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LOBOHEDLEOLIDE AND (7Z)-LOBOHEDLEOLIDE, NEW CEMBRANOLIDES FROM THE SOFT CORAL *LOBOPHYTUM HEDLEYI* WHITELEGGE

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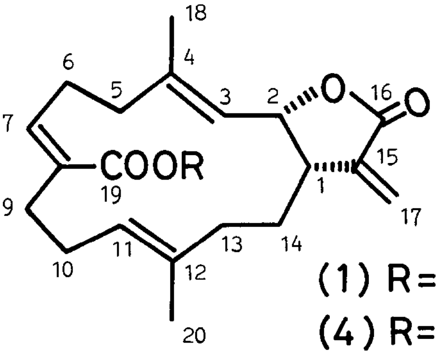
Summary: The structures of lobohedleolide (1) and (7Z)-lobohedleolide (2), two new cembranolides containing the α,β-unsaturated carboxylic acid system isolated from the Japanese soft coral *Lobophytum hedleyi* Whitelegge, were elucidated from spectral and chemical evidence, and the absolute configuration of (1) was determined by X-ray analysis of its *p*-bromophenacyl ester (9). Lobohedleolide (1) showed growth inhibition of the *in vitro* Hella cells.

In the course of bioactive and structural studies of metabolites in soft corals, we have recently reported[1] the isolation of two new cembrane diterpenes, cembrenene and mayol, and cembrene of marine origin, from *Sinularia mayi* Lüttschw. Subsequently, we investigated the terpenoid constituents of the soft coral *Lobophytum hedleyi* Whitelegge,[2] collected in the coral reefs of Yayeyama islands of Okinawa, Japan, and isolated two new cembranoid lactonic acids, named lobohedleolide (1) and (7Z)-lobohedleolide (2). In this communication we report the isolation and structures of these two compounds. The dichloromethane-soluble material from a methanolic extracts of *L. hedleyi* was chromatographed over Si gel with CH_2Cl_2-MeOH (95:5) to obtain the major cembranolide (1) and the minor one (2) in 0.3 and 0.002% from dried animal, respectively.

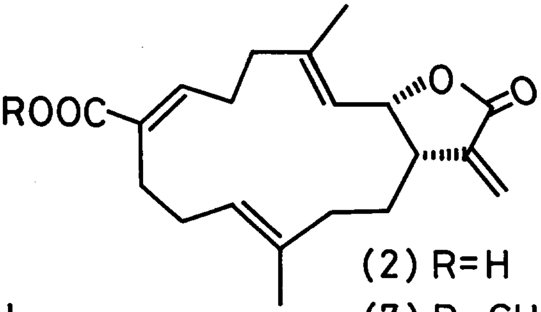
Lobohedleolide (1) was isolated as colorless prism, m.p. 183.0-184.0 °C (EtOH), $\{\alpha\}_D$ +104.2° (c 1.12, $CHCl_3$). The molecular formula $C_{20}H_{26}O_4$ was established by elemental analysis (Found: C, 72.43; H, 8.03. Calcd: C, 72.70; H, 7.93%); $M^{+\cdot}$ 330. The IR ($CHCl_3$), UV (EtOH) and ^1H NMR ($CDCl_3$) spectra indicated the presence of an α,β-unsaturated carboxyl group (ν 3000-2500, 1685 and 1630 cm^{-1}; λ_{max} 217 nm, ε 9800) and an α-methylene α,β-unsaturated γ-lactone (ν 1760 cm^{-1}; δ 5.55, 1H, d, J=2.5 Hz and δ 6.27, 1H, d, J=3.0 Hz) as evidenced by formation of the following derivatives. Treatment of (1) with ethereal CH_2N_2 furnished a crystalline pyrazoline methyl ester (3),[3] $C_{22}H_{30}O_4N_2$ ($M^{+\cdot}$ 386), m.p. 106.5-107.0 °C (MeOH), $\{\alpha\}_D$ -35.1° (c 1.06, $CHCl_3$), and also reaction of the silver salt[4] of (1) with CH_3I in refluxing benzene afforded a methyl ester (4),[3] $C_{21}H_{28}O_4$ ($M^{+\cdot}$ 344), m.p. 85.5-86.5 °C (EtOH), $\{\alpha\}_D$ +102.6° (c 1.33, $CHCl_3$). The ^1H NMR spectrum of (1) furthermore exhibited signals for two methyl-bearing trisubstituted double bonds (δ 1.55, 3H, br s; 1.73, 3H, br s; 4.97, 1H, br t, J=7.0 Hz; 5.06, 1H, br d, J=10.0 Hz), a vinylic proton (δ 5.76, 1H, dd, J=8.0 and 4.0 Hz) due to the β-proton on an α,β-unsaturated carboxyl group, a highly deshielded lactonic methine proton (δ 5.44, 1H, dd, J=10.0 and 7.5 Hz), and two allylic protons (δ 3.11, 2H overlapping, m). Decoupling experiments showed that the



(1) R=H

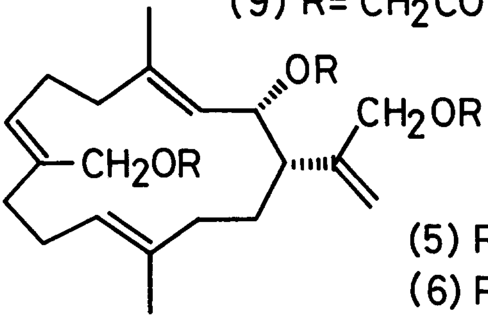
(4) R= CH_3

(9) R= $CH_2COC_6H_4Br(p)$



(2) R=H

(7) R= CH_3



(5) R=H

(6) R=Ac

methyls at δ 1.55 and 1.73 were allylically coupled to the vinylic protons at δ 4.97 and 5.06, respectively, and the lactonic methine proton at δ 5.44 was mutually coupled to both the vinylic proton at δ 5.06 and one of the protons at δ 3.11 in the values of J=10.0 and 7.5 Hz, respectively. The latter one proton of δ 3.11 in turn exhibited clear long-range couplings to a pair of exo-methylene protons at δ 5.55 (J=2.5 Hz) and 6.27 (J=3.0 Hz), thus confirming the allylic nature and the ring size of the lactone. Based on the above spectral and chemical properties, structure (1) was deduced for lobohedleolide which was a new cembranolide diterpene with an α,β-unsaturated carboxyl group. This was further corroborated by its ^{13}C NMR spectrum ($CDCl_3$): δ 15.3q, 16.1q, 24.9t, 26.7t, 27.0t, 35.1t, 36.1t, 39.8t, 43.0d, 78.1d, 120.6d, 120.6t, 122.9d, 129.1s, 135.4s, 138.8s, 142.0s, 148.2d, 170.7s and 173.2s.

Reduction of the methyl ester (4) with $(i\text{-}Bu)_2AlH$ in toluene (-78 °C), after quenched with a MeOH, followed by addition of ethanolic $NaBH_4$, afforded a monocarbocyclic triol (5), $C_{20}H_{32}O_3$ ($M^{+\cdot}$ 320), $\{\alpha\}_D$ +98.2° (c 1.02, $CHCl_3$), which on acetylation with Ac_2O/Py gave a triacetate (6), $C_{26}H_{38}O_6$ ($M^{+\cdot}$ 446), $\{\alpha\}_D$ +37.3° (c 1.07, $CHCl_3$); δ 1.98 (3H, s) and 2.06 (6H, s). The secondary alcoholic methine protons of the triol (5) and its acetate (6) appeared as a double doublets at δ 4.43 (J=8.5 and 1.0 Hz) and δ 5.58 (J=9.0 and 1.5 Hz), respectively, whose chemical shifts and coupling constants were of the same order magnitudes as the values reported for related cembranoids, mukulol and its acetate,[5] hence supporting the allylic nature and *cis*-junction of the γ-lactone ring of (1). Micro-ozonolysis[6] of the methyl ester (4) in AcOEt at -78 °C and successive GC-MS analysis of the products mixture afforded 4-oxopentanal[7] and methyl 2,5-dioxopentanoate[7] in a ratio of 1 : 1 (as determined from the GLC peak areas). This result established the locations of the carboxyl group (C-19) and the trisubstituted double bonds of (1). The *trans* geometry of the trisubstituted double bonds and about the α,β-unsaturated carboxyl group could be deduced from the chemical shifts of the vinylic methyl carbons and the β-vinylic proton on the unsaturated carboxyl group.

The second cembranolide (2) was isolated as an oil, $\{\alpha\}_D$ +61.4° (c 1.01, $CHCl_3$), and was found to possess the same molecular formula of $C_{20}H_{26}O_4$ ($M^{+\cdot}$ 330.1839. Calcd. 330.1829) as (1). IR ($CHCl_3$) ν 3000-2500, 1760, 1685 and 1630 cm^{-1}; UV (EtOH) λ_{max} 215 nm (ε 12500); ^1H NMR ($CDCl_3$) δ 1.56 (3H, br s), 1.84 (3H, br s), 3.10 (2H, m), 4.95 (1H, br t, J=8.0 Hz), 4.99 (1H, br d, J=10.0 Hz), 5.43 (1H, dd, J=10.0 and 8.0 Hz), 5.52 (1H, d, J=2.5 Hz), 6.27 (1H, d, J=3.0 Hz) and 6.89 (1H, m); ^{13}C NMR ($CDCl_3$) δ 15.0q, 17.3q, 24.9t, 26.3t, 27.5t, 28.6t, 37.1t, 39.5t, 43.3d, 77.9d, 120.9t, 121.1d, 123.1d, 130.4s, 135.5s, 138.5s, 142.2s, 146.9d, 170.7s and 172.9s. These spectral features were highly similar to those of (1) except for the large downfield shift of one vinylic proton (δ 5.76 in (1) and δ 6.89 in (2)). Like lobohedleolide, (2) gave a pyrazoline methyl ester (7),[3] $C_{22}H_{30}O_4N_2$ ($M^{+\cdot}$ 386), $\{\alpha\}_D$ -31.0° (c 1.40, $CHCl_3$) and a methyl ester (8),[3] $C_{21}H_{28}O_4$ ($M^{+\cdot}$ 344), $\{\alpha\}_D$ +73.3° (c 0.90, $CHCl_3$), hence containing the same functional groups as (1). Moreover, since micro-ozonolysis of the methyl ester (8) also afforded 4-oxopentanal and methyl 2,5-dioxopentanoate, the positions of the double bonds and the carboxyl group must be the same as (1). The low field ^1H signal at δ 6.89 in (2) was readily assignable to the β-proton on the α,β-unsaturated carboxyl group with *cis* geometry. On the basis of the above facts, the second cembranolide (2) was determined to be the 7Z geometrical isomer of (1).

The absolute configuration of (1) was determined by X-ray crystallographic analysis[8] of its *p*-bromophenacyl ester (9), $C_{28}H_{31}O_5Br$, m.p. 130.0-131.0 °C (EtOH). Crystal data: monoclinic, space group $P2_1$ with a=10.370(2), b=8.463(1) and c=14.975(4)Å, β=94.85(2)°, ρ_c=1.34 gcm^{-3} and Z=2. The crystal structure was solved by heavy atom method and refined by full-matrix least-squares calculations to R=0.060 on 1496 reflections and 0.067 for its enantiomorph, respectively, thus establishing the absolute configuration shown in Figure 1 to (9). Therefore, the absolute configuration of lobohedleolide should be represented by (1). That of (2) was not defined, however, it is presumed to be the same chirality as (1). Lobohedleolide (1) and (7Z)-lobohedleolide (2) are the first carboxylic acid cembranolides from marine sources. It is interesting that the closely related lactonic acid containing the *trans* fused γ-lactone ring, anisomeric acid,[9] has been isolated from the South Indian medicinal plant *Anisomeles malabarica*. In preliminary bioactive test, lobohedleolide of the major metabolite displayed growth inhibition of the *in vitro* Hella cells at 5 μg/ml.

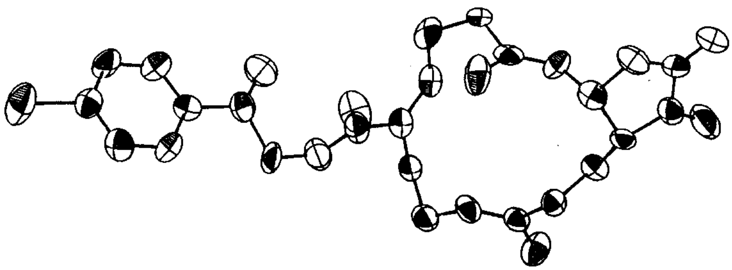


FIGURE 1. MOLECULAR STRUCTURE OF (9)

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References and Notes

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2. The same species of Australian coral contained non-cembranoid diterpene: B. F. Bowden, J. C. Coll, N. Liyanage, S. J. Mitchell, G. C. Stokie and I. A. Van Altena, Aust. J. Chem., 31, 163 (1978).
3. The ^{1}H NMR spectra ($CDCl_3$) of the compounds are as follows: (3); δ 1.48 (3H, br s), 1.78 (3H, d, J=1.0), 3.38 (1H, m), 3.74 (3H, s), 4.94 (1H, br t, J=7.0), 5.20 (1H, br d, J=10.0), 5.61 (1H, dd, J=9.0, 4.0), 5.77 (1H, dd, J=10.0, 7.5). (4); δ 1.53 (3H, br s), 1.73 (3H, d, J=1.0), 3.07 (1H, m), 3.73 (3H, s), 4.94 (1H, t, J=8.0), 5.04 (1H, br d, J=10.5), 5.44 (1H, dd, J=10.5, 8.0), 5.54 (1H, d, J=3.0), 5.59 (1H, dd, J=9.0, 3.0), 6.26 (1H, d, J=3.5). (7); δ 1.42 (3H, br s), 1.86 (3H, d, J=1.0), 3.37 (1H, m), 3.70 (3H, s), 4.86 (1H, br t, J=8.0), 5.12 (1H, br d, J=10.5), 5.72 (1H, dd, J=10.5, 7.5), 6.64 (1H, m). (8); δ 1.51 (3H, br s), 1.82 (3H, d, J=1.5), 3.06 (1H, m), 3.72 (3H, s), 4.92 (1H, br t, J=8.0), 4.99 (1H, br d, J=11.0), 5.41 (1H, dd, J=11.0, 8.0), 5.52 (1H, d, J=3.0), 6.27 (1H, d, J=3.5), 6.73 (1H, m). The spectra of (3) and (4) were also analogous to those of (7) and (8), respectively, except for the wide differences of the β-proton on the α,β-unsaturated carboxyl group (δ 5.61 in (3) and δ 6.64 in (7), and δ 5.59 in (4) and δ 6.73 in (8)).
4. Neutralization of (1) with 0.1N methanolic KOH in EtOH followed by addition of a soln. of 2% $AgNO_3$ in EtOH yielded the silver salt as a precipitate.
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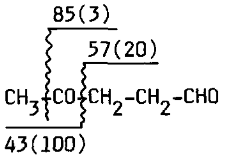
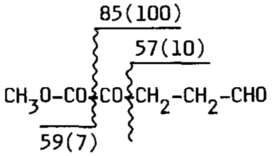
7. 4-Oxopentanal (A), MS: m/z (%) 100 ($M^{+\cdot}$, $C_5H_8O_2$, 0.4). The fragmentation pattern and the GC retention time were identical with those obtained by ozonolysis of geraniol. Methyl 2,5-dioxopentanoate (B), MS: m/z (%) 144 ($M^{+\cdot}$, $C_6H_8O_4$, 0.2), 116 (M-CO, 0.9), 85 (100), 59 (7), 57 (10).

(A):



$CH_3-CO-CH_2-CH_2-CHO$

(B):



$CH_3O-CO-CO-CH_2-CH_2-CHO$

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