7-Epidenticulatolide, a New Cembranolide with a Cyclic Peroxide Function from the Soft Coral *Lobophytum denticulatum*

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Synopsis. A new cembranolide diterpene, 7-epidenticulatolide, was isolated from a soft coral, *Lobophytum denticulatum*. The structure was deduced spectroscopically and determined by X-ray crystallography.

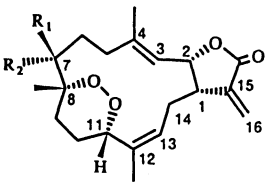
In connection with our continuing chemical investigation of soft corals,[1a–i] we have previously reported the structure of a new cembranolide, denticulatolide (**1**),[1g] as the major metabolite of the soft coral *Lobophytum denticulatum* (Tixie–Durivault, 1956). In this paper we report on the structural determination of an additional highly oxidized cembranolide, 7-epidenticulatolide (**2**), which was obtained as a minor metabolite of *L. denticulatum*.

7-Epidenticulatolide (**2**) was isolated as a crystalline compound and recrystallized from ether as rectangular plates; mp 125.0—126.0 °C, and showed $[\alpha]_D$ −58.4° (*c* 2.3, $CHCl_3$). The molecular formula $C_{22}H_{30}O_6$ was established by a high-resolution mass measurement (M^+, 390.2075, Calcd MW=390.2042) and ^{13}C NMR spectrometry.

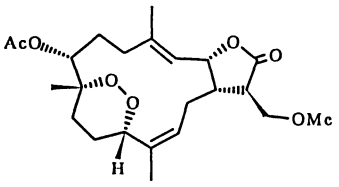
A comparison of the ^1H NMR (400 MHz) spectral data for **2** with those of **1** clearly showed their similarity, and allowed assignments for each proton in **2** as follows: A tertiary methyl group (δ=1.12, 3H, s), two olefinic methyl groups (δ=1.73, 3H, br s; 1.81, 3H, br s), an acetoxyl group (δ=2.09, 3H, s), a pair of allylic methylene protons (δ=2.60, 1H, diffused br d, *J*=15.8 Hz; 2.93, 1H, ddd, *J*=15.8, 10.4, 10.4 Hz), an allylic proton (δ=3.30, 1H, m), a cyclic peroxide methine proton (δ=4.47, 1H, dd, *J*=10.7, 3.9 Hz), two olefinic protons (δ=5.23, 1H, br d, *J*=8.4 Hz; 5.50, 1H, br dd, *J*=10.4, 4.8 Hz), a methine proton (δ=5.33, 1H, dd, *J*=8.4, 7.1 Hz) on a carbon bearing a lactone oxygen, *exo*-methylene protons (δ=5.78, 1H, d, *J*=2.0 Hz; 6.27, 1H, d, *J*=2.9 Hz) conjugated with the lactonic carbonyl group, and a methine (δ=6.04, 1H, dd, *J*=10.3, 3.2 Hz) bearing an acetoxyl group. ^1H NMR decoupling studies clarified the relative locations of the two double bonds as well as the lactone group (experimental). Treatment of **2** with sodium in methanol yielded compound **3**, $C_{23}H_{34}O_7$, resulting from a conjugate addition of methanol to the *exo*-methylene group of the α,β-unsaturated lactone.[2] Based on the above-mentioned spectral and chemical data, compound **2** was assumed to have a similar structure and the location of its functional groups similar to those of denticulatolide (**1**).

An extensive NMR spectral analysis demonstrated that **2** is a stereoisomer of **1** about the trisubstituted double bonds and/or the acetoxyl group, since one of the olefinic protons observed in **1** at δ=5.50 (H-3), was observed at a higher field in the spectrum of **2** at δ=5.23, while another olefinic proton of **1** at δ=5.36 (H-13) was shifted in **2** at δ=5.50; a doublet (*J*=9.5 Hz) of the methine proton on a carbon bearing the acetoxyl group observed in **1** at δ=5.78 appeared as a double doublet (*J*=10.3, 3.2 Hz) in **2** at δ=6.04. An analysis of the available NMR data did not unambiguously suggest the structure or the relative stereochemistry for **2** due to the flexible cembrane ring. Hence, the structure of **2** was solved by a single-crystal X-ray crystallographic method.

Crystals of **2** recrystallized from diethyl ether were monoclinic, space group $P2_1$ with cell dimensions of *a*=9.575(2), *b*=34.111(3), *c*=6.563(1) Å, β=91.14(1)°, and *Z*=4. Although attempts to solve the X-ray crystal



1 R_1=OAc, R_2=H
2 R_1=H, R_2=OAc



3

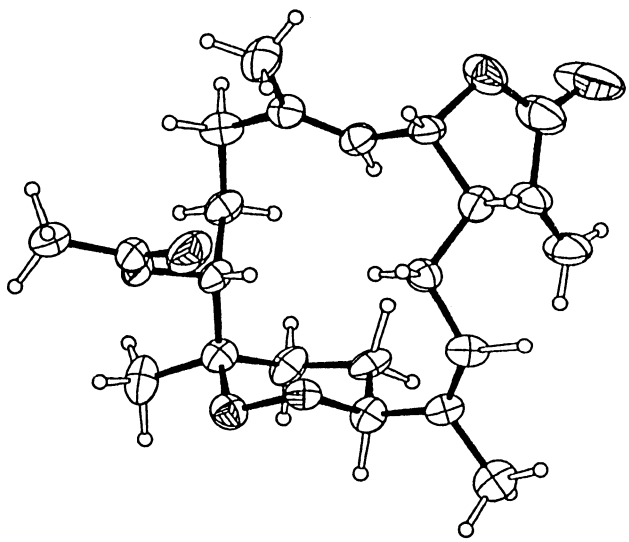


Fig. 1. Crystal structure of 7-epidenticulatolide (**2**).

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structure with MULTAN-78[3] were unsuccessful, the structure was eventually determined by using a Monte Carlo direct method,[4] and refined by block-diagonal least-squares calculations[5] to the final crystallographic residual of 0.066 for 2889 unique reflections with $|F_o|>3\sigma(F_o)$.[6] The computer-generated perspective drawing of the final X-ray model of **2** given in Fig. 1 shows that **2** differs from **1** only regarding the C-7 configuration. The X-ray analysis thus defined the structure of **2** and gave the relative stereochemistry of the molecule in this experiment.

Experimental

Measurements. The NMR spectra were recorded using a Hitachi R-90H (90 MHz) or a JEOL GX-400 (400 MHz) spectrometer. Mass spectra were determined on a Hitachi RMU-6L mass spectrometer. A high resolution-mass measurement was performed with a Hitachi RMU-7L mass spectometer. X-ray intensity data were collected on a Syntex P3 four-circle diffractometer operating in the ω–2θ scan mode, and the intensities were measured for $2\theta \leq 55°$.

Extraction and Isolation of 7-Epidenticulatolide (2) from Lobophytum Denticulatum. The fresh soft coral (2.8 kg, wet weight), collected by scuba diving along a coral reef near Miyako island of Okinawa, was chipped and extracted several times with methanol. The metanol extract was partitioned between brine and dichloromethane, and the dichloromethane extract was subsequently dried over $MgSO_4$, which was filtered and evaporated to afford a crude organic extract (72 g). A part of the extract (30 g) was subjected to rapid fractionation using a preparative Medium Pressure LC (MPLC) on silica gel (230—400 mesh) with solvents of gradually increasing polarity from dichloromethane through methanol. Elution with dichloromethane gave fractions containing a mixture of cembranolides, **1** and **2**. These fractions were rechromatographed on silica-gel by MPLC with hexane–EtOAc (5 : 1) to separate the less polar cembranolide **1** (*ca.* 1000 mg) and the more polar cembranolide **2**. The later compound **2** was further purified by repetition of MPLC with the same solvent mixture and preparative TLC with petroleum ether (35—45 °C)–diethyl ether (2 : 1) to give colorless crystals (50 mg). Compound **2** exhibited the following spectral features: IR ($CHCl_3$) 1755, 1730, 1660, 1235, and 890 cm^{-1}; ^{13}C NMR (22.6 MHz, $CDCl_3$) δ=18.8 (q), 19.9 (q), 21.0 (q), 24.2 (t), 24.7 (q), 26.3 (t), 28.9 (t), 31.5 (t), 33.6 (t) 42.6 (d), 70.9 (d), 78.6 (d), 82.2 (s), 82.8 (d), 120.7 (d), 122.6 (t), 126.6 (d), 135.0 (s), 138.6 (s), 140.3 (s), 170.0 (s), and 170.9 (s); ^1H NMR (400 MHz, $CDCl_3$) δ=1.12 (3H, s, 8-Me), 1.73 (3H, br s, 4-Me), 1.81 (3H, br s, 12-Me), 2.09 (3H, s, OAc), 2.60 (1H, br d, J=15.8 Hz, 14-H), 2.93 (1H, ddd, J=15.8, 10.4, and 10.4 Hz, 14-H′), 3.30 (1H, m, 1-H), 4.47 (1H, dd, J=10.7 and 3.9 Hz, 11-H), 5.23 (1H, br d, J=8.4 Hz, 3-H), 5.33 (1H, dd, J=8.4 and 7.1 Hz, 2-H), 5.50 (1H, br dd, J=10.4 and 4.8 Hz, 13-H), 5.78 (1H, d, J=2.0, 16-H), 6.04 (1H, dd, J=10.3 and 3.2 Hz, 7-H), and 6.27 (1H, d, J=2.9 Hz, 16-H′); double resonance experiments: irradiation of the olefinic proton at δ=5.50 sharpened a broad singlet of the olefinic methyl at δ=1.81 and simultaneously changed a pair of an allylic methylene protons at δ=2.60 and 2.93 to a broad double doublet (J=15.8, 5.6 Hz) and a double doublet (J=15.8, 10.4 Hz), respectively; irradiation of the methyl at δ=1.81 reduced long range coupling from the olefinic proton at δ=5.50 to become a sharp double doublet (J=10.4, 4.8 Hz) and caused only one proton signal at δ=2.60 of the allylic methylene group to become clear doublets of a double doublet (J=15.8, 5.6,4.8 Hz); irradiation of the allylic proton at δ=3.30 collapsed a pair of doublets at δ=5.78 and 6.27 to two sharp singlets and a double doublet of the methine carrying the lactone oxygen at δ=5.33 to a doublet (J=8.4 Hz) and further decoupled the allylic methylene signals at δ=2.60 and 2.93 to change a broad double doublet (J=15.8, 4.8 Hz) and a double doublet (J=15.8 Hz, J=10.4 Hz), respectively. MS (EI, 70 eV) m/z 390 (0.4%), 372 (0.6), 330 (1.1), 147 (11), 133 (13), 131 (13), 122 (10), 121 (16), 119 (14), 109 (19), 107 (15), 105 (21), 99 (21), 97 (10), 95 (20), 94 (12), 93 (28), 91 (31), 84 (13), 83 (19), 81 (32), 79 (25), 77 (22), 71 (12), 67 (13), 55 (29), 53 (19), 43 (100), and 41 (29).

Formation of the Methanol Adduct 3. Compound **2** (20 mg) was dissolved in methanol (10 ml) and minute amounts of sodium (ca. 1 mg) were added; the reaction mixture was then boiled under reflux for 1 h. After cooling, the solvent was evaporated and the residue partitioned between ether and water. The ether solution was washed with 0.5 mol dm^{-3} HCl, sat $NaHCO_3$ and dried over Na_2SO_4. The solvent was evaporated to give a mixture which was chromatographed on preparative TLC with SiO_2 and 1% MeOH–$CHCl_3$ to afford unchanged **1** (2 mg) and the main reaction product **3** (12 mg) as a crystalline solid: Mp 139.5—140.0 °C; $[\alpha]_D$ −108.7° (*c* 0.70, $CHCl_3$); M^+ 422; IR ($CHCl_3$) 1760, 1735, and 1230 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$); δ=1.13 (3H, s), 1.60 (3H, br s), 1.72 (3H, br s), 1.81 (3H, t, J=1.7 Hz), 2.09 (3H, s), 2.39 (1H, br d, J=15.4 Hz), 2.94 (1H, ddd, J=15.4, 11.7, and 11.7 Hz), 3.06 (1H, m), 3.35 (3H, s, OMe), 3.51 and 3.81 (each 1H, dd, J=9.5 and 3.4 Hz and dd, J=9.5 and 2.2 Hz, $-CH_2OMe$), 4.42 (1H, dd, J=13.4 and 3.2 Hz), 5.25 (1H, br d, J=9.0 Hz), 5.35 (1H, dd, J=9.0 and 7.6 Hz), 5.45 (1H, br dd, J=11.7 and 4.0 Hz), and 6.12 (1H, dd, J=11.5 and 2.0 Hz). The structure and relative stereochemistry of the methanol adduct **3** was also confirmed by X-ray crystallography on the crystals.[7]

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5) The crystallographic calculations were done using the UNICSIII Programs, T. Sakurai and K. Kobayashi, *Rep. Inst. Phys. Chem. Res. (Jpn.)*, **55**, 69 (1979).

6) Final crystallographic data have been deposited as Document No. 8985 at the Office of the Editor of Bull. Chem. Soc. Jpn.

7) Crystals of **3** recrystallized from CH_2Cl_2–hexane has the following crystallographic data: Monoclinic, space group $C2$, a=17.373(2), b=7.041(1), c=19.010(2) Å, β=95.41(2)°, and Z=4. The crystal structure was solved using the MULTAN-78 programs[3] and refined by the block-diagonal least-squares calculations[5] to the final R value of 7.46% on 2505 reflections. Final crystallographic data have been deposited as Document No. 8985 at the Office of the Editor of Bull. Chem. Soc. Jpn.