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DITERPENES FROM THE MARINE PULMONATE *TRIMUSCULUS RETICULATUS*

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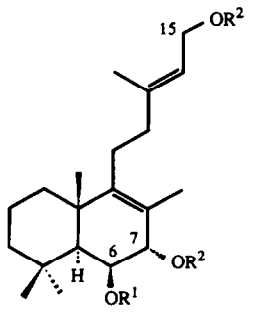
Abstract. The marine pulmonate *Trimusculus reticulatus* contains 6β-isovaleroxylabda-8,13-dien-7α,15-diol (**1**) and 2α,7α-diacetoxy-6β-isovaleroxylabda-8,13-dien-15-ol (**2**). The diol **1** is found in the mucus produced by *T. reticulatus* that repels predatory sea stars.

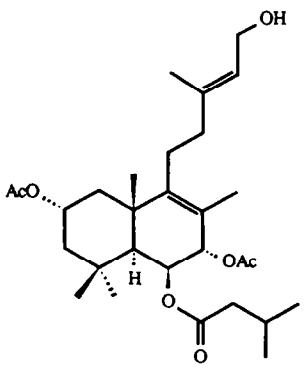
The marine pulmonate limpet *Trimusculus reticulatus*[1] is unusual in both habitat and behavior. It hangs inverted from the roofs of caves or from the upper surfaces of crevices in the intertidal zone along the Pacific coast of North America.[2] Because the low light intensity of this habitat does not allow the growth of algae or attached diatoms upon which limpets usually graze, *T. reticulatus* remains sessile throughout its life and filter feeds by producing a mucous net that traps suspended phytoplankton.[3] When disturbed, *T. reticulatus* produces copious amounts of a milky white mucus which was found to repel predatory starfish.[4] Extracts of both the whole animals and the mucus contained 6β-isovaleroxylabda-8,13-dien-7α,15-diol (**1**) but 2α,7α-diacetoxy-6β-isovaleroxylabda-8,13-dien-15-ol (**2**) was only isolated as a minor constituent of the animals.

The dichloromethane extract from 115 freeze dried animals was chromatographed by LC on Partisil to obtain the diol **1** (1.75 mg/animal) and the diacetate **2** (0.035 mg/animal). The mucus from 180 animals was treated with acetone to precipitate mucopolysaccharides; the remaining material was chromatographed as before to obtain the diol **1** (0.05 mg/animal).

The diol **1** was isolated as an oil, $[\alpha]_D = +38.7°$. The molecular formula, $C_{25}H_{42}O_4$, was deduced from a combination of the molecular ion determined by chemical ionization mass spectrometry and the exact mass of a fragment ion corresponding to loss of isovaleric acid. The ^{13}C NMR spectrum contained signals for an ester carbonyl at δ 172.8 (s), a trisubstituted olefin at 139.5 (s) and 123.3 (d), a tetrasubstituted olefin at 145.3 (s) and 124.5 (s), two secondary alcohols (one esterified) at 73.4 (d) and 72.4 (d) and a primary alcohol at 59.1 (t). The facile loss of a $C_5H_{10}O_2$ fragment in the mass spectrum, together with ^1H NMR signals at δ 0.94 (d, 6 H, J = 6.3 Hz) indicated the presence of an isovaleric ester. The diterpene skeleton of the diol **1** contained two olefinic bonds and two rings. In the ^1H NMR spectrum the primary alcohol proton signals at δ 4.17 (d, 2 H, J = 6.7 Hz) were coupled to an olefinic signal at 5.44 (t, 1 H, J = 6.7 Hz) that was in turn allylically coupled to a methyl signal at 1.72 (br s, 3 H). These data define a $-C(CH_3)=CHCH_2OH$ moiety. The tetrasubstituted double bond has one methyl substituent [(δ 1.75 (br s, 3 H)] and must therefore be placed in the bicyclic ring system. Since the ^1H NMR spectrum also contained three methyl singlets at δ 1.00 (s, 6 H) and 1.27 (s, 3 H), a labda-8,13-dien-15-ol skeleton was proposed.

The positions of the remaining alcohol and the ester functionality were assigned with some difficulty. The ^1H NMR system contained three broad singlets that, with resolution enhancement, appeared as sharp signals at δ 1.57 (d, 1 H, J = 1.5 Hz), 5.27 (t, 1 H, J = 1.5 Hz) and 3.67 (d, 1 H, J = 1.5 Hz) that were assigned to a bridgehead proton at C-5, a proton adjacent to an ester at C-6





1 R^1 = $-COCH_2CH(CH_3)_2$ R^2 = H
3 R^1 = $-COCH_2CH(CH_3)_2$ R^2 = $-CONHCOCCl_3$
4 R^1 = R^2 = H

2

Table 1. ^1H (360 MHz, $CDCl_3$) and ^{13}C NMR (50 MHz, $CDCl_3$) data for diol **1**[a] and diacetate **2**.

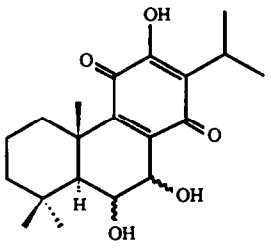
Carbon #	Diol 1 ^1H [δ (mult., int., *J*)]	Diol 1 ^{13}C [δ (mult.)]	Diacetate 2 ^1H [δ (mult., int., *J*]	Diacetate 2 ^{13}C [δ (mult.)]
1		39.4 (t)		43.6 (t)
2		19.0 (t)	5.13 (m, 1 H)	68.5 (d)
3		42.9 (t)		47.8 (s)
4		33.4 (s)		34.4 (s)
5	1.57 (d, 1 H, *J* = 1.5 Hz)	47.9 (d)	1.59 (bs, 1 H)	48.8 (d)
6	5.27 (t, 1 H, *J* = 1.5 Hz)	73.4 (d)	5.33 (bs, 1 H)	73.1 (d)
7	3.67 (d, 1 H, *J* = 1.5 Hz)	72.4 (d)	5.01 (bs, 1 H)	69.1 (d)
8		124.5 (s)		122.3 (s)
9		145.3 (s)		146.7 (s)
10		39.6 (s)		40.6 (s)
11		26.9 (t)		26.9 (t)
12		39.1 (t)		39.2 (t)
13		139.5 (s)		139.5 (s)
14	5.44 (t, 1 H, *J* = 6.7 Hz)	123.3 (d)	5.44 (t, 1 H, *J* = 6.4 Hz)	123.4 (d)
15	4.17 (d, 2 H, *J* = 6.7 Hz)	59.1 (t)	4.18 (d, 2 H, *J* = 6.4 Hz)	59.4 (t)
16	1.72 (s, 3 H)	16.2 (q)	1.73 (s, 3 H)	16.4 (q)
17	1.75 (s, 3 H)	17.4 (q)	1.63 (s, 3 H)	17.0 (q)
18	1.27 (s, 3 H)	21.2 (q)	1.37 (s, 3 H)	‡21.5 (q)
19	1.00 (s, 3 H)	23.4 (q)	1.04 (s, 6 H)	‡22.0 (q)
20	1.00 (s, 3 H)	33.1 (q)		33.1 (q)
21		172.8 (s)		*171.8 (s)
22		44.0 (t)		44.0 (t)
23		25.4 (d)		25.9 (d)
24	0.94 (d, 6 H, *J* = 6.3 Hz)	22.2 (q)	0.98 (d, 6 H, *J* = 6.4 Hz)	22.4 (q)
25		22.3 (q)		22.3 (q)
2-OAc			2.05 (s, 3 H)	*169.6 (s)
				‡22.4 (q)
7-OAc			2.05 (s, 3 H)	*170.6 (s)
				‡23.7 (q)

a) ^1H and ^{13}C NMR assignments are based on one, two and three bond couplings observed in a 2D long range carbon-proton correlation experiment.

*,‡ - signals may be interchanged.

and a *CH*OH proton at C-7. Observation of the ^1H NMR spectrum obtained by treatment of the diol **1** with trichloroacetyl isocyanate to form the corresponding bis-urethane **3** confirmed the location of the hydroxyl groups. Hydrolysis of diol **1** gave a triol **4** and sodium isovalerate. The ^1H NMR spectrum of the triol **4** was unusual because the allylic alcohol proton (C-7) was at δ 3.72 (br s, 1 H) while the homoallylic alcohol proton (C-6) was further downfield at δ 4.25 (br s, 1 H). Proton signal assignments in the diol **1** were confirmed by one, two and three-bond carbon-hydrogen NMR correlation experiments. Specifically, the H-7 signal at δ 3.67 was coupled to carbon signals at 72.4 (C-7), 47.9 (C-5), 145.3 (C-9) and 17.4 (C-17) while the H-6 signal at δ 5.27 was coupled to carbon signals at 73.4 (C-6), 39.6 (C-10), 124.5 (C-8) and 172.8 (C0). The relative stereochemistry in ring B was determined by comparison of the $J_{5,6}$ and $J_{6,7}$ coupling constants observed for diol **1** and triol **4** with those measured for the four possible isomers of 6,7-dihydroxyroyleanone (**5**).[5]

Oxidation of diol **1** with Jones' reagent gave a diketone **6** and the corresponding epoxide **7**. The diketone **6** has the expected α,β-unsaturated ketone and a methyl ketone that results from oxidation of the terminal allylic alcohol at C-13 with loss of a two carbon fragment. The epoxide **7** arises from oxidation of the 8,9-olefinic bond from the α-face, directed by the 7α-hydroxyl in the diol **1**. The $J_{5,6}$ coupling constants in the diketone **6** ($J_{5,6}$ = 3.2 Hz) and epoxide **7** ($J_{5,6}$ = 8.2 Hz) differ quite markedly because the conformation of ring B changes from pseudo-chair in **6** to pseudo-boat in **7**.



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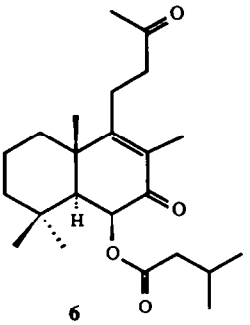

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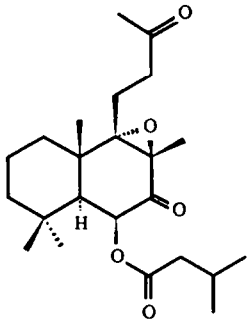

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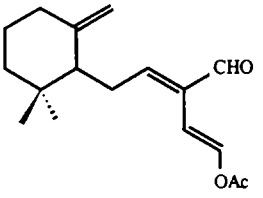

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The diacetate **2** was obtained as an oil, $[\alpha]_D$ = +41.2°. The molecular formula, $C_{29}H_{46}O_7$, was determined by fast-atom bombardment mass spectrometry. The ^{1}H NMR spectrum (Table 1) contained signals at δ 2.05 (s, 6 H) assigned to two acetate esters and at 0.98 (d, 6 H, *J* = 6.4 Hz) assigned to an isovalerate ester. Signals at δ 4.18 (d, 2 H, *J* = 6.4 Hz), 5.44 (t, 1 H, *J* = 6.4 Hz) and 1.73 (br s, 3 H) indicated the presence of the -C(CH_3)=CHCH_2OH moiety at the end of the side chain, while the signals at 5.01 (br s, 1 H) and 5.33 (br s, 1 H) suggested 6β-isovaleroxy-7α-acetoxy substitution in ring B. The remaining downfield signal at δ 5.13 (tt, 1 H, *J* = 12, 4 Hz) was assigned to an axial proton at C-2, implying the presence of a 2α-acetoxy group.

Pulmonate molluscs of the Family Siphonaridae typically contain polypropionate metabolites[6] while *Onchidella binneyi*,[7] *O. borealis* and *O. patelloides*[8] all contained the sesquiterpene onchidal (**8**). *Trimusculus reticulatus* from the Family Trimusculidae is the first pulmonate found to contain diterpenoids. Although the diol **1** is the only non-polar metabolite found in the mucus of *T. reticulatus*, we have been unable to demonstrate that it is the active constituent that deters starfish. This could well be due to our inability to prepare a formulation that will stick to the tube feet of the starfish in the manner of a mucus. Specimens from three collections of *T. reticulatus* from San Nicholas Island, Monterey and San Diego all contained the same metabolites in approximately the same quantities.

Experimental

115 animals were collected intertidally from crevices between boulders on San Nicholas Island, CA. Whole animals were freeze dried and sequentially extracted with hexane, dichloromethane and ethyl acetate. The dichloromethane extract was separated by LC on silica using 9:9:2 hexane/ethyl acetate/isopropanol followed by LC using 45% hexane/ethyl acetate to obtain 6β-isovaleroxylabda-8,13-dien-7α,15 diol (**1**, 201 mg, 1.7 mg/animal) and 2α,7α-diacetoxy-6β-isovaleroxylabda-8,13-dien-15-ol (**2**, 4 mg, 0.3 mg/animal). The mucus from 180 animals collected was diluted with water (500 mL) and acetone (100 mL). The resulting white precipitate was removed by filtration, the acetone was evaporated and the aqueous residue freeze dried. The dichloromethane-soluble material from the lyophilized residue was chromatographed as detailed above to obtain the diol **1** (9 mg, 0.05 mg/animal).

6β-isovaleroxylabda-8,13-dien-7α,15-diol (1): oil; $[\alpha]_D$ = +38.7° ($CHCl_3$, c 0.83); IR ($CHCl_3$) 3600, 1725 cm^{-1}; ^{1}H and ^{13}C NMR see Table 1; HRMS obsd, *m/z* 304.2408 (M^+ - $C_5H_{10}O_2$), $C_{20}H_{32}O_2$ requires 304.2402.

2α,7α-diacetoxy-6β-isovaleroxylabda-8,13-dien-15-ol (2): oil; $[\alpha]_D$ = +41.2° ($CHCl_3$, c 0.35); IR ($CHCl_3$) 3600, 1730 (broad) cm^{-1}; ^{1}H NMR and ^{13}C NMR see Table 1; FABMS obsd, *m/z* 529.3124, $C_{29}H_{46}O_7Na^+$ (MNa^+) requires 529.3141.

Treatment of diol 1 with trichloroacetyl isocyanate. A solution of diol **1** (7 mg) in $CDCl_3$ (0.5 mL) was treated with trichloroacetyl isocyanate (0.05 mL). After five minutes, the ^{1}H NMR spectrum showed complete conversion to the corresponding bisurethane **3**. ^{1}H NMR ($CDCl_3$) δ 0.96 (d, 3 H, *J* = 6.7 Hz), 0.97 (d, 3 H, *J* = 6.7 Hz), 0.99 (s, 3 H), 1.01 (s, 3 H), 1.31 (s, 3 H), 1.53 (bs, 1 H), 1.70 (s, 3 H), 1.81 (s, 3 H), 2.2 (m, 8 H), 4.83 (d, 1 H, *J* = 7.1 Hz), 5.04 (bs, 1 H), 5.44 (m, 2 H), 6.0 (bs, 1 H), 6.64 (bs, 1 H), 8.38 (s, 1 H), 8.40 (s, 1 H).

Hydrolysis of diol 1. Aqueous sodium hydroxide solution (20%, 1 mL) was added to a solution of the diol **1** (20 mg) in methanol (3 mL) and the mixture was heated under nitrogen for 18 hours. The reaction mixture was evaporated to dryness and the residue partitioned between water and ether. The aqueous layer yielded the sodium salt of isovaleric acid. ^{1}H NMR (D_2O) δ 1.00 (s, 3 H), 1.01 (s, 3 H), 2.03 (m, 1 H), 2.16 (d, 2 H, *J* = 7.5 Hz).

The ether extract was dried over anhydrous sodium sulfate and the solvent was evaporated to obtain white crystals of the triol **4** (8 mg): mp = 134-5°C; $[\alpha]_D$ = +73.0° (MeOH, c 0.43); IR ($CHCl_3$) 3600, 3450 br cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.01 (s, 3 H), 1.22 (s, 3 H), 1.31 (s, 3 H), 1.34 (bs, 1 H), 1.72 (s, 3 H), 1.80 (s, 3 H), 2.08 (m, 4 H), 3.72 (bs, 1 H), 4.17 (d, 2 H, *J* = 6.9 Hz), 4.25 (bs, 1 H), 5.44 (t, 1 H, *J* = 6.9 Hz). FABMS obsd, *m/z*, 345.2413, $C_{20}H_{34}O_3Na^+$ (MNa^+), requires 345.2406.

Oxidation of diol 1 with Jones' Reagent. 8 N Jones' reagent (0.5 mL) was added dropwise to a solution of the diol **1** (20 mg) in acetone (3 mL). The mixture was stirred at 0°C for 2 hours then quenched with an excess of isopropanol, diluted with water (5 mL) and extracted with ethyl acetate (4 x 5 mL). The combined extracts were washed with water and evaporated to give a yellow oil (7.6 mg) which was separated by LC using 60% hexane/ethyl acetate as eluant to obtain the diketone **6** (2.1 mg) and the epoxide **7** (3.7 mg).

Diketone 6: IR ($CHCl_3$) 2950, 1735, 1720, 1660 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.94 (d, 6 H, *J* = 6.4 Hz), 1.03 (s, 3H), 1.05 (s, 3 H), 1.18 (m, 1 H), 1.22 (m, 1 H), 1.25 (m, 1 H), 1.41 (s, 3 H), 1.59 (s, 3 H), 1.72 (d, 1 H, *J* = 2.8 Hz), 1.76 (s, 3H), 2.19 (s, 3 H), 2.57 (m, 2 H), 5.81 (d, 1 H, *J* = 2.8 Hz). CIMS obsd *m/z* 377.2661, $C_{23}H_{37}O_4$ (MH^+), requires *m/z* 377.2692.

Epoxide 7: IR ($CHCl_3$) 2950, 1735, 1720 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.95 (s, 3 H), 1.00 (s, 3 H), 1.01 (d, 3 H, *J* = 6.5 Hz), 1.02 (d, 3 H, *J* = 6.5 Hz), 1.11 (s, 3 H), 1.24 (m, 2 H), 1.39 (s, 3 H), 1.75 (m, 1 H), 1.93 (m, 1 H), 2.16 (s, 3H), 2.20 (m, 1 H), 2.26 (d, 1 H, *J* = 7.5 Hz), 2.34 (m, 1 H), 2.43 (d, 1 H, *J* = 7.2 Hz), 2.57 (t, 1 H, *J* = 7.5 Hz), 5.68 (d, 1 H, *J* = 7.2 Hz). CIMS obsd *m/z* 393.2642, $C_{23}H_{37}O_5$ (MH^+), requires 393.2641.

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References and Notes

[1]Beeman, R.D.; Williams, G.C. in "Intertidal invertebrates of California", Morris, R.H.; Abbott, D.P.; Haderlie, E.C., Eds.; Stanford Univ. Press: Stanford, CA, 1980; p. 341.

[2]Yonge, C.M. *Proc. Malacol. Soc. London* **1958**, *33*, 31-37.

[3]Walsby, J.R. *Veliger* **1975**, *18*, 139-145.

[4]Rice, S.H. *J. Exp. Mar. Biol. Ecol.* **1985**, *93*, 83-89.

[5]Meier, H.; Ruedi, P.; Eugster, C.H. *Helv. Chim. Acta* **1981**, *64*, 630-642. [All 5α hydrogen, 8,9 unsaturated: 6α,7α-diol, $J_{5,6}$ = 12 Hz, $J_{6,7}$ = 5 Hz; 6α,7β-diol, $J_{5,6}$ = 12 Hz, $J_{6,7}$ = 8 Hz; 6β,7α, $J_{5,7}$ = 1-2 Hz, $J_{6,7}$ = 2 Hz; 6β,7β-diol, $J_{5,6}$ = 1 Hz, $J_{6,7}$ = 5.5 Hz.

[6]For recent reviews see, Faulkner, D.J. *Nat. Prod. Rep.* 1984, *1*, 551-598. Faulkner, D.J. *Nat. Prod. Rep.* **1986**, *3*, 1-33.

[7]Ireland, C.M.; Faulkner, D.J. *Bioorg. Chem.* **1978**, *7*, 125-131.

[8]Manker, D.C. Unpublished data from this laboratory.