

6 β -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 2 α ,3 β ,6 β -trihidroiolean-12-en-28-óico (ácido 6 β -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 eritrodíol, ácidos ursólico e oleanólico e os ácidos 2 α ,3 β -dihidroxiurs-12-en-28-óico e o respectivo isômero oleanólico (ácido maslínico ou cratególico). Nos frutos, a bellericagenina A e o seu éster (28 \rightarrow 1) β -D-glicopiranosílico (bellericasídeo A) estão presentes em elevados teores.

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

Keywords: *Vochysia ferruginea*, Vochysiaceae, pentacyclic triterpenes, 6 β -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 2 α ,3 β ,19 α -trihydroxy-24-oxo-urs-12-en-28-oic acids from *V. pyramidalis*².

From the stem bark of *V. divergens* we isolated the terpenoids β -sitosterol, betulinic, sericic, divergioic and 24-hydroxytormentonic acids, and the (28 \rightarrow 1) β -D-glucopyranosyl ester of later³⁻⁵. Sericic acid (2 α ,3 β ,19,24-tetrahydroxyolean-12-en-28-oic acid) presented antifungal³ and antibacterial⁴ activities. 24-Hydroxytormentonic acid showed antinociceptive action⁶.

V. ferruginea is a tree commonly found in wet soils of Venezuelan Amazon⁷. In this communication we report the structure elucidation of 6 β -hydroxymaslinic acid, **1**, a novel

triterpenoid of the oleanic series obtained from the leaves and the fruits of *V. ferruginea*. β -sitosterol-glucoside and mixtures of uvaol and erythrodiol, ursolic and oleanolic acids, 2 α ,3 β -dihydroxyurs-12-en-28-oic acid and its respective oleanolic isomer (maslinic or crategolic acid) were also obtained. Bellericagenin A and its (28 \rightarrow 1) β -D-glucopyranosyl ester (bellericaside A) were present in high amount in the fruits.

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. ¹H and ¹³C-NMR spectra were recorded in CDCl₃ or in pyridine-d₅, using TMS as internal reference, employing a Varian Gemini 300 (¹H, 300 MHz; ¹³C, 75 MHz) spectrometer. Optical rotations were measured in a Polamat A (Carl Zeiss) polarimeter.

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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 Biología, 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 $2\alpha,3\beta$ -dihydroxyurs-12-en-28-oic and $2\alpha,3\beta$ -dihydroxyolean-12-en-28-oic acids (maslinic or crategolic acid, 220 mg). After repeated chromatography, 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 Soxhlet 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 (50 mg). Methylation of **1** with ethereal diazomethane afforded the monomethyl ester **1a**.

6b-Hydroxymaslinic acid, **1**

Mp 190-192 °C; $[\alpha]_D^{20} +35^\circ$ ($c = 3$, MeOH); ^1H NMR of **1** (300 MHz, $\text{C}_5\text{D}_5\text{N}$), 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 (75 MHz) of **1** in $\text{C}_5\text{D}_5\text{N}$ (Table 1);

Methylation of **1** with ethereal diazomethane, 0 °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); ^1H 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).

2\alpha,3\beta,7\alpha,23-Tetrahydroxyolean-12-en-28-oic acid (Bellericagenin A)

^1H NMR (300 MHz, $\text{C}_5\text{D}_5\text{N}$) 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 $\text{C}_5\text{D}_5\text{N}$); ^{13}C NMR (75 MHz, $\text{C}_5\text{D}_5\text{N}$) 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

^1H NMR (300 MHz, $\text{C}_5\text{D}_5\text{N}$) 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 $\text{C}_5\text{D}_5\text{N}$); ^{13}C NMR (75 MHz, $\text{C}_5\text{D}_5\text{N}$) 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 6 β -hydroxymaslinic (**1**), maslinic¹¹, terminolic¹² and 2 α ,3 β ,6 β ,19 α -tetrahydroxyurs-12-en-28-oic acids¹³ (compound A) in C₅D₅N.

Position	6 β -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

^aInterchangeable values

Other known compounds

Uvaol and erythrodiol, ursolic and oleanolic acids, and 2 α ,3 β -dihydroxyurs-12-en-28-oic acid and 2 α ,3 β -dihydroxyolean-12-en-28-oic acid (maslinic acid or crategolic acid) were identified by the comparison of ¹³C NMR data with those reported in the literature⁸. Oleanolic acid and β -sitosterol glucoside by comparison with authentic samples available in our laboratory.

Results and Discussion

Upon repeated column chromatography the CHCl₃ extract of the leaves afforded β -sitosterol glucoside and isomeric mixtures of uvaol/erythrodiol, ursolic/oleanolic acids, and 2 α ,3 β -dihydroxyurs-12-en-28-oic/maslinic acids, as well as a single compound characterized as 6 β -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 C₃₀H₄₈O₅. The complete ¹H and ¹³C assignments for 6 β -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 6 β -hydroxymaslinic acid (**1**) rather than that of an urs-12-en derivative⁹. The ¹H NMR spectrum disclosed the presence of a doublet (*J* 9.5 Hz) at δ 3.42 (H-3 α) suggesting that two of hydroxy groups are in the 2 α ,3 β positions¹⁰. This assumption was confirmed by the comparison of ¹³C NMR parameters for ring A carbons with those of 2 α ,3 β -dihydroxyolean-12-en-28-oic acid¹¹ (maslinic acid; Table 1). Analogously, the third hydroxyl was assigned 6 β comparing the ¹³C NMR data for B-E ring carbons with those of 2 α ,3 β ,6 β ,23-tetrahydroxyolean-12-en-28-oic acid¹² (terminolic acid; Table 1) and 2 α ,3 β ,6 β ,19 α -tetrahydroxyurs-12-en-28-oic acid¹³ (compound A, Table 1). Signals of carbons 24, 25 and 26 in ¹³C 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 6 β . Therefore, 6 β -hydroxymaslinic acid, **1**, was characterized as 2 α ,3 β ,6 β -trihydroxyolean-12-en-28-oic acid.

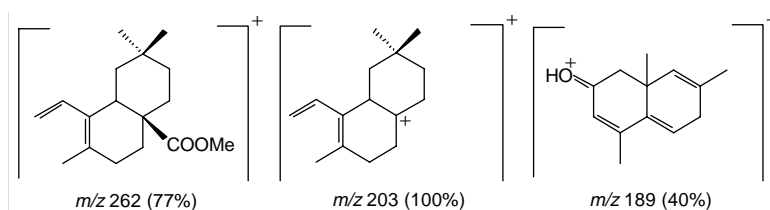


Figure 1. Mass fragments of 6 β -hydroxymaslinic acid monomethyl ester, **1a**.

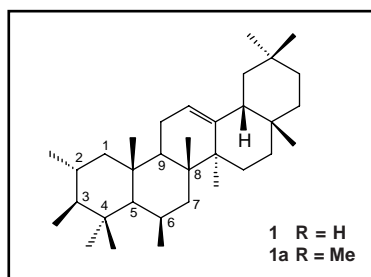


Table 2. Long-Range NMR connectivities for 6 β -hydroxymaslinic acid (**1**)^{*}.

Irradiated resonance	Hydrogen	Connected carbons	
		³ J	² J
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-6 α) 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-6 α , as well as among H-2 β , 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 pattern¹⁴ (Figure 1). The ¹H NMR and ¹³C 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 \rightarrow 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 bellericageninA have been reversed with respect to literature¹⁵.

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