

Divergioic Acid, a Triterpene from *Vochysia divergens*

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Um novo triterpeno lupânico, ácido divergioico, além de β -sitosterol, ácidos betulínico, serícico e 24-hidroxitormêntico, e o éster (28 \rightarrow 1) β -D-glucopiranosílico do último, foram obtidos a partir da casca do caule de *Vochysia divergens*. A estrutura do ácido divergioico foi elucidada por meio de técnicas espectroscópicas, e caracterizada como sendo o ácido 2 α ,3 β ,6 β -trihidroxi-lup-20(29)-en-28-óico.

A new lupane triterpene, divergioic acid, has been isolated from the stem bark of *Vochysia divergens* together with β -sitosterol, betulinic, sericic and 24-hydroxytormentic acids and the (28 \rightarrow 1) β -D-glucopyranosyl ester of the latter. The structure of divergioic acid was elucidated by spectroscopic techniques and characterized as 2 α ,3 β ,6 β -trihydroxy-lup-20(29)-en-28-oic acid.

Keywords: *Vochysia divergens*, *Vochysiaceae*, *lupene*, *divergioic acid*

Introduction

Vochysia divergens Pohl (Vochysiaceae) is a tree commonly found in wet soils of the "pantanal" of Mato Grosso do Sul, Brazil, and used in folk medicine against infections and asthma¹. In previous reports we described the isolation of β -sitosterol, betulinic and sericic acids from the stem bark, as well as the antifungal² and antibacterial³ activities of sericic acid. In this communication we report the structure elucidation of divergioic acid, a novel lupene. 24-Hydroxytormentic acid and its glucopyranosyl ester were also isolated.

Results and Discussion

In addition to β -sitosterol, betulinic and sericic acids, the reinvestigation of the EtOH extract from the stem bark afforded 24-hydroxytormentic acid and its glucopyranosyl ester in high yield (see Experimental), and a new triterpene, which was named divergioic acid (**1**). The molecular formula C₃₀H₄₈O₅ was deduced from the NMR data and the molecular ion (M⁺ at *m/z* 488) in the mass spectrum. The ¹H-NMR spectrum exhibited signals at δ 4.97 (1 H), δ 4.80 (1H) and δ 1.81 (3 H), characteristic of triterpenes with a lupene skeleton. In addition, five methyl singlets and signals for three methines on hydroxyl bearing carbons (δ 4.86, br s; δ 4.30, dt and δ 3.44, d) were present. By

irradiation at δ 4.30, the signal at δ 3.44 became a singlet. These findings suggested a lup-20(29)-ene gross structure with two OH in position 2 and 3, and an additional hydroxyl. In accordance, the ¹³C-NMR spectra (Table 1) showed the appropriate signals for the isopropylene moiety and for three hydroxylated carbons. Comparison of ¹³C-NMR data of **1** with those of betulinic acid, **2**⁴ (Table 1) revealed a good agreement of the values for the carbons of rings C/D/E. Furthermore, the signals due to A/B ring carbons were found to be very similar to those of 2 α ,3 β ,6 β ,19-tetrahydroxy-urs-12-en-28 oic acid, **6**⁵. Table 2 reports the main long-range C-H and H-C connectivities found in the long-range HETCOR spectrum and by selective INEPT experiments, respectively, which are in agreement with the structure proposed for **1**. Therefore, divergioic acid (**1**) was assigned the structure 2 α ,3 β ,6 β -trihydroxy-lup-20(29)-en-28-oic acid and is the first example of a lupane possessing the rare 6-OH group.

Experimental

Plant material

Vochysia divergens Pohl (Vochysiaceae) was collected in Corumba (Mato Grosso do Sul, Brazil) and identified by G. A. Damasceno Jr. (DAM/CEUC/UFMS) and Arnildo Pott (CPAP/EMBRAPA). A voucher specimen is depos-

Table 1. NMR data of compounds **1**, **2**⁴ and **6**⁵ (75 MHz, pyridine-d₅).

Carbon	1	2	6
1	50.2	38.5	50.3
2	68.9	28.2	69.6
3	84.0	78.1	84.7
4	38.5	39.4	38.8
5	56.5	55.9	57.4
6	67.7	18.7	68.8
7	42.5	34.7	41.8
8	40.5	41.0	41.2
9	51.7*	50.9	49.1
10	37.4	37.5	40.3
11	21.4	21.1	24.7
12	26.1	26.0	129.6
13	37.7	39.2	139.4
14	42.9	42.8	42.1
15	30.2	30.2	29.5
16	32.7	32.8	27.8
17	56.5	56.6	49.7
18	51.6*	49.7	55.1
19	47.6	47.7	73.6
20	151.1	151.4	43.1
21	31.1	31.1	26.6
22	37.4	37.4	39.0
23	28.6	28.5	29.0
24	19.1	16.2	16.6
25	18.8	16.3	18.5
26	17.0	16.2	18.8
27	15.0	14.8	24.8
28	178.6	179.0	182.2
29	109.6	110.0	27.1
30	19.3	19.4	18.5

*These values may be interchanged.

ited in the herbarium of the Centro Universitario de Corumba/UFMS (Corumba, MS, Brazil) under number 0500.

Extraction and isolation

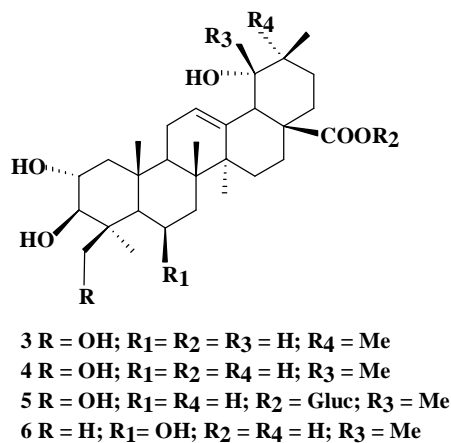
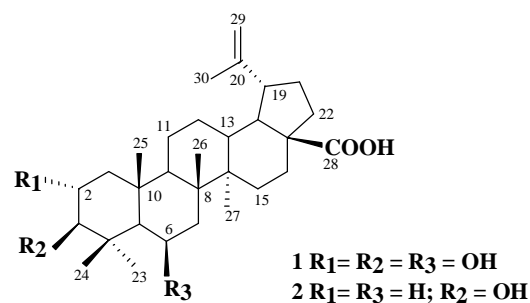
The powdered stem bark (4.0 kg) was exhaustively extracted with cold EtOH. After evaporation, a mixture of MeOH/H₂O, 95:5 (250 mL) was added to a part (20 g) of the EtOH extract, and filtered. Evaporation of the soluble portion (14 g) and washing with cold CHCl₃ yielded two fractions, B (soluble, 8 g) and C (insoluble, 6 g). CC of

Table 2. Long-Range NMR connectivities of compound **1**.

Irradiated proton	H-C*	C-H [#]	
	Connected carbons	Carbon	Connected protons
Me-23	C-3, C-4, Me-24	C-2	H-3
Me-24	C-4, C-5	C-4	Me-24
Me-26	C-9, C-14	C-7	H-6
Me-27	C-8, C-14	C-8	Me-26
Me-30	C-20	C-14	Me-26
H-6	C-4, C-8	C-20	Me-30

*Selective INEPT experiments.

[#]Long-Range HETCOR.



fraction B on SiO₂ yielded β-sitosterol (30 mg), betulinic acid, **2** (300 mg), by elution with a gradient of EtOAc in hexane; impure **1**, a mixture of sericic (**3**) and 24-hydroxy-tormentic (**4**) acids and the impure glucoside of **4**, **5** (4.2 g) were obtained by elution with a gradient of MeOH in EtOAc. CC of fraction C (SiO₂; gradient of MeOH in EtOAc) gave **2** (20 mg), impure **1** (80 mg) and a mixture (2 g) of **3**, **4** and **5**. Extended chromatography (SiO₂, hexane/EtOAc, 7:3) of the pooled impure **1** afforded divergioic acid (42 mg). Repeated CC (SiO₂; CHCl₃/MeOH/H₂O 19.5:8.5:2.3, and EtOAc/MeOH, 9:1) of the fractions containing impure **3**, **4** and **5** gave pure **3**

(500 mg), pure **4** (700 mg), a mixture of **3** and **4** (3.2 g) and the (28 → 1) β-D-glucopyranosyl ester **5** (300 mg). ¹³C-NMR data for **3** (2α,3β,19α,24-tetrahydroxyolean-12-en-28 oic acid) were not available in the literature, and the signals were attributed by comparison with the respective data for rings A/B of **4** (2α,3β,19,24-tetrahydroxyurs-12-en-28 oic acid)⁶ and for rings C/D/E of arjungenin (2α,3β,19α,23-tetrahydroxyolean-12-en-28 oic acid)⁷. Reaction of **3** with diazomethane afforded the respective methyl ester, ¹H-NMR data were in agreement with data previously published⁸.

Divergioic acid, 1 Mp 155-6 °C; [α]_D²⁰ -7° (c 3.0, MeOH); ¹H-NMR (300 MHz, Pyridine-*d*₅), δ: 4.97 and 4.80 (br s, H₂-29), 4.86 (brs, H-6), 4.30 (dt, H-2), 3.57 (m, H-19), 3.44 (d, J = 9.3 Hz; H-3), 2.90 (m, H-13), 2.63 (m, H-16α), 2.27 and 2.25 (m, H-22α and H-21α), 1.81 (s, Me-30), 1.77 (s, Me-25), 1.69 (s, Me-26), 1.60 (s, Me-24), 1.46 (s, Me-23), 1.08 (s, Me-27); ¹³C-NMR (75 MHz, Pyridine-*d*₅) see Table 1.

Known triterpenes

Sericic acid, 3: [α]_D²⁰ +33° (c 0.3, MeOH), Mp 280 °C (dec.), IV and MS data were comparable to those in Ref. 8. ¹³C-NMR (75 MHz, Pyridine-*d*₅), δ: **C-1** 47.2; **C-2** 68.4; **C-3** 85.6; **C-4** 43.7; **C-5** 56.5; **C-6** 19.2; **C-7** 33.5; **C-8** 39.9; **C-9** 48.3; **C-10** 38.3; **C-11** 28.9; **C-12** 123.5; **C-13** 144.6; **C-14** 41.9; **C-15** 29.1; **C-16** 28.2; **C-17** 45.9; **C-18** 44.6; **C-19** 81.2; **C-20** 35.5; **C-21** 28.2; **C-22** 33.4; **C-23** 23.9; **C-24** 65.4; **C-25** 17.2; **C-26** 17.0; **C-27** 24.6; **C-28** 180.5; **C-29** 28.5; **C-30** 24.7.

24-Hydroxytormentonic acid, 4: [α]_D²⁰ +24° (c 0.3, MeOH), NMR data in agreement with those previously reported were obtained⁶;

24-Hydroxytormentonic acid (281)-D-glucopyranosyl ester, 5: [α]_D²⁰ -9° (c 0.7, MeOH), NMR data in agreement with those previously reported were obtained⁶.

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