[Chem. Pharm. Bull. 27(10)2394—2397(1979)]

UDC 547.597.02.05 : 593.6.05

Studies on Marine Natural Products. I. 13-Membered Carbocyclic Cembranolide Diterpenes from the Soft Coral *Lobophytum pauciflorum* (Ehrenberg)

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(Received April 2, 1979)

Two cembranolide diterpenes were isolated from the Japanese soft coral *Lobophytum pauciflorum* (Ehrenberg). The structures of these diterpenes were identified as I and II, involving a 13-membered carbocyclic ring system unique in the soft coral literature.

Keywords——soft coral; *Lobophytum pauciflorum* (Ehrenberg); cembranolide diterpenes; γ-lactone; 13-membered carbocyclic ring system

A number of marine cembranolide diterpenes have recently been isolated from soft coral,[2)] and some of these diterpenes show interesting biological properties,[2b,3)] such as an anticancer activity. The major members of this class of diterpenes possess a 14-membered carbocyclic ring system, to which a γ- or δ-lactone moiety is attached in many cases.

In the course of our investigations on the biologically active constituents of the Japanese soft coral *Lobophytum pauciflorum* (Ehrenberg) (Coelenterata, Anthozoa, Alcyonaria, Alcyonacea), two cembranolide diterpenes containing a unique 13-membered carbocyclic ring system have been isolated. The present paper deals with the isolation and structural study of these diterpenes.

Isolation was achieved by the procedure shown in Fig. 1. Wet coral, *Lobophytum pauciflorum* (Ehrenberg),[4)] collected from the coral reefs of Ishigaki Island (Okinawa Prefecture, Japan), was crushed in methanol and extracted successively with methanol and acetone. The combined extract was suspended in water and the mixture was extracted with ether. Repeated silica gel column chromatography of the ether-soluble fraction gave two crystalline substances; the major substance (I) ($C_{22}H_{30}O_5$, mp 119°, 0.23% yield) was eluted first, followed by the minor substance (II) ($C_{20}H_{28}O_4$, mp 147—149°, 0.012% yield).

The physical properties of these compounds are shown in Table I. The infrared (IR), proton nuclear magnetic resonance (^{1}H–NMR) and carbon-13 nuclear magnetic resonance (^{13}C–NMR) spectra show that the compounds are closely related, but the acetoxyl group in I is replaced by a hydroxyl group in II.

Inspection of the spectroscopic data suggested a bicyclic structure for both compounds, consisting of an α-methylene-γ-lactone ring and a 13-membered carbocyclic ring carrying a

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2) Recent examples are shown below; *a)* Y. Kashman and A. Groweiss, *Tetrahedron Lett.*, **1977**, 1159; *b)* A.J. Weinheimer, J.A. Matson, M.B. Hossain, and D. van der Helm, *Tetrahedron Lett.*, **1977**, 2923; *c)* B.F. Bowden, J.A. Brittle, J.C. Coll, N. Liyanage, S.J. Mitchell, and G.J. Stokie, *Tetrahedron Lett.*, **1977**, 3661; *d)* J.C. Coll, S.J. Mitchell, and G.J. Stokie, *Aust. J. Chem.*, **30**, 1859 (1977); *e)* B.F. Bowden, J.C. Coll, S.J. Mitchell, and G.J. Stokie, *Aust. J. Chem.*, **31**, 1303 (1978); *f)* P. Kazlauskas, P.T. Murphy, R.J. Wells, P. Schonholzer, and J.C. Coll, *Aust. J. Chem.*, **31**, 1817 (1978).

3) J.R. Rice, C. Papastephanou, and D.G. Anderson, *Biol. Bull.*, **138**, 334 (1970).

4) S. Shirai, "Ecological Encyclopedia of the Marine Animals of the Ryukyu Islands," Shinsei-Tosho, Okinawa, 1977, p. 444.

methylketone, an acetoxyl (or hydroxyl) group and two trisubstituted carbon-carbon double bonds ($-CH=C<^{CH_3}$).

The following chemical transformations and ^1H-NMR decoupling experiments led to the structures (I) for the major compound and II for the minor compound, as illustrated in Fig. 2.

The ^1H-NMR decoupling experiments of I clarified the relationship of the protons at the C-3a, 14a and 14 positions. Irradiation at δ 6.20 ppm (Ha) converted the multiplet at 3.02 ppm into a octet (J=10.0, 8.3, 3.5 Hz). A similar change was also observed upon irradiation at 5.47 ppm (Hb), showing that the signal at 3.02 ppm was assignable to the angular C-3a proton. Irradiation at 3.02 ppm collapsed a pair of doublets at 5.47 and 6.20 ppm into singlets and also converted the quartet at 5.36 ppm (lactonic proton at C-14a) into a doublet (J=10.0 Hz), which coupled with the C-14 proton (5.06 ppm), with J=10.0 Hz.

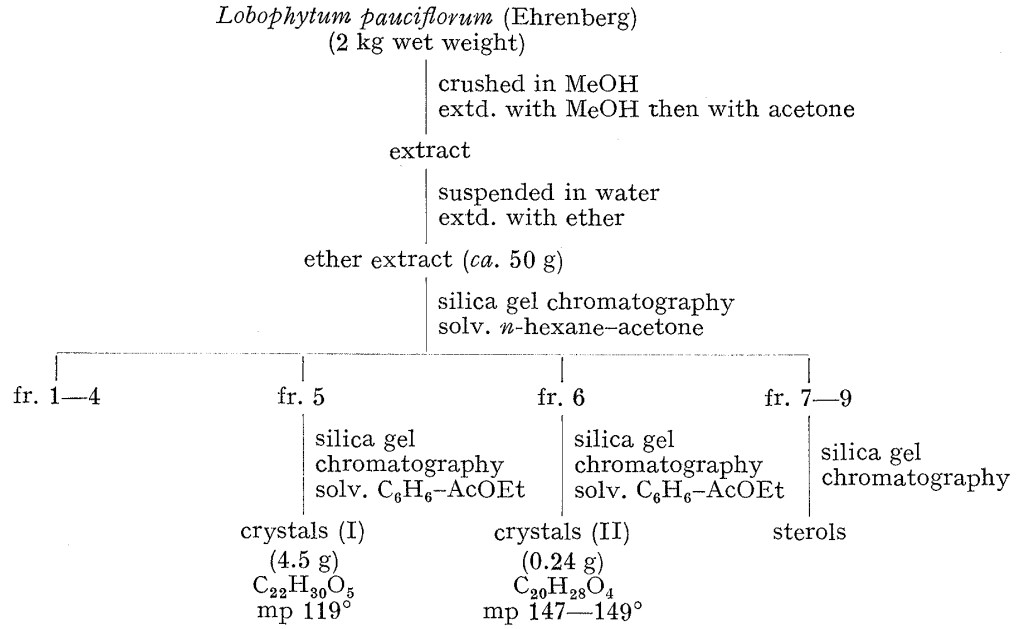


Fig. 1. Isolation of Cembranolide Diterpenes

TABLE I. Physical Data of I and II

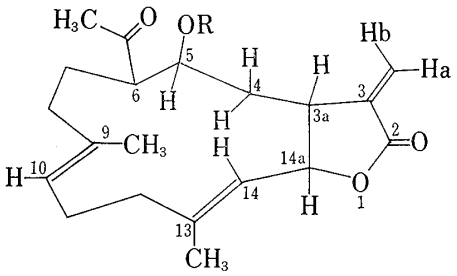
	I	II
Molecular formula	$C_{22}H_{30}O_5$	$C_{20}H_{28}O_4$
mp	119°	147—149°
$[\alpha]_D^{20}$	−82° (c=1.7, $CHCl_3$)	−12.5° (c=0.8, $CHCl_3$)
ν_{max}^{KBr}	1755, 1750 (γ-lactone), 1730 (OAc)	3400 (OH), 1760 (γ-lactone)
	1695 (methylketone), 1660 (C=CH_2)	1687 (methylketone), 1660 (C=CH_2)
^1H-NMR $\delta_{ppm}^{CDCl_3}$	1.60 (3H, brs), 1.88 (3H, brs), 2.14 (3H, s)	1.64 (3H, brs), 1.79 (3H, d, J=1.0)
	2.20 (3H, s), 3.02 (1H, m,C_{3a}-H)	2.21 (3H, s), 3.50 (1H, m, C_{3a}–H)
	5.06 (1H, brd, J=10.0, C_{14}–H), 5.10 (1H, m)	3.65 (1H, m, C_5–H), 5.00 (1H, m, C_{10}–H)
	5.18 (td, J=3.2, 9.0, C_5–H)	5.11 (1H, brd, J=10.0, C_{14}–H)
	5.36 (1H, dd, J=10.0, 8.3, C_{14a}–H)	5.31 (1H, dd, J=10.0, 7.0, C_{14a}–H)
	5.47 (1H, d, J=3.0, Hb)	5.51 (1H, d, J=2.5, Hb)
	6.20 (1H, d, J=3.5, Ha)	6.21 (1H, d, J=3.0, Ha)
^{13}C-NMR $\delta_{ppm}^{CDCl_3}$	15.1(q), 15.5(q), 21.0(q)	14.7(q), 15.7(q), 23.4(t)
	23.6(t), 24.9(t), 29.2(q)	25.0(t), 30.8(q), 37.9(t)
	34.5(t), 38.1(d), 38.9(t)	38.1(d), 39.9(t), 40.0(t)
	40.0(t), 57.6(d), 70.4(d)	56.2(d), 68.7(b), 76.2(d)
	76.2(d), 119.1(d), 121.3(dd)	119.5(d), 121.3(dd), 126.0(d)
	126.1(d), 133.6(s), 139.2(s)	134.2(s), 140.0(s), 144.1(s)
	145.5(s), 169.7(s), 170.8(s)	170.1(s), 211.0(s)
	208.8(s)	

Mild base treatment of I gave a conjugated enone (III) ($C_{21}H_{30}O_4$, UV λ_{max}^{EtOH} 232 mμ) by elimination of acetic acid accompanied by addition of methanol to the α-methylene-γ-lactone moiety. This clearly showed that the methylketone and the acetoxyl groups were arranged vicinally on the 13-membered carbocyclic ring. On the other hand, oxidation of I with selenium dioxide yielded another conjugated enone (IV) ($C_{22}H_{28}O_6$, mp 154—155°, UV λ_{max}^{EtOH} 234 mμ). ^{1}H-NMR decoupling experiments of IV placed the acetoxyl and the methylketone groups at C-5 and C-6, respectively. Irradiation of the multiplet at 3.00 ppm (C_{3a}–H) collapsed the octet at 1.66 ppm (assignable to one of the methylene protons at C-4) into a quartet (J=15.0, 3.0 Hz). Irradiation at 1.66 ppm converted the multiplet at 5.07 ppm (assignable to the proton attached to the carbon bearing the acetoxyl group) into a quartet (J=10.0, 3.0 Hz).

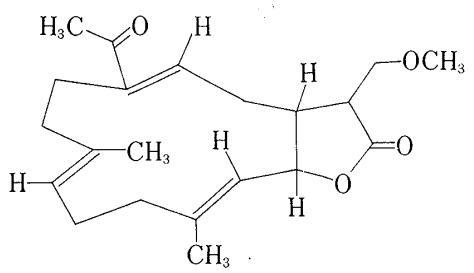
Ozonolysis of I gave laevulinaldehyde, placing the two trisubstituted carbon-carbon double bonds at the positions illustrated.

Acetylation of II gave the compound (I). Accordingly, it was confirmed unambiguously that the minor compound (II) is a desacetyl derivative of I.

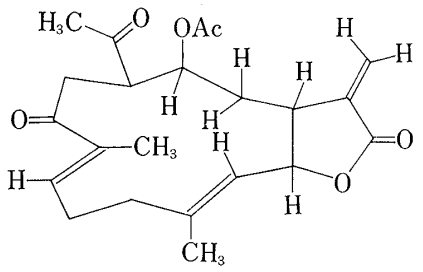
The E-type geometry of the trisubstituted carbon-carbon double bonds was strongly suggested by the ^{13}C-NMR chemical shifts (*ca.* 15 ppm[5]) of the olefin methyl carbons. The stereochemistry at the C-3a, 5, 6 and 14a positions and the absolute configuration are currently under investigation.



I : R=$COCH_3$
II : R=H



III



IV

Fig. 2

It is of interest that these diterpenes, involving a unique 13-membered carbocyclic ring system, which is presumably biosynthesized from the usual 14-membered carbocyclic cembranolide by ring contraction, were major constituents of soft coral.

The compound (II) is a new cembranolide diterpene, although Coll *et al.* recently reported the isolation[1e] of the compound represented by the structure (I) as an oily substance from Australian soft coral (genus *Lobophytum*).

The biological activities of these diterpenes will be described in a separate paper.

5) J.B. Stothers, "Carbon-13 NMR Spectroscopy," Academic Press, New York, 1972.

Experimental

All melting points are uncorrected. Optical rotations were taken with a JASCO DIP-SL automatic polarimeter. IR and UV spectra were obtained with Hitachi 215 and Hitachi 323 spectrometers, respectively. ^{1}H-NMR spectra were measured at 100 MHz on a JEOL PS-100 spectrometer and ^{13}C-NMR spectra were measured at 25.0 MHz on a JEOL FX-100 spectrometer. Chemical shifts are given as δ (ppm) with tetramethylsilane as an internal standard. Mass spectra (MS) were recorded on a Hitachi RMU-7L spectrometer. Column chromatography was carried out with silica gel 60 (70—230 mesh, Merck).

Isolation of the Cembranolide Diterpenes (I) and (II)——Wet corals of *Lobophytum pauciflorum* (Ehrenberg) (2 kg), which were collected at the coral reefs of Ishigaki Island in Okinawa Prefecture, were crushed in MeOH (20 l) and extracted with MeOH (20 l) then with acetone (20 l) at room temperature overnight. The combined extract was suspended in water (3 l) and extracted twice with ether (2 l). The ether extract (*ca.* 50 g) was chromatographed on silica gel (800 g, 60 mm × 700 mm), eluting with an *n*-hexane–acetone gradient of increasing polarity (20: 1 to 1: 1). Elution with *n*-hexane–acetone (5: 1) gave fraction 5 (9.3 g) and elution with *n*-hexane–acetone (2: 1) gave fraction 6 (0.9 g). Further silica gel chromatography (300 g, 50 mm × 320 mm) of fraction 5, eluting with C_6H_6–AcOEt (20: 1, then 10: 1) gave a crystalline substance (6 g). Recrystallization from MeOH gave I (4.5 g, 0.23% yield) as colorless plates: *Anal.* Calcd. for $C_{22}H_{30}O_5$; C, 70.69; H, 8.02. Found: C, 70.62; H, 8.22. MS *m/e*: 374 (M^+).

Silica gel chromatography (100 g, 26 mm × 320 mm) of fraction 6, eluting with C_6H_6–AcOEt (5: 1) gave a crystalline substance (0.5 g). Recrystallization from MeOH gave II (0.24 g, 0.012% yield) as colorless rods: *Anal.* Calcd. for $C_{20}H_{28}O_4$; C, 72.24; H, 8.50. Found: C, 72.25; H, 8.53. MS *m/e*: 332 (M^+).

The physical properties of I and II are listed in Table I.

Base Treatment of I——A solution of I (500 mg) in 40 ml of 1% methanolic NaOH was stirred at 5° overnight. The reaction mixture was neutralized with 0.5 N HCl at 0° and the solvent was removed *in vacuo*. The residue was extracted with AcOEt (200 ml) and the organic solution was washed with water. After removal of the solvent, a pale yellow oily substance (450 mg) was obtained. Silica gel chromatography (30 g), eluting with *n*-hexane–acetone (14: 1) gave the enone (III) (245 mg) as a colorless amorphous substance: MS *m/e*: 346 (M^+), UV λ_{max}^{EtOH} 232 mμ, IR $\nu_{max}^{CHCl_3}$ 1735, 1655 cm^{-1}, ^{1}H-NMR $\delta_{ppm}^{CDCl_3}$ 1.62 (3H, brs), 1.70 (3H, brs), 2.23 (3H, s), 2.90 (1H, m), 3.37 (3H, s), 3.70 (2H, m), 4.70 (1H, br), 5.08 (1H, brd, J=10.0), 5.43 (1H, dd, J=10.0, 8.0), 6.40 (1H, brt); ^{13}C-NMR $\delta_{ppm}^{CDCl_3}$ 16.8 (q), 17.6 (q), 24.2 (t), 24.7 (t), 25.8 (q), 29.1 (t), 37.4 (t), 37.9 (t), 40.5 (d), 46.1 (d), 57.3 (q), 70.3 (t), 77.7 (d), 118.7 (d), 125.8 (d), 133.8 (s), 140.9 (d), 142.3 (s), 143.0 (s), 176.4 (s), 198.8 (s).

Selenium Dioxide Oxidation of I——A solution of selenium dioxide (100 mg) in 3 ml of 95% EtOH was added to a solution of I (300 mg) in 6 ml of 95% EtOH, and the mixture was refluxed for 24 hr. The reaction mixture was filtered and the solvent was removed to give an orange-yellow amorphous substance (350 mg). Silica gel chromatography (10 g) of the products eluted with $CHCl_3$–AcOEt (2: 1) gave an orange-yellow amorphous substance (110 mg). High-speed liquid chromatography [silica gel 60 prepacked column (Merck)], eluting with $CHCl_3$–AcOEt (5: 1) gave the crystalline conjugated enone (IV) (50 mg). Recrystallization from AcOEt–*n*-hexane gave pale yellow needles: mp 154—155°. MS *m/e*: 388 (M^+), UV λ_{max}^{EtOH} 234 mμ, IR $\nu_{max}^{CHCl_3}$ 1755, 1730 (sh), 1710, 1650 cm^{-1}, ^{1}H-NMR $\delta_{ppm}^{CDCl_3}$ 1.66 (1H, ddd, J=3.0, 9.0, 15.0), 1.80 (3H, brs), 1.85 (3H, brs), 2.20 (3H, s), 2.25 (3H, s), 3.00 (1H, m), 4.82 (1H, brd, J=10.0), 5.07 (1H, m), 5.36 (1H, dd, J=10.0, 8.0), 5.50 (1H, d, J=3.0), 6.26 (1H, d, J=3.5), 6.55 (1H, brt, J=6.5), ^{13}C-NMR $\delta_{PPm}^{CDCl_3}$ 11.4 (q), 16.3 (q), 20.8 (q), 25.1 (t), 30.7 (q), 32.6 (t), 34.7 (t), 38.0 (d), 38.9 (t), 55.1 (d), 70.9 (d), 76.7 (d), 120.5 (d), 121.2 (dd), 138.0 (s), 138.9 (s), 142.7 (s), 144.7 (d), 169.6 (s), 170.0 (s), 200.0 (s), 207.2 (s).

Ozonolysis of I——A solution of I (500 mg) in CH_2Cl_2 (35 ml) and pyridine (0.17 ml) was treated with O_3 at −65° for 1.5 hr. Zinc dust (0.68 g) and acetic acid (6.8 ml) were added, and the mixture was vigorously stirred at room temperature for 30 min. The reaction mixture was filtered and the filtrate was washed with saturated $NaHCO_3$ solution then water. Removal of the solvent gave a yellow oil, whose ^{1}H-NMR spectrum showed signals corresponding to those of laevulinaldehyde. Gas chromatography of the oil showed that the main peak was laevulinaldehyde, as determined by comparison with an authentic sample obtained by ozonolysis of geraniol under the same conditions.

Acetylation of II——A solution of II (50 mg) in pyridine (1 ml) and acetic anhydride (0.5 ml) was kept at room temperature for 21 hr. The reaction mixture was poured into excess ice-water, then extracted with AcOEt (60 ml). Removal of the solvent gave a pale yellow crystalline product (47 mg). Recrystallization from $CHCl_3$–*n*-hexane gave pale yellow plates (35 mg), whose physical properties coincided in every respect with those of I.

Acknowledgement The authors are grateful to Professor Kiyoshi Yamazato, Department of Biology, University of the Ryukyus, for identification of the origin.