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Article

The Use of ^{13}C and ^{1}H-NMR in the Structural Elucidation of a New *Nor*-Lupane Triterpene

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Um triterpeno *nor*-lupânico, inédito, foi isolado das folhas de *Eugenia florida* DC (Myrtaceae). Tomando-se por base as evidências espectroscópicas, RMN ^{1}H, ^{13}C, experimentos de RMN 2D, em especial correlações homonuclear ^{1}H-^{1}H, heteronuclear ^{1}H-^{13}C e correlações a várias ligações (HMBC), sua estrutura foi elucidada como: 29-hidroxiplatan-28-ato de β-D-glicosila.

The new *nor*-lupane triterpene, isolated from the leaves of *Eugenia florida* DC (Myrtaceae) was identified on the basis of its spectroscopic data, ^{13}C and ^{1}H-NMR-2D, specially COSY ^{1}H-^{1}H, COSY ^{1}H-^{13}C and HMBC correlations, as 28-O-β-D-glucopyranosyl ester of 29-hydroxyplatanic acid.

Keywords: Nor-*lupane triterpene, ^{13}C and ^{1}H-NMR, HMBC, COSY,* Eugenia florida, *Myrtaceae*

Introduction

The triterpenes are a large group of plant substances with a wide spectrum of biological activities, such as anti-HIV[1]. The study of the *Eugenia florida* DC (Myrtaceae) leaves, which occur in the areas of tropical and subtropical[2] climate, is underway in our laboratories. In this work an unpublished triterpene with skeleton *nor*-lupane was identified. The identification of its structure was made through the use of spectroscopic methods. The homonuclear ^{1}H-^{1}H and one bond and multiple bond heteronuclear ^{1}H-^{13}C correlations experiments were used as the main tools to identify this new compound.

Results and Discussions

The structural determination of **1** was done on the basis of a comparative analysis of its ^{1}H and ^{13}C-NMR 1D and 2D data with those reported in the literature for platanic acid[1,3,4]. In the ^{1}H-NMR spectrum of **1**, it was observed the presence of 5 methyl signals, δ 0.77, 0.85, 0.94, 0.96 and 1.07 (Table 1). The signal at δ 2.21 of the CH_3-29, which is characteristic of platanic acid[1] was not detected. At δ 3.26, it was observed a triplet (J = 7.6 Hz) of the H_{ax} bounded to C-3, which was coupled with H-2. The multiplet appearing at δ 3.36-3.43 corresponds to H-19, coupled with H-18 and H-21. The COSY ^{1}H-^{1}H spectrum of **1** (Fig. 1) also exhibited several important correlations to establish the triterpene skeleton and the attribution of the ^{1}H chemical shifts (Table 1) was supported by these data. From the DEPT 135 experiment it was possible to determine that **1** was a primary alcohol, which justifies the fact that **1** showed only 5 methyl groups, instead of 6 as in platanic acid. The location of the hydroxyl group at CH_2-29 (δ 68.6), whose ^{1}H signals appeared as two doublets at δ 4.40 and 4.32 (J = 18.8 Hz) and the attribution of the chemical shifts of all methyl groups, was carried out by analysis of the HMBC spectrum (Figs. 2 and 3). It was noticed the unequivocal correlation of the two methyl groups, H-23 (δ 1.07) and H-24 (δ 0.85) with C-3 at δ 78.1 (^{3}J, Table 1), and with the carbons C-5 at δ 55.7, and C-4 at δ 39.2 (^{2}J). It was also noticed the correlation of the methyl H-25 (δ 0.77) with C-10 at δ 37.4 (^{2}J) and of two methyl groups, H-26 and H-27 with C-8 at δ 40.9 (respectively ^{2}J and ^{3}J). H-29 showed correlation with the carbonyl at δ 214.2 (C-20)[3].

The presence of the sugar moiety in the molecule was verified by the signal of the anomeric hydrogen detected at

δ 5.95 (d, J = 8.4 Hz), confirmed by other signals between δ 3.70 and 4.36. The inspection of the COSY ^{1}H-^{1}H spectrum (Fig. 1), relatively to the glucosyl unit, allowed to establish the correlation between the hydrogens H-1' to H-6'. The inspection of the HMQC spectrum of **1** (Fig. 2, Table 1) showed correlation of H-1'-H-6' signals with the signals at δ 95.5, 73.6, 78.3, 70.9, 78.2 and 62.0, C-1' to C-6' respectively. These signals and these correlations are the same registered in the literature for β-D-glucose[7]. After having assigned the ^{1}H and ^{13}C chemical shifts of the sugar moiety, it was still remaining to locate this unit in the *nor*-lupane skeleton at the position C-3, C-28 or C-29. The ^{13}C-NMR spectrum showed a carbonyl carbon signal at δ 174.7, shielded by 4.0 ppm, which occurs when C-28 is an ester function[5,6]. This functionality was confirmed by the HMBC spectrum (Table 1, Fig. 3), whose ^{1}H signal at δ 5.95 was correlated to the carbonyl carbon at δ 174.7 (^{3}J). This unequivocally confirmed that the position C-28 was indeed esterified with β-D-glucose.

Finally, the mass spectrum of **1**, obtained by FAB/MS, revealed the peak corresponding to the molecular ion (*m/z* 637, M+1, 3%), and the peaks 636 (5), 473 (20), 275 (32), 183 (100) Daltons.

These data allowed to attribute the structure of **1** as being 28-O-β-D-glucopyranosyl ester of 29-hydroxyplatanic acid which is now described for the first time in the literature.

Experimental

General experimental procedure

The ^{1}H and ^{13}C-NMR experiments were recorded in a Bruker spectrometer ARX, operating respectively at 400 MHz for ^{1}H and 100 MHz for ^{13}C, using deutero pyridin

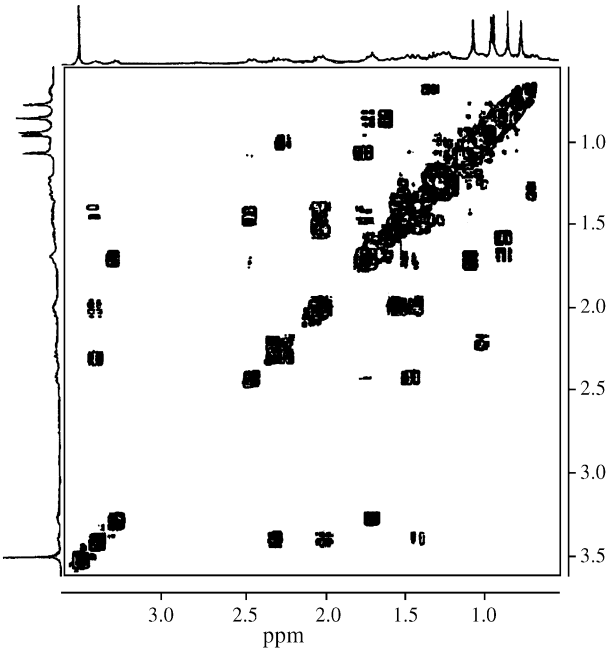

Figure 1. ^{1}H x ^{1}H-COSY spectrum of **1**.

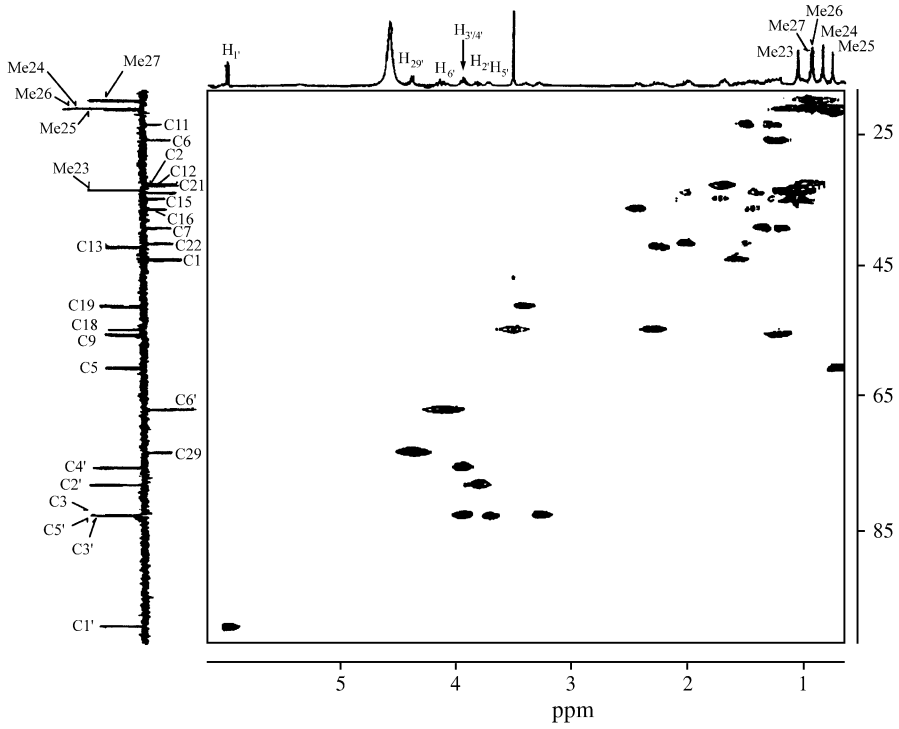

Figure 2. HMQC spectrum of **1**.

Table 1. ^{1}H and ^{13}C-NMR data of 1 and platanic acid.[1,3] The H-1 and C-13 chemical shift assignments were obtained from 2D spectra (HMBC, HMQC and COSY ^{1}H-^{1}H) and 1 D (fully decoupled from ^{1}H and DEPT). The spectra were recorded in pyridine, and the chemical shifts referenced to internal TMS.

^{1}H or ^{13}C	**1** (δ)	HMBC	**1** (δ)	platanic acid (δ)
1 ax.	0.82-0.91 (m)		39.1	39.3
1 eq.	1.50-1.60 (m)			
2	1.63-1.73 (m)		27.9	28.2
3α	3.26 (t, J = 7.6)		78.1	77.8
4			39.2	39.5
5	0.69 (d, J = 9.6)		55.7	55.7
6	1.25-1.38 (m)		21.0	21.1
7	1.30*		34.4	34.6
8			40.9	40.9
9	1.27*		50.7	50.7
10			37.4	37.7
11	1.40*		18.6	18.8
12	1.00*		27.6	27.7
13	2.22-2.28 (m)		37.2	37.7
14			42.5	42.5
15 ax.	1.04-1.12 (m)		29.9	30.2
15 eq.	1.70-1.77 (m)			
16 ax.	1.40-1.50 (m)		31.4	32.3
16 eq.	2.44 (dl, J = 12.4)			
17			56.6	56.4
18	2.30 (t, J = 6.7)		49.9	49.7
19	3.36-3.43 (m)		46.3	52.0
20			214.2	211.6
21 ax.	1.38-1.44 (m)		29.0	28.7
21 eq.	1.95-2.03 (m)			
22 ax.	1.45-1.56 (m)		36.8	37.4
22 eq.	1.95-2.03 (m)			
23 αCH_3	1.07 (s)	C-3 and C-4, ($^{3}J/^{2}J$) /C-5, (^{3}J)	28.5	28.6
24 βCH_3	0.85 (s)	C-3, (^{3}J) / C-4 and C-23, ($^{2}J/^{3}J$)	16.1	16.3
25 βCH_3	0.77 (s)	C-10, (^{2}J) /C-5, (^{3}J)	16.4	16.3
26 βCH_3	0.94 (s)	C-8, (^{2}J)	16.1	16.3
27 αCH_3	0.96 (s)	C-8, (^{3}J)	14.9	14.8
28			174.7	178.7
29 a	4.40 (d, J = 18.8)	C-20, (^{2}J)	68.1	29.6
29 b	4.32 (d, J = 18.8)	C-20, (^{2}J)		
1' ax	5.95 (d, J = 8.4)	C-28, (^{3}J)	95.0	95.0
2' ax	3.81 (t, J = 8.4)		73.6	73.6
3' ax	3.91 (t, J = 8.4)		78.3	78.3
4' ax	3.95 (t, J = 8.4)		70.9	70.9
5' ax	3.70-3.79 (m)		78.2	78.2
6' a	4.15 (dd, J = 2.8/12)		62.0	62.0
6' b	4.07 (dd, J = 4.4/12)			

*Center of the signal correlated.

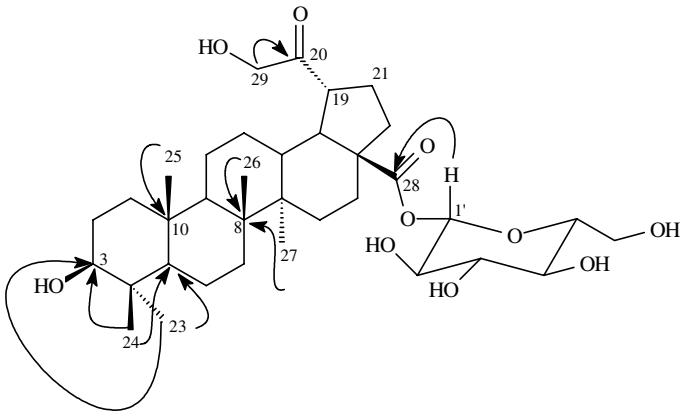

Figure 3. Main correlations detected in the HMBC spectrum of 28-O-β-D-glucopyranosyl ester of 29-hydroxyplatanic acid (**1**).

(C_5D_5N) as solvent, with TMS as internal standard. HMQC and HMBC data were acquired using the microprogram *invbtp* (J = 145 and 9 Hz respectively). Melting point: Kofler block (Rochester) with one non calibrated thermometer. FT/IR: Bomen spectrometer, model M 102, KBr (1% of sample). For the chromatographic analysis a droplet countercurrent chromatograph Eyela DCC-300 model, equipped with 300 columns (42.0 x 0.3 cm) and also a R-HPLC Shimadzu, model CR4A equipped with UV detector Shimadzu model SPD-6AV were used.

Plant material

Leaves of *Eugenia florida* DC, were collected in February of l991, in the Ecological reserve of the Luís Antônio - São Paulo, Brazil and identified by Drs. José Rubens Pirani (USP) and Marcos Sobral (UFRGS). The respective voucher was deposited in the herbarium of the Instituto de Biociências of University of São Paulo-SP (SPF 75745). The leaves were dried separately in a stove with circulating air at 40 °C and grounded in a Willey mill.

Extraction and isolation of the chemical constituents

The leaves (541 g) were extracted consecutively with hexane (3 x 1 L), dichloromethane (3 x 1 L) and methanol (3 x 1 L) with intervals of 3 days between each extraction at room temperature. After distillation of the solvents, it was obtained in each extract 3.9 g, 4.7 g and 5.1 g of solid residues. The residue obtained from the MeOH extraction (5.1 g) was submitted to droplet countercurrent chromatography, using $CHCl_3$:MeOH:H_2O (5:5:3 v/v) as solvent, with the organic layer being the mobile one. After 48 h of analysis, 200 fractions, 13 mL each, were collected and combined into 8 groups based on the results from analytical TLC. The fraction 67-74 (15 mg) was crystallized in methanol:acetone (9:1) and submitted to recycling-HPLC. Solutions of 7.5 mg mL^{-1} in MeOH were prepared and 2 mL of these solutions were chromatographed on polymeric packing column (Asahipak GS-310 P, 21.5 cm ID x 50.0 cm L) using MeOH for elution with a flow rate of 8 mL min^{-1}. The UV detector was set at 215 nm. Three cycles of 60 min afforded compound **1** (9 mg, *28-O-β-D-glucopyranosyl ester of 29-hydroxyplatanic acid*), solid, mp 240-241 °C. IR ν_{max} KBr disc cm^{-1}: 3398, 2944, 2869, 1744, 1714, 1459, 1378, 1065, 589. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz): Table 1. FAB/MS, *m/z* (relative intensity, %): 637 (M+1, 3), 636 (5), 473 (20), 275 (32), 183 (100).

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