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TERPENOIC ACID GLYCERIDES FROM THE DORID NUDIBRANCH ARCHIDORIS MONTEREYENSIS

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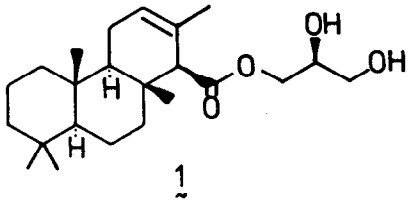
SUMMARY: Extracts of the dorid nudibranch Archidoris montereyensis contain a diterpenoic acid glyceride 1 whose structure has been determined by x-ray diffraction analysis. The structure of a minor metabolite, the sesquiterpenoic acid glyceride 2, was determined by chemical correlation.

Many dorid nudibranchs apparently utilize chemical antifeedants as one component of their defensive arsenal[1]. Our ongoing search for unusual metabolites in extracts of British Columbia nudibranchs has led to the discovery of compounds belonging to a variety of structural types; including degraded monoterpenes[2], sesquiterpenes[1b], a degraded sesterterpene[1b], steroids[3], and a diacylguanidine[4]. In this paper we wish to report the structure of two terpenoic acid glycerides from the dorid Archidoris montereyensis. We have previously reported that extracts of Archidoris odhneri, a closely related species, contained the farnesic acid glycerides 3 to 5[5].

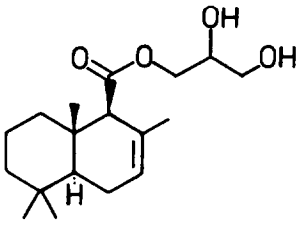
Specimens of A. montereyensis were collected in Barkley Sound, British Columbia and La Jolla, California. Freshly collected animals were extracted whole in methanol for one to two days. The organic soluble portion of the methanol extract was fractionated in a stepwise fashion by flash chromatography (Silica gel, 100% EtOAc), radial thin layer chromatography (Harrison Chromatatron, Silica gel, 5% MeOH/95% $CHCl_3$) and HPLC (Partisil PXS5, 50% EtOAc/50% Hexane) to yield a number of pure metabolites.

The major metabolite (≈2 mg/animal), glyceride 1, was an optically active ($[\alpha]_D$ = -12.5°, $CHCl_3$) crystalline compound (mp 125-126°, Hexane, Diethyl ether) which had a molecular formula of $C_{23}H_{38}O_4$ (HRMS: M^+; m/z 378.2772, calc'd 378.2770). Its IR spectrum ($CHCl_3$) showed hydroxyl (3700 → 3300 cm^{-1}) and ester (1730 cm^{-1}) absorption bands. A ^1H NMR spectrum (400 MHz, $CDCl_3$) of 1 showed four aliphatic methyl singlets at δ 0.82 (s, 3H), 0.87 (s, 3H), 0.92 (s, 3H), and 0.96 (s, 3H), a vinyl methyl at 1.61 (bs, 3H), a deshielded singlet at 2.96 (1H), resonances appropriate for a 2,3-dihydroxypropyl residue (2.48(bs, 2H, exchangable), 3.63

(dd, 1H, J=12, 6Hz), 3.70 (dd, 1H, J=12, 4 Hz), 3.95 (m, 1H), 4.15 (dd, J = 12, 7 Hz), 4.22 (dd, 1H, J=12, 5 Hz)) and a single olefinic proton at 5.54 (bs, 1H) ppm. The spectral data for 1 indicated that it was a tricyclic diterpenoic acid glyceride[6]. Limited quantities of 1 precluded the determination of the structure by chemical and spectral means.



1



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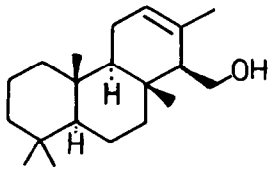

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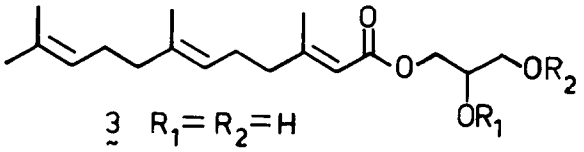
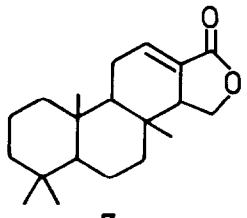


3 $R_1=R_2=H$

4 $R_1=Ac$ $R_2=H$

5 $R_1=H$ $R_2=Ac$



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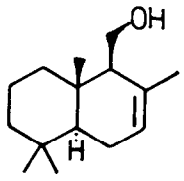

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The structure of 1 was therefore determined by a single-crystal x-ray diffraction experiment. Preliminary x-ray photographs of glyceride 1 showed monoclinic symmetry and accurate lattice parameters of a=51.73(2), b=7.321(2), c=11.207(3)Å, and β=85.48(2)° were calculated from a least squares fit of fifteen diffractometer measured 2θ-values. The systematic extinctions, crystal density and presence of chirality were uniquely consistent with space group C2 with two molecules of $C_{23}H_{38}O_4$ forming the asymmetric unit. All unique diffraction maxima with $2\theta < 100°$ were collected using a variable speed, 1.5° omega-scan and graphite monochromated CuKα radiation (1.54178Å). After correcting for Lorentz, polarization and background effects, 1776 (73%) of the 2441 reflections were judged observed ($|F_o| > 3\sigma(F_o)$). A multi-solution tangent formula approach was used to find a phasing model[7]. Initial maps revealed relatively little but successive tangent formula recycling of plausible molecular fragments eventually revealed all of the heavy atoms. All hydrogens were included at calculated positions. Full matrix least squares refinements have converged to a current crystallographic residual of 0.0925 for the observed data.

A computer generated perspective drawing of the final x-ray model of glyceride 1 is presented in Figure 1. The two molecules that comprise the asymmetric unit have identical stereostructures but the glyceride portions adopt different conformations. The x-ray experiment defined only the relative stereochemistry so the absolute configuration shown was chosen as that corresponding to alcohol 6. Bond distances and angles agree well with generally accepted values[8].

Reduction of glyceride 1 (DIBAL) gave alcohol 6 in high yield. Comparison of the specific rotation observed for 6 ($[\alpha]_D$ = -9°) with the literature value [9]($[\alpha]_D$ = -9°) indicated that its absolute configuration was as shown. The carbon skeleton of the diterpenoid acyl residue in 1 is rather rare in nature despite being biogenetically straightforward. The only other

known examples are the family of sponge derived metabolites related to isoagatholactone (7)[9].

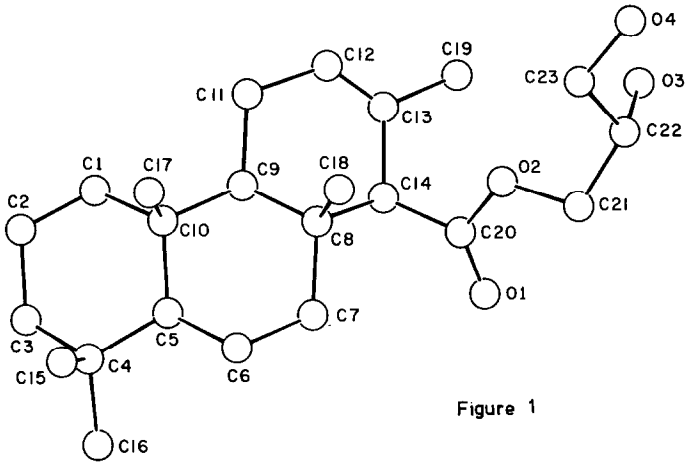

Figure 1

A very minor component (≈0.2 mg/animal) of the A. montereyensis extracts was shown by mass spectrometry to have a molecular formula of $C_{18}H_{30}O_4$ (HRMS: M^+; m/z 310.2142, calc'd 310.2144). Its ^1H NMR spectrum (400 MHz, $CDCl_3$) showed three aliphatic methyl singlets at δ 0.89 (s, 3H), 0.92 (s, 3H) and 0.98 (s, 3H), a vinyl methyl at 1.62 (bs, 3H), a deshielded singlet at 2.96 (1H), resonances appropriate for a 2,3-dihydroxypropyl residue (2.48 (bs, 2H, exchangable), 3.63 (dd, 1H, J = 12, 6 Hz), 3.70 (dd, 1H, J = 12, 4 Hz), 3.95 (bm, 1H), 4.15 (dd, J = 12, 7 Hz), 4.22 (dd, 1H, J = 12, 5 Hz)) and a single olefinic proton at 5.57 (bs, 1H) ppm. The spectral data for this minor metabolite suggested that it should have the structure 2. Verification of this proposal was accomplished by reducing (DIBAL) the glyceride 2 to alcohol 8, which was shown to be identical to an authentic sample[10] by tlc, G.C., ^1H NMR and MS comparison. Its specific rotation ($[\alpha]_D$ = -20°, $CHCl_3$) was identical to the literature value for (-) drimenol[10b] ($[\alpha]_D$ = -20.5°, $CHCl_3$) indicating that the terpenoid portion of both 2 and 8 have the absolute configurations indicated.

The discovery of terpenoic acid glycerides in extracts of two species of dorid nudibranchs belonging to the same genus suggested that the compounds were being synthesized by the nudibranchs. We have been able to show via feeding experiments that ^{14}C labelled mevalonic acid is incorporated into the terpenoid portion of the glycerides 1 and 3 by A. montereyensis and A. odhneri respectively[11]. The complete details of these experiments will be reported elsewhere.

Acknowledgements

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6) Additional spectral data for 1: mass spectrum, m/z 378, 363, 347, 286, 258, 192 (base peak); ^{13}C NMR (100 MHz, $CDCl_3$) 15.63(q), 15.73(q), 18.52(q), 18.73(q), 21.15(t), 21.68(t), 22.74(t), 33.20(s), 33.43(q), 36.70(s), 37.53(s), 40.01(t, 2C), 41.95(t), 54.45(d), 56.58(d), 62.76(d), 63.64(t), 65.17(t), 70.47(d), 124.32(d), 128.60(s), 173.35(s).

7) All crystallographic calculations were done on a PRIME 850 computer operated by the Cornell Chemistry Computing Facility. Principal programs employed were: REDUCE and UNIQUE, data reduction programs by M.E. Leonowicz, Cornell University, 1978; MULTAN 78, a system of computer programs for the automatic solution of crystal structures from x-ray diffraction data (locally modified to perform all Fourier calculations in-cluding Patterson syntheses) written by P. Main, S.E. Hull, L. Lessinger, G. Germain, J.P. Declercq and M.M Woolfson, University of York, England, 1978; NQEST, CYBER 173 version of the negative quartets figure of merit estimate written by C.M. Weeks at the Medical Foundation of Buffalo, Inc., 1976; BLS78A, an anisotropic block diagonal least squares refinement written by K. Hirotsu, and E. Arnold, Cornell University, 1980; CRYSTALS, a crystallographic system written by D.J. Watkin and J.R. Carruthers, Chemical Crystallography Laboratory, University of Oxford, 1981; ORTEP, a crystallo-graphic illustration program written by C.K. Johnson, Oak Ridge National Laboratory (ORNL-3794), 1970; PLUTO78, a crystallographic illustration program by W.D.S. Mother-well, Cambridge Crystallographic Data Centre, 1978; and BOND, a program to calculate molecular parameters and prepare tables written by K. Hirotsu, Cornell University, 1978.

8) Crystallographic data have been deposited with the Cambridge Crystallographic Data Centre.

9) Cimino, G.; De Rosa, D.; De Stefano, S.; Minale, L. Tetrahedron 1974, 30, 645.

10a) Kindly provided by Rosemary J. Armstrong, Chemistry Department, U.B.C.--see Ph.D. thesis 1983.

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11) Cimino et al. have recently demonstrated the incorporation of ^{14}C mevalonic acid into the sesquiterpenoid polygodial by the dorid Dendrodoris limbata. Cimino, G.; De Rosa, S.; De Stephano, S.; Sodano, G.; Villani, G. Science, 1983, 219, 1237.

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