Three New Butenolide Lipids from the Caribbean Gorgonian *Pterogorgia anceps*

Yue-Wei Guo, Margherita Gavagnin,* Ernesto Mollo, Enrico Trivellone, and Guido Cimino

Istituto per la Chimica di Molecole di Interesse Biologico,† CNR, via Toiano 6, 80072−Arco Felice (Na), Italy

Received March 22, 1999

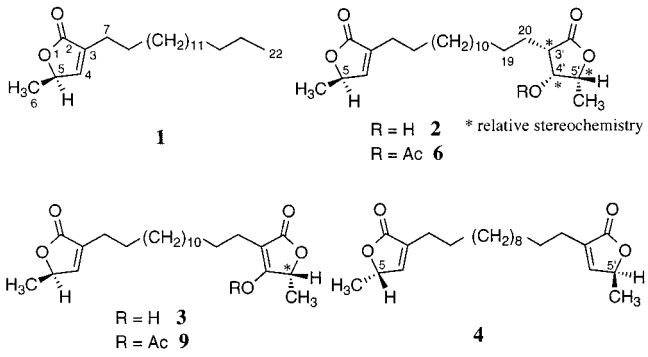
Three novel fatty acid derivatives (**1**−**3**), containing one or two butenolide moieties, were isolated from the Caribbean gorgonian *Pterogorgia anceps* and chemically characterized by spectroscopic methods and comparison with known compounds. The new molecules were structurally related to ancepsenolide (**4**), a typical metabolite from *Pterogorgia* species, which was not detected in this collection of *P. anceps*.

Gorgonians have been studied extensively by marine natural product chemists and have yielded a plethora of terpenes and acetogenins, the latter including prostaglandins and fatty acid derivatives.[1] It has been suggested that such secondary metabolites are probably involved in the defensive mechanisms of the animals, which appear to be relatively free from predation.[2] Antifeedant properties of fatty acid derivatives from gorgonians have been demonstrated.[3] Crude extracts of many gorgonians have also shown interesting antimicrobial and cytotoxic activities.

In the course of our investigations on the chemical ecology of Caribbean benthic invertebrates, we have examined the gorgonian *Pterogorgia anceps* Pallas (order Gorgonacea, family Gorgoniidae), collected off Puerto Morelos, Mexico. Three novel butenolide lipids (**1**−**3**) have been isolated from the ethereal extract. We report here the structure elucidation of these new metabolites mainly on the basis of their spectral properties and correlation with known compounds.

Specimens of *P. anceps* were extracted exhaustively with acetone, and the extract was partitioned between water and Et_2O. The Et_2O-soluble fraction was analyzed by TLC, revealing the presence of a main compound (R_f 0.34, $CHCl_3$−MeOH, 96:4), along with two less abundant components (R_f 0.50, petroleum ether−Et_2O, 7:3 and R_f 0.41, petroleum ether−Et_2O, 2:8). The Et_2O extract was fractionated on a Si gel column eluting with petroleum ether and increasing amounts of Et_2O, and then with $CHCl_3$−MeOH (9:1), obtaining, in order of increasing polarity, compounds **1** (25.1 mg), **2** (7.6 mg), and **3** (110 mg). A preliminary NMR analysis revealed that the three molecules contained butenolide partial moieties and were structurally related to ancepsenolide (**4**), previously isolated from the same Caribbean gorgonian species[4] and from *Pterogorgia guadalupensis*[5] and *Pterogorgia citrina*.[6]

Compound **1** was an amorphous and optically active powder {$[\alpha]_D$ −12.7° (dioxane)}, with elemental composition $C_{21}H_{38}O_2$, determined by HREIMS measurements on the molecular ion at *m/z* 322. ^{1}H NMR spectrum displayed signals for a vinylic proton at δ 6.97 (br s, H-4), a methyl signal at δ 1.40 (d, J = 6.8 Hz, H_3-6), and a methine at δ 4.98 (br q, J = 6.7 Hz, H-5), suggesting an α,β unsaturated γ-lactone system, which was confirmed by the presence in the ^{13}C NMR spectrum of signals at δ 173.6 (s, C-2), 134.2 (s, C-3), 148.8 (d, C-4), and 77.4 (d, C-5) and in the IR spectrum of a strong absorption at 1748 cm^{-1}. In addition, the ^{1}H NMR spectrum of **1** exhibited a triplet methyl signal at δ 0.88 (J = 6.6 Hz, H_3-22) attributable to a terminal methyl group and a strong broad singlet at δ 1.26 due to protons in a long methylene chain. These data led us to assign a structure, containing a methyl butenolide moiety linked to an unbranched alkyl chain, as formulated in **1**. The absolute configuration at C-5 was suggested to be *R*, opposite that established for ancepsenolide (**4**),[7] by comparing the negative $[\alpha]_D$ value of **1** with that reported in the literature for the enantiomeric compound **5** {$[\alpha]_D$ +26.7° (dioxane)}, an intermediate obtained in the synthesis of 3,4,5-trisubstituted furanones by Ortuño et al.[8] Comparison of the $[\alpha]_D$ sign of **1** with those of related compounds[9] supported the assigned *R* configuration.



Compound **2** was obtained as a white, optically active solid, {$[\alpha]_D$ −4.7° ($CHCl_3$)}. HREIMS and ^{13}C NMR spectral analysis established the molecular formula of **2** as $C_{24}H_{40}O_5$ (*m/z* 408.2876). The ^{1}H NMR spectrum showed some analogies with that of **1**, in particular exhibiting the same series of signals at δ 6.98 (1H, br s, H-4), 4.99 (1H, br q, J = 6.8 Hz, H-5), and 1.40 (3H, d, J = 6.8 Hz) due to a substituted butenolide ring, the same as in **1**. In addition, the ^{1}H NMR spectrum displayed a signal at δ 4.45 (1H, dq, J = 6.4 and 2.9 Hz, H-5′), due to a proton linked to a carbon bearing a methyl group resonating at δ 1.43 (3H, d, J = 6.4 Hz), further coupled to a methine at δ 4.31 (1H, m, H-4′), which showed, in turn, coupling with another methine at δ 2.57 (1H, dt, J = 4.8 and 9.8 Hz, H-3′), linked to a methylene chain. The ^{13}C NMR spectrum contained, in addition to the signals at δ 173.9 (s), 148.9 (d), 134.3 (s), and 77.4 (d) attributable to the butenolide moiety as in compound **1**, resonances at δ 177.4 (s), 78.7 (d), 71.2 (d), and 47.4 (d), which were consistent with the presence in the molecule of a further saturated γ-lactone ring bearing

* To whom correspondence should be addressed. Tel.: +39 081 8534247. Fax: +39 081 8041770. E-mail: marghe@trinc.icmib.na.cnr.it.

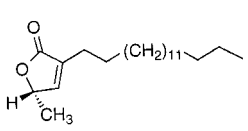
† Associated with National Institute for the Chemistry of Biological Systems (CNR).

10.1021/np9901231 CCC: $18.00
Published on Web 06/30/1999

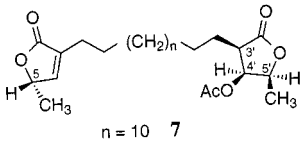
a hydroxyl and a methyl group. This was supported by two broad absorption bands at 1736 cm^{-1} and 3429 cm^{-1} in the IR spectrum. On this basis, a structure containing both a butenolide and a butanolide moiety was suggested for compound **2**. The relative cis stereochemistry of all substituents at the three contiguous stereogenic centers in the 3′-alkyl-4′-hydroxy-5′-methyl-γ-lactone ring was inferred by analysis of coupling constants and by comparison of ^{13}C NMR data with those of model compounds[6,10,11] and further supported by some NOE experiments. In fact, diagnostic NOE effects were observed between H-3′ (δ 2.57) and H-5′ (δ 4.45), clearly indicating a cis relationship between them. A cis-orientation could be suggested also for H-4′. In fact, very strong positive enhancements of the signals at δ 2.57 (H-3′) and 4.45 (H-5′) were induced by irradiation of H-4′ (δ 4.31), supporting a very close spatial distance between H-4′ and the two vicinal protons at C-3′ and C-5′. To confirm the proposed structure, **2** was converted to the corresponding acetate **6**, which showed NMR data identical with those reported for homoancepsenolide acetate (**7**), previously isolated from the Caribbean gorgonian *P. citrina*,[6] exhibiting the same relative stereochemistry in the butanolide ring.

The absolute stereochemistry at C-5 in the butenolide moiety of **2** was suggested to be *R*, the same as compound **1**, by comparison of the CD spectra, which revealed identical profiles, indicating the same chiral butenolide chromophore. To determine the absolute configuration of the hydroxy butanolide ring of **2**, we tried to apply the Mosher method, but unfortunately, the reaction of **2** with (*R*)- and (*S*)-MTPA chloride did not occur, preventing us from assigning rigorously the stereochemistry at the chiral centers C-3′, C-4′, and C-5′ in the saturated γ-lactone ring. However, by analogy with ancepsenolide (**4**), exibiting the same absolute stereochemistry at C-5 and C-5′, the *R* configuration at C-5′, the same as at C-5, should be suggested for compound **2**. Some further comment could be made by analyzing literature data. Compound **6**, the acetyl derivative of **2**, displayed an $[\alpha]_D$ opposite that of homoancepsenolide acetate (**7**) {$[\alpha]_D$ −5.4° ($CHCl_3$)},[6] indicating a different absolute stereochemistry. Although the absolute configuration of **7** has not been determined, it could be suggested to be 5*S*,3′*R*,4′*S*,5′*S*, by analogy with the co-occurring homologue ancepsenolide acetate (**8**). In fact, the reported chemical conversion of **8** to ancepsenolide (**4**)[6] implies the *S* absolute configuration at the chiral centers C-5 and C-5′ of **8**, the same as **4**, the stereochemistry of which has been secured by synthesis.[7] Therefore, the 3′*S*,4′*R*,5′*R* stereochemistries could be suggested for compound **2**.

Compound **3**, a white amorphous powder {$[\alpha]_D$ −27.2° ($CHCl_3$)} was the most polar and abundant metabolite of *P. anceps*. It had a molecular formula $C_{24}H_{38}O_5$ as deduced from HREIMS (*m/z* 406.2719, M^+). A strong, sharp absorption at 1743 cm^{-1} with a shoulder at 1730 cm^{-1}, in the IR spectrum of **3**, suggested the presence of two butenolide rings, which was further supported by ^1H and ^{13}C NMR data. In particular, the proton spectrum of **3** in $CDCl_3$ displayed resonances at δ 7.06 (1H, d, J = 1.4 Hz, H-4), 5.06 (1H, m, H-5), and 1.43 (3H, d, J = 6.8, H_3-6), attributable to the same butenolide system as in **1** and **2**, and in addition signals at δ 4.77 (1H, q, J = 6.7 Hz, H-5′) and 1.48 (3H, d, J = 6.7 Hz, H_3-6′), due to an isolated methine bearing a methyl group, of a second γ-lactone moiety. Comparison of ^1H NMR spectrum of **3** with that of **2** revealed that **3** lacked the two vicinal protons at C-3′ and C-4′, and, therefore, it was the 3′,4′-dehydroderivative of **2**. ^1H and ^{13}C NMR assignments of **3** were achieved by detailed interpretation of 2D ($^1H-^1H$ COSY, TOCSY, HMQC, HMBC) NMR spectra in C_6D_6 containing ca. 1% CD_3OD (**3** in $CHCl_3$ was very unstable). Compound **3** was acetylated with Ac_2O−pyridine to afford the expected acetate **9**, which was characterized by NMR, confirming the proposed assignment. Analogously with **2**, the absolute stereochemistry at C-5 and C-5′ of **3** was suggested to be *R*, *R*, as supported by the CD curve, similar to those of **1** and **2**.



5



n = 10 **7**
n = 8 **8**

Previous chemical studies conducted on different species of *Pterogorgia* from the Caribbean Sea revealed that ancepsenolide (**4**) was the most abundant metabolite from each of them. Surprisingly, no trace of **4** was detected in the extract of this collection of *P. anceps*. In addition, it is worth noting that, although monobutenolide lipids are widely present in terrestrial organisms,[9,11] they seem to be almost rare in marine animals.

Experimental Section

General Experimental Procedures. Optical rotations were measured in $CHCl_3$ and in dioxane on a JASCO DIP 370 digital polarimeter, and CD curves were recorded in EtOH solution on a JASCO 710 spectropolarimeter. The IR spectra were obtained on a Bio-Rad FTS 7 spectrometer and UV spectra on a Varian DMS 90 double beam spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on DRX 500 MHz, AM 400 MHz, and DPX 300 MHz Bruker spectrometers. Chemical shifts are reported in parts per million referenced to $CHCl_3$ as internal standard (δ 7.26 for proton and δ 77.0 for carbon) and C_6H_6 (δ 7.17 for proton and 128.7 for carbon). ^1H and ^{13}C NMR assignments were supported by $^1H-^1H$ COSY, TOCSY, HMQC, and HMBC experiments. EIMS and HREIMS were recorded on a Carlo Erba TRIO 2000 VG and a ZAB T mass spectrometers, respectively. Commercial Merck Si gel 60 (70−230 mesh ASTM) was used for column chromatography. Merck precoated Si gel plates were used for TLC. The chromatograms were sprayed with 0.1% $Ce(SO_4)_2$ in 2N H_2SO_4 and heated at 80° for 5 min to detect the spots.

Animal Material. *P. anceps* was collected in the Bay of Puerto Morelos (Mexico) by scuba at a depth of 10 m, during April 1995. The material was immediately frozen and transferred to ICMIB, where it was kept at −80 °C until extraction. A voucher specimen is stored for inspection at the ICMIB (voucher no. MexG7).

Extraction and Isolation. The gorgonian material (2 colonies, 110 g, dry wt after extraction) was extracted with Me_2CO (2.5 L). After concentration, the aqueous residue was extracted with Et_2O (3 × 150 mL). The combined ether extracts were taken to dryness, yielding an oily residue (1.5 g) that was chromatographed on Si gel column using a petroleum ether−Et_2O gradient, as eluent. The fractions 24−28, eluted by petroleum ether−Et_2O, 8:2, yielded crude butenolide **1**, which was rechromatographed on a Si gel column (petroleum ether−Et_2O, 8:2) to afford pure **1** (25.1 mg). The fractions 45−47, eluted by petroleum ether−Et_2O, 1:9, and the fractions 58−60, eluted with $CHCl_3$−CH_3OH, 9:1, contained pure compounds **2** (7.6 mg) and **3** (110 mg), respectively.

Compound 1: amorphous powder, $[\alpha]_D$ −12.7° (*c* 1.9, dioxane); $[\alpha]_D$ −11.9° (*c* 2.5, $CHCl_3$); UV (EtOH) λ_{max} 214 nm (ϵ 3800); CD $[\theta]_{209}$ (EtOH) −1900; IR (liquid film) ν_{max} 2923, 2857, 1748, 1467,1374, 1081 cm^{-1}; ^1H NMR and ^{13}C NMR, see

Table 1. ^{1}H and ^{13}C NMR Data[a] of Compounds **1**−**3**

	1[b]		**2**[b]		**3**[c]	
position no.	δ, ^{1}H[d] (mult., J in Hz)	δ, ^{13}C[e] (mult.)	δ, ^{1}H[d] (mult., J in Hz)	δ, ^{13}C[e] (mult.)	δ, ^{1}H[d] (mult., J in Hz)	δ, ^{13}C[e] (mult.)
1						
2		173.6 (s)		173.9 (s)		174.9 (s)
3		134.2 (s)		134.3 (s)		134.7 (s)
4	6.97 (br s)	148.8 (d)	6.98 (br s)	148.9 (d)	6.21 (d, 1.2)	149.7 (d)
5	4.98 (br q, 6.7)	77.4 (d)	4.99 (br q, 6.8)	77.4 (d)	4.34 (m)	78.2 (d)
6	1.40 (d, 6.8)	19.2 (q)	1.40 (d, 6.8)	19.2 (q)	0.89 (d, 6.8)	19.4 (q)
7	2.26 (br t, 7.3)	25.1 (t)	2.26 (t, 7.5)	25.1 (t)	2.10 (t, 7.4)	26.1 (t)
8	1.56 (m)	27.3 (t)	1.54 (m)	27.4 (t)	1.37 (m)	28.3 (t)
19			1.55 (m)	27.5 (t)	1.68 (m)	29.2 (t)
20	1.30 (m)	31.9 (t)	1.67,1.82 (m)	23.3 (t)	2.41 (t, 7.5)	22.3 (t)
21	1.30 (m)	22.6 (t)				
22	0.88 (t, 6.6)	14.0 (q)				
1′						
2′				177.4 (s)		177.1 (s)
3′			2.57 (dt, 4.8,9.8)	47.4 (d)		101.6 (s)
4′			4.31 (m)	71.2 (d)		176.8 (s)
5′			4.45 (dq, 2.9,6.4)	78.7 (d)	4.52 (q, 6.7)	75.2 (d)
6′			1.43 (d, 6.4)	13.7 (q)	1.29 (d, 6.7)	18.6 (q)

[a] Bruker AMX 500, AM 400, and DPX 300 MHz; assignments were deduced by analysis of 1D and 2D spectra. [b] $CDCl_3$. [c] C_6D_6 + ca. 1% CD_3OD. [d] Methylene protons not reported appeared as a large signal at δ 1.26−1.30. [e] Methylene carbons not reported resonated between δ 29.8 and 29.0.

Table 1; EIMS *m*/*z* 322 (M^+); HREIMS *m*/*z* 322.2868 (calcd for $C_{21}H_{38}O_2$, 322.2872).

Compound 2: white amorphous powder, $[\alpha]_D$ −4.7° (*c* 0.3, $CHCl_3$); UV (EtOH) λ_{max} 213 nm (ϵ 5200); CD $[\theta]_{209}$ (EtOH) −7743; IR (liquid film) ν_{max} 3429, 2919, 2857, 1735, 1189, 1027 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 408 (M^+, 5%), 390 (10%); HREIMS *m*/*z* 408.2878 (calcd for $C_{24}H_{40}O_5$, 408.2876).

Compound 3: white amorphous powder, $[\alpha]_D$ −27.2° (*c* 5.6, $CHCl_3$); UV (EtOH) λ_{max} 210 (ϵ 15000); CD $[\theta]_{209}$ (EtOH) −6431; IR (liquid film) ν_{max} 2919, 2849, 1742, 1735, 1656, 1390, 1073 cm^{-1}; ^{1}H and ^{13}C NMR (C_6D_6 + CD_3OD), see Table 1; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.06 (1H, d, J = 1.4 Hz, H-4), 5.06 (1H, m, H-5), 4.77 (1H, q, J = 6.7 Hz, H-5′), 2.27 (2H, t, J = 7.7 Hz, H_2-7), 2.19 (2H, t, J = 7.3 Hz, H_2-20), 1.48 (3H, d, J = 6.7 Hz, H_3-6′), 1.43 (3H, d, J = 6.8 Hz, H_3-6), 1.26−1.30 (24H, m, methylenes); EIMS *m*/*z* 406 (M^+, 5%), 378 (100%), 360 (90%); HREIMS *m*/*z* 406.2721 (calcd for $C_{24}H_{38}O_5$, 406.2719).

Acetylation of 2. Compound **2** (0.5 mg) was dissolved in 0.3 mL of anhydrous pyridine and 1 drop of Ac_2O was added. The reaction mixture was stirred at room temperature for 2 h. Then the solvent was evaporated, and the residue was purified by Si gel chromatography in a pasteur pipet, eluting with petroleum ether−Et_2O, 6:4 to afford pure **6** (0.3 mg).

Compound 6: white amorphous powder, $[\alpha]_D$ +3.8° (*c* 0.05, CH_3Cl); UV (EtOH) λ_{max} 212 nm (ϵ 2700); IR (liquid film) ν_{max} 2919, 2857, 1753, 1382, 1277, 1119, 1019 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.98 (1H, br s, H-4), 5.59 (1H, m, H-4′), 4.99 (1H, m, H-5), 4.56 (1H, m, H-5′), 2.70 (1H, m, H-3′), 2.26 (2H, t, J = 7 Hz, H_2-7), 1.40 (3H, d, J = 7 Hz, H_3-6), 1.32 (3H, d, J = 7 Hz, H_3-6′), 1.26 (26H, m, methylenes); EIMS *m*/*z* 450 (M^+, 3%), 432 (10%), 390 (15%).

Acetylation of 3. Compound **3** (5.0 mg) was acetylated in a similar manner as described above for **2**, obtaining the acetate **9** (2.3 mg).

Compound 9: amorphous powder, UV (EtOH) λ_{max} 232 nm (ϵ 2800); IR (liquid film) ν_{max} 2927, 2857, 1757, 1691, 1382, 1174, 1073 cm^{-1}; ^{1}H NMR (C_6D_6, 500 MHz) δ 6.07 (1H, br s, H-4), 4.25 (1H, br q, J = 6.9 Hz, H-5), 4.95 (1H, q, J = 7.0 Hz, H-5′), 0.99 (3H, d, J = 6.8 Hz, H_3-6′), 0.83 (3H, d, J = 6.8 Hz, H_3-6), 2.13 (2H, t, J = 7.7 Hz, H_2-7), 2.22 (2H, t, J = 7.3 Hz, H_2-20); ^{13}C NMR (C_6D_6, 100 MHz) δ 173.6, 171.5, 167.5, 166.2, 148.8, 135.0, 117.17, 77.36, 74.95, 30.66, 30.58, 30.35, 30.20, 29.90, 29.83, 29.17, 28.42, 27.94, 26.27, 23.81, 23.62, 20.27, 19.73, 18.21, 17.35, 17.16; EIMS *m*/*z* 448 (M^+, 1%), 420 (15%), 378 (100%), 360 (80%).

Acknowledgment. We are grateful to Prof. J. Ortea of University of Oviedo, Spain, for giving us the opportunity to participate in the expedition in Puerto Morelos, Mexico, in April 1995. We also thank Mr. F. Castelluccio, Mr. G. Scognamiglio, and Mr. R. Turco for their valuable technical help. The NMR and EIMS spectra were obtained at the "Servizio NMR ICMIB e Area di Ricerca di Napoli" and "Servizio di Spettrometria di Massa del CNR e dell'Università di Napoli', respectively, the staff of both of which are acknowledged. We are finally grateful to Dr. G. Pocsfalvi for HREIMS measurements and to Dr. P. Lopez-Gonzalez for the identification of *P. anceps*. Dr. Y-W. Guo thanks the Ministry of Foreign Affairs of Italy for the financial support in the frame of the Italian−Chinese VII Program of Scientific and Technological Cooperation. This research was partly funded by the Italian−Spanish bilateral project "Studio Biologico e Chimico di Invertebrati Bentonici da Coste Mediterranee e Atlantiche".

References and Notes

(1) Faulkner, D. J. *Nat. Prod. Rep.* **1998**, *15*, 113−158, and previous reports in this series.
(2) Paul, V. J. In *Ecological Roles of Marine Natural Products*, Paul V. J., Ed., Comstock Publishing Associates: Ithaca, NY, 1992; pp 164−188.
(3) Pawlik, J. R.; Fenical, W. *Mar. Ecol. Prog. Ser.* **1992**, *87*, 183−188.
(4) (a) Schmitz, F. J.; Kraus, K. W.; Ciereszko, L. S.; Sifford, D. H.; Weinheimer, A. J. *Tetrahedron Lett.* **1966**, 97−104. (b) Schmitz, F. J.; Lorance, E. D.; Ciereszko, L. S. *J. Org. Chem.* **1969**, *34*, 1989−1990.
(5) Schmitz, F. J.; Lorance, E. D. *J. Org. Chem.* **1971**, *36*, 719−721.
(6) Rodriguez, A.; Ramirez, C. *J. Nat. Prod.* **1994**, *57*, 339−347.
(7) Trost, B. M.; Muller, T. J. *J. Am. Chem. Soc.* **1994**, *116*, 4985−4986.
(8) Ortuño, R. M.; Bigorra, J.; Font, J. *Tetrahedron* **1988**, *44*, 5139−5144.
(9) Takeda, K.; Sakurami, K., Ishii, H. *Tetrahedron* **1972**, *28*, 3757−3766.
(10) Chaves, M. H.; Roque, N. F. *Phytochemistry* **1997**, *44* (3), 523−528.
(11) Pupo, M. T.; Vieira, P. C.; Fernandes, J. B.; Silva, M. F. G. F. *Phytochemistry* **1998**, *48* (2), 307−310.

NP9901231