Chem. Pharm. Bull.
28(7)2035—2038(1980)

Studies on Marine Natural Products. III.[1] Two New Cembranolides from the Soft Coral *Lobophytum pauciflorum* (Ehrenberg)

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(Received January 11, 1980)

Two new epoxy cembranolide diterpenes were isolated from the Japanese soft coral *Lobophytum pauciflorum* (Ehrenberg). The structures of these diterpenes were elucidated as I and II, mainly on the basis of spectroscopic data. The compound (II) is a possible biogenetic precursor of the 13-membered carbocyclic cembranolides.

Keywords——soft coral; Alcyonaria; *Lobophytum pauciflorum* (Ehrenberg); epoxy cembranolide diterpenes; α-methylene-γ-lactone

Our previous studies on the chemical constituents of the Japanese soft coral *Lobophytum pauciflorum* (Ehrenberg) have led to the isolation of 13-membered carbocyclic cembranolides,[3] which were the major constituents of this soft coral. The unique 13-membered carbocyclic ring system is of interest from a biosynthetic point of view, since usual cembrenoids possess a 14-membered carbocyclic ring. Our interest in the biosynthesis of the 13-membered carbocyclic cembranolides as well as in the biological behavior of cembranolides[4] led us to investigate other cembranolides from the same source. This paper describes the isolation and structure elucidation of two new cembranolides obtained during this investigation.

The extract from the soft coral *Lobophytum pauciflorum* (Ehrenberg) had already been separated into nine fractions by the procedure described in the previous papers.[1,3] Thin-layer chromatography (TLC) of fraction 5, which contained the 13-membered carbocyclic cembranolide, revealed the presence of two additional less polar substances as minor constituents. The following re-examination of fraction 5 resulted in the isolation of these minor substances in crystalline form. Fraction 5 was chromatographed on a silica gel column using a less polar solvent system than that used for the isolation of the 13-membered carbocyclic cembranolide. Elution with benzene–ethyl acetate (20: 1) gave I ($C_{20}H_{28}O_3$, mp 121—124°, 0.02% yield) and II ($C_{22}H_{30}O_5$, mp 181—184°, 0.039% yield) in that order of elution. These substances were considered to be cembranolides on the basis of their physical properties. The physical data for I and II are summarized in Table I.

The infrared (IR), proton nuclear magnetic resonance (^{1}H-NMR) and carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra of the compounds are very similar, except for the absorptions due to an acetoxyl group in II. These spectral data indicate that I and II are new cembranolides, each with a tricyclic system which consists of an α-methylene-γ-lactone ring, a trisubstituted epoxide and a 14-membered carbocyclic ring.

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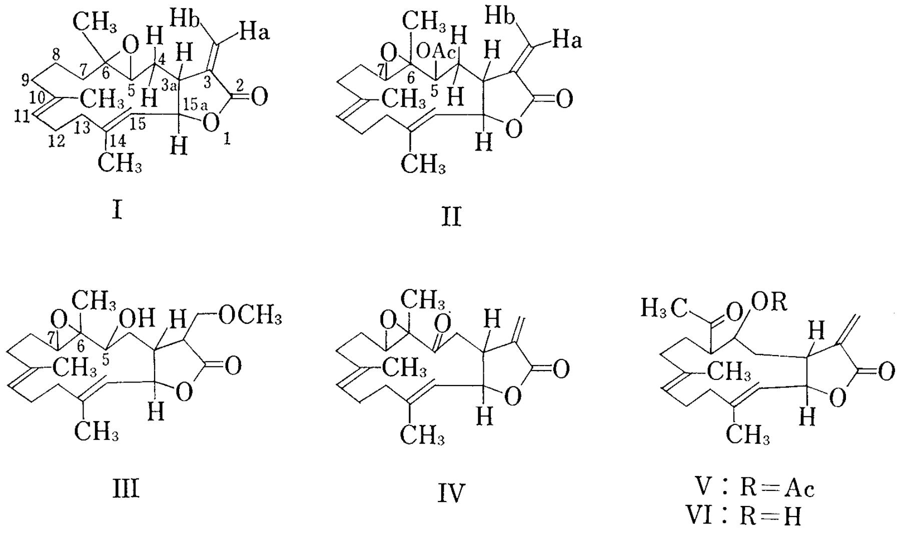
Table I. Physical Data for I and II

	I	II
Molecular formula	$C_{20}H_{28}O_3$	$C_{22}H_{30}O_5$
mp	121—124°	181—184°
$[\alpha]_D^{20}$	+80° (c=1.0, $CHCl_3$)	+86° (c=1.0, $CHCl_3$)
ν_{max}^{KBr} cm^{-1}	1760 (γ-lactone), 1665 (C=CH_2)	1775 (γ-lactone), 1725 (OAc), 1665 (C=CH_2)
^{1}H-NMR $\delta_{ppm}^{CDCl_3}$	1.18 (3H, s), 1.32 (1H, ddd, J=12, 9, 3 Hz, C_4–H), 1.61 (3H, brs), 1.72 (3H, brs), 2.75 (1H, dd, J=9, 3 Hz, C_5–H), 3.06 (1H, m, C_{3a}–H), 4.98 (1H, m, C_{11}–H), 5.06 (1H, brd, J=10 Hz, C_{15}–H), 5.28 (1H, dd, J=10, 8 Hz, C_{15a}–H), 5.52 (1H, d, J=3 Hz, Hb), 6.24 (1H, d, J=3 Hz, Ha)	1.15 (3H, s), 1.44 (1H, ddd, J=14, 12, 2 Hz, C_4–H), 1.59 (3H, brs), 1.86 (3H, brs), 2.16 (3H, s), 2.78 (1H, dd, J=9, 2.5 Hz, C_7–H), 2.95 (1H, m, C_{3a}–H), 4.95 (1H, m, C_{11}–H), 4.93 (1H, brd, J=10 Hz, C_{15}–H), 4.97 (1H, dd, J=10, 2 Hz, C_5–H), 5.42 (1H, dd, J=10, 8 Hz, C_{15a}–H), 5.47 (1H, d, J=4 Hz, Hb), 6.22 (1H, d, J=4 Hz, Ha)
^{13}C-NMR $\delta_{ppm}^{CDCl_3}$	15.3(q), 15.6(q), 18.6(q), 24.1(t), 24.1(t), 24.6(t), 32.9(t), 36.9(t), 40.0(t), 41.9(d), 60.5(d), 60.5(s), 77.8(d), 120.0(d), 121.4 (dd), 125.2(d), 134.2(s), 138.4(s), 141.4 (s), 170.2(s)	15.4(q), 15.4(q), 15.5(q), 20.7(q), 23.6(t), 24.5(t), 27.1(t), 36.7(t), 38.3(t), 39.6(t), 60.0(d), 61.0(s), 71.9(d), 77.2(d), 119.6 (d), 120.7(dd), 125.5(d), 134.0(s), 138.0 (s), 143.6(s), 169.9(s), 170.9(s)



I

II

III

IV

V : R=Ac
VI : R=H

Fig. 1

^{1}H-NMR decoupling experiments with I clarified the relationships among the protons located at the C-3a, 4, 5, 15a and 15 positions. Irradiation at δ 3.06 (1H, m, C_{3a}–H) caused the following changes. A pair of doublets at δ 6.24 (Ha) and 5.52 (Hb) were converted into singlets. Simultaneously an octet at δ 1.32 (J=12, 9, 3 Hz, C_4–H) was changed into a quartet (J=9, 3 Hz) and a quartet at δ 5.28 (J=10, 8 Hz, C_{15a}–H) into a doublet, whose coupling constant (J=10 Hz) was the same as that of the signal at δ 5.06 (brd, J=10 Hz) due to the olefinic C-15 proton. Similar relationships among the protons (C-3a, 4, 5, 15a and 15 positions) in II were also established by irradiation at δ 2.95 (C_{3a}–H). A pair of doublets δ 6.22 (Ha) and 5.47 (Hb) were converted into singlets. Simultaneously an octet at δ 1.44 (J=14, 12, 2 Hz, C_4–H) was converted into a quartet (J=14, 2 Hz) and a quartet at δ 5.42 (J=10, 8 Hz, C_{15a}-H) into a doublet (J=10 Hz).

Ozonolysis of both compounds gave levulinaldehyde, showing the presence of a structural unit between the C-10—C-15 positions.

The position of the trisubstituted epoxide in I was also determined to be between C-5 and C-6 by an ^{1}H-NMR decoupling experiment. Irradiation at δ 2.75 (dd, J=9, 3 Hz), assignable to the epoxy-methine proton, converted the octet at δ 1.32 (C_4–H) into a quartet (J=12, 9 Hz). On the basis of these results the planar structure of I was established as shown in Fig. 1.

The position of the acetoxyl group at C-5 in II was also decided by decoupling experiments. Irradiation of the signal at δ 4.97 (dd, J=10, 2 Hz) due to a proton attached to a carbon bearing the acetoxyl group converted an octet at δ 1.44 (C_4–H) into a quartet (J=14, 12 Hz), while irradiation at δ 1.44 converted a quartet at δ 4.97 into a doublet (J=10 Hz).

The position of the trisubstituted epoxide between C-6 and C-7 in II was elucidated by comparison of the ^{13}C-NMR spectra of II and its derivative (III) (mp 167—168°), which was obtained by treatment of II with methanolic potassium hydroxide. On going from II to III, the signal at δ 61.0 (s) due to the quaternary carbon of the epoxide shifted markedly to low field (δ 63.5), in addition to the high field shift of the C-5 carbon signal (71.9 to 70.8),[5] showing clearly that the epoxy quaternary carbon was located at the position (C-6) adjacent to C-5, carrying the acetoxyl group. Thus the planar structure of II was established as shown in Fig. 1.

The *E*-type stereochemistry of two trisubstituted carbon-carbon double bonds and a trisubstituted epoxide in both I and II was decided on the basis of the ^{13}C-NMR chemical shifts of the methyl carbons (15—19 ppm).[6] The stereochemistry at the C-3a, 5 (in II) and 15a positions and the absolute configurations are currently under investigation.

The co-occurrence of II with the 13-membered carbocyclic cembranolides (V) and (VI)[3] in the soft coral is of interest in connection with the biosynthesis of the latter cembranolides. Presumably the desacetyl derivative of II is transformed by oxidation into an epoxy ketone (IV),[7] from which V and VI can be derived by epoxide opening followed by ring contraction[8] and reduction.

Experimental

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

Isolation of I and II——The wet soft coral of *Lobophytum pauciflorum* (Ehrenberg) (2 kg), collected from the coral reefs of Ishigaki Island in Okinawa Prefecture, was crushed in MeOH (20 l) and extracted with MeOH (20 l) and 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 a silica gel column (800 g, 60 mm × 700 mm) eluted with *n*-hexane–acetone mixtures of increasing polarity (20: 1 to 1: 1) followed by MeOH: fr. 1 (20: 1, 4 l), fr. 2 (10: 1, 2 l), fr. 3 (10: 1, 2 l), fr. 4 (5: 1, 2 l), fr. 5 (5: 1, 2 l), fr. 6 (2: 1, 2 l), fr. 7 (2: 1, 2 l), fr. 8 (1: 1, 4 l), and fr. 9 (MeOH), respectively. Fraction 5 (5.3 g) was further chromatographed on a silica gel column (300 g, 50 mm × 320 mm) eluted with C_6H_6–AcOEt (20: 1), giving two fractions.

The crystalline substance obtained from the first fraction was recrystallized from MeOH to give the compound (I) (400 mg, 0.02% yield) as colorless rods, *Anal.* Calcd for $C_{20}H_{28}O_3$: C, 75.90; H, 8.92. Found: C, 75.66; H, 8.92. MS *m/e* 316 (M^+).

The crystalline substance obtained from the second fraction was recrystallized from MeOH to give the compound (II) (780 mg, 0.039% yield) as colorless rods, *Anal.* Calcd for $C_{22}H_{30}O_5$: C, 70.59; H, 8.02. Found: C, 70.69; H, 8.18. MS *m/e* 374 (M^+).

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Elution with C_6H_6–AcOEt (10: 1) gave the 13-membered carbocyclic cembranolide (V) as reported previously.[3]

The physical properties of I and II are listed in the table.

Ozonolysis of I and II——A solution of I (100 mg) in CH_2Cl_2 (10 ml) and pyridine (0.1 ml) was treated with O_3 at -62 — $-60°$ for 2 hr. Zinc dust (164 mg) and AcOH (1.7 ml) were added to the reaction mixture and the whole was vigorously stirred at room temperature for 2 hr. The reaction mixture was filtered and the filtrate was washed with saturated $NaHCO_3$ solution and then water. Removal of the solvent gave a yellow oil, whose ^{1}H-NMR spectrum showed signal corresponding to those of levulinaldehyde. Gas chromatography of the oil showed that the main peak was levulinaldehyde, as determined by comparison with an authentic sample (obtained by ozonolysis of geraniol) under the same conditions. Similar ozonolysis of II also gave levulinaldehyde.

Methanolysis of II——A solution of II (100 mg) in Et_2O (20 ml) was treated with 1% methanolic potassium hydroxide (22 ml), and the mixture was stirred at room temperature for 14 hr. After neutralization of the mixture with 0.5 N HCl, the solvent was removed *in vacuo* and the residue was extracted three times with AcOEt (30 ml). The AcOEt extract was washed with water and dried over anhydrous Na_2SO_4. Removal of the solvent gave colorless crystals (85 mg). Recrystallization from *n*-hexane–$CHCl_3$ gave the compound (III) as colorless plates (59 mg), mp 167—168°. MS *m/e* 364 (M^+). IR ν_{max}^{KBr} 3580, 1775 cm^{-1}. ^{1}H-NMR $\delta_{ppm}^{CDCl_3}$ 1.22 (3H, s), 1.61 (3H, brs), 1.69 (3H, brs), 2.70 (1H, dt, $J=9$, 4 Hz), 2.80 (1H, m), 2.87 (1H, dd, $J=13$, 3 Hz), 3.35 (3H, s), 3.50 (1H, dd, $J=9$, 3 Hz), 3.74 (2H, d, $J=4$ Hz), 5.00 (1H, m), 5.18 (1H, brd, $J=10$ Hz), 5.32 (1H, dd, $J=10$, 7 Hz). ^{13}C-NMR $\delta_{ppm}^{CDCl_3}$ 15.6(q), 15.9(q), 15.9(q), 24.0(t), 25.0(t), 33.6(t), 36.5(t), 38.1(d), 39.6(t), 45.4(d), 59.2(q), 60.3(d), 63.5(s), 70.4(t), 70.8(d), 78.1(d), 121.3(d), 125.8(d), 134.0(s), 140.6(s), 177.2(s).

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