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LANKALAPUOL A AND B: TWO CIS-EUDESMANES FROM THE SEA HARE APLYSIA DACTYLOMELA

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Abstract: The marine mollusk *Aplysia dactylomela* is the source of two new brominated sesquiterpenes, lankalapuol A and B (1, 2). Characterization is based on the spectral data of their acetates (3, 4). The absolute stereochemistry of lankalapuol A acetate, (6R) acetoxy-(1R)bromo-(5R,7R,10R)eudesm-3-ene, is based on X-ray analysis. The two lankalapuols are antipodal cis-fused eudesmanes.

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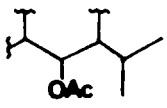
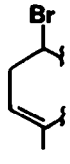
Sea hares are herbivorous gastropod mollusks, some of which feed on red algae, *Laurencia* spp. Both biota, which have been studied extensively, have many secondary metabolites in common, predominantly halogenated sesquiterpenes and C_{15} acetogenins.[1,2] *Aplysia dactylomela* from Hawaii has yielded several sesquiterpenes previously described from *Laurencia* spp.[3] Two specimens of *A. dactylomela* from Puttalam Lagoon, Sri Lanka, yielded two new cis-fused eudesmane sesquiterpenes, lankalapuol A and B (1, 2).[4]

The crude organic extract (120 mg) from two animals was repeatedly chromatographed on silica gel and eluted with petroleum ether/dichloromethane (2:1). A major fraction (80 mg) eluted as a 2:1 mixture of two closely related compounds, which could not be resolved on HPLC (silica gel, petroleum ether/dichloromethane, 2:1), and hence was acetylated. The acetates were separated by HPLC (silica gel, petroleum ether/ethyl acetate, 95:5).

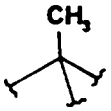
Lankalapuol A acetate (15 mg) crystallized from aqueous ethanol, mp 83.5-84°C,. Its molecular formula, $C_{17}H_{27}BrO_2$, was established by high resolution EIMS. It is UV-transparent. Its ^{1}H NMR spectrum displays five methyl resonances, an acetoxy methyl at δ2.04, two methyls of an isopropyl at δ0.78 and 0.89, coupled by J = 7Hz to a methine at δ1.60. Two remaining methyls are singlets, one aliphatic at δ0.96, one vinylic at δ1.64. One low field ^{1}H NMR signal, a broad triplet at δ5.21 is coupled to the vinyl methyl. The acetoxy methine triplet at δ4.90 (J = 10.5 Hz) was unambiguously assigned by comparison with the ^{1}H NMR spectrum of the alcohol mixture, which exhibits two triplet hydroxy methines (δ3.4, 3.5, both J = 9.9 Hz, 2:1). The third low field signal was assigned to a bromomethine (δ4.56, dd, J, = 6.9, 3.5 Hz). These data, together with two olefinic signals in the ^{13}C NMR spectrum at δ134.7(s) and 122.2(d) delineate laulakapuol A as a bicyclic sesquiterpene.



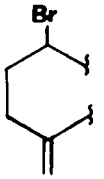
a



b



c

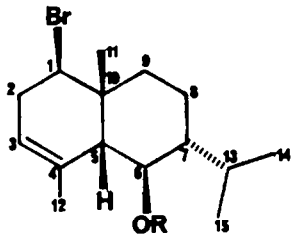


d

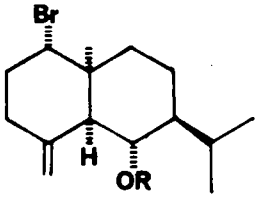
Part structure a follows from ^{1}H NMR decoupling experiments, which show H-6 coupled to two non-equivalent methines, H-5 (δ1.95, 1H, d, J = 10.7 Hz) and H-7 (δ1.39, 1H, dt, J = 2.4, 10.7 Hz). H-7 is also coupled to the isopropyl methine and to overlapping signals in the high field region of the spectrum. Partial structure b is derived from decoupling and 2D-COSY NMR experiments, which show that two non-equivalent allylic methines at C-2 (δ2.85, br d, J = 18.1 Hz and 2.55, multiplet) are coupled to the bromomethine (H-1). The gross structure of lankalapuol A acetate (3) combines these two part structures with a quaternary methyl (c) and two methylenes.

Lankalapuol B acetate, (7mg), mp 93.5–94°C, $[\alpha]_D$ -93°, $C_{17}H_{27}BrO_2$, is an isomer of lankalapuol A acetate. Obvious differences in the ^{1}H NMR spectrum of B are two rather than one olefinic signals (δ4.77, 1H, br d, J = 2 Hz; 4.66, 1H, br d, J = 2 Hz) and only four methyl signals. Partial structure a was present in lankalapuol B acetate, as shown by decoupling and COSY experiments. However, the bromomethine was present in a different isolated spin system, part structure d. This allowed assignment of the gross structure of lankalapuol B acetate (4) as the $\Delta^{4,12}$isomer of lankalapuol A acetate (3).

Relative stereochemistry was inferred from coupling constants and NOE experiments. The *anti* relationship between H-5 and H-6, and between H-6 and H-7 was based on coupling constants of 10.5 Hz[5] Upon irradiation of H-6, a nuclear Overhauser enhancement of H-1 provided the unexpected result that the ring fusion must be cis. This NOE also establishes that the bromomethine must be axial. The relative stereochemistry, of both metabolites, must be $1R^*$, $5R^*$, $6R^*$, $7R^*$, $10R^*$.



1 R=H



2 R=H

3 R=Ac

4 R=Ac

Single crystal X-ray diffraction data (vide infra) coupled with ^{1}H NMR data establish that both lankalapuols are cis-eusdesmanes, apparently unique among *Laurencia* - *Aplysia* metabolites. More remarkable is the opposite and nearly equal optical rotation of the two isomers which necessitates that all five chiral centers must be enantiomeric and that 3 and 4 possess opposite absolute configuration. This has been previously encountered in different *Laurencia*-*Aplysia* sesquiterpenes, e.g. heterocladol from *L. filiformis* forma *heteroclada*[6] and brasudol from *A. brasiliana*,[7] which are similar in structure but possess opposite absolute configuration. In the lankalapuol case the antipodes arise from the same organism, *A. dactylomela*.

The absolute stereochemistry of lankalapuol A acetate was established by X-ray analysis of crystals grown from ethanol/water. The positions of the two bromine atoms in the asymmetric unit were determined using MULTAN-80.[8] Three cycles of full-matrix least-squares refinement of two isotropic bromine atoms using SHELX-76[9] led to $R_1 = (\Sigma||F_o|-|F_c||)/\Sigma|F_o| = 0.365$. Then 32 additional non-hydrogen atoms were found in a difference Fourier function. The isotropic refinement of the bromine atoms and of these 32 non-hydrogen atoms led to $R_1 = 0.251$. The subsequent anisotropic refinement of the two bromine atoms with the other non-hydrogens isotropic led to $R_1 = 0.121$. The 6 remaining non-hydrogen atoms were found in an ensuing difference Fourier function. After two cycles of full-matrix least-squares anisotropic refinement[10] of all non-hydrogen atoms, $R_2 = [\Sigma w(|F_o|-|F_c|)^2/\Sigma w|F_o|^2]^{1/2} = 0.0923$. Repeating these refinements with the inverted structure led to $R_2 = 0.0948$. One more cycle of refinement of all anisotropic non-hydrogen atoms, with 24 calculated hydrogen positions held fixed, led to $R_2 = 0.0925$ for the inverted structure and $R_2 = 0.0879$ for the original structure, which was therefore taken to be correct. A difference Fourier function with phases calculated using 40 anisotropic non-hydrogen positions revealed all hydrogen positions. Full-matrix least-squares with anisotropic thermal parameters for all non-hydrogen atoms and isotropic ones for all hydrogen atoms converged with $R_1 = 0.0584$, $R_2 = 0.0528$, and "goodness-of-fit" $= [\Sigma w(|F_o|-|F_c|)^2/(m-n)]^{1/2} = 2.88$. The number of observations used in least-squares refinement is m (1567), and n (577) is the total number of parameters. The overdetermination ratio (m/n) is therefore 2.7. In practice, non-hydrogen and hydrogen atoms were refined in alternate cycles (361 and 217 parameters, respectively).

The maximum shift-to-error ratios in the final cycle of least squares are 0.20 for non-hydrogens and 1.31 for hydrogens. In the final difference Fourier function (e.s.d. = 0.093 $e^-/Å^3$), the ten largest peaks (0.34-0.46 $e^-/Å^3$) were all at chemically implausible positions.

The molecular structure of lankalapuol A acetate is presented Figures 1, 2, and 3. Two independent molecules are present in the unit cell. Figures 1 and 2 illustrate the differences in their structure and in their thermal ellipsoids. The two molecules, of course, have the same absolute configuration. The A ring is a half-chair form: the C(4) atom is 0.642 Å out of the least-squares plane of this ring. C(7)-C(8) by its length is a double bond. Ring B exists as a boat form. The absolute stereochemistry, then, of lankalapuol A acetate is 1R, 5R, 6R, 7R, 10R.

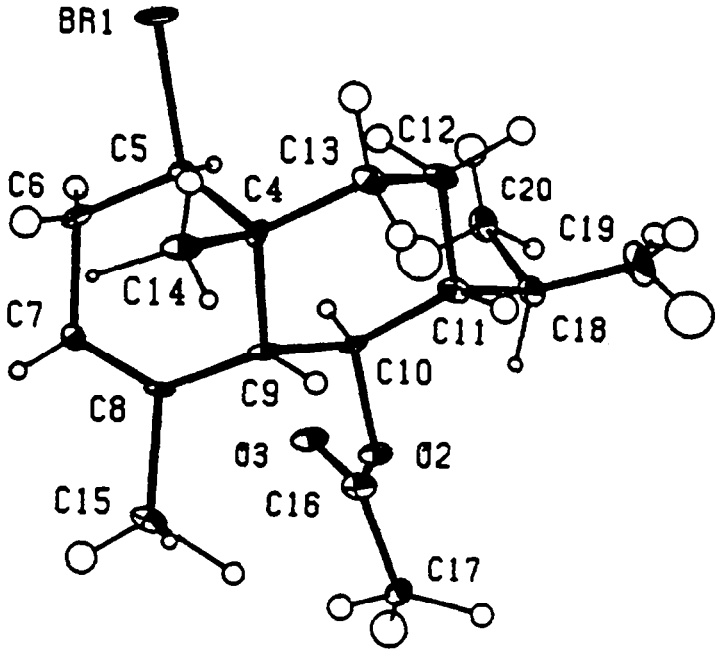


Figure 1. Molecule I in the crystal structure of lankalapuol A acetate (3). Ellipsoids of 5% probability are used.[11] The view is perpendicular to the Br(1), C(6), C(12), and C(15) plane.

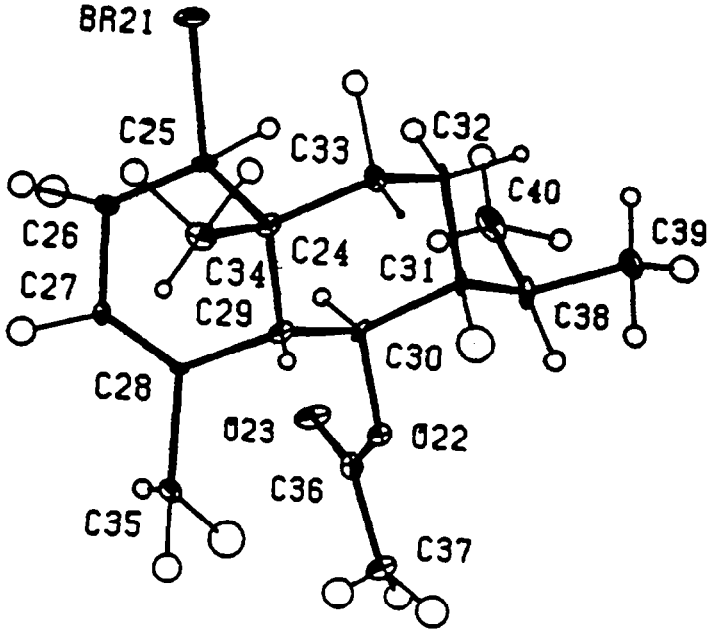


Figure 2. Molecule II in the crystal structure of lankalapuol A acetate (3). Ellipsoids of 5% probability are used.[11] The view is perpendicular to the Br(21), C(26), C(32), and C(35) plane.

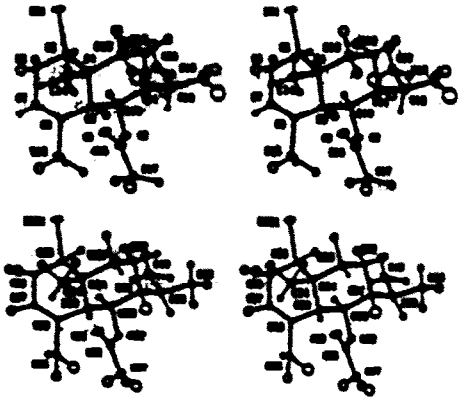


Figure 3. Stereoview[11] of molecules I and II in the crystal structure of lankalapuol A acetate (3). Ellipsoids of 5% probability are used. The views are along the same directions as in Figures 1 and 2.

Experimental Section

Melting points were obtained on a Fisher-Johns apparatus and are uncorrected. Optical rotations were recorded on a Rudolph 1600 polarimeter. IR spectra were recorded on a Nicolet MX-S FTIR or a Perkin Elmer 467 instrument. UV spectra were recorded on a Hewlett-Packard DU-7 spectrophotometer. EI mass spectra were recorded on a MAT 311 spectrometer. A Nicolet NM 300 spectrometer was used to record 300 MHz ^{1}H and 75 MHz ^{13}C spectra with residual protons of the solvent used as internal standards.

Isolation of Lankalapuol A and B Acetates. Two specimens of *A. dactylomela* were obtained from fishermen at Puttalam Lagoon, Sri Lanka, in April, 1983. The animals were frozen and taken to the laboratory where the whole animals were extracted with dichloromethane/methanol. The crude organic extract (120 mg) was repeatedly chromatographed on a silica gel column eluted with petroleum ether/dichloromethane (2:1). A major fraction (80 mg) eluted as a mixture of two closely related compounds. Since the components of this mixture could not be resolved on HPLC (silica gel, petroleum ether/dichloromethane, 2:1), the mixture was subjected to acetylation. Purification of the acetates was achieved by HPLC (silica gel, petroleum ether/ethyl acetate, 95:5), yielding 15 mg of lankalapuol A acetate and 7 mg of lankalapuol B acetate.

Acetylation of the lankalapuols: The mixture of alcohols (30 mg) was dissolved in pyridine (2 mL). To the stirred mixture, 1 mL of freshly distilled Ac_2O was added and the solution was stirred at room temperature overnight. Solvent was removed *in vacuo* and the residue purified by HPLC on silica gel (petroleum ether/ethyl acetate, 95:5, yielding 15 mg of lankalapuol A acetate (3) and 7 mg of lankalapuol B acetate (4).

Lankalapuol A acetate (3). White crystals (ethanol/water): mp 83.5-84.0°C; $[\alpha]_D$= +96° (c 0.47, MeOH); IR (film): ν_{max} 3100, 3000, 2900, 1700, 1400, 1300, 1200cm^{-1}; UV ($CHCl_3$), end absorption only; EIMS (70 eV): m/z (%) 342/344 (0.3), 282/284 (29), 239/241 (42), 203/205 (33), 159/161 (38), 43 (100); HREIMS on m/z 282 (M^+ - HOAc) 282.0983, calculated for $C_{15}H_{23}Br$, 282.0984; ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.21 (1H, H-3, br s), 4.90 (1H, H-6, dd, J = 10.5, 10.5 Hz), 4.56 (1H, H-1, dd, J = 6.9, 3.5 Hz), 2.85 (1H, H-2a, br d, J = 18.1 Hz), 2.55 (1H, H-2b, m), 2.13 (1H, H-9a, dd, J = 10.5,3 Hz), 2.04 (3H, H_3-OAc, s), 1.95 (1H, H-5,d,d=10.7 Hz, m), 1.64 (3H, H_3-12, brs), 1.60(1H, H-13, m), 1.49 (1H, H-8a, m), 1.39 (1H, H-7, dt, J = 2.4, 10.7 Hz), 1.19 (2H, H-9b, -8b, m), 0.96 (3H, H_3-11, s), 0.89 (3H, H_3-14, d, J = 6.9 Hz), 0.78 (3H, H_3-15, d, J = 6.9 Hz; ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.2, 134.7, 122.2, 77.2, 55.0, 53.6, 49.1, 39.6, 37.1, 36.2, 26.0, 24.6, 21.8, 21.4, 21.2, 17.6, 16.1.

Lankalapuol B acetate (4). White crystals (ethanol/water); mp 93.5-94.5°C; $[\alpha]_D$ - 93° (c = 1.0, MeOH); IR (film): ν_{max} 3000, 2930, 1730, 1370, 1240, 1030, 890; UV ($CHCl_3$), end absorption only; EIMS (70 eV); m/z (%) 342/344 (0.5), 282/284 (38), 239/241 (38), 203 (58), 159 (39), 43 (100); HREIMS on m/z 282 (M^+ - HOAc), 282.0983, calculated for $C_{15}H_{23}Br$, 282.0984; ^1H NMR (300 MHz, $CDCl_3$): δ 5.06 (1H, H-6, dd, J = 10.7, 10.7 Hz), 4.77 (1H, H-12a, br d, J = 2 Hz), 4.66 (1H, H-12b, br d, J = 2 Hz), 4.49 (1H, H-1, dd, J = 4.8, 12.1 Hz), 2.50 (1H, H-2a, br dt), 2.24 (1H, H-2b, br dd) 2.13 (1H H-5, d, J = 10.7 Hz), 2.05 (4H, H-3a, -3b, -8a, -9a, m), 1.95 (3H, H_3-OAc, s), 1.74 (1H, H-13, dsep, J = 1.5, 7 Hz), 1.40 (1H, H-7 br ddt), 1.25 (1H, H-9b, m), 1.1 (1H, H-8b, m), 1.0 (3H, H_3-11, s), 0.90 (3H, H_3-14, d, J = 7.0 Hz), 0.81 (3H, H_3-15, d, J = 7.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.3, 144.0, 133.7, 70-.8, 59.0, 57.6, 47.8, 41.3, 37.2, 34.1, 32.1, 26.8, 23.3, 20.9, 20.8, 18.0, 16.4.

Crystallographic analysis: Clear colorless crystals of lankalapuol A acetate were grown from ethanol/water solution. Their density as determined by flotation in NH_4Br solution is 1.2 g cm^{-3}, so therefore Z = 4. The crystal used for intensity measurements was cylindrical with dimensions 0.4 X 0.4 X 0.2 mm. Preliminary experiments showed this material to be monoclinic with the following cell constants: a = 10.275(5), b = 9.5861(29), c = 20.446(12) Å, β = 117.81(4)°, V = 1781.3(14) $Å^3$, Z = 4, F(000) = 720, molecular weight = 343.286 amu. The space group may be either $P2_1$ or $P2_1/m$ on the basis of systematic absences, k odd for (0k0). The structure was solved in $P2_1$; the space group for such a chiral natural product must be non-centric.

All diffraction intensities were observed on a Syntex $P\bar{1}$ automated diffractometer using Mo Kα radiation [$K\alpha_1$, λ=0.70930 Å; $K\alpha_2$, λ=0.71359 Å] and a graphite monochromator. The cell constants and their standard deviations were determined by a least-squares treatment of the angular coordinates of 15 independent reflections with 2θ < 21.75°. The θ-2θ scan mode was used with a constant scan rate (ω) in 2θ of 2° min^{-1}. The background time to scan time ratio used was 1.0. The scan range varied from 1° (2θ) below the $K\alpha_1$ peak to 1° above that of $K\alpha_2$. Two standard reflections, measured after each 97 reflections during the course of data collection, showed a regular decrease of about 23% in intensity due to the deterioration of the crystal; the appropriate correction was made.

Standard deviations were assigned to the intensities according to the formula:

$$\sigma(I) = [(CT + B_1 + B_2)\omega^2 + (pI)^2]^{1/2}$$

where CT is the total integrated count, B_1 and B_2 are the background counts, and the intensity is $I = \omega (CT - B_1 - B_2)$. A value of 0.02 was assigned to the empirical parameter p to account for physical and instrumental inaccuracies.[12] The weights w used in least-squares refinement of the structural parameters were the reciprocal squares of $\sigma(F_o)$. Of the 3291 unique reflections measured, those for which 2θ < 50.0°, 1567 had intensities greater than three times their standard deviations; only those were used in subsequent calculations. The intensities measured were corrected for Lorentz and polarization effects,[13] but not for absorption (μ = 22.8 cm^{-1}).[14] The monochromator crystal was assumed to be half perfect and half mosaic in character in the polarization correction. The atomic scattering factors for Br^0, O^0, C^0 (bonded), and H^0 (bonded) were used;[14] all but the last were corrected for the effects of anomalous dispersion.[14]

Acknowledgment. We thank Prof. E. A. Kay for identification of *Aplysia dactylomela* and the Natural Resources, Energy and Science Authority of Sri Lanka for grant RG/83/24 (to EDdS and LMUT) for financial support.

Supplementary data available: Tables of atomic and thermal parameters with e.s.d.'s, bond distances and angles, deviations of atoms from least-squares planes, and anisotropic thermal parameters with e.s.d.'s for non-hydrogen atoms. See Notice to Authors, *Tetrahedron* 40 (2), 11(1984).

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