hz, H-7), 2.09 (3H, s, -OAc), 1.96 (4H, m, H-12, 15), 1.74 (1H, br m, H-20), 1.48 (3H, m, H-8, 20′), 1.36 (3H, d, *J*=6.6 Hz, Me-6), 1.29 (14H, br s), 1.28 (3H, d, *J*=6.6 Hz, Me-6′); ^{13}C nmr ($CDCl_3$, 75 MHz) see Table 1; hrfabms *m/z* $[M+H]^+$ 449.2885 (100%) (calcd for $C_{26}H_{41}O_6$, 449.2904).

HYDROGENATION OF 13,13′-DEHYDROHOMOANCEPSENOLIDE [**7**].—A solution of bis-butenolide **7** (11.01 mg, 0.028 mmole) in EtOAc (10 ml) was stirred at 25° under hydrogen at atmospheric pressure in the presence of catalytic amounts of 5% Pd on activated carbon. After the H_2 uptake ceased (30 min) the catalyst was removed by filtration and the excess solvent evaporated under reduced pressure. The crude product was taken up with hexane-EtOAc (70:30) and passed through a short column of Si gel. The white solid recovered exhibited spectral (ir, uv, ms, ^1H- and ^{13}C-nmr), mp, and tlc properties identical to those of homoancepsenolide [**4**].

HYDRO-ACETOXY ELIMINATION OF ANCEPSENOLIDE ACETATE [**2**].—To a solution of acetate **2** (19.0 mg) in dry THF (10 ml) at room temperature was added with constant stirring 2 to 3 drops of 1,5-diazabicyclo[5.4.0]undecene-5 (DBU, Aldrich Chemical Company). The resulting solution was warmed to 35° and stirred for approximately 12 h. After removal of the solvent *in vacuo* the crude residue was passed through a short column of Si gel and eluted with hexane-EtOAc (70:30). The material recovered (17.1 mg) exhibited spectral (ir, uv, ms, ^1H- and ^{13}C-nmr) and tlc properties identical to those of ancepsenolide [**1**]. Following the same general procedure, we successfully converted acetate **3a** into ancepsenolide [**1**], lactones **5** and **6** were converted into homoancepsenolide [**4**], and butenolide **8** was converted into 13,13′-dehydrohomoancepsenolide [**7**].

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