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LEMNALOL, A NEW SESQUITERPENOID FROM THE SOFT CORAL LEMNALIA TENUIS VERSEVELDT

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Summary: The structure and absolute stereochemistry of lemnalol (1), a new ylangene-type sesquiterpenoid isolated from the soft coral Lemnalia tenuis Verseveldt, has been established by spectroscopic, chemical, and x-ray crystallographic analyses.

Marine terpenoids continue to be of considerable interest because of the frequent occurrence of unusual chemical features and biological activity.[1] We wish to report that our continuing investigations[2] have yielded a new sesquiterpenoid, lemnalol, from the Japanese soft coral Lemnalia tenuis Verseveldt. Lemnalol is the first example of a ylangene-type sesquiterpenoid[3] isolated from a marine source and the first oxygenated ylangene-type sesquiterpenoid from any source.

The methanol extract of Lemnalia tenuis Verseveldt (wet weight 900 g) collected at the coral reefs of Ishigaki Island (Okinawa, Japan) was extracted with ethyl acetate. The ethyl acetate extract was chromatographed on a silica gel column using n-hexane-acetone (40:1) as an eluant to give lemnalol[4] as colorless crystals (1.37 g); mp 46-47°C, $[\alpha]_D^{20}$ -9.3°(c 0.01, $CHCl_3$), $C_{15}H_{24}O$.

The presence of an allylic alcohol system in lemnalol (1) was indicated by the IR($CHCl_3$) [3580, 1640 and 900 cm^{-1}], ^{1}H-NMR(200 MHz, $CDCl_3$)[δ_{ppm} 4.42(1H, brd, J=8 Hz, >CHOH), 4.86(1H, brs) and 5.04(1H, brs)] and ^{13}C-NMR(25.0 MHz, $CDCl_3$)[δ_{ppm} 66.5(d), 111.4(t) and 154.8(s)] spectra. This was further supported by chemical conversions: acetylation of 1 with acetic anhydride in pyridine gave a monoacetate (2),[5] oxidation of 1 with manganese dioxide yielded an α,β-unsaturated ketone (3),[6] and ozonolysis of the monoacetate (2) gave a ketoacetate[IR($CHCl_3$) 1730 and 1703 cm^{-1}]. The ^{13}C-NMR spectrum of 1 showed the signals of three methyl carbons(δ_{ppm} 19.4, 20.0 and 20.2), three methylene carbons(21.4, 34.0 and 36.5), five methine carbons(32.3, 42.0, 44.3, 47.2 and 47.6) and one quaternary carbon (42.3) in addition to the carbons of the allylic alcohol

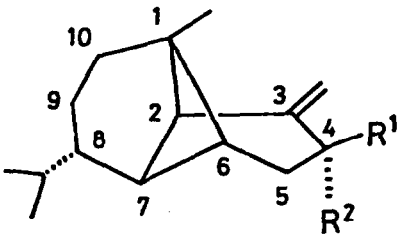
moiety. The ^{1}H NMR spectrum of 1 showed the signals due to a tertiary methyl group at 0.63(3H,s) and an isopropyl group at 0.87(6H,d,J=6 Hz) in addition to the signals at 1.85(1H,ddd,J=1.5,4,14 Hz), 2.23(1H,ddd,J=2,8,14 Hz), 2.23(1H,s) and 2.61(1H,d,J=6 Hz).

^{1}H-NMR decoupling experiments[7] of 1 revealed the relationship of the protons at C-2, -4, -5 and -6 positions and the stereochemistry of the secondary hydroxyl group at C-4 position. A large long-range coupling (J=6 Hz) observed between H-2(δ_{ppm} 2.61) and H-6 indicated the presence of a bridged cyclobutane system.[8] Furthermore no coupling was observed between H-7 [2.23(s)] and the adjacent protons (H-2, H-6 and H-8) suggesting a dihedral angle of approximately 90° between these protons. This defines the stereochemistry at the isopropyl bearing carbon, C-8, to be as shown. The α-configuration of the secondary alcohol at C-4 was supported by the reduction of 3 with sodium borohydride to give exclusively the epimeric alcohol (4),[9] which would be expected by attack of a hydride from the less hindered face of the carbonyl. These findings led to the formulation of lemnalol as 4-α-hydroxy-β-ylangene (1).

The absolute configuration of the secondary hydroxyl group at C-4 in 1 was deduced by applying a ^{1}H-NMR lanthanide induced shift (LIS) method[10] for the diastereomeric α-methoxy-α-trifluoromethylphenylacetyl(MTPA) esters (5a) and (5b). Acylation of 1 with R-(+)-and S-(-)-MTPA chlorides gave the esters (5a)[11] and (5b),[12] respectively. The R-configuration at C-4 was suggested by the positive sign of the ΔLIS_{OMe} value[10] (+0.36 ppm) for the R-(+)-MTPA ester (5a) (LIS_{OMe} 7.23 ppm) and the S(-)-MTPA ester (5b) (LIS_{OMe} 6.87 ppm) at a molar ratio of Eu(fod)$_3$/ester of 0.84:1 in CCl_4.

The full structure of 1 was confirmed by an x-ray crystallographic analysis of the p-bromobenzoate (6).[13] A cubic crystal of 6, 0.35 mm on an edge, was used for the single crystal x-ray diffraction experiment. It belonged to space group $P2_12_12_1$ with $\underset{\sim}{a}$ = 13.117(6), $\underset{\sim}{b}$ = 19.876(4 and $\underset{\sim}{c}$ = 7.597(2)Å and one molecule of composition $C_{22}H_{27}BrO_2$ formed the asymmetric unit. All unique data with $2\theta \leq 46°$, including Friedel pairs, were collected on a computer controlled four-circle diffractometer using graphite monochromated MoKα̃ (0.71069Å) radiation and variable speed, 1° ω-scans. 3499 reflections were collected of which 2904 (83%) were judged observed ($|F_o| \geq 3\sigma(F_o)$) after correction for Lorentz, polarization and background effects. The structure was solved using only half of the data by standard heavy atom methods.[14] After full-matrix least-squares refinements with anisotropic nonhydrogen atoms, fixed isotropic hydrogens and anomalous scattering for bromine, the conventional crystallographic residual was 0.055 for all of the data and the structure shown in Figure 1. The enantiomer only refined to a residual of 0.073.[15] This absolute configuration for lemnalol corresponds to that found for the ylangenes produced by higher plants, and is enantiomeric to that found in the liverworts.[1,3]

Lemnalol (1) rendered murine peritoneal exudate cells cytotoxic for syngeneic tumor cells (DBA/MC fibrosarcoma) *in vitro* and produced the highest levels (≥ 75%) of growth inhibition of the tumor cells at a concentration of 20 μg/ml, although significant levels (30%) of inhibition were observed at a concentration of 5 μg/ml.



1 R^1=H R^2=OH

2 R^1=H R^2=OAc

3 R^1, R^2=O

4 R^1=OH R^2=H

5a R^1=H R^2=OCO-C(OCH$_3$)(Ph)(CF$_3$)

(R-(+)-MTPA ester)

5b R^1=H R^2=OCO-C(Ph)(OCH$_3$)(CF$_3$)

(S-(-)-MTPA ester)

6 R^1=H R^2=OCOC$_6$H$_4$Br(p)

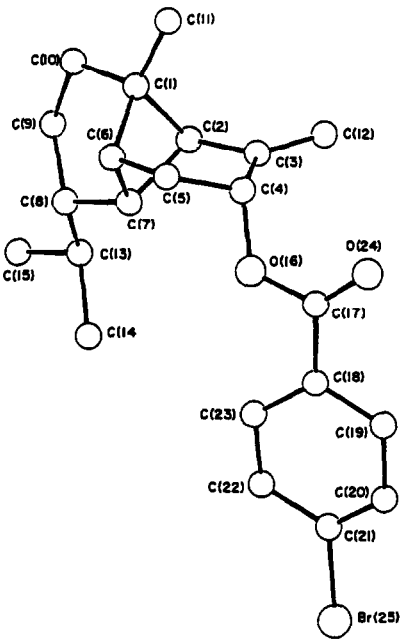


Fig.1. X-Ray structure of the p-bromobenzoate (6).

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References and Notes

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4. All new compounds gave satisfactory elemental analyses or high resolution mass spectra.

5. 2; colorless oil, $[\alpha]_D$+30.2°(c 0.005, $CHCl_3$). IR($CHCl_3$) 1720, 1635, 900 cm^{-1}. ^{1}H-NMR(100 MHz, $CDCl_3$) δ_{ppm}0.68(3H,s), 0.88(6H,d,J=6 Hz), 2.06(3H,s), 2.13(1H,s), 2.36(1H,ddd,J=2, 8,15 Hz), 2.62(1H,d,J=6 Hz), 4.90(1H,brs), 5.05(1H,brs), 5.72(1H,brd,J=8 Hz).

6. 3; colorless oil, $[\alpha]_D$+7.8°(c 0.01, $CHCl_3$). IR($CHCl_3$) 1695, 1618 cm^{-1}. ^{1}H-NMR(90 MHz, $CDCl_3$)δppm 0.80(3H,s), 0.86(3H,d,J=6 Hz), 0.88(3H,d,J=6 Hz), 2.62(1H,s), 2.86(1H,d, J=7 Hz), 5.04(1H,d,J=1.5 Hz), 6.01(1H,d,J=1.5 Hz).

7. The result of the ^{1}H-NMR decoupling experiment of 1 is summarized as follows:

irradiated proton(δ, ppm)	observed protons and changes (J in Hz)
H-4(4.42)	H-5 (1.85,ddd) → dd J=4,14, H-5 (2.23,ddd) → dd J=2,14
H-5(2.23)	H-4(4.42,brd) → brs
H-6*	H-5 (brdd) → brd J=14, H-5 (ddd) → dd J=8,14
	H-2(d) → s

*H-6 proton signal, which is overlapped with other signals near 1.6 ppm, can be observed by adding Eu(fod)$_3$ to a $CDCl_3$ solution.

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9. 4; mp 96-98°C, $[\alpha]_D$+66.4°(c 0.01, $CHCl_3$). IR($CHCl_3$) 3580, 3350, 1638, 890 cm^{-1}. ^{1}H-NMR (90 MHz, $CDCl_3$) δppm 0.67(3H,s), 0.82(6H,d,J=6 Hz), 2.02(1H,s), 2.59(1H,d,J=7 Hz), 4.49(1H,m), 4.76(1H,brd,J=1.5 Hz), 5.06(1H,d,J=1.5 Hz).

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11. 5a: colorless oil, $[\alpha]_D$+31.6°(c 0.027, $CHCl_3$). IR($CHCl_3$) 1735 cm^{-1}. ^{1}H-NMR(90 MHz, CCl_4) δppm 0.70(3H,s), 0.74(3H,d,J=6 Hz), 2.16(1H,s), 2.58(1H,d,J=5 Hz), 3.50(3H,s), 4.98(1H, brs), 5.23(1H,brs), 5.61(1H,d,J=7 Hz), 7.25-7.60(5H,m), 0.83(3H,d,J=6 Hz).

12. 5b: colorless oil, $[\alpha]_D$-15.8°(c 0.024, $CHCl_3$). IR($CHCl_3$) 1735 cm^{-1}. ^{1}H-NMR(90 MHz, CCl_4) δppm 0.67(3H,s), 0.70(3H,d,J=6 Hz), 0.76(3H,d,J=6 Hz), 2.08(1H,s), 2.51(1H,d,J=6 Hz), 3.52(3H,s), 4.89(1H,brs), 5.17(1H,brs), 5.69(1H,d,J=8 Hz), 7.2-7.55(5H,m).

13. 6: mp 65-66°C, $[\alpha]_D$-27.9(c 0.028, $CHCl_3$). IR($CHCl_3$) 1705 cm^{-1}. ^{1}H-NMR(100 MHz, $CDCl_3$)δppm 0.72(3H,s), 0.90(6H,d,J=6 Hz), 2.30(1H,s), 2.40(1H,ddd,J=2,8,16 Hz), 2.65(1H,d,J=6 Hz), 4.92(1H,brs), 5.12(1H,brs), 5.72(1H,brd,J=8 Hz), 7.50(2H,d,J=8 Hz), 7.81(2H,d,J=8 Hz).

14. All crystallographic calculations were done on a Prime 400 computer operated by the Materials Science Center and the Department of Chemistry, Cornell University. The principal programs used were REDUCE and UNIQUE, data reduction programs, M.E. Leonowicz, Cornell University, 1978; BLS78A, anisotropic block-diagonal least squares refinement, K. Hirotsu and E. Arnold, Cornell University, 1980; XRAY76, the X-ray System of Crystallographic Programs, edited by J. M. Stewart, University of Maryland, Technical Report TR-445, March, 1976; ORTEP, crystallographic illustration program, C.K. Johnson, Oak Ridge, ORNL-3794; BOND, molecular metrics program, K. Hirotsu, Cornell University, 1978; MULTAN-78, "A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data". University of York, England. Principal author P. Main. For literature description of MULTAN see: G. Germain, P. Main, M.M. Woolfson, Acta Crystallogr., Sect B, 26, 274 (1970) and M.M. Woolfson, Acta Crystallogr., Sect A, 33, 219 (1977).

15. Fractional coordinates and thermal parameters have been deposited with the Cambridge Crystallographic Data Centre.

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