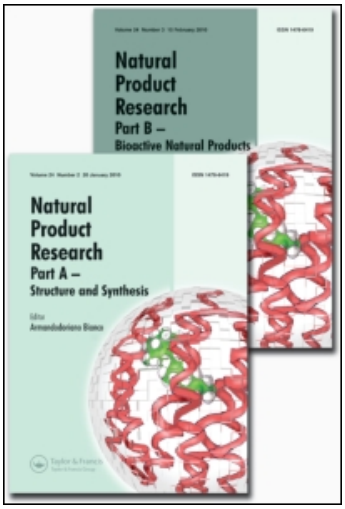

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New diterpenes from the marine pulmonate *Trimusculus peruvianus*

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The family Trimusculidae produce labdane diterpenes, which differ in the degree and type of esterification with acetoxy and isovaleroyl ester, predominantly. In this article, we describe the isolation from the marine pulmonate *Trimusculus peruvianus*, collected on intertidal rocks of Chilean coasts, of two new labdane diterpenes closely related to the above mentioned characteristics. Their structures were determined by spectroscopic data and correspond to 6β-hydroxy-labda-8, 13-dien-15-ol and 3, 19-isovaleroyl-6β, 9α-dihydroxylabda-$\Delta^{8,17}$, 13-dien-15-oic acid.

Keywords: marine mollusc; diterpenes; labdane diterpenoids; *Trimusculus peruvianus*

1. Introduction

Shell bearing molluscs such as intertidal pulmonate limpets of the family Trimusculidae seem to produce only *de novo* secondary metabolites (Manker & Faulkner, 1987). They produce diterpenes, which appear to be repellent to predatory fish (Gray, Davies-Coleman & McQuaid, 1998), and also toxic to larvae settling around them (Manker and Faulkner, 1996), in order to avoid the presence of other invertebrates nearby.

Four species of *Trimusculus* have been studied (Manker & Faulkner, 1996; Ravirosa, Quezada & San-Martín, 1992), all having a common feature: they produce labdane diterpenes, which differ in the degree and type of esterification with acetoxy and isovaleroyl ester, predominantly. Previously, we reported the isolation and structure elucidation of five diterpenes from *T. peruvianus* (San-Martin, Quezada, Soto, Palacios & Rovirosa, 1996; Diaz-Marrero et al., 2003). In this article, we describe the isolation, from the same source, of two new diterpenoids closely related to the above mentioned compounds.

2. Experimental section

IR spectra were recorded on a Perkin Elmer System 2000 FTIR spectrophotometer in $CHCl_3$ solutions. EIMS and HRMS data were taken on a Micromass Autospec

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spectrometer. The ^{1}H, ^{13}C-NMR spectra as well as ^{1}H–H COSY, ^{13}C (DEPT), HMQC (optimised for $^1J_{H\text{-}C} = 140$ Hz), HMBC (mixing time of 75 msec) and ROESY (mixing time of 250 msec) data were obtained on Bruker AM-400 and AM-500 spectrometers. All chemical shifts are reported with respect to TMS ($\delta = O$). Two-dimensional NMR spectra were obtained with the standard Bruker software. The gel filtration column (Sephadex LH-20) used hexane-MeOH–CH_2Cl_2 (3: 1: 1) as solvent. Merck Si gels 7734 and 7729 were used in column chromatography. The spray reagent for TLC was H_2SO_4–H_2O–AcOH (1: 4: 20). Solvents were of analytical grade.

2.1. *Biological material*

Specimens of *T. peruvianus* were collected on intertidal rocks near Pichidangui, IV Region, Chile, during November 2005. The organism was identified by Prof. C. Osorio, Universidad de Chile. A voucher specimen has been deposited at the Facultad de Ciencias, Universidad de Chile collection.

2.2. *Extraction and isolation*

Five-hundred and sixty freeze-dried specimens of *T. peruvianus* were extracted with ethyl acetate at room temperature and concentrated to give a dark residue (26.0 g). The extract was separated by flash chromatography on Si gel, using hexane–ethyl acetate mixtures, followed by filtration column and HPLC, obtaining compounds **1** and **3**.

2.2.1. *6β-hydroxy-labda-8, 13-dien-15-ol (**1**)*

Colourless oil; $[\alpha]_D^{25}$ 28 (*c* 0.12 $CDCl_3$). IR (film)υ_{max}($CHCl_3$): 3450, 2950,1660,1280,1210. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.98 (3H, s, Me-19), 1.30 (3H, s, Me-16), 1.35 (3H, s, Me-20), 1.16 (1H, d, $J = 3.3$ Hz, H-5), 1.52 (2H, ddd, $J = 3.4$, 6.8, 14.0 Hz, H_2-2), 1.66 (3H, s, Me-17), 1.80 (3H, s, Me-18), 4.14 (2H, d, $J = 7.1$ Hz, H-15), 4.44 (1H, brd, $J = 5.0$ Hz, H-6), 5.39 (1H, t, $J = 6.7$ Hz, H-14). ^{13}C-NMR (100 MHz, $CDCl_3$) $\boldsymbol{\delta}$: 19.2 (CH_2, C-2), 19.9 (CH_3, C-17), 21.4 (CH_3, C-20), 23.4 (CH_3, C-18), 23.9 (CH_3, C-16), 26.8 (CH_2, C-11), 32.7 (CH_2, C-12), 33.6 (CH_3, C-19), 34.0 (C, C-4), 38.8 (C, C-10), 40.0 (CH_2, C-1), 43.1 (CH_2, C-3), 43.9 (CH_2, C-7), 53.9 (CH, C-5), 59.2 (CH_2, C-15), 66.0 (CH, C-6), 122.3 (C, C-8), 123.8(CH, C-14), 140.1(C, C-9), 140.7 (C, C-13). MS (70 eV) *m/z*: 306[M^+, $C_{20}H_{34}O_2$](2), 288[$M^+ - H_2O$]$^+$(5), 270[$M^+ - 2H_2O$]$^+$(8), 211[$M - C_5H_9O$]$^+$(5), 207[$M^+ - C_4H_7O$–H_2O]$^+$(36), 193[$M - C_5H_9O$–H_2O]$^+$(8), 133(40), 119(100).

2.2.2. *3,19-isovaleroyl-6β,9α-dihydroxylabda-$\Delta^{8,17}$,13-dien-15-oic acid (**3**)*

Colourless oil; $[\alpha]_D^{25}$ 131 (c 0.29, $CDCl_3$). IR (film) ν_{max}: 3503, 2956, 1710, 1646 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.96[a] (3H, d, $J = 6.6$ Hz, H-5′), 0.98[a] (3H, d, $J = 6.6$ Hz, H-4′), 0.98[a] (6H, d, $J = 6.6$ Hz, H-4″ and H-5″), 1.08 (3H, s, H-18), 1.14 (3H, s, H-20), 1.21 (1H, d, $J = 12.2$ Hz, H-1β), 1.80 (1H, m, H-2), 1.87 (1H, m, H-11), 1.94 (1H, m, H-2′), 1.97 (1H m, H-11), 2.00 (1H, m, H-1α), 2.13 (1H, m, H-7β), 2.20 (2H, m, H-3′and H-3″), 2.21 (3H, s, H-16), 2.23 (4H, m, H-2′and H-2″), 2.27 (1H, m, H-12), 2.34 (1H, m, H-12), 2.40 (1H, d, $J = 1.3$ Hz, H-5), 2.97 (1H, dd, $J = 3.1$, 13.6 Hz, H-7α), 4.32 (1H, brs, H-6), 4.49 (1H, d, $J = 11.7$ Hz, H-19), 4.75 (1H, d, $J = 11.7$ Hz, H-19), 4.88 (1H, s, H-17b), 5.02 (1H, brs, H-3), 5.14 (1H, s, H-17a), 5.74 (1H, s, H-14). ^{13}C-NMR (125.7 MHz, $CDCl_3$) $\boldsymbol{\delta}$: 19.4 (CH_3, C-

16), 20.5 (CH_3, C-20), 22.4[a] (CH_3, C-4′), 22.4[a] (CH_3, C-5′), 22.5[a] (CH_3, C-4″), 22.5[a] (CH_3, C-5″), 22.8 (CH_3, C-18), 23.1 (CH_2, C-2), 27.7 (CH_2, C-11), 25.7 (CH, C-3′), 25.7 (CH, C-3″), 26.9 (CH_2, C-1), 35.4 (CH_2, C-12), 41.9 (C, C-4), 42.2 (CH_2, C-7), 43.5[b] (CH_2, C-2′), 44.0 (C, C-10), 44.0[b] (CH_2, C-2″), 44.6 (CH, C-5), 67.3 (CH_2, C-19), 68.2 (CH, C-6), 73.2 (CH, C-3), 79.7 (C, C-9), 113.8 (CH_2, C-17), 114.7 (CH, C-14), 143.7 (C, C-8), 163.2 (C, C-13), 170.3 (C, C-15), 172.5 (C, C-1′), 173.1 (C, C-1″)[a, b] signals may be reversed. MS (70 eV) *m/z*: 536 $[M]^+$ (2), 518 $[M - H_2O]^+$ (2), 500 (1), 434 $[M - C_5H_{10}O_2]^+$ (4), 416 $[M - C_5H_{10}O_2–H_2O]^+$ (7), 314 (18), 251 (46), 85 (91), 57 (100). HREIMS *m/z*: $[M]^+$ 536.3382 (Calcd for $C_{30}H_{48}O_8$, 536.3349), 518.3239 (Calcd for $C_{30}H_{46}O_7$, 518.3244), 434.2734 (Calcd for $C_{25}H_{38}O_6$, 434.2668), 416.2618 (Calcd for $C_{25}H_{36}O_5$, 416.2563).

3. Results and discussion

Compound **1** has close similarities with 6β-acetoxylabda-8, 13-dien-15-ol (**2**), a labdane type diterpene isolated in an earlier investigation from this source (San-Martin et al., 1992). Comparison of the ^{1}H-NMR spectral data of compounds **1** and **2** showed that they have the following signals in common: two olefinic methyl groups at C-8 and C-13, a primary hydroxyl group at C-15, and three tertiary methyl groups at C-4 and C-10 positions. The only differences found are the absence of the signal due to the methyl of an acetate group in **1**, and that the signal corresponding to the proton geminal to the acetate in **2** is shifted from δ 5.45 in **2** to δ 4.44 in **1**. The mass spectra of **2** showed a difference of 42 mass units, suggesting that **1** is the nonacetylated version of **2**. This fact was corroborated with the HMBC experiments, where the signal at δ 4.43 (δ_C 66.0) is correlated with the carbons at δ 43.9 (C-7) and 53.9 (C-5). Thus, compound **1** corresponds to 6β-hydroxy-labda-8, 13-dien-15-ol (Figure 1).

Compound **3** was isolated as a colourless oil, whose mass spectrum showed a molecular ion at $[M]^+$ at *m/z* 533, in accordance with the molecular formula $C_{30}H_{48}O_8$ (HRMS at 536.3382), indicating that there are seven insaturations. The ^{13}C-NMR spectrum of **3** exhibited signals for 30 carbon atoms; a methylene at δ 113.8, a methine sp^2 at δ 114.7, two quaternary olefinic carbons at δ 143.7 and 163.2, three carbonylic carbons at δ 170.3, 172.5 and 173.1, while the IR spectrum displayed absorptions at 3503, 1710, 1646 cm^{-1} indicating the presence of hydroxyl and carbonyl functions. So the molecule must be a bicyclic diterpenoid of the labdane type esterified. Comparison of the NMR data of compound **3** with those of **4** (Diaz-Marrero et al., 2003), indicated that the C-3 and C-19 are esterified with isovaleric moieties ($\delta_{H\text{-}3}$ 5.02 brs, $\delta_{C\text{-}3}$ 73.2 and $\delta_{H\text{-}19}$ 4.49 (d, $J = 11.7$ Hz), $\delta_{H\text{-}19}$ 4.75 (d, $J = 11.7$ Hz) $\delta_{C\text{-}19}$ 67.3, respectively); two deshielded carbon resonances at δ_C 113.8 (CH_2) and 143.7(C) and two coupled proton resonances at δ_H 4.88 s and 5.14 s were assigned to an exocyclic double bond in **3**. Furthermore, the disappearance of a signal at δ 138.1 in **4** and the appearance of a signal at δ 79.7 in **3** indicated that there is an oxidation of the C-9 position with a tertiary hydroxyl. All these features were corroborated with HMBC, HSQC and ^{1}H-^{1}HCOSY experiments. 2D NOESY experiment of **3** established the relative configuration of C-3, C-4 and C-6, due to the NOE effects observed between Me-18 and H-3, H-5 and H-6 and between H_2-19 with Me-20; therefore the relative stereochemistry of compound **3** is coincident with that of compound **4** (Diaz-Marrero et al., 2003). The configuration at C-9 was determined by the following features: the observation of a NOE effect between H-5 and one of the protons of the methylene H_2-7 permitted the differentiation between these two protons H-7α and H-7β; a NOE effect between H-7β and one of the protons of the exocyclic methylene H-17a and the

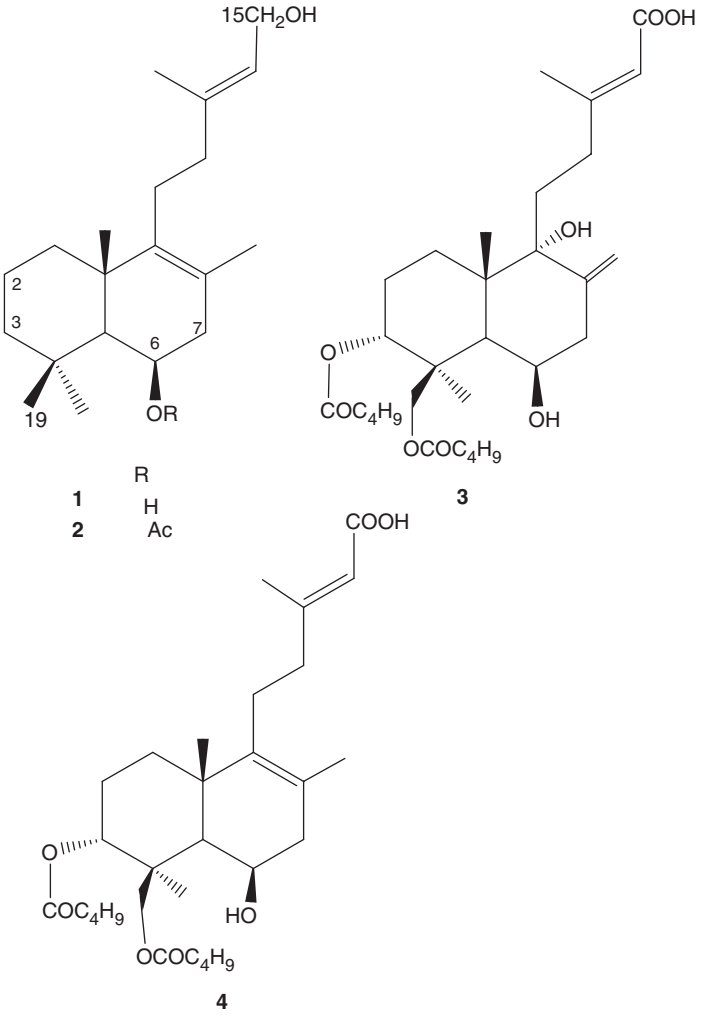

Figure 1. Metabolities isolated from *T. Peruvianus*.

observation of a NOE effect between H-17b with one of the protons of C-11 and one proton of C-12 indicated that the configuration of the lateral chain in C-9 must be β. The configuration for the trisubstituted double bond of the lateral chain must be E, due to the value for Me-16 and C-12 (δ_C 19.4 and 35.4, respectively); furthermore, a NOE effect between H-14 and H-12 is observed. These data support the structure proposed for **3** as 3,19-isovaleroyl-6β,9α-dihydroxylabda-$\Delta^{8,17}$-13-dien-15-oic acid (Figure 1).

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