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Short Report

A New Tormentic Acid Derivative from *Luehea divaricata* Mart. (Tiliaceae)

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Um extrato metanólico das folhas de *Luehea divaricata* (Tiliaceae), vulgarmente conhecida no Brasil como "açoita-cavalo", forneceu um novo triterpeno caracterizado como ácido 3β-*p*-hidroxibenzoiloxitormentico [ácido 3β-(*p*-hidroxibenzoiloxi)-2α-hidroxiurs-12-en-28-óico], juntamente com uma mistura contendo o ácido maslínico, um triterpeno conhecido. As estruturas dos compostos foram estabelecidas por métodos espectroscópicos.

A methanolic extract from leaves of *Luehea divaricata* (Tiliaceae), known in Brazil as "açoita-cavalo", yielded two triterpene: a novel characterized as 3β-*p*-hydroxybenzoyloxytormentic acid [3β-(*p*-hydroxybenzoyloxy)-2α-hydroxyurs-12-en-28-oic acid] and a mixture containing the maslinic acid. The new compound's structure was established by spectroscopic methods.

Keywords: *Luehea divaricata*, 3β-*p*-hydroxybenzoyloxytormentic acid, maslinic acid

Introduction

The Tiliaceae family has not been extensively studied yet. α-Amyrin derivatives have already been isolated from the genus *Corchorus*.[1] There is no studies about the chemical composition of this plant and no reports were found on the genus *Luehea* as well. *Luehea divaricata* Mart. (Tiliaceae), known in Brazil as "açoita-cavalo", is a tree which grows in Brazil, Argentina and Paraguay.[2,3] The *L. divaricata* is used in Brazilian folk medicine for different purposes: the leaves are used as diuretic, the stems as anti-inflammatory,[4] the bark and aerial parts are used for healing skin wounds, pimples, and for vaginal washes.[2] Also the *L. divaricata* was assayed for antifungal properties and exhibited a broad spectrum of activity against dermatophytes.[2] The aqueous extract of *L. divaricata* presented genotoxic activity in the Ames test (*Salmonella*/microsome) with microsomal activation.[5] However, a phytochemical screening of *L.divaricata* reported the presence of flavonoids, tannins and saponins.[4] In this paper we report the isolation and the structure elucidation of a new α-amyrin derivative, which was characterized as 3β-*p*-hydroxybenzoyloxytormentic acid and a mixture containing the maslinic acid.

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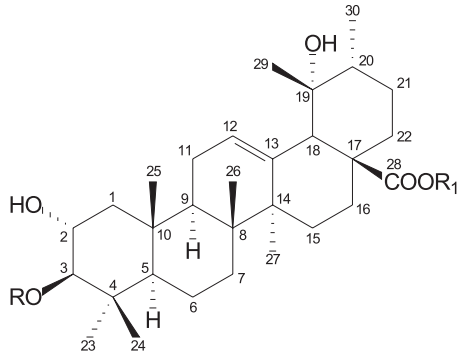
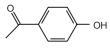
Results and Discussion

Structural elucidation and NMR signal assignments of 3β-p-hydroxybenzoyloxytormentic acid **(1)**

Compound **1** was isolated as white crystals. Its EIMS spectrum showed a molecular peak at *m/z* 608 in agreement with $C_{37}H_{52}O_7$ molecular formula. It also showed a base peak at *m/z* 121 attributed to *p*-hydroxybenzoyl cation and also the peaks at *m/z* 563 (10.8%, $[M - COOH]^+$) and 562 (17.0%, $[M- HCOOH]^{+\bullet}$). The peak at *m/z* 146 (61.9%, $[C_{11}H_{14}]^{+\bullet}$) is a characteristic of a tertiary hydroxyl function presence at C-19 in the urs-12-ene skeleton.[6] Other important peaks were noticed at *m/z* 246 (14.0%), 219 (7.9%), 218 (14.0%) and 201 (5.8%). Some low relative abundance peaks at *m/z* 179 and 264 were detected, as previously related to triterpenes from ursane class.[6] The ^{1}H chemical shifts of **1** (CD_3OD), in the range of δ 0.82-1.37, showed six singlets from methyl groups and one doublet, partly superposed by the singlets in agreement with an ursane type compound. The ^{1}H NMR spectrum of an oleanane type compound has seven methyl singlets and no doublets.[7] The ^{1}H NMR spectrum also showed two carbinolic methine hydrogens at δ 3.90 (td, *J* 10.3 Hz, 10.3 Hz, 4.0 Hz, H-2β) and 4.70 ppm (d, *J* 10.3 Hz, H-3α) and one olefinic hydrogen at δ 5.30 (br s, H-12). These data suggested **1** to be a triterpene with an ursane skeleton.

The ^{1}H NMR spectral data were compared to those from



1 R =  , R^1 = H

3 R = H, R^1 = β-D-glucopyranosyl

4 R = R^1 = H

tormentic acid,[8] and a strong agreement was observed, except the chemical shift corresponding to H-3. The difference ($\Delta_{\delta H}$ = 1.79 ppm) of the H-3 chemical shift (δ_H 4.70) in respect to tormentic acid[8] was justified by the presence of a *p*-hydroxybenzoyl group linked to the oxygen of C-3 in compound **1**, which induces an electron density reduction by inductive and resonance withdrawal effects of the *p*-hydroxybenzoyloxy group. The ^{1}H and ^{13}C NMR spectra's compound had signals for a *p*-hydroxybenzoate group [δ 7.92 (2H, dd, *J* 9.0 Hz, 2.1 Hz) and δ 6.83 (2H, dd, *J* 9.0 Hz, 2.1 Hz), δ 168.7 (C-7'), 163.6, (C-4'), 133.0 (CH-2' and CH-6'), 123.0 (C-1'), 116.2 (CH-3' and CH-5')]. The presence of this group was supported by the observation of a strong peak in the mass spectrum at *m/z* 121 corresponding to $[C_7H_5O_2]^{+\bullet}$. The correlation between H-3 and C-7' observed in HMBC spectrum was very important because it was possible to establish a link between the *p*-hydroxybenzoyl group and the triterpene skeleton from the structure **1** in the C-3 position. Initially, compound **1** ^{13}C NMR spectrum was run in CD_3OD, however it was also necessary to run in C_5D_5N to confirm the presence of the signals that were superposed by the solvent signal in the range of δ 48-50 (Table 1). The ^{13}C NMR spectrum of **1** confirmed that is a triterpene skeleton with an ursolic acid type (C-12 and C-13 at δ 129.3 and 140.4). The ^{13}C NMR spectral data of **1** were compared with those from tormentic acid ester glucoside.[9] The coupling constant ($J_{2,3}$) of 10.3 Hz is typical to an antiperiplanar (axial-axial) relationship between H-2 and H-3 (Table 1). The NOE difference NMR experiment was also performed to confirm the *p*-hydroxybenzoyloxy group orientation at C-3. Irradiation of H-2β signal at δ 3.90 produced an enhancement in the methyl hydrogens resonance at δ 1.01 (3H-24) and 1.08 (3H-25) which showed a coaxial relationship between 3H-24 and 3H-25. In the same irradiation, it wasn't observed NOE enhancement at H-3 or H-5, showing an antiperiplanar relationship between H-2 / H-3 and H-2 / H-5.

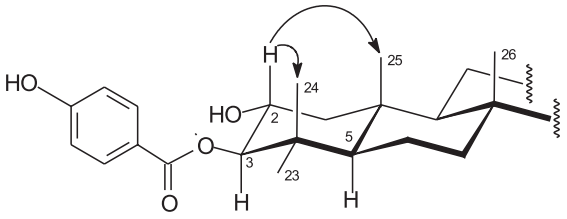
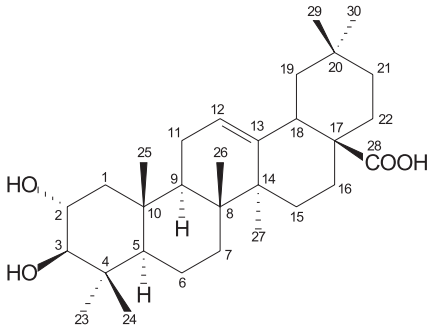


Figure 1. Selected ^{1}H-^{1}H dipolar correlation of **1** observed through NOE difference spectra experiment.

The complete and unequivocal ^{1}H and ^{13}C chemical shifts assignments of **1** were assisted by DEPT, COSY (^{1}H x ^{1}H), HSQC (^{13}C x ^{1}H) and HMBC (^{13}C x ^{1}H) spectra (Table 1).

Assignments of maslinic acid **(2)**

The 1D ^{1}H NMR from the mixture showed signals for methyl groups (δ 0.80–1.16), for two carbinolic methine hydrogens at δ 2.90 (d, *J* 9.9Hz, H-3α) and 3.61 (ddd, *J* 9.9 Hz, 9.9 Hz, 3.9 Hz, H-2β) and for one olefinic hydrogen at δ 5.24 (t, *J* 3.6 Hz). These data suggested **2** to be a triterpene with an oleanane skeleton.[8] The ^{13}C NMR spectrum showed the signals at δ 145.5, 123.6, 84.5 and 69.5 confirming that is olean-12-ene-2α, 3β -diol.[10] Some aspects of the maslinic acid structure was made by comparison of its ^{1}H NMR and ^{13}C NMR data with those proposed to a similar compound.[8,10,11]



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Experimental

General

EIMS: 70 eV; ^{1}H (300 or 500 MHz) and ^{13}C (75.5 or 125 MHz) NMR spectra were obtained in pyridine-d_5 or methanol-d_4 with TMS as internal reference; column chromatography (CC): silica gel 60 (70-230 mesh); thin-layer chromatography (TLC): silica gel F254 (0.25 mm in thickness).

Table 1. ^{1}H, ^{13}C and 2D NMR spectral data for 3β-*p*-hydroxybenzoyloxytormentic acid (**1**), ^{13}C NMR data for tormentic acid ester glucoside (**3**)[9] and for tormentic acid (**4**),[8] ^{1}H NMR in CD_3OD [**1** (300 MHz) and **4** (400 MHz)] and ^{13}C NMR in CD_3OD [**1** (75.5 MHz)] and C_5D_5N [**3** (50 MHz)][a]

C (DEPT)	δ ^{13}C	δ ^{1}H		COSY (^{1}H x ^{1}H)	HMBC (^{13}C x ^{1}H)
	1 (**1**[b], **3**)	**1**	**4**	**1** (^{2}J and ^{3}J)	**1** (^{2}J, ^{3}J and ^{4}J)
1 (CH_2)	48.6 (48.4, 48.0)	1.10 / 2.04 nd		H-1b / H-1a; H-2	H-5; H-25
2 (CH)	67.7 (66.3, 68.6)	3.90 td (10.3, 10.3, 4.0)	3.62 ddd (9.8; 9.8; 3.5)	H-1; H-3	H-3; H-25
3 (CH)	85.9 (85.2, 83.8)	4.70 d (10.3)	2.91 d (9.8)	H-2	H-23; H-24
4 (C)	41.1 (40.2, 38.5)				H-3; H-5; H-23; H-24
5 (CH)	56.5 (55.4, 56.0)	1.04 nd		H-6	H-23; H-24; H-25
6 (CH_2)	19.5 (18.6, 19.1)	1.59 nd		H-5; H-7	H-5; H-25
7 (CH_2)	34.0 (33.1, 33.5)	1.37 / 1.64 nd		H-6	H-26
8 (C)	40.8 (40.2, 40.6)				H-27
9 (CH)	48.6 (47.5, 47.9)	1.82 nd		H-11	H-5; H-25; H-26
10 (C)	39.2 (38.3, 39.9)				H-25
11 (CH_2)	24.7 (23.9, 24.2)	2.04 nd		H-12; H-9	
12 (CH)	129.3 (127.7, 128.2)	5.30 br s	5.28 t (3.2)	H-11	H-18
13 (C)	140.4 (140.1, 139.5)				H-18; H-27
14 (C)	42.7 (42.2, 42.3)				H-27; H-26
15 (CH_2)	29.6 (29.1, 29.3)	1.02 / 1.85 nd		H-15b / H-15a; H-16	H-27
16 (CH_2)	27.3 (26.7, 26.8)	1.28 / 1.75 nd		H-16b / H-16a; H-15	H-18
17 (C)	48.6 (48.1, 48.6)				H-18
18 (CH)	55.1 (54.5, 54.4)	2.51 s	2.50 s		H-29
19 (C)	73.7 (72.6, 72.6)				H-18; H-29; H-30
20 (CH)	43.1 (42.0, 42.2)	1.37 nd		H-30	H-30; H-29; H-18
21 (CH_2)	26.6 (26.2, 26.1)	1.54 / 2.59 nd		H-21b / H-21a	H-30
22 (CH_2)	39.0 (38.2, 37.8)	1.75 nd			
23 (CH_3)	29.2 (28.8, 29.5)	0.90 s			H-3; H-24
24 (CH_3)	18.3 (16.5, 16.8)	1.01 s			H-3; H-23
25 (CH_3)	17.0 (16.6, 17.1)	1.08 s			
26 (CH_3)	17.5 (18.1, 17.8)	0.82 s			
27 (CH_3)	24.8 (24.5, 24.6)	1.37 s			
28 (C)	182.7 (180.8, 176.9)				H-18
29 (CH_3)	27.0 (26.9, 27.0)	1.20 s			
30 (CH_3)	16.5 (17.0, 17.5)	0.93 d (6.6)		H-20	
1' (C)	123.0 (122.7, -)				H-3'; H-5'
2', 6' (CH)	133.0 (132.5, -)	7.92 dd (9.0, 2.1)		H-3', H-5'	H-6', H-2'
3', 5' (CH)	116.2 (116.0, -)	6.83 dd (9.0, 2.1)		H-2', H-6'	H-5', H-3'
4' (C)	163.6 (163.4, -)				H-3'; H-5'; H-2'; H-6'
7' (C)	168.7 (167.0, -)				H-3; H-2'; H-6'

[a] Values are in ppm (δ). Coupling constants (*J*), in parentheses, are in Hz; [b] ^{13}C NMR in C_5D_5N (75.5 MHz).

Plant material

The plant was collected in April 1999, Mandacaru stream, Maringá city, State of Paraná, Brazil and identified by Dr. Maria Conceição de Souza, Universidade Estadual de Maringá. A voucher specimen (HUM 9057) was kept at the herbarium of the Biological Department of Universidade Estadual de Maringá.

Isolation

Air-dried and powdered leaves (600 g) of *L. divaricata* Mart., were extracted with MeOH at room temp. The MeOH extract was concentrated in vacuum and yielded 74 g of crude methanolic extract. Part of the crude methanolic extract (38 g) was partitioned with n-hexane (600 mL), chloroform (600 mL), ethyl acetate (600 mL) and methanol (100 mL), yielding 5.8 g (15.3%), 2.9 g (7.6%), 4.9 g (12.9%), 24 g (63.2%) respectively. The choroformic fraction was subjected to CC on silica gel (70 g) and eluted with different rations of n-hexane, $CHCl_3$ and MeOH. The appropriate frs (monitored by TLC analysis) were combined resulting in 22 frs. Fr 14 (160 mg), eluted with chloroform-methanol (90:10), was subjected to repeated CC on silica gel, eluted with n-hexane, chloroform and methanol mixts of increasing polarity to give 3β-*p*-hydroxybenzoyloxytormentic acid **(1)** (2.8 mg-1.8%) and a mixture containing maslinic acid **(2)** (4.3 mg-2.7%).

3β-p-hydroxybenzoyloxytormentic acid (**1**). White crystals. EIMS *m/z* (rel. int.): $[M]^{+\bullet}$ 608, 563 (10.8), 562

(17.0), 264 (< 3.0), 246 (14.0), 219 (7.3), 218 (14.1), 201 (5.8), 189 (31.8), 187 (15.6), 179 (< 3.0), 146 (61.9), 121 (100).

Maslinic acid **(2)**. White crystals. ^{13}C NMR (75.5 MHz, CD_3OD): 48.1 (C-1), 69.5 (C-2), 84.5 (C-3), 40.5 (C-4), 56.7 (C-5), 19.5 (C-6), 33.9 (C-7), 39.2 (C-8), 49.0 (C-9), 39.2 (C-10), 24.0 (C-11), 123.6 (C-12), 145.5 (C-13), 42.6 (C-14), 28.8 (C-15), 24.0 (C-16), 47.7 (C-17), 42.7 (C-18), 47.2 (C-19), 31.6 (C-20), 34.9 (C-21), 33.8 (C-22), 29.3 (C-23), 17.0 (C-24), 17.1 (C-25), 17.4 (C-26), 23.9 (C-27), 180.0 (C-28), 33.5 (C-29), 23.9 (C-30).

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