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A triterpenoid saponin isolated from *Lafoensia glyptocarpa*

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Abstract

From the leaves of *Lafoensia glyptocarpa* Koehne (Lytraceae) was isolated a triterpenoid saponin, 3β-*O*-β-L-arabnopyranosylolean-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl ester, along with the known compound 3β-*O*-β-D-glucopyranosylsitosterol. The structures of both compounds were elucidated with spectral data of the natural products and their acetyl derivatives, including 2D NMR spectroscopic experiments. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Lafoensia glyptocarpa; Lytraceae; Triterpene saponin; Spectral data

1. Introduction

Lafoensia glyptocarpa Koehne (Lytraceae) is a tree known in Brazil as 'mirindiba rosa, mirindiba bagre, mirinduva, louro de São Paulo'. It occurs in the eastern part of Brazil and has been found more frequently in the states of Bahia, Espirito Santo, Rio de Janeiro and São Paulo (Correa, 1984).

2. Results and discussion

Saponins **1** and 3β-*O*-β-D-glucopyranosylsitosterol were isolated from the MeOH extract obtained from the leaves of *L. glyptocarpa*, using the procedure described in the Experimental section.

The known natural glycoside 3β-*O*-β-D-glucopyranosylsitosterol was identified mainly by ^{1}H and ^{13}C NMR spectral data, including the peracetyl derivative, and by comparison with literature data (Braz-Filho et al., 1986; Guevara et al., 1989).

The negative FAB mass spectrum of **1** revealed quasi-molecular ion peak at *m/z* 751 ($[M+H]^-$), consistent with the molecular formula $C_{41}H_{66}O_{12}$ (750 Da), and prominent peaks due to the loss of $C_6H_{12}O_5$ [*m/z* 587(100%)] followed by $C_5H_8O_4$ [*m/z* 455(12%)], corresponding to one hexose and one pentose moiety, respectively. The peak at *m/z* 179 (55%), attributed to fragment $[C_6H_{12}O_6]^-$, also suggested the presence of a hexose moiety.

The ^{1}H NMR spectrum of **1** displayed singlet signals for seven tertiary methyl groups in the aglycone moiety, suggesting a pentacyclic skeleton of the oleanane type containing a carbonyl ester (ν 1734 cm^{-1}). In addition, it was possible to observe an olefinic hydrogen signal at δ 5.26 (br s, H-12) and a signal typical of H-3ax [δ 3.18 (br d, J = 11.0 Hz)] consistent with the presence of a β-OR group at C-3 position.

The hydrogen broad band decoupled (HBBD) ^{13}C NMR spectrum of **1** showed 41 signals. Comparative analysis of HBBD- and DEPT ^{13}C NMR spectra was used to recognise the signals corresponding to eight quarternary carbons (including two sp^2), 14 methine (including one sp^2, eight monoxygenated and two dioxygenated), 12 methylene (including two monoxygenated) and seven methyl carbons atoms. The 30 ^{13}C-NMR resonances of the aglycone were deduced by subtracting the 11 sugar carbon (one pentose and one hexose) signals from the total (41 signals).

Thus, these spectral data and comparison with ^{13}C

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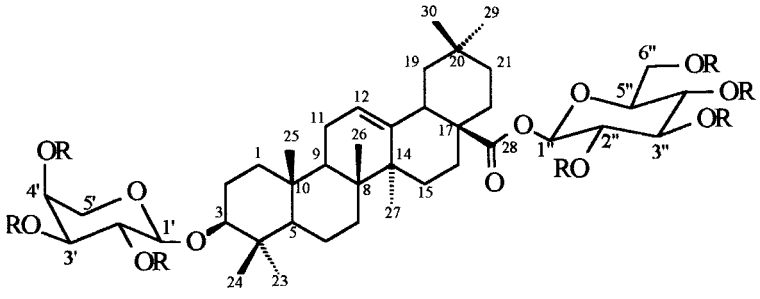
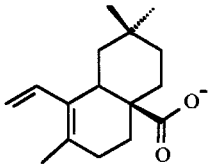


1 R=H {[M + H]$^-$, 751 (1%)}
1a R=Ac



1b *m/z* 247 (12%)

NMR spectroscopic values described in the literature for olean-12-en-28-oic acid (Mahato and Kundu, 1994) corresponding to aglycone moiety suggested two structural possibilities: 3β-*O*-β-L-arabinopyranosylolean-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl ester (**1**) or 3β-*O*-β-D-glucopyranosylolean-12-en-28-oic acid 28-*O*-3β-β-L-arabinopyranosyl ester (**1**). The characterization and location of an -*O*-β-D-glucopyranosyl moiety at C-28 was based on comparison with literature data (Hamed et al., 1996) and afforded the following results: (a) the chemical shifts of the H-1″ [**1**: δ 5.38 (d, J = 8.0 Hz); **1a**: δ 5.56 (d, J = 8,0 Hz), H-1″ ax] showed significant deshielding when compared with the signal of H-1′ [**1**: δ 4.26 (d, J = 7.2 Hz); **1a**: δ 4.43 (d, 8.8 Hz), H-1′ ax]; (b) the cross-peaks belongs to direct coupling ($^1J_{CH}$) between hydrogen H-1″ [δ 5.38 (**1**) and 5.56 (**1a**)] and carbon CH-1″ [δ 95.84 (**1**) and 91.44 (**1a**)] observed in the 2D ^{1}H,^{13}C-HMQC-$^1J_{CH}$ spectra of **1** and **1a**; (c) the 2D ^{1}H,^{13}C-HMBC-$^nJ_{CH}$ (n = 2 and 3) spectrum of **1a** revealed long-range coupling between hydrogen H-1″ (δ 5.56) and carbon C-28 (δ 175.46, $^3J_{CH}$); (d) homonuclear 2D ^{1}H,^{1}H-COSY spectrum of **1a**, which revealed all the spin–spin interactions after identification of the H-1″ signal (δ 5.56).

Consequently, the remaining ^{1}H and ^{13}C signals corresponding to the carbinolic hydrogen and carbon atoms were used to identify a β-L-arabinopyranosyl moiety at β-position of carbon CH-3 [**1**: δ_C 90.83 and δ_H 3.18 (br d, J = 11.0 Hz, H-3ax). **1a**: δ_C 89.97 and δ_H 3.16 (dd, J = 11.2 and 4.4 Hz, H-3ax)]: one anomeric [**1**: δ_C 107.31 and δ_H 4.26 (d, J = 7.2 Hz); **1a**: δ_C 103.18 and δ_H 4.43 (d, J = 8.0 Hz), CH-1′], three methyne and one methylene groups. This deduction was confirmed by the presence of cross-peaks corresponding to spin–spin interaction ($^3J_{CH}$) of H-1′ [δ 4.26 (**1**) and 4.43 (**1a**) and CH-3 [δ 90.83 (**1**) and 89.97 (**1a**)] observed in the ^{1}H,^{13}C-HMBC-$^nJ_{CH}$ spectra of **1** and **1a**. The unambiguous ^{1}H and ^{13}C chemical shift assignments of **1** and **1a** were obtained by the heteronuclear 2D ^{1}H,^{13}C-HMQC-$^1J_{CH}$ and ^{1}H,^{13}C-HMBC-$^nJ_{CH}$ (n = 2 and 3) spectra, as summarised in the Experimental section.

Thus, the structure of the new triterpenoid saponin isolated from *L. glyptocarpa* was established as 3β-*O*-L-arabinopyranosylolean-12-en-28-oic acid 28-*O*-β-D-glucopyranosyl ester (**1**).

3. Experimental

3.1. General procedure

Mps are uncorr. The ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Varian UN 400 spectrometer, in MeOH-d_4 (**1**) and $CDCl_3$ (**1a**). Varian pulse sequences were used to obtain homonuclear 2D ^{1}H,^{1}H-COSY and heteronuclear 2D ^{1}H,^{13}C-HMQC-$^1J_{CH}$ (modulated with $^1J_{CH}$ = 140 Hz) and ^{1}H,^{13}C-HMBC-$^nJ_{CH}$ (n = 2 and 3, modulated with $^1J_{CH}$ = 9.0 Hz). Mass spectra were obtained with a VG Quattro instrument. FT-IR spectra were recorded on a Perkin-Elmer model 1420 spectrometer. Chromatography was performed using silica gel (Aldrich) with suitable granulation for column and preparative TLC.

3.2. Plant material

L. glyptocarpa Koehe was collected in February 1992, in Horto Florestal de Seropédica, RJ, Brazil. Authentication was performed by Professor José Aguiar Sobrinho by comparing it with a herbarium specimen (#379) kept at the Herbarium of the Horto Florestal de Seropédica, Seropédica-RJ, Brazil.

3.3. Extraction and isolation

Air dried and powdered leaves (1052 g) were extracted exhaustively with hexane and MeOH with maceration at room temperature. MeOH was removed under vacuum to yield a residue (132.2 g). This residue

was partitioned between CH_2Cl_2 and MeOH–H_2O (95:5). The residue obtained (23.7 g) of the CH_2Cl_2 solution was chromatographed on silica gel column using CH_2Cl_2–MeOH as eluents in increasing polarities to afford 31 fractions of 125 mL each. Fraction 8, eluted with CH_2Cl_2–MeOH (9:1), gave 3β-*O*-β-D-glucopyranosylsitosterol (110 mg, mp 270°). Fraction 16, eluted with CH_2Cl_2–MeOH (1:1) furnished **1** (240 mg, mp 220°).

3.4. Acetylation of compounds 3β-O-β-D-glucopyranosylsitosterol and 1

Compounds 3β-*O*-β-D-glucopyranosylsitosterol (140 mg) and **1** (50 mg) were separately dissolved in a mixture of pyridine and Ac_2O (1:1) and the solution was allowed to stand for 24 h at room temperature. The usual work-up gave a residue which was dried under vacuum and purified through chromatography on silica gel column to yield peracetyl derivative of 3β-*O*-β-D-glucopyranosylsitosterol (100 mg, mp 130°) and **1a**: (50 mg, mp 160°), $[\alpha]_D^{25}$ +16.62° (MeOH, *c*, 0.00572). IR (KBr) ν_{max} (cm^{-1}): 1740, 1200, 1050, 800. ^1H ($CDCl_3$): 5.56 (d, 8.0, H-1′), 5.30 (br s, H-12), 5.20 (m, H-3″), 5.18 (m, H-4′), 5.16, (dd, 10.0, 8.0, H-2′), 5.11 (m, H-2″), 5.09 (t, 9.6, H-4″), 4.43 (d, 8.0, H-1′) 4.26 (dd, 12.4, 4.4, H-6″), 4.02 (dd, 12.4, 3.2, H-6″), 3.98 (dd, 12.8, 2.4, H-5′), 3.77 (m, H-5″), 3.50 (dd, 12.8, 1.2, H-5′) 3.16 (dd, 11.2, 4.4, H-3), 2.79 (dd, 11.0, 3.1, H-18), 2.10-1.99 (s, H_3CCO), 1.84 (m, H-9, 11), 1.68 (H-7), 1.61 (H-7), 1.60 (H-19), 1.50 (H-16), 1.46 (H-6), 1.42 (H-15, 22), 1.32 (H-21), 1.30 (H-16), 1.28 (H-21), 1.20 (H-22), 1.19 (H-2, 15), 1.15 (H-19), 1.09, 0.90, 0.89, 0.89, 0.88, 0.74. 0.70 (s, H_3C-27, 23, 25, 30, 29, 24, 26, respect.), 0.68 (H-5); ^{13}C NMR ($CDCl_3$), δ_C 175.46, 142.72, 170.50–168.80, 46.69, 41.57, 39.22, 38.79, 36.61, 31.64 (C-28, 13, H_3C–*C*O, 17, 14, 8, 4, 10, 20), δ_{CH}: 122.76, 103.18, 91.44, 89.97, 72.73, 72.33, 70.30, 69.82, 69.48, 67.89, 67.79, 55.39, 47.46, 40.91, (C-12, 1′, 1″, 3, 3″, 5″, 3′, 2″, 2′, 4″, 4′, 5, 9, 18)), δ_{CH_2}: 63.16, 61.41, 45.63, 41.57, 33.64, 32.90, 32.90, 25.71, 27.63, 23.34, 22.74, 18.06 (C-5″, 6″, 19, 14, 21, 7, 22, 2, 15, 16, 11, 6), δ_{CH_3}: 32.94, 27.63, 25.70, 23.35, 20.87–20.46, 16.87, 16.27, 15.05 (C-29, 23, 27, 30, H_3C–CO, 26, 24, 25).

Compound **1**

Mp 220°, $[\alpha]_D^{25}$ +10.30° (Me0H, *c* 0.0033). IR (KBr) ν_{max} (cm^{-1}): 3415, 2943, 1734, 1625, 1453, 1388, 1257, 1166, 1070, 918, 757. ^1H (MeOH-d_4): 5.38 (d, 8.0, H-1″), 5.24 (br s, H-12), 4.26 (d, 7.2, H-1′), 3.80 (m, H-4′, 6″), 3.67 (dd, 11.6, 3.6, H-6″) 3.58 (brd, H-2′), 3.57 (m, H-5′), 3.51 (dd, 8.0, 4.8, H-5′), 3.50 (m, H-3′), 3.34 (m, H-4″, 5″), 3.31 (m, H-2″), 3.26 (m, H-3″), 3.18 (br d, 11.0, H-3), 2.84 (br d, 10.0, H-18), 1.82 (H-2, 15), 1.74 (H-7), 1.64 (H-15, 2), 1.58 (H-7), 1.54 (H-6), 1.52 (H-19) 1.48 (H-22), 1.35 (H-6), 1.32 (H-21), 1.28 (H-22), 1.20 (H-21), 1.12 (H-19) 1.03, 0.97, 0.94, 0.92, 0.90, 0.84, 0.79 (s, CH_3, 23, 27, 25, 30, 29, 24, 26, respectively), 0.88 (H-16), 0.79 (m, H-5), ^{13}C NMR (MeOH-d_4): δ_C: 178.18, 144.99, 47.26, 40.86, 40.37, 38.04, 36.11, 31.69 (C-28, 13, 17, 14, 8, 4, 10, 20), δ_{CH}: 123.94, 107.31, 95.84, 90.83, 78.85, 78.45, 74.47, 74.06, 72.96, 71.29, 69.68, 57.18, 42.71, 24.10 (C-12, 1′, 1″, 3, 5″, 3″, 2′, 2″, 3′, 4″, 4′, 5, 18, 24), δ_{CH_2}: 66.55, 62.54, 47.30, 40.86, 34.99, 34.05, 33.25, 27.12, 24.82, 24.66, 24.10, 19.50 (5′, 6″, 19, 1, 21, 22, 7, 15, 2, 16, 11, 6), δ_{CH_3}: 33.60, 28.66, 26.40, 24.06, 17.83, 17.07, 16.12 (29, 23, 27, 30, 26, 24, 25), respectively. FABMS [Glycerol matrix, negative ion model *m/z* (rel. int.): 751 ($[M+H]^-$, 1), 587 ($[M\text{-}C_6H_{11}O_5]^-$, 100), 455 ($[M\text{-}C_6H_{11}O_5\text{–}C_5H_8O_4]^-$, 12), 247 (**1b**, 12), 179 ($[C_6H_{11}O_6]^-$, 55).

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