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Article

6β-Hydroxymaslinic Acid, a Triterpene from *Vochysia ferruginea*

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Um novo ácido triterpenóide pentacíclico foi isolado a partir de folhas e frutos de *Vochysia ferruginea*. A estrutura do novo composto foi elucidada por espectroscopia de RMN como sendo o ácido 2α,3β,6β-triidroxiolean-12-en-28-óico (ácido 6β-hidroximaslínico, **1**). Além do novo triterpenóide, foram isolados das folhas e frutos o glicosídeo de β-sitosterol e três misturas contendo os triterpenóides conhecidos uvaol e eritrodiol, ácidos ursólico e oleanólico e os ácidos 2α,3β-diidroxiurs-12-en-28-óico e o respectivo isômero oleanólico (ácido maslínico ou cratególico). Nos frutos, a bellericagenina A e o seu éster (28 → 1) β-D-glicopiranosílico (bellericasídeo A) estão presentes em elevados teores.

A novel oleane acid was isolated from the leaves and the fruits of *Vochysia ferruginea*. The structure of the new triterpenoid was elucidated by NMR spectroscopy as 2α,3β,6β-trihydroxy-olean-12-en-28-oic acid (6β-hydroxymaslinic acid, **1**). In addition, β-sitosterol-glucoside and three mixtures containing known triterpenoids, uvaol and erythrodiol, ursolic and oleanolic acids, 2α,3β-dihydroxyurs-12-en-28-oic acid and its respective oleanolic isomer (maslinic or crategolic acid), were isolated from the leaves and the fruits of *Vochysia ferruginea*. In the fruits, bellericageninA and its (28 → 1)β-D-glucopyranosyl ester (bellericasideA) were present in high amount.

Keywords: *Vochysia ferruginea*, Vochysiaceae, pentacyclic triterpenes, 6β-hydroxymaslinic acid

Introduction

The genus *Vochysia* (Vochysiaceae) seems to be an abundant source of triterpenoids. Bartogenic and vismiaefolic acids have been reported from *V. vismiaefolia*[1]; betulinic, 4-epi-vismiaefolic, and 2α,3β,19α-trihydroxy-24-oxo-urs-12-en-28-oic acids from *V. piramidalis*[2].

From the stem bark of *V. divergens* we isolated the terpenoids β-sitosterol, betulinic, sericic, divergioic and 24-hydroxytormentic acids, and the (28 → 1) β-D-glucopyranosyl ester of later[3-5]. Sericic acid (2α,3β,19,24-tetrahydroxyolean-12-en-28-oic acid) presented antifungal[3] and antibacterial[4] activities. 24-Hydroxytormentic acid showed antinociceptive action[6].

V. ferruginea is a tree commonly found in wet soils of Venezuelan Amazon[7]. In this communication we report the structure elucidation of 6β-hydroxymaslinic acid, **1**, a novel triterpenoid of the oleane series obtained from the leaves and the fruits of *V. ferruginea.* β-sitosterol-glucoside and mixtures of uvaol and erythrodiol, ursolic and oleanolic acids, 2α,3β-dihydroxyurs-12-en-28-oic acid and its respective oleanolic isomer (maslinic or crategolic acid) were also obtained. Bellericagenin A and its (28 → 1) β-D-glucopyranosyl ester (bellericaside A) were present in high amount in the fruits.

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Experimental

General experimental procedures

Melting points were determined using a Kofler hot-stage instrument and were uncorrected. IR spectra were measured on a Perkin-Elmer 1320 spectrometer. ^{1}H and ^{13}C-NMR spectra were recorded in $CDCl_3$ or in pyridine-d_5, using TMS as internal reference, employing a Varian Gemini 300 (^{1}H, 300 MHz; ^{13}C, 75 MHz) spectrometer. Optical rotations were measured in a Polamat A (Carl Zeiss) polarimeter.

Mass spectra were recorded on a HP 5988A apparatus (EI, 70 eV). Isolation procedures were monitored by employing thin-layer chromatography on pre-coated silica gel plates (Merck, Kieselgel 60 F-254).

Plant material

Vochysia ferruginea Mart (Vochysiaceae) was collected in the margins of Cataniapo River near Puerto Ayacucho (Amazona State, Venezuela) and identified by Dr. A. Castillo. A voucher specimen is deposited in the Herbarium of the Escuela de Biologia, Universidad Central de Venezuela, Caracas, under number AC 3301.

Extraction and isolation

Air dried and powdered leaves (400 g) were exhaustively extracted at room temperature with $CHCl_3$ to give after evaporation a residue (8.0 g), which was purified by CC (SiO_2 ; $CHCl_3$ with increasing amounts of MeOH). The first three fractions (eluted with $CHCl_3$ - MeOH, 49:1, 19:1, and 9:1, respectively) gave unseparated mixtures of uvaol and erythrodiol (38 mg), of ursolic and oleanolic acids (280 mg), and of 2α,3β-dihydroxyurs-12-en-28-oic and 2α,3β-dihydroxyolean-12-en-28-oic acids (maslinic or crategolic acid, 220 mg). After repeated chromathography, the fourth fraction also eluted with $CHCl_3$-MeOH, 9:1, yielded β-sitosterol-glucoside (200 mg) and 6β-hydroxymaslinic acid, **1** (92 mg).

The crushed fruits (196 g) were exhaustively extracted with hot $CHCl_3$-EtOH,1:1 in Soxlet apparatus. The residue (5.7 g) was suspended in cold MeOH and filtered. By crystallization (MeOH) the solid material gave bellericagenin A (2.8 g). The MeOH soluble portions (1.6 g) were purified by CC (SiO_2 ; EtOAc with increasing amounts of MeOH). Oleanolic acid (20 mg) and **1** (40 mg) were eluted with EtOAc, further bellericagenin A (1.1 g; total yield, 2% of the fruits) with EtOAc-MeOH, 97:3. The last eluted fraction (EtOAc-MeOH,19:1) yielded bellericaside A after washing with cold acetone (50mg). Methylation of **1** with ethereal diazomethane afforded the monomethyl ester **1a**.

6b-Hydroxymaslinic acid, **1**

Mp 190-192 ºC; $[\alpha]_D^{20}$ +35º (*c* = 3, MeOH); ^{1}H NMR of **1** (300 MHz, C_5D_5N), δ 1.83 and 1.29 (H-1a and H-1b), 4.28 (H-2, dt, *J* 9.5 and 4.5 Hz), 3.42 (H-3, d, *J* 9.5 Hz), 1.18 (H-5), 4.85 (H-6), 1.86 (H-7), 1.99 (H-9), 5.57 (H-12, br t), 2.20 (H-16), 3.33 (H-18, dd, *J* 14 and 4 Hz), 2.32 and 1.40 (H-19a and H-19b), 1.85 (H-22), 1.46 (H-23, s), 1.78 (H-24, s), 1.71 (H-25, s), 1.61 (H-26, s), 1.30 (H-27, s), 0.96 (H-29), 1.02 (H-30); ^{13}C NMR (75MHz) of **1** in C_5D_5N (Table 1); Methylation of **1** with ethereal diazomethane, 0º C, afforded the mono methyl ester **1a**. EIMS of **1a** (70 eV), (*m/z*, %): 502 (M^+, 5), 484 (M-H_2O, 4), 442 (M-HCOOMe, 6), 262 (77), 249 (12), 203 (100), 189 (40) (Figure 1); ^{1}H NMR of **1a** (300 MHz,$CDCl_3$) δ 5.33 (H-12), 4.53 (H-6), 3.73 (H-2), 3.63 (OMe), 2.95 (H-3), 2.89 (H-18), 1.37, 1.25, 1.20, 1.10, 1.08, 0.93 and 0.90 (7Me); ^{13}C NMR of **1a** (75 MHz, $CDCl_3$) δ 178.2 (C-28), 142.9 (C-13), 122.5 (C-12), 83.7 (C-3), 68.7 (C-2), 68.4 (C-6), 55.6 (C-5), 51.5 (OMe), [a]48.4 (C-19), 47.8 (C-9), [a]46.6 (C-1), 45.7 (C-17),42.1 (C-14), 41.2 (C-18), 40.6 (C-7), 39.7 (C-8), 38.4, 37.8 (C-10; C-4), 33.8 (C-21), 33.1 (Me-29), 32.3 (C-22), 30.6 (C-20), 28.3 (Me-23), 27.5 (C-15), 25.9 (Me-27), 23.5 (Me-30), 23.3 (C-16), 22.9 (C-11), 18.1 (Me-24, Me-25, Me-26) ([a] Interchangeable values).

2α,3β,7α,23-Tetrahydroxyolean-12-en-28-oic acid (Bellericagenin A)

^{1}H NMR (300 MHz, C_5D_5N) δ 5.57 (br s, H-12), 5.10 (br s, H-7), 4.41 (m, H-2, H-23 a), 4.25 (d, *J* 10.4 Hz, H-3), 4.08 (d, *J* 11.8 Hz, H-23 b), 3.32 (br dd, H-18), 1.78 (Me-25), 1.74 (Me-24), 1.63 (Me-26), 1.23 (Me-27), 1.00 (Me-30), 0.93 (Me-29) (data not available in the literature, in C_5D_5N); ^{13}C NMR (75 MHz, C_5D_5N) δ 180.0 (s, C-28), 144.0 (s, C-13), 122.7 (d, C-12), 78.1 (d, C-3), 68.8 (d, C-2), 67.4 (d, C-7), 65.3 (t, C-23), 49.9 (t, C-1), 48.7 (d, C-5), 48.6 (d, C-9), 46.5 (s, C-17), 46.2 (t, C-19), 44.4 (s, C-4), 42.6 (s, C-14), 41.9 (d, C-18), 39.1 (s, C-8), 38.0 (s, C-10), 34.0 (t, C-21), 33.1 (q, Me-29), 33.1 (t, C-22), 30.8 (s, C-20), 28.1 (t; C-6, C-15), 26.1 (q, Me-27), 23.9 (t, C-11), 23.6 (q, Me-30), 23.5 (t, C-16), 18.8 (q, Me-25), 18.4 (q, Me-26), 15.8 (q, Me-24). Some selective INEPTL experiments: irradiation at δ 5.57 (H-12) gave response for δ 42.6 (C-14), 48.6 (C-9) and 41.9 (C-18); irradiation at δ 3.32 (H-18) gave response for δ 144.0 (C-13) and 46.5 (C-17); irradiation at δ 1.74 (Me-24) gave response for δ 44.4 (C-4), 78.0 (C-3) and 65.9 (C-23); irradiation at δ 1.63 (Me-26) gave response for δ 42.6 (C-14) and 39.1 (C-8); irradiation at d 1.23 (Me-27) gave response for δ 144.0 (C-13), 42.6 (C-14) and 39.1 (C-8).

Bellericaside A

^{1}H NMR (300 MHz, C_5D_5N) δ 5.62 (H-12), 5.52 (H-1'), 5.09 (H-7), 4.44-3.95 (H2-23), 3.22 (H-18), 1.80 (Me-25), 1.76 (Me-24), 1.73 (Me-23), 1.19 (Me-27), 0.88, 0.87 (Me-30, Me-29) (data not available in the literature, in C_5D_5N); ^{13}C NMR (75 MHz, C_5D_5N) δ 176.2 (C-28), 143.3 (C-13), 123.6 (C-12), 95.6 (C-1'), 79.0, 78.5 (C-3', C-5'), 73.9 (C-2'), 70.9 (C-4'), 61.9 (C-6'), 46.8 (C-17), 46.0 (C-19), 41.6 (C-18), 32.3 (C-22) and the other values practically identical to those of bellericagenin A.

Table 1. NMR data for 6β-hydroxymaslinic (**1**), maslinic[11], terminolic[12] and 2α,3β,6β,19α-tetrahydroxyurs-12-en-28-oic acids[13] (compound A) in C_5D_5N.

Position	6b-hydroxymaslinic acid	Maslinic acid	Terminolic acid	Compound A
1	[a]46.5	47.8	46.9	50.3
2	68.2	68.6	68.8	69.6
3	83.9	83.8	78.3	84.7
4	39.2	39.8	43.1	38.8
5	56.4	55.9	48.1	57.4
6	67.4	18.9	67.6	68.8
7	41.1	33.2	39.2	41.8
8	40.6	40.2	40.0	41.2
9	48.6	48.2	48.9	49.1
10	38.3	38.5	38.4	40.3
11	23.9	23.9	23.8	24.7
12	122.7	122.5	122.5	129.6
13	144.0	144.9	144.0	139.4
14	42.6	42.2	42.2	42.1
15	28.1	28.3	28.1	29.5
16	23.6	23.6	23.3	27.8
17	43.3	46.7	43.0	49.7
18	41.9	42.6	41.9	55.1
19	[a]49.9	46.4	46.1	73.6
20	30.8	30.9	30.5	43.1
21	34.1	34.2	33.8	26.6
22	33.1	33.2	32.5	39.0
23	29.0	29.3	66.6	29.0
24	19.1	16.9	15.3	16.6
25	18.4	17.5	17.5	18.5
26	18.3	17.7	18.5	18.8
27	26.2	26.2	23.7	24.8
28	180.0	180.2	179.3	182.2
29	33.2	33.2	33.1	27.1
30	23.6	23.1	23.9	18.5

[a]Interchangeable values

Other known compounds

Uvaol and erythrodiol, ursolic and oleanolic acids, and 2α,3β-dihydroxyurs-12-en-28-oic acid and 2α,3β-dihydroxyolean-12-en-28-oic acid (maslinic acid or crategolic acid) were identified by the comparison of ^{13}C NMR data with those reported in the literature[8]. Oleanolic acid and β-sitosterol glucoside by comparison with authentic samples available in our laboratory.

Results and Discussion

Upon repeated column chromatography the $CHCl_3$ extract of the leaves afforded b-sitosterol glucoside and isomeric mixtures of uvaol/erythrodiol, ursolic/oleanolic acids, and 2α,3β-dihydroxyurs-12-en-28-oic/maslinic acids, as well as a single compound characterized as 6β-hydroxymaslinic acid, **1**, which was obtained as a dextrorotatory powder. The molecular ion *m/z* 502 of monomethyl ester **1a** and NMR data of **1** are consistent with the molecular formula $C_{30}H_{48}O_5$. The complete ^1H and ^{13}C assignments for 6β-hydroxymaslinic acid, **1**, are reported in Experimental and in Table 1 as a result of APT and HETCOR experiments and some INEPT measurements. Preliminary inspection of the data disclosed the presence of seven tertiary methyls, three oxymethine groups (δ_C 83.9, 68.2 and 67.4) and a trisubstituted double bond (δ_C 144.0 and 122.7; δ_H 5.57, br t). These findings clearly suggested a trihydroxyolean-12-en-28-oic gross structure for 6β-hydroxymaslinic acid (**1**) rather than that of an urs-12-en derivative[9]. The ^1H NMR spectrum disclosed the presence of a doublet (*J* 9.5 Hz) at δ 3.42 (H-3α) suggesting that two of hydroxy groups are in the 2α,3β positions[10]. This assumption was confirmed by the comparison of ^{13}C NMR parameters for ring A carbons with those of 2α,3β-dihydroxyolean-12-en-28-oic acid[11] (maslinic acid; Table 1). Analogously, the third hydroxyl was assigned 6β comparing the ^{13}C NMR data for B-E ring carbons with those of 2α,3β,6β,23-tetrahydroxyolean-12-en-28-oic acid[12] (terminolic acid; Table 1) and 2α,3β,6β,19a-tetrahydroxyurs-12-en-28-oic acid[13] (compound A, Table 1). Signals of carbons 24, 25 and 26 in ^{13}C NMR spectrum of **1** are deshielded in respect to maslinic acid (Table 1), which can be justified by δ effect of the hydroxyl group on 6β. Therefore, 6β-hydroxymaslinic acid, **1**, was characterized as 2α,3β,6β-trihydroxyolean-12-en-28-oic acid.

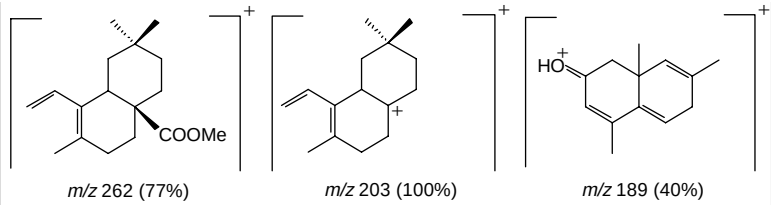

Figure 1. Mass fragments of 6β-hydroxymaslinic acid monomethyl ester, **1a**.

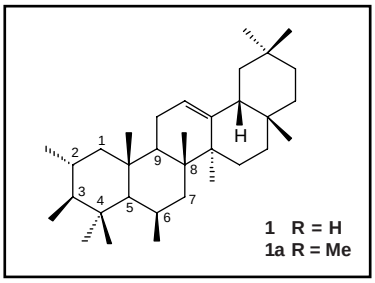

Table 2. Long-Range NMR connectivities for 6β- hydroxymaslinic acid (**1**)*.

Irradiated resonance	Hydrogen	Connected carbons ^3J	^2J
3.42	H-3	Me-24	
4.85	H-6	C-4, C-10	
5.57	H-12	C-9, C-14, C-18	
3.33	H-18		C-13, C-17
1.46	Me-23	C-3, C-5, Me-24	C-4
1.78	Me-24	C-3	
1.71	Me-25		C-10
1.61	Me-26		C-8
1.30	Me-27	C-8	
0.96	Me-29	Me-30	C-20
1.02	Me-30		C-20

*Selective INEPTL experiments

Most of the assignments of Experimental and Table 1 have been confirmed by selective INEPT experiments (Table 2); in particular, the selective irradiation of the broad singlet at δ 4.85 (H-6α) gave a response on the signals at 39.2 and 38.3 ppm, C-4 and C-10, respectively.

Finally, the relative configuration of the molecule was checked by Difference NOE spectra. Mutual enhancement of the signals were observed among H-3, Me-23, H-5 and H-6α, as well as among H-2β, Me-24 and Me-25.

Methylation of **1** with ethereal diazomethane afforded the monomethyl ester **1a**. MS spectrum of **1a** presented the expected Retro Diels-Alder fragmentation pattern[14] (Figure 1). The ^{1}H NMR and ^{13}C NMR spectra (see Experimental) were also in accordance with the structure proposed for **1a**.

Examination of the fruits of *Vochysia ferruginea* led to the isolation in high yield of bellicagenin A (see Experimental) and its (28 → 1) β-D-glucopyranosyl ester (bellericaside A) previously found in *Terminalia bellerica* (Combretaceae)[15]. On the basis of HETCOR, APT and INEPTL experiments the signals for C-1, C-9, C-17, C-19, C-4, C-14 and C-18 of bellericagenin A have been reversed with respect to literature[15].

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