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DENTICULATOLIDE, AN ICHTHYOTOXIC PEROXIDE-CONTAINING CEMBRANOLIDE FROM THE SOFT CORAL *LOBOPHYTUM DENTICULATUM*

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Summary: Denticulatolide, an ichthyotoxic cembranoid diterpene bearing acetoxy, α-methylene γ-lactone, and cyclic peroxide functions, has been isolated from the soft coral *Lobophytum denticulatum*. The structure (**1**) has been determined by spectral and chemical evidence and X-ray crystallographic method.

During our continuing search[1] for biologically active compounds of soft corals, we examined *Lobophytum denticulatum* (Tixie-Durivault, 1956), collected near Miyako island of Okinawa, Japan, and isolated a new cembranolide which was toxic to the Medaka (*Oryzias latipes*) at a concentration of 5 μg/ml in laboratory test. In this paper we report on the isolation and structure of the new cembranolide which we have named denticulatolide.

Dichloromethane extraction of the freeze dried soft coral and subsequent preparative medium-pressure liquid chromatography on silica gel (230-400 mesh) using 5% methanol in dichloromethane yielded an amorphous solid **1** (ca. 1% dry weight). Denticulatolide (**1**) was recrystallized from hexane-dichloromethane as colorless prisms, mp 129.0-130.5 °C, $[\alpha]_D$ +1.4° (c 0.5, $CHCl_3$). High resolution mass spectrometry established the molecular formula of $C_{22}H_{30}O_6$ ($M^{+\cdot}$ 390.2065, calcd. 390.2042), indicating eight degrees of unsaturation. The ^{13}C NMR ($CDCl_3$) spectrum showed the presence of two ester carbonyl carbons (δ 170.3s, 170.5s), six olefinic carbons (δ 121.1t, 123.3d, 125.4d, 135.5s, 138.4s, 140.2s) due to one exo-methylene and two trisubstituted double bonds, four oxygen-bearing carbons (δ 70.1d, 79.3d, 80.0s, 84.2d), one methine carbon (δ 44.5d), five methylene carbons (δ 24.7t, 26.0t, 28.0t, 31.0t, 35.5t), and four methyl carbons (δ 16.4q, 19.5q, 21.0q, 24.3q), therefore **1** was tricyclic. The IR ($CHCl_3$) and ^1H NMR ($CDCl_3$, 90 MHz) spectra indicated the presence of an acetoxy group (ν 1730 cm^{-1}; δ 2.10, 3H, s) and an α-methylene γ-lactone (ν 1760 cm^{-1}; δ 5.63 and 6.23, both 1H, d, J=1.0 Hz). These data suggested that **1** possessed a 14-membered monocarbocyclic ring of the cembrane skeleton, a γ-lactone ring and a third extra ring. Since the IR spectrum of **1** showed no hydroxyl absorption and four of the six oxygens in **1** were accounted for with the acetoxy and lactone groups, the remaining two oxygens appeared to be present as part of a cyclic peroxide which was the third ring. The peroxide was chemically confirmed by positive KI test.

The ^1H NMR spectrum also revealed the presence of a tertiary methyl group (δ 1.09, 3H, s, H-19), two methyl-bearing trisubstituted double bonds (δ 1.68, 3H, br s, H-18; 1.77, 3H, t, J=

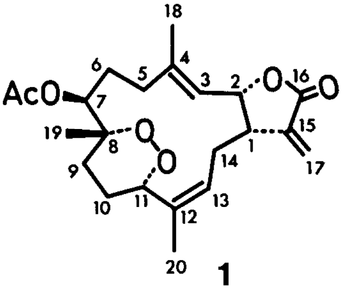
1.5 Hz, H-20; 5.36, 1H, m, H-13; 5.50, 1H, br d, J=7.0 Hz, H-3), one allylic methine proton (δ 2.81, 1H, m, H-1), one proton of an allylic methylene group (δ 3.09, 1H, ddd, J=14.0, 11.0, 2.5 Hz, H-14a), an oxygen-carrying methine proton (δ 4.33, 1H, dd, J=12.5, 2.5 Hz, H-11), a lactonic methine proton (δ 5.20, 1H, dd, J=7.0, 6.5 Hz, H-2) and an acetoxy methine proton (δ 5.78, 1H, d, J=9.5 Hz, H-7). Decoupling experiments of well separated signals in the solvent-shifted spectrum (C_6D_6)[2] of **1** revealed the following interproton relationships (Table 1) which established the locations of the two trisubstituted double bonds and γ-lactone moiety.

Table 1. ^{1}H NMR decoupling data for **1** (C_6D_6, 90 MHz)

Irradiated proton	Observed proton	Change of signals (J, Hz)
2.55 (H-1)	3.11 (H-14a)	ddd → dd (14.0, 11.0)
	4.86 (H-2)	dd → d (7.0)
	5.19 and 6.17 (exo-methylene)	two doublets → two singlets
3.11 (H-14a)[a]	2.55 (H-1)	m → br dd (12.0, 6.5)[b]
	5.18 (H-13)	m → br s[c]
4.86 (H-2)	2.55 (H-1)	m → br d (12.0)[b]
	5.71 (H-3)	br d → br s[d]
5.18 (H-13)	3.11 (H-14a)	ddd → dd (14.0, 2.5)
	1.54 (H-20)	t → d (1.5)
5.71 (H-3)	4.86 (H-2)	dd → d (6.5)
	1.41 (H-18)	br s → sharpening

[a] Irradiation at δ 3.11 also detected the signal of H-14b proton overlapping with a different methylene protons near δ 1.80 (3H, complex m) and reverse irradiation at δ 1.80 changed the signal patterns of three mutually coupled protons as follows: δ 2.55 (H-1, m → br d, J=6.5 Hz), 3.11 (H-14a, ddd → dd, J=11.0, 2.5 Hz), and 5.18 (H-13, m → br d, J=11.0 Hz).
[b] Broadened by allylic coupling with the exo-methylene. [c] Broadened by allylic coupling with the methyl (H-20). [d] Broadened by allylic coupling with the methyl (H-18).

Reduction of **1** with Zn in AcOH resulted in cleavage of the peroxide bond to obtain the diol **2**,[3] $C_{22}H_{32}O_6$ ($M^{+\cdot}$ 392), mp 79.0-80.0 °C, $[\alpha]_D$ +1.6° (c 1.5, $CHCl_3$); IR ($CHCl_3$) 3620, 3300 cm^{-1}, which on oxidation with pyridinium dichromate in CH_2Cl_2 yielded the hydroxy α,β-unsaturated ketone **3**,[4] $C_{22}H_{30}O_6$ ($M^{+\cdot}$ 390), $[\alpha]_D$ +4.6° (c 1.1, $CHCl_3$); IR ($CHCl_3$) 3400, 1680 cm^{-1}; UV (EtOH) λ_{max} 238 nm (ε 3480), thus indicating that the peroxide carbons are tertiary and allylic secondary. Oxidation of **2** with pyridinium chlorochromate in CH_2Cl_2, however, gave a 2',5',5'-trisubstituted dihydrofuran derivative **4**,[5] $C_{22}H_{28}O_5$ ($M^{+\cdot}$ 372), $[\alpha]_D$ -200° (c 0.9, $CHCl_3$), which showed in its ^{1}H NMR spectrum ($CDCl_3$) two methylene protons of the dihydrofuran system at δ 2.23 and 2.44 (both 1H, dd, J=16.0, 2.0 Hz), which on irradiation at δ 4.58 (1H, t, J=2.0 Hz) due to the vicinal olefinic proton were converted to an AB quartet (J=16.0 Hz). Apparently the formation of **4** was accounted for with a six-membered peroxide ring in **1**. Reduction of the lactone of **1** with Al(*i*-Bu)$_2$H (1.5 eq.) in toluene at -78 °C followed by treatment of the resulting aldol with ethanolic $NaBH_4$ at 0 °C gave the diol **5**, $C_{22}H_{34}O_6$ ($M^{+\cdot}$ 394), mp 164.0-165.0 °C, $[\alpha]_D$ +21.8° (c 1.0, $CHCl_3$); IR ($CHCl_3$) 3610, 3350 cm^{-1}. In the ^{1}H NMR spectrum ($CDCl_3$) of **5**, the secondary alcohol methine proton appeared at δ 4.46 (1H, dd, J=7.0, 1.0 Hz), whose chemical shift and coupling mode approximated those of the related diol obtained by the same way from



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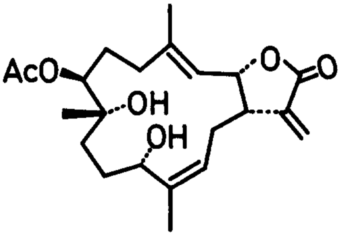

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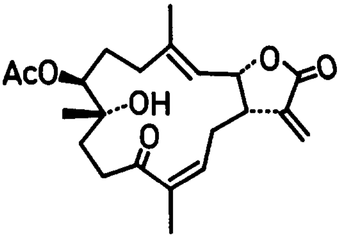

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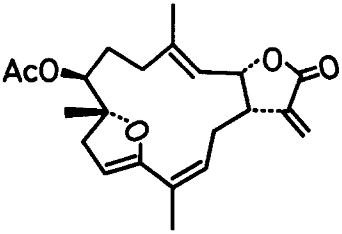

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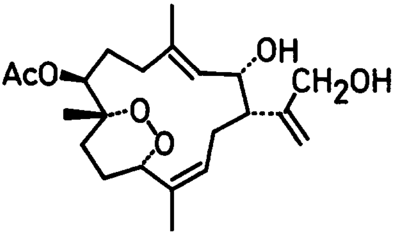
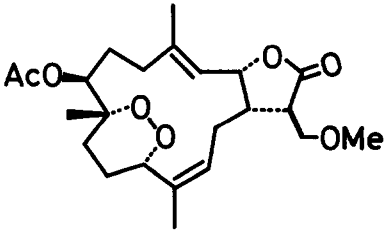


5

a *cis*-fused cembranolide isolated from the soft coral *Sinularia mayi*.[1c] On the basis of these spectral and chemical data, the cembranolide structure **1**, which contained an acetoxy group and a cyclic peroxide, was deduced for denticulatolide. However, the location of the acetoxy group and the stereochemistry of the trisubstituted double bonds and of the chiral centers of the peroxide moiety could not be defined from the available data. A single-crystal X-ray analysis was therefore carried out on suitable derivative **6**, since crystalline denticulatolide did not form adequate crystals for the analysis.

Treatment of **1** with Na in MeOH yielded the crystalline compound **6**, $C_{23}H_{34}O_7$ ($M^{+\cdot}$ 422), mp 188.5-189.5 °C (acetone-water). The crystals belonged to the orthorhombic system, space group $P2_12_12_1$, with a=18.103(4), b=12.236(6), c=10.404(4)Å, D_c =1.22 gcm^{-3} and Z=4. The structure was solved using the MULTAN-78 programs[6] and refined by the block-diagonal least-squares calculations to the final R-value of 7.22%[7] on 1916 reflections with $|Fo|>3\sigma(Fo)$.[8] Figure 1 is a computer generated perspective drawing of the final X-ray model of **6**, hence giving structure **1** for denticulatolide: the acetoxy group is located at C-7; the geometry of two trisubstituted double bonds are C-3(E) and C-12(Z) and the relative configurations are C-7(S*), C-8(R*), and C-11(S*).



6

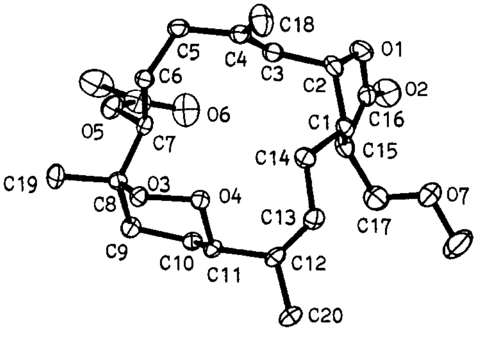


Figure 1. Crystal structure of compound **6**.

Cyclic peroxides are rare natural products[9] and denticulatolide (**1**) is the first example of diterpenoid containing a cyclic peroxide from marine sources, although cyclic peroxides of nordi- and norsesterterpenoids have been isolated to date as sponge metabolites.[10]

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References and Notes

1. a) Y. Uchio, H. Nabeya, M. Nakayama, S. Hayashi and T. Hase, *Tetrahedron Letters*, 22, 1689 (1981). b) Y. Uchio, J. Toyota, H. Nozaki, M. Nakayama, Y. Nishizono and T. Hase, *Tetrahedron Letters*, 41, 4089 (1981). c) Y. Uchio, S. Eguchi, M. Nakayama and T. Hase, *Chemistry Letters*, 1982, 277. d) Y. Uchio, M. Nitta, M. Nakayama, T. Iwagawa and T. Hase, *Chemistry Letters*, 1983, 613. e) Y. Uchio, M. Nitta, H. Nozaki, M. Nakayama, T. Iwagawa and T. Hase, *Chemistry Letters*, 1983, 1719. f) M. Aoki, T. Kato, Y. Uchio, M. Nakayama and M. Kodama, *Bull. Chem. Soc. Jpn.*, 58, 779 (1985).
2. **1**: ^{1}H NMR (C_6D_6): δ 0.98 (3H, s, H-19), 1.41 (3H, br s, H-18), 1.54 (3H, t, J=1.5 Hz, H-20), 1.68 (3H, s, AcO), 2.55 (1H, m, H-1), 3.11 (1H, ddd, J=14.0, 11.0, 2.5 Hz, H-14a), 4.23 (1H, dd, J=12.0, 2.0 Hz, H-11), 4.86 (1H, dd, J=7.0, 6.5 Hz, H-2), 5.18 (1H, m, H-13), 5.19 and 6.17 (both 1H, d, J=1.0 Hz, exo-methylene), 5.71 (1H, br d, J=7.0 Hz, H-3), 5.86 (1H, d, J= 9.0 Hz, H-7).
3. **2**: ^{1}H NMR ($CDCl_3$): δ 1.19 (3H, s, H-19), 1.64 (3H, br s, H-18), 1.71 (3H, br s, H-20), 2.06 (3H, s, AcO), 3.00 (1H, m, H-1), 3.04 and 3.80 (both 1H, broad D_2O exchangeable peak), 4.24 (1H, dd, J=9.5, 3.0 Hz, H-11), 5.09 (1H, d, J=8.0 Hz, H-7), 5.27 (1H, dd, J=9.0, 6.0 Hz, H-3), 5.29 (1H, m, H-13), 5.48 (1H, br d, J=9.0 Hz, H-2), 5.60 (1H, d, J=1.0 Hz, H-17a), 6.22 (1H, d, J=1.5 Hz, H-17b).
4. **3**: ^{1}H NMR ($CDCl_3$): δ 1.21 (3H, s, H-19), 1.63 (3H, br s, H-18), 1.84 (3H, t, J=1.5 Hz, H-20), 2.07 (3H, s, AcO), 2.91 (1H, m, H-1), 3.26 (1H, ddd, J=20.0, 12.0, 3.0 Hz, H-10a), 3.72 (1H, br s, D_2O exchangeable peak), 4.82 (1H, d, J=8.0 Hz, H-7), 5.23 (1H, dd, J=8.0, 6.0 Hz, H-2), 5.44 (1H, br d, J=8.0 Hz, H-3), 5.58 (1H, m, H-13), 5.64 (1H, d, J=1.0 Hz, H-17a), 6.31 (1H, d, J=1.5 Hz, H-17b). On irradiation at δ 1.84 the signal at δ 5.58 was transformed into a double doublet (J=10.0, 4.0 Hz). ^{13}C NMR ($CDCl_3$): δ 15.8q, 20.1q, 21.2q, 23.8q, 26.3t, 29.1t, 32.4t, 36.2t, 38.3t, 44.4d, 72.3s, 73.3d, 78.5d, 122.5t, 122.8d, 126.3d, 138.9s, 140.1s, 140.1s, 170.1s, 170.9s, 213.0s.
5. Because of acidic nature of the oxidizing reagent (PCC), the resultant keto-alcohol produced a hemiacetal as intermediate and subsequent dehydration would lead to the formation of the dihydrofuran ring. **4**: ^{1}H NMR ($CDCl_3$): δ 1.38 (3H, s, H-19), 1.60 (3H, d, J=1.0 Hz, H-18), 1.79 (3H, t, J=1.0 Hz, H-20), 2.06 (3H, s, AcO), 3.24 (1H, m, H-1), 4.96 (1H, dd, J=6.0, 1.0 Hz, H-7), 5.00 (1H, br d, J=11.0 Hz, H-3), 5.26 (1H, m, H-13), 5.29 (1H, dd, J=11.0, 7.0 Hz, H-2), 5.53 (1H, d, J=3.0 Hz, H-17a), 6.27 (1H, d, J=3.5 Hz, H-17b); ^{13}C NMR ($CDCl_3$): δ 15.7q, 19.0q, 20.7q, 21.2q, 27.0t, 27.8t, 37.7t, 39.4t, 44.7d, 71.7d, 78.7d, 86.3s, 99.8d, 120.0t, 120.8d, 127.4d, 130.3s, 138.1s, 142.1s, 154.4s, 170.9s, 171.1s.
6. P. Main, S. E. Hall, L. Lessinger, G. Germain, J.-P. Declercq and M. M. Woolfson, MULTAN-78, *A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data*, University of York, England, 1978.
7. The crystallographic calculations were done using the UNICS III programs, T. Sakurai and K. Kobayashi, *Rep. Inst. Phys. & Chem. Res.* (Japan), 55, 69 (1979).
8. Crystallographic data have been deposited with the Cambridge Crystallographic Data Center.
9. W. Adam and A. J. Bloodworth, *Ann. Rep. Prog. Chem.*, 75B, 342 (1978).
10. a) Y. Kashman and M. Rotem, *Tetrahedron Letters*, 1979, 1707.
    b) S. Sokoloff, S. Halevy, V. Usieli, A. Colorni and S. Sarel, *Experientia*, 38, 337 (1982).
    c) M. Albericci, J. C. Braekman, D. Daloze and B. Tursch, *Tetrahedron*, 38, 1881 (1982).
    d) L. V. Manes, G. J. Bakus and P. Crew, *Tetrahedron Letters*, 25, 931 (1984).

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