

11 β , 12 β -EPOXYPUKALIDE, A FURANOCEMBRANOLIDE FROM THE GORGONIAN *LEPTOGORGIA SETACEA*

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ABSTRACT.—A furanocembranolide, 11 β , 12 β -epoxypukalide (**1**), C₂₁H₂₄O₇, was isolated in trace quantities from the gorgonian *Leptogorgia setacea*. The structure was determined from detailed ¹H-nmr and ¹³C-nmr analyses and comparison of these spectral data with those of the closely related compounds pukalide (**2**) and lophotoxin (**3**). The ultimate origin of **1** is discussed briefly.

The gorgonian *Leptogorgia setacea* Pallas, dubbed "spaghetti" by Gulf Coast fishermen, occurs along parts of the western Atlantic Ocean from Chesapeake Bay to Brazil, including the Gulf of Mexico (1). In 1959, one of us (LSC) isolated from *L. setacea* a lactone distinguished from several others obtained from Caribbean gorgonians by its higher melting point and higher frequency carbonyl absorption in the ir (2). Lack of material prevented a resolution of the structure at that time. We have now reinvestigated this problem and report here the structure of the new lactone.

High resolution mass spectral analysis corroborated by ¹³C-nmr and ¹H-nmr data (see Table 1) established the molecular formula C₂₁H₂₄O₇ for **1**, a composition corresponding to 10 degrees of unsaturation. An α,β -unsaturated methyl ester moiety was deduced from ir (1710, 1070, 1225 cm⁻¹), ¹H-nmr (δ 3.78, s) and ¹³C-nmr data (δ 164.03, s and 51.39, q). This confirmed that the parent structure possessed only 20 carbons and immediately suggested consideration of a cembrane framework for the new lactone, since cembranolides are common gorgonian metabolites. A review of the literature revealed a close similarity of the spectral data of **1** and the furanocembranolides, pukalide (**2**) (3), lophotoxin (**3**) (4), and pseudopterolide (**4**) (5). In particular, ¹³C-nmr resonances at δ 160.04, 114.5, 148.27 (all s), and 107.55 (d) combined with a broad singlet in the ¹H-nmr spectrum at 6.48 and ir absorption at 1710 cm⁻¹ indicated that **1** possessed a 1,3-dialkylated-2-carbomethoxyfuran moiety as in pukalide and pseudopterolide. Similarly, an α,β -epoxy- γ -lactone moiety was indicated by spectral absorptions similar to those observed for lophotoxin: ir, 1782 cm⁻¹; ¹³C-nmr, δ 171.20 (s, carbonyl), and ¹H-nmr, 4.73 (one proton). An isopropenyl group was revealed by ir absorption at 890 cm⁻¹, ¹H-nmr absorptions at δ 1.75 (3H, br s) for the vinyl methyl group, and 4.91 and 5.11 (broad singlets) for the terminal methylene protons, and ¹³C-nmr signals at δ 113.54 (t), 145.40 (s), and 18.90 (q). ¹H-nmr decoupling confirmed the isopropenyl proton assignments. The remaining oxygen was assigned to a trisubstituted epoxide unit, inasmuch as spectral data indicated that two such epoxides were present in **1**, (carbon signals at 62.57, s; 63.36, d; 56.31, s; 55.63, d; proton signals at 4.01 and 4.05), and one was already assigned to the α,β -epoxy- γ -lactone moiety. The spectral data for both of these epoxides correspond closely with data for similar epoxides in lophotoxin (5). These functionalities account for 9 of the 10 degrees of unsaturation in **1** and indicate that it contains one carbocyclic ring as in a cembranolide.

Although the close correspondence of the foregoing spectral data with those of pukalide, lophotoxin, and pseudopterolide permitted the formulation of structure **1** for the new lactone, independent evidence for the carbon connectivity in the skeleton was obtained from proton decoupling and nOe effects. The C-7,-8,-9 connection was confirmed by the observation that the methyl signal at δ 1.14 is W-coupled ($J=1$ Hz) to both H-7 and one of the H-9 protons; the C-9,-10 connection was evident from vicinal

TABLE 1. Nmr Data of 11 β ,12 β -Epoxy pukalide (1)

Carbon	¹³ C-nmr ^a	¹ H-nmr ^b	
		Recorded in CDCl ₃ ^c	Recorded in C ₆ H ₆ ^c
1	40.68 d	3.47 dddd, (11.5, 9.5, 5.1, 2.2).	3.65 ddt (11.9, 10.5, 3.4).
2	32.13 t	3.06 dd (18.4, 9.5).	3.09 dd (18.3, 11.9).
		2.95 dd (18.4, 5.1).	2.90 dd (18.3, 3.4).
3	160.04 s		
4	114.50 s		
5	107.55 s	6.48 br s.	6.56 br s.
6	148.27 s		
7	55.63 d	4.05 br s.	3.95 br s.
8	56.31 s		
9	39.27 t	2.48 dd (15.3, 2.9).	1.51 dd (16.5, 3.5).
		2.04 br dd (15.3, 4.3).	1.44 dd (16.5, 4).
10	76.84 d	4.73 dd (4.3, 2.9).	3.95 dd (4, 3.4).
11	63.36 d	4.01 s.	3.03 s.
12	62.57 s		
13	26.71 t	2.56 br dd (15.2, 11.5).	2.59 ddd (16, 10.3, 2.2).
		1.42 m.	1.11 ddd (16, 8.2, 2).
14	23.99 t	1.60-1.74 m.	1.31 m.
		1.60-1.74 m.	1.24 m.
15	145.40 s		
16	113.54 t	5.11 br d (1.9).	5.28 br d (1.8).
		4.94 br q (1.9).	4.92 br q (1.8).
17	18.90 q	1.75 br s.	1.58 br s.
18	164.03 s		
19	20.30 q	1.14 s.	0.71 s.
20	171.20 s		
21	51.39 q	3.78 s.	3.39 s.

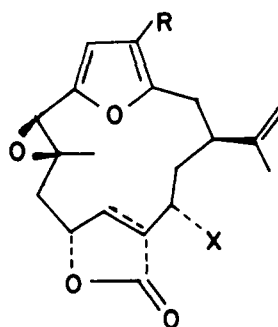
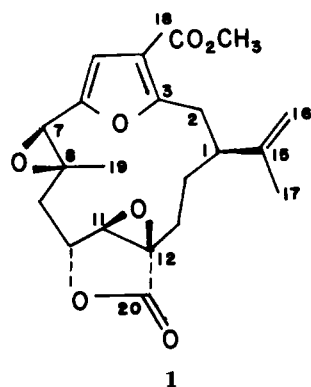
^aRecorded in CDCl₃ at 75.40 MHz. Assignments were made by comparison to **2**, **3**, **4**.

^bFigures in parentheses are coupling constants expressed in Hz.

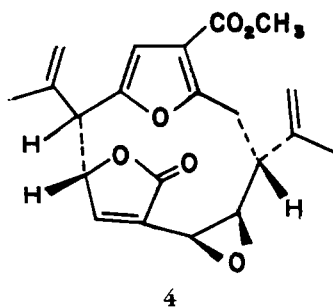
^cRecorded at 300 MHz. Assignments established by spin-decoupling.

couplings (see Table 1). The C-10,-11 connection was evidenced by a very small coupling between H-10,-11, indicative of a dihedral angle approaching 90° as was observed in lophotoxin and several synthetic α,β -epoxy- γ -lactones (**6**) with *trans* β,γ -protons. A small coupling ($J=1$ Hz) between H-11 and one of the H-13 protons was discerned by double irradiation and this was attributed to a W-coupling through the quaternary carbon, C-12, thus extending the evidence for the carbon skeleton from C-11 to C-13. Vicinal and geminal couplings (Table 1) confirmed the C-13 to C-2 carbon array. Sharpening of the H-1 proton signal (δ 3.47) upon irradiation of the isopropenyl proton at δ 5.11 confirmed the connection of C-1 to C-15. Determination of an allylic coupling between the furan proton at δ 6.48 (H-5) and H-7 (see Table 2) established the C-6, -7 connection, as did the observation of mutual nOe enhancements between H-5 and -7. The nOe enhancement of the H-2 signals upon irradiation of the methyl ester protons verified the C-2, -3 connection. Hence, the overall structure of **1** is confirmed.

The stereochemical features of **1** follow from decoupling and nOe effects. The *trans* arrangement of H-10 and -11 follows from the small coupling between these protons, *vide supra*. The E-configuration of the 7,8-epoxide was confirmed by observation of nOe enhancement between H-9' and H-7 and lack of enhancement of H-7 by irradiation of H-19. Both H-5 and -11 show enhancement when H-19 is irradiated, verifying that H-19 and -11 are both directed to the inside of the carbocyclic ring and are on the same face (α) of the molecule. Correspondingly, both epoxide oxygens are on the same



- 2 R=CO₂CH₃; X=H; Δ¹¹
 3 R=CHO; X=O₂CCH₃;
 11β, 12β-oxido



side (β) of **1**, facing the periphery of the carbocyclic ring. The relative configuration of the isopropenyl group in **1** is assigned by analogy to that of pukalide since the nmr chemical shifts for both H-1 and C-1 in the two compounds are very similar, δ 3.47 and 3.55, and 40.68 and 40.7, respectively. ¹³C-nmr chemical shifts have proven to be a particularly sensitive probe for stereochemical identities in other series of natural products (7).

L. setacea does not appear to contain zooxanthellae (L.S.C., personal observations), as do most other octocorals that yield cembranolides. Hence, it can be added to a growing list of gorgonians that lack zooxanthellae but from which terpenoid metabolites have been isolated (8-12). Because only trace quantities of 11β, 12β-epoxy pukalide were found in several isolation efforts, it seems possible that it is of dietary origin, although it might also be produced by the animal polyps. Cembranolides are often major metabolites from other gorgonians containing zooxanthellae (13-14).

EXPERIMENTAL

¹H-nmr spectra were recorded at 300 MHz and ¹³C-nmr spectra at 75.4 MHz on a Varian XL-300 spectrometer; chemical shifts are reported in parts per million (δ) downfield from internal tetramethylsilane. Ir spectra were recorded on a Perkin-Elmer model 298 spectrophotometer. Uv spectra were measured with a Perkin-Elmer Lambda 3 spectrophotometer. Low resolution mass spectra were recorded on a Hewlett-Packard 5985B mass spectrometer; high resolution mass spectra were taken on a CED (DuPont, Monrovia, CA) 110 instrument. Optical rotation was measured on a Perkin-Elmer 141 polarimeter.

L. setacea was collected in the surf, near the jetty on Mustang Island near Port Aransas, Texas, as it was being washed up onto the beach after a storm. The animals were freed of adhering debris, washed in sea water, drained, and spread out on a clean concrete walkway to dry in the sun. Drying was rapid, as the animals are very slender and are composed largely of calcite spicules.

The cortex of the dried, yellow *L. setacea* was stripped off, ground in a blender, and extracted in a large continuous extractor with redistilled *n*-pentane for several days. The precipitate, obtained by allowing the extract to stand at room temperature for a week, was filtered off on a sintered glass funnel, washed with pentane, and extracted with CHCl₃. 11β, 12β-Epoxy pukalide crystallized when pentane was added to the

CHCl₃ solution:¹ [α]_D -5.33 (c .6, MeOH); uv (EtOH) λ max 238 nm (ε=5424); ir (CHCl₃) 3090, 3030, 2950, 2860, 1782, 1708, 1640, 1575, 1440, 1380, 1350, 1285, 1255, 1210, 1070, 900, 890, 860, 820 cm⁻¹; for ¹H-nmr and ¹³C-nmr see Table 1; hrms: observed *m/z* (composition, interpretation, calculated millimass) 388.15316 (C₂₁H₂₄O₇, M⁺, 388.15221), 373.12938 (C₂₀H₂₁O₇, M⁺-Me: 373.12873), 356.12796 (C₂₀H₂₀O₆, M⁺-MeOH, 356.12599), 328.13280 (C₁₉H₂₀O₅, M⁺-MeOH-CO: Gal 35B JNP N-D C575 File 17 Disk V47N6-5 84138

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¹Only 3 mg of pure **1** were isolated. Unfortunately, no mp is available because it was not taken at the outset, and after prolonged exposure to solvents for spectral measurements, the sample had decomposed. It was evident from comparison of ir spectra that the sample of **1** isolated in reference 2 was not pure, and hence that mp cannot be used.