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THYRSIFEROL: A SQUALENE-DERIVED METABOLITE OF LAURENCIA THYRSIFERA

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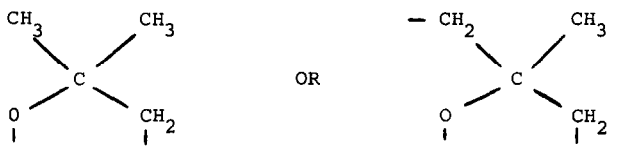
Previous investigations of red algae of the genus Laurencia (Rhodomelaceae, Rhodophyta) have established the structures of many interesting metabolites based in the main on fatty acid, sesquiterpenoid or diterpenoid skeletons.[1] We now report the isolation and identification of a squalene-derived metabolite from a Laurencia species.

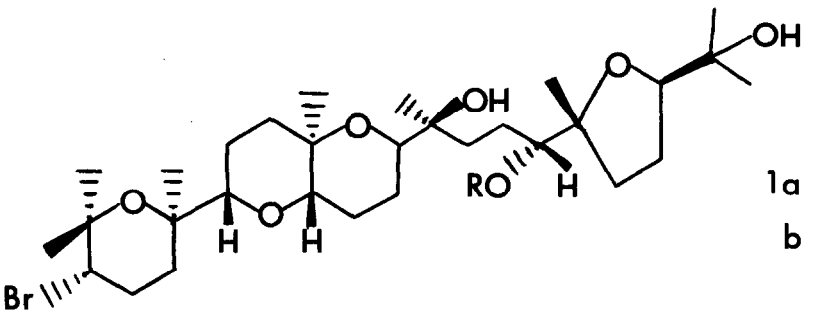
Laurencia thyrsifera (Hook) was collected from the intertidal zone at Seal Reef, Kaikoura, N.Z.[2] Chromatography of the methanol extract (4.5% of dry weight) on alumina gave a complex mixture of fatty acid methyl esters,[3] cholesterol[4] and the polar compound thyrsiferol (1a) as a mixture with two other metabolites (ene-ynes). After acetylation[5] of this mixture thyrsiferol (1a) was isolated as its mono-acetate (1b), m.p. 104.5-106°, RD (in cyclohexane, c = .0002) $[\alpha]_{400}$ + 89°, $[\alpha]_{350}$ + 111°, $[\alpha]_{300}$ 220°, $[\alpha]_{225}$ 400°. The molecular formula was inferred from high resolution mass measurement of the [M-H_2O] doublet and the [M-HBr] peak, as the molecular ion doublet (m/e 646/648) was of low intensity. The ^{13}C nmr spectrum[7] confirmed that the acetate (1b) was highly oxygenated as eleven C-O type resonances ($\delta_{^{13}C}$ 60-80) were discernible. The molecular formula, ^{13}C offset resonance decoupling experiments[8] and ^{13}C LIS studies established that thyrsiferyl acetate (1b) was a saturated, non-carbocyclic, dihydroxy acetate with 4 ether bridges. In addition, some 8 methyl groups were resolved (excluding the acetate methyl) and these were in similar structural environments:



The structure of thyrsiferyl acetate (1b) was determined by X-ray crystallography. Preliminary photographs indicated that thyrsiferyl acetate belonged to the monoclinic crystal class and systematic absences (0k0 with k odd) together with the chirality of the compound suggested that the space group was $P2_1$. Refined cell constants were a = 12.348(4) Å, b = 12.055(5) Å, c = 12.166(5) Å and β = 107.31(4)°, D_c = 1.24 g cm^{-3} and Z = 2.[8] The best crystal available for intensity collections was of high mosaicity (0.36-0.48°) with dimensions

of (0.250 x 0.075 x 0.063)mm^3. A total of 1931 unique reflections ($\theta<50^o$) were collected using Ni-filtered Cu Kα radiation (λ = 1.5418 Å) with θ-2θ scans on a Hilger and Watts four-circle automatic diffractometer. Of these reflections 1103, for which $F_o^2 > 3\sigma(F_o^2)$, were used in all structure solving calculations. Lorentz and polarisation corrections were applied, but no absorption corrections were made (μ = 21.53 cm^{-1}).

The crystal structure was determined by conventional heavy atom methods and refined using the full-matrix least-squares procedure with isotropic temperature factors for all carbon and oxygen atoms and anisotropic temperature factors for the bromine atom.[9] The scattering factors of Cromer and Mann[10] with the anomalous dispersion corrections of Cromer[11] for bromine were applied. Hydrogen atoms were not included in the structure factor calculations. This model was refined to conventional agreement factors R = 0.144 and R_w = 0.182. The centrosymmetric distribution of bromine atoms in an otherwise non-centrosymmetric array, together with the poor crystal quality, severely hindered attempts to determine the absolute configuration. The correct enantiomer has not been conclusively established but the weighted R-factor and the standard error in an observation of unit weight were marginally better for the enantiomer shown. Final position and thermal parameters are given in Table 1.[12]



1a R = H
b R = Ac

The conformation of thyrsiferyl acetate in the crystal studied is shown in Fig. 1. Ring C is in a twist-boat conformation in which strong 1,3-diaxial interactions between C(27) and C(15) are avoided. The molecular packing is dominated by intermolecular H-bonding (2.80(4) Å) between O(4) and O(6) in adjacent molecules, and there is close intra-molecular contact between O(7) and O(6) (2.82(4) Å).

Limited biological testing[13] of a crude sample of thyrsiferol (1a) and thyrsiferyl acetate (1b) indicated that they were biologically inactive.

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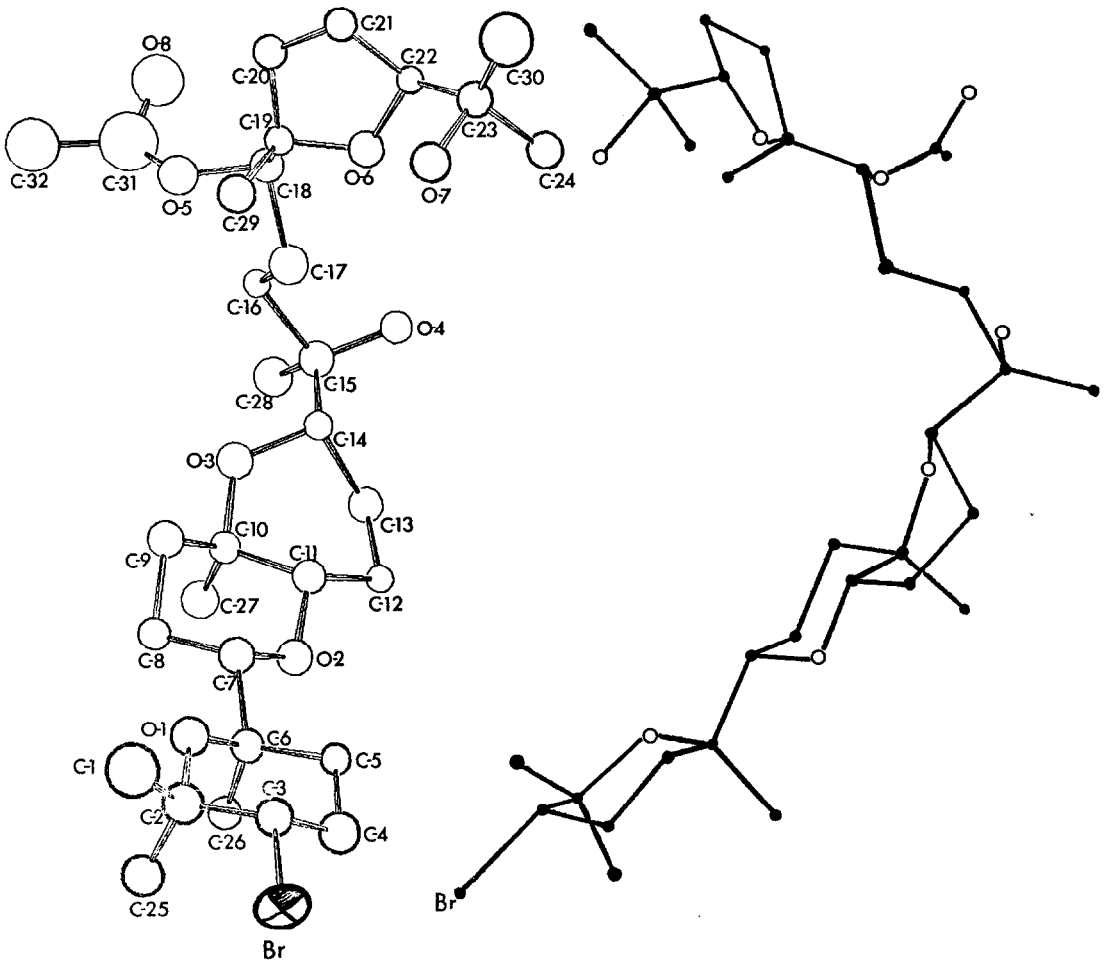

Fig. 1: Two perspective views of thyrsiferyl acetate (1b).

	x	y	z	U
Br	0.2389(4)	0.2500	0.0263(5)	
O(1)	0.517(2)	0.438(2)	0.232(2)	0.060(9)
O(2)	0.540(2)	0.734(3)	0.249(2)	0.057(9)
O(3)	0.818(2)	0.874(3)	0.346(2)	0.061(9)
O(4)	0.855(2)	1.133(2)	0.204(2)	0.049(8)
O(5)	1.185(3)	0.879(3)	0.274(3)	0.07(1)
O(6)	1.067(2)	0.934(2)	-0.014(2)	0.056(9)
O(7)	0.991(2)	0.807(3)	-0.218(2)	0.063(9)
O(8)	1.314(4)	1.000(4)	0.349(4)	0.12(1)
C(1)	0.511(5)	0.240(7)	0.186(5)	0.11(2)
C(2)	0.441(4)	0.334(4)	0.199(4)	0.06(1)
C(3)	0.354(3)	0.366(4)	0.080(4)	0.06(1)
C(4)	0.292(4)	0.484(4)	0.075(4)	0.07(1)
C(5)	0.383(3)	0.571(3)	0.108(3)	0.04(1)
C(6)	0.467(3)	0.549(4)	0.232(4)	0.05(1)
C(7)	0.582(4)	0.615(4)	0.249(4)	0.06(1)
C(8)	0.671(3)	0.602(3)	0.363(4)	0.05(1)
C(9)	0.774(4)	0.687(4)	0.357(4)	0.06(1)
C(10)	0.725(3)	0.803(4)	0.357(4)	0.04(1)
C(11)	0.625(4)	0.809(4)	0.237(4)	0.05(1)
C(12)	0.572(3)	0.928(3)	0.216(3)	0.04(1)

	x	y	z	U
C(13)	0.672(4)	1.014(4)	0.265(4)	0.06(1)
C(14)	0.796(3)	0.958(4)	0.252(3)	0.04(1)
C(15)	0.879(4)	1.062(4)	0.300(4)	0.06(1)
C(16)	1.007(3)	0.996(3)	0.302(3)	0.03(1)
C(17)	1.000(4)	0.944(4)	0.195(4)	0.07(1)
C(18)	1.129(4)	0.943(4)	0.171(4)	0.05(1)
C(19)	1.143(3)	0.884(3)	0.087(3)	0.04(1)
C(20)	1.249(3)	0.886(4)	0.052(3)	0.05(1)
C(21)	1.223(3)	0.891(4)	-0.079(3)	0.04(1)
C(22)	1.109(3)	0.949(3)	-0.116(3)	0.03(1)
C(23)	1.033(4)	0.922(4)	-0.236(4)	0.05(1)
C(24)	1.073(5)	0.902(6)	-0.339(5)	0.11(2)
C(25)	0.389(4)	0.312(4)	0.291(4)	0.07(1)
C(26)	0.412(3)	0.572(4)	0.328(4)	0.05(1)
C(27)	0.688(4)	0.831(4)	0.453(4)	0.06(1)
C(28)	0.897(4)	1.112(4)	0.425(4)	0.07(1)
C(29)	1.109(3)	0.741(5)	0.075(3)	0.06(1)
C(30)	0.926(4)	0.999(4)	-0.260(4)	0.05(1)
C(31)	1.264(7)	0.913(8)	0.364(7)	0.15(2)
C(32)	1.332(5)	0.845(6)	0.468(6)	0.12(2)

	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
Br	0.069(3)	0.044(3)	0.119(5)	-0.040(3)	0.013(3)	-0.034(4)

Table 1: Position and thermal parameters for thyrsiferyl acetate (1b)

References and Notes:

1. D. J. Faulkner, Tetrahedron, 33, 1421 (1977), W. Fenical, J. Phycol, 11, 245 (1975) and references cited therein.

2. Collected at low tide on 2 February, 1975.

3. Identified by glc retention time on DEGS and by gc/ms: 12:0, 14:0, 16:0, 16:1ω7, 18:0, 18:1ω9, 18:1ω10, 18:2ω6, 18:2ω3, 18:3ω3, 18:4ω3, 20:3ω9, 20:3ω6, 20:4ω6.

4. Identified on the basis of mp, mixed mp, ^{1}H nmr, ^{13}C nmr, ms.

5. Pyridine/acetic anhydride (1:1).

6. AEI MS-902C, Varian-MAT CH-5.

7. Varian CFT-20. Spectrum acquired in $CDCl_3$, referenced to TMS at $\delta_{^{13}C}$ 0.

8. Standard deviations for the last significant digit are given in parentheses.

9. Calculations were carried out on a Burroughs B6718 computer using local modifications of the well-known programs ORFLS and ORFFE (W. R. Busing, K. O. Martin and H. A. Levy) and FORDAP (A. Zalkin).

10. D. T. Cromer and J. B. Mann, Acta Cryst., A-29, 321, (1968).

11. D. T. Cromer, Acta Cryst., 18, 17 (1965).

12. Isotropic thermal parameters in $\AA^2$. Anisotropic parameters (U's), also in $\AA^2$, are coefficients in:
$\exp -2\pi^2(U_{11}h^2\underline{a}^{*2} + U_{22}k^2\underline{b}^{*2} + U_{33}l^2\underline{c}^{*2} + 2U_{12}hk\underline{a}^*\underline{b}^* + 2U_{13}hl\underline{a}^*\underline{c}^* + 2U_{13}hl\underline{a}^*\underline{b}^* + 2U_{23}kl\underline{b}^*\underline{c}^*)$

13. Test organisms used were: Bacillus subtilis, Staphyloccus aureus, Pseudomonas aeruginosa, Aspergillus niger, Saccharomyces cerevisiae.