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ENT-LABDANE GLYCOSIDES FROM *HETEROTHALAMUS ALIENUS*

JOSÉ R. SAAD, OSCAR S. GIORDANO,

Departamento de Química Orgánica, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Chacabuco y Pedernera, 5700 San Luis, Argentina

CARLOS M. CERDA-GARCÍA-ROJAS, JOEL J. TRUJILLO-SERRATO, and PEDRO JOSEPH-NATHAN*

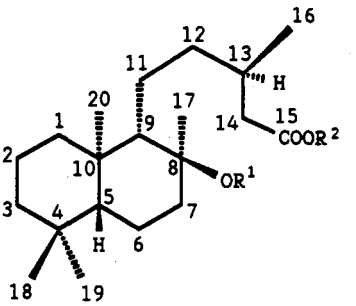
Departamento de Química, Centro de Investigación y de Estudios Avanzados, Instituto Politécnico Nacional, Apartado 14-740, México, D. F., 07000 México

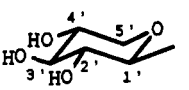
ABSTRACT.—Two new diterpene glycosides, 8-*O*-β-D-xylopyranosyl-*ent*-8β-hydroxylabdan-15-oic acid [**1**] and 8-*O*-β-D-glucopyranosyl-*ent*-8β-hydroxylabdan-15-oic acid [**2**], were isolated from *Heterothalamus alienus*. Their structures were elucidated by nmr spectroscopy, and their absolute configurations were determined by tranformation of both natural products into methyl *ent*-8β-hydroxylabdan-15-oate. The sugar residues were identified after acid hydrolyses of the two natural products.

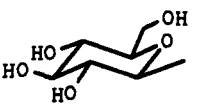
The small genus *Heterothalamus* is composed of ca. seven species which are primitives from South America (1,2), and to our knowledge there are only limited studies of this genus (3,4). This paper describes the chemical study of *Heterothalamus alienus* (Spreng.) O.Kuntze (Compositae), a very ramified shrub which grows in the mountains near San Luis and Comechingones, Argentina and is often used as an ornamental (1,2).

RESULTS AND DISCUSSION

The MeOH extracts of *H. alienus* afforded two new diterpene glycosides: 8-*O*-β-D-xylopyranosyl-*ent*-8β-hydroxylabdan-15-oic acid [**1**] and 8-*O*-β-D-glucopyranosyl-*ent*-8β-hydroxylabdan-15-oic acid [**2**].



1 R^1=  , R^2=H

2 R^1=  , R^2=H

3 R^1=  , R^2=Me

The ir spectrum of compound **1** indicated the presence of hydroxyl and carboxyl groups. The ^{1}H-nmr spectrum, measured in DMSO-d_6 in the presence of D_2O (Table 1), shows six signals at the characteristic chemical shifts and with the coupling constants of a β-xylopyranosyl residue (5). The 8β-hydroxylabdan-15-oic acid moiety of **1** was evident when the ^{13}C-nmr spectrum (Table 2) was compared with the reported chemical shifts for methyl 8β-hydroxylabdan-15-oate (6). Acetylation of **1** followed by treatment with CH_2N_2 afforded compound **3** which shows three acetyl singlets and one carboxymethyl singlet in its ^{1}H-nmr spectrum (Table 1). Furthermore, the agreement between the ^{13}C-nmr chemical shifts of compound **3** in $CDCl_3$ and those of methyl 8β-hydroxylabdan-15-oate (6) is indicative of the diterpene moiety in **3**.

Compound **2** shows bands for hydroxyl and carboxyl groups. Its ^{1}H-nmr spectrum, in DMSO-d_6 +D_2O (Table 1), shows characteristic signals for the β-glucopyranosyl fragment (7). The structure of **2** was deduced by comparison of the ^{13}C-nmr spectrum with those of **1**, **3** (Table 2), and methyl 8β-hydroxylabdan-15-oate (6).

In order to confirm the structures of **1** and **2** and to have evidence for their absolute configurations, both compounds were subjected to acid hydrolysis. While treatment of **1** and **2** with aqueous HCl in MeOH under reflux gave methyl *ent*-labd-8-en-15-oate (8,9), a similar treatment of both substances using diluted aqueous HCl at room temperature afforded methyl *ent*-8β-hydroxylabdan-15-oate, identical in all respects to the previously described compound (6,10), including ^{13}C-nmr measurements and optical rotation.

The D-xylose and D-glucose residues were isolated as their corresponding anomeric mixtures from the aqueous layers are left after the acid hydrolyses and indentified by tlc and ^{1}H nmr. The absolute configurations of the sugar moieties were determined by optical rotations of the isolated sugars from **1** and **2** and by comparison with mutarotated samples obtained from authentic β-D-xylose and β-D-glucose, respectively. In each case the rotations were identical. Thus, the sugars attached to **1** and **2** are the D enantiomers.

TABLE 1. ^{1}H-nmr Data (300 MHz) of *ent*-Labdane Derivatives **1–3**.[a]

Proton	Compound		
	1[b]	**2**[b]	**3**[c]
H_a-14	2.24 (6,15)	2.25 (6,15)	2.38 (6,15)
H_b-14	1.92 (9,15)	1.96 (9,15)	2.18 (9,15)
Me-16	0.86 (6)	0.88 (6)	0.92 (6)
Me-17	1.10	1.12	1.12
Me-18	0.83	0.84	0.84
Me-19	0.76	0.76	0.77
Me-20	0.78	0.79	0.80
H-1′	4.28 (8)	4.34 (8)	4.64 (8)
H-2′	2.87 (8,9)	2.90 (8,9)	4.87 (8,10)
H-3′	3.09 (9,9)	3.18 (9,10)	5.18 (10,10)
H-4′	3.23 (5,9,11)	3.04 (10,10)	4.96 (6,10,11)
H-5ax	2.98 (11,11)	3.06 (1,5,10)	3.27 (11,12)
H-5eq	3.58 (5,11)	—	4.05 (6,12)
H_a-6′	—	3.42 (5,12)	—
H_b-6′	—	3.63 (1,12)	—

[a]Chemical shifts are in ppm with TMS as the internal reference. Coupling constants in parentheses are in Hz.

[b]In DMSO-d_6 +a drop of D_2O.

[c]In $CDCl_3$. Ac: 2.04, 2.03 and 2.02 ppm. OMe: 3.66 ppm.

TABLE 2. ^{13}C-nmr Chemical Shifts of *ent*-Labdane Derivatives **1**–**3**.[a]

Carbon	Compound		
	1[b]	**2**[b]	**3**[c]
C-1	39.8	39.8	39.9
C-2	17.9	17.9	18.4
C-3	41.5	41.4	41.5
C-4	32.7	32.7	33.2
C-5	55.4	55.3	55.8
C-6	19.5	19.5	20.0
C-7	39.7	—[d]	39.6
C-8	79.9	79.8	82.3
C-9	60.1	60.2	60.6
C-10	39.7	—	39.2
C-11	22.3	22.3	23.2
C-12	40.2	40.2	40.2
C-13	30.4	30.3	31.4
C-14	41.5	41.4	42.0
C-15	174.3	174.4	174.0
C-16	19.7	19.7	19.6
C-17	20.0	19.7	20.3
C-18	33.1	33.2	33.4
C-19	21.3	21.2	21.5
C-20	15.5	15.4	15.7
C-1′	96.5	95.7	94.4
C-2′	73.6	73.8	71.7
C-3′	76.8	77.1	72.6
C-4′	69.6	70.2	69.3
C-5′	65.2	76.4	62.3
C-6′	—	61.1	—

[a]In ppm at 75.4 MHz.
[b]In DMSO-d_6.
[c]In $CDCl_3$. Acetates: 170.3, 169.8, 169.0, 20.7, 20.7 and 20.7 ppm. MeO: 51.2 ppm.
[d]Obscured by the DMSO-d_6 signal.

It should be noted that *ent*-labdane derivatives related to the two new substances **1** and **2** have been isolated from South American species of the genus *Baccharis* (5,11,12).

EXPERIMENTAL

PLANT MATERIAL, EXTRACTION, AND ISOLATION.—Specimens of *H. alienus* were collected in El Suyuque, San Luis, Province of Argentina in March 1990 and identified by botanist Luis A. del Vitto. A voucher specimen (5335 UNSL) is deposited at the Herbario de la Universidad Nacional de San Luis. The air-dried plant material (2.1 kg) was extracted with hot MeOH. The combined extracts were evaporated to dryness, and the residue (150 g) was chromatographed on Si gel 60G. The fractions eluted with C_6H_6-EtOAc (1:1) and C_6H_6-EtOAc (4:6) were separately rechromatographed to afford **1** (2.2 g) and **2** (1.6 g), respectively.

8-O-β-D-Xylopyranosyl-ent-8β-hyrdoxylabdan-15-oic acid [**1**].—Recrystallizations from EtOH provided pure **1** as white needles: mp 199–200°; $[\alpha]_{589}$ −2.6, $[\alpha]_{578}$ −2.6, $[\alpha]_{546}$ −2.8, $[\alpha]_{436}$ −4.6, $[\alpha]_{365}$ −6.6, (DMSO, c=5.0); ir (KBr) ν max cm^{-1} 3420 and 1030 (OH), 3300 and 1715 (COOH); ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

8-O-β-D-Glucopyranosyl-ent-8β-hyrdoxylabdan-15-oic acid [**2**].—Recrystallization from MeOH/H_2O provided pure **2** as white needles: mp 178–180°; $[\alpha]_{589}$ +3.0, $[\alpha]_{578}$ +4.0, $[\alpha]_{546}$ +4.5, $[\alpha]_{436}$ +7.5, $[\alpha]_{365}$ −13.0, (EtOH, c=2.0); ir (KBr) ν max cm^{-1} 3400, 1060 and 1015 (OH), 3400 and 1708 (COOH); ^{1}H nmr see Table 1; ^{13}C nmr see Table 2.

Methyl-ent-8β-hydroxylabdan-15-oate.—Solutions of **1** or of **2** (100 mg) in MeOH (67 ml) were stirred in solutions of concentrated aqueous (HCl-H_2O (1:3) (four additions of 1.4 ml each 24 h). After 72 h, the reaction mixtures were neutralized wtih $NaHCO_3$ and evaporated under vacuum. In each case the residue

was extracted with CH_2Cl_2, the organic layer was washed with H_2O and aqueous $NaHCO_3$ and H_2O, dried over Na_2SO_4, filtered, and evaporated under vacuum. Each residue was chromatographed on Si gel (230–400 mesh). The fractions eluted with hexane-EtOAc (9:1) yielded a solid which was recrystallized from MeOH/H_2O to give methyl *ent*-8β-hydroxylabdan-15-oate (8 mg) as white needles: mp 70–72° [lit. (10) 72–74°]; $[\alpha]_{589}$ +7.0, $[\alpha]_{578}$ +9.0, $[\alpha]_{546}$ +10.5, $[\alpha]_{436}$ +18.0, $[\alpha]_{365}$ +29.0 ($CHCl_3$, c=1.0) [lit. (10) $[\alpha]_{589}$ +10°].

Methyl 8-O-[3',4',5'-tri-O-acetyl-β-D-xylopyranosyl]-ent-*8β-hydroxylabdan-15-oate* [**3**].—Treatment of **1** with Ac_2O followed treatment with CH_2N_2 under standard conditions afforded **3** as an amorphous solid. Recrystallization from MeOH/H_2O provided pure **3** as white needles: mp 158–159°; $[\alpha]_{589}$ −22.0, $[\alpha]_{578}$ −22.0, $[\alpha]_{546}$ −24.0, $[\alpha]_{436}$ −40.0, $[\alpha]_{365}$ −62.0° ($CHCl_3$, c=0.5); ir (KBr) ν max cm^{-1} 1740 (COOR); ^1H nmr see Table 1; ^{13}C nmr see Table 2.

Methyl ent-*labd-8-en-15-oate.*—Solutions of **1** or of **2** (200 mg) in MeOH/H_2O (45 ml and 45 ml) were treated with a solution of concentrated aqueous HCl (20 ml). The reaction mixtures were refluxed during 2 h, concentrated to one-half volume, and extracted with CH_2Cl_2. In each case the organic layer was washed with H_2O, aqueous $NaHCO_3$, and H_2O, dried over Na_2SO_4, filtered, and evaporated under vacuum. Each residue was then chromatographed on alumina. The fractions eluted with hexane yielded the pure methyl *ent*-labd-8-en-15-oate (8,9) (80 mg) as a colorless oil.

IDENTIFICATION OF THE SUGAR RESIDUES.—The aqueous layers left from the acid treatments of **1** and of **2**, described above, were neutralized with concentrated aqueous $NaHCO_3$ and evaporated to dryness. The solid residues were chromatographed on alumina. The fractions eluted with EtOAc-MeOH (7:3) afforded D-xylose and D-glucose, respectively. These samples were identified after tlc, ^1H nmr, and optical rotation comparisons with authentic samples, which were previously stored overnight as aqueous solutions.

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LITERATURE CITED

1. M.J. Dimitri, "Enciclopedia Argentina de Agricultura y Jardinería. Descripción de las Plantas Cultivadas," Acme, Buenos Aires, 1980, Vol. 2, p. 1033.
2. D.L. Anderson, J.A. Del Aguila, and A.E Bernardón, *Rev. Invest. Agropec., Ser. 2*, **7**, 153 (1970).
3. R. Boeker, J. Jakupovik, F. Bohlmann, and R.M. King, *Rev. Latinoam. Quím.*, **17**, 47 (1986).
4. H. Beck, *An. Assoc. Bras. Quim.*, **10**, 295 (1951).
5. C. Zdero, F. Bohlmann, R.M. King, and H. Robinson, *Phytochemistry*, **25**, 2841 (1986).
6. H. Hirota, T. Nakamura, T. Tsuyuki, and T. Takahashi, *Bull. Chem. Soc. Jpn.*, **61**, 4023 (1988).
7. E. Vidal-Ollivier, A. Babadjamian, R. Faure, R. Chemli, K. Boukef, G. Balansard, and E.J. Vincent, *Spectrosc. Lett.*, **22**, 579 (1989).
8. M.T. Davies-Coleman and D.J. Faulkner *Tetrahedron*, **47**, 9743 (1991).
9. G. Hugel, A.C. Oehlschlager, and G. Ourisson, *Tetrahedron*, Suppl. 8, Part 1, 203 (1966).
10. C.A. Henrick, P.R. Jefferies, and R.S. Rosich, *Tetrahedron Lett.*, 3475 (1964).
11. C. Zdero, F. Bohlmann, and H.M. Niemeyer, *Phytochemistry*, **29**, 2611 (1990).
12. F. Faini, C. Labbe, and M.I. Polanco, *Phytochemistry*, **31**, 3274 (1992).

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