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Note

Complete Assignments of ^{1}H and ^{13}C-NMR Spectra of the 3,4-*seco*-Triterpene Canaric Acid isolated from *Rudgea jasminoides*

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O ácido canárico **1** foi isolado das folhas de *Rudgea jasminoides*. A substância isolada é um derivado triterpênico do tipo *seco*-lupano e teve sua estrutura elucidada com base nos dados espectrais, principalmente em experimentos de RMN a 1D e 2D. O sitosterol, o estigmasterol e os ácidos ursólico e oleanólico também foram isolados.

The canaric acid 1 was isolated from the leaves of *Rudgea jasminoides*. Compound **1** is a *seco*-lupane derivative and its structure was established on the basis of spectral data, mainly by 1D and 2D NMR experiments. Sitosterol, stigmasterol and ursolic and oleanolic acids were also isolated.

Keywords: Rudgea jasminoides, *Rubiaceae, seco-A-ring lupane triterpene*

Introduction

The genus *Rudgea* is widely distributed along the Brazilian East Coast, where some species are used as a remedy to treat rheumatism, syphilis and swelling of the members relief[1]. *Rudgea jasminoides* is a small tree, especially impressive due to the pleasant jasmine smell of its wonderful white flowers, during the blooming. Several triterpenes and saponins have been isolated from other species, *R. viburnioides*[2]. However, *seco*-lupane triterpene derivatives have never been isolated from the Rubiaceae family.

As a part of our study on the constituents of plants from Rubiaceae[2-5], we report the isolation of the canaric acid **1** from the leaves of a specimen of *R. jasminoides*. The canaric acid is a rare *seco*-ring-A-triterpene derivative, previously isolated from *Dacryoides edulis, Canarium zeylanicum and C. muelleri* from the Burseraceae family[6,7,8]. However, no detailed studies of ^{13}C-NMR, and high-resolution ^{1}H-NMR spectra of the compound **1** have been carried out. Herein we report application of these NMR spectral data in the structural elucidation of this triterpene. In addition, sitosterol, stigmasterol and two known triterpenes ursolic and oleanolic acid were isolated.

Experimental

IR spectra were obtained on a Perkin-Elmer Model 1600 spectrometer; ^{1}H-NMR (200, 300 and 500 MHz) and ^{13}C-NMR (75 and 125 MHz) spectra were registered on a Bruker AC 200, DPX 300 and ARX 500, at 25°, in $CDCl_3$; HRFABMS: Central Analítica, Instituto de Química, Universidade de São Paulo, SP, Brasil.

Plant material

Leaves of *Rudgea jasminoides* were collected from preserved areas of Atlantic forest in the biological reserve of the "Ilha do Cardoso", Cananéia, SP, Brazil. A voucher specimen SP 161 348 was deposited in the Herbarium Maria Eneida de Fidalgo, Instituto de Botânica de São Paulo.

Extraction and isolation of the constituents

Dried and powdered leaves (1.0 kg) of *R. jasminoides* were successively extracted with acetone and EtOH at room temp. to yield the acetonic (11.0 g) and EtOH (3.0 g) extracts respectively. Half of the acetone extract (5,5 g) was submitted to flash column chromatography eluting with a mixture of hexane and EtOAc. Twenty fractions (25 mL) were collected and analyzed by TLC on silica gel in hex-

Dedicated to the memory of professor W. Saffioti.

ane-EtOAc (7:3) and $CHCl_3$-MeOH (9:1 and 9.5:0.5). Fractions 2-3 were submitted to prep. TLC (hexane-EtOAc, 7:3) yielding sitosterol (21 mg) and stigmasterol (5 mg). Fractions 12-16 (200 mg) containing the crude triterpene **1** as a mixture with oleanane and ursane acid were further purified by a sequence prep. TLC with hexane-

Table 1. ^{13}C and ^{1}H-NMR spectral data for compound 1 and observed ^{1}H-^{13}C-HMQC-$^{1}J_{CH}$ and ^{1}H-^{13}C-HMBC-$^{n}J_{CH}$ (n = 2 and 3).

atom	δ_C[a,b]	δ_H[c]	Selected HMBC to
1	33.90 (t)	H_a 1.42 m H_b 1.59 m	
2	28.36 (t)	H_a 1.99 ddt (2.45, 8.0, 14.0); H_b 2.45 (m)	C-3, C-10
3	179.91 (s)	-	
4	147.58 (s)	-	
5	40.74 (d)	2. 65 dd (10.5, 4.8)	C-10, C-7, C-23, C-24
6	24.19 (t)	Hα 2.10 dddd (19.0, 4.8, 6.4, 2.0) Hβ 2.26 ddd (19.0, 10.5, 5.0)	C-4, C-8
7	33.94 (t)	Hα 1. 98 ddd (10.5, 2.0, 16.5) Hβ 2.06 ddd (16.5, 6.4, 5.0)	
8	39.90 (s)	-	
9	50.39 (d)	1.77 dd (10.0, 8.4)	C-5, C-11, C-12, C-25, C-26
10	39.24 (s)	-	
11	21.70 (t)	Hα 1.13 m Hβ 1.26 m	
12	27.45 (t)	Hα 1.56 m Hβ 1.36 m	
13	38.10 (d)	2.01 m	C-14, C-17, C-18, C-27
14	43.19 (s)	-	
15	27.45 (t)	Hα, Hβ 1.25 m	
16	35.51 (t)	Hα, Hβ 1.48 m	
17	43.19 (s)	-	
18	48.22 (d)	2.05 br, d (8.8)	C-13, C-14, C-20, C-22, C-28
19	47.93 (d)	1.90 m	C-13, C-17, C-18, C-22, C-29, C-30
20	150.78 (s)	-	
21	29.70 (t)	Hα 1.49 m Hβ 1.68 m	
22	40.55 (t)	Hα 1.25 m Hβ 1.76 dd (11.5, 9.5)	
23	20.09 (q)	1.61 s	C-4, C-5, C-24
24	113.38 (t)	H_a 4.77 br, s; 4.57 br, s	C-4, C-5, C-23
25	23.22 (q)	1.08 s	C-1, C-5, C-10, C-9
26	16.00 (q)	0.72 s	C-7, C-8, C-9, C-14
27	14.54 (q)	0.78 s	C-8, C-13, C-14
28	18.01 (q)	0.95 s	C-16, C-17, C-18
29	109.50 (t)	H_a 4.50 br s; 4.61 br s	C-19, C-20, C-29, C-30
30	19.21(q)	1.65 s	C-19, C-20, C-29

[a]125 MHz, $CDCl_3$; Multiciplity in ^{13}C obtained by DEPT 135° and 90° experiments.
[b]Chemical shifts assigned from HMQC and HMBC. [c]500 MHz, $CDCl_3$; Coupling constants (Hz) in parentheses.

EtOAc (7:3) and $CHCl_3$-MeOH (9:1) to give compound **1** (43 mg) and oleanolic and ursolic acids (102 mg) in a mixture (2:1). The EtOH extract was diluted with H_2O and extracted with $CHCl_3$ at room temp. The $CHCl_3$ soluble portion was chromatographed on a silica gel column with a mixture of $CHCl_3$-MeOH. From the $CHCl_3$-MeOH (98:2) eluate was obtained a mixture of triterpenes and steroids. This mixture furnished the compounds sitosterol in a mixture with stigmasterol (12 mg) and ursolic acid (9 mg) by repeated column chromatography and prep. TLC.

*Canaric acid (**1**)*

Colorless crystalline powder, mp. 215-217° [lit.(8) mp. 215-217°, $[\alpha]_D^{25}$ +43° (0.5, $CHCl_3$) [lit.(8) $[\alpha]_D^{25}$ +57]; IR ν_{max} cm^{-1}: 1690, 1646, 895, 880; FABMS *m/z*: 463 $[M+Na]^+$; ^{1}H-NMR ($CDCl_3$): Table 1. ^{13}C-NMR ($CDCl_3$): Table 1.

Methylation of canaric acid **(1)**

Compound **1** was methylated with CH_2N_2, affording a methyl canarate **(1a)**. Gum, $[\alpha]_D^{25}$ +038 (0.6, $CHCl_3$); IR ν_{max} cm^{-1}: 1715, 1640, 1100, 880; FABMS m/z: 477 $[M+Na]^+$; ^{1}H-NMR ($CDCl_3$) δ: 4.76 (1H, s, H_a-24), 4. 60 (1H, s, H_a-29), 4.55 (1H, s, H_b-24), 4.50 (1H, s, H_b-29), 3.53 (3H, s, OCH_3), 2.53 (1H, br s, H-18), 0,75 (3H, s, H-26), 0.78 (3H, s, H-27), 0.98 (3H, s, H-28), 1.10 (3H, s, H-25), 1. 65 (3H, s, H-H-30), 1.63 (3H, s, H-23); ^{13}C-NMR ($CDCl_3$) δ: 178.0 s (C-3), 150. 9 s (C-20), 148.1 s (C-4), 113.5 t (C-24), 109.0 t (C-29), 51.3 q (OCH3), 50.7 d (C-9), 48.6 d (C-18), 48.0 d (C-19), 42.9 s (C-17), 42.5 s (C-14), 40.9 d (C-5), 40.5 t (C-22), 40.0 s (H-8), 39.0 s (H-10), 38.5 d (H-13), 36.0 t (H-16), 33.9 t (C-7), 32.9 t (C-1), 29.9 t (C-21), 27.5 t (C-12), 27.0 t (C-15), 24.4 t (C-6), 23.6 q (C-25), 21.7 t (C-11), 20.0 q (C-23), 19.0 q (C-30), 18.3 q (C-28), 16.1 q (C-26), 14.3 q (C-27).

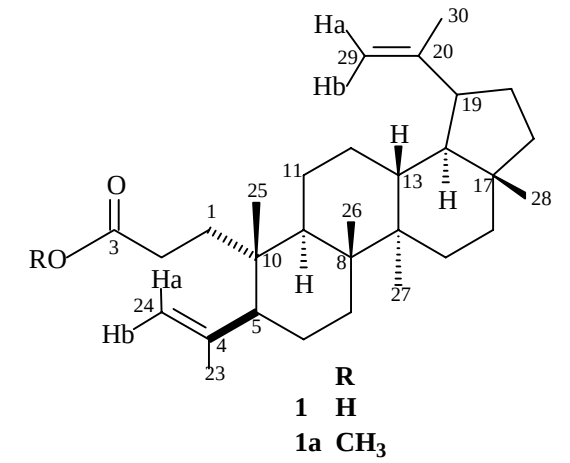
Results and Discussion

The acetone extract of the leaves of *R. jasminoides* was chromatographed on a silica gel flash column and the fractions containing the mixture of triterpenes were further purified by prep. TLC to obtain the triterpene **1**. All compounds isolated are known and were identified by spectroscopic means and comparison with authentic samples.

Compound **1** gave a positive test with Liebermann Buchard and ceric sulfate. The FABMS of **1** revealed the peak $[M+Na]^+$ at m/z 463 consistent with the same molecular formula $C_{30}H_{48}O_2$ (m/z 440 $[M]^{+\cdot}$) of canaric acid[6,7,8]. The assignments of the NMR data of **1** and **1a** were confirmed using a combination of DEPT, COSY, HMBC and NOESY experiments and comparison with data of known lupene and lupane derivatives[9-11]. The ^{13}C chemical shifts of **1** (Table 1) and **1a** (see Experimental), especially those corresponding to C-20 and C-29 at δ 150.78 and 109.50 **(1)** and 150.9 and 109.0 **(1a)**, clearly corroborate the lupane structure of the compound **1**. The combined ^{1}H and ^{13}C-NMR data, together with DEPT and HMQC experiments (Table 1) suggested the presence of six tertiary methyl groups, two of them located at sp^2 carbons; twelve methylenes, five methines and seven quaternary carbons in the molecule of **1**. The ^{1}H-NMR spectrum (Table 1) showed that **1** has six singlet methyls (δ 0.72, .0.78, 0.95, 1.08, 1.61 and 1.65; two attributable to methyl groups at sp^2 carbons δ 1.61 and 1.65) and four hydrogens typical of terminal double bonds at δ 4.50, 4.57, 4.61 and 4.77. The ^{13}C-NMR spectra suggested the presence of a carboxylic acid (δ 179.91) and two terminal double bonds (δ 150.78, 109.50 and 147.58, 113.38 respectivelly). ^{1}H and ^{13}C-NMR spec-

Table 2. NOE data for compound 1.

Irradiated H	Observed H (%)
H_b-2	H_a-1 (12.5), H_b-2 (2.0)
H-5	H_a-1 (38.0), 3H-23(11), Hα-6 (15.0), H-9 (15.2), Hα-7 (17.8)
Hα-6	H-5 (6.0), Hα-7 (10.8)
Hβ-6	H_a-24 (5.6), 3H-25(20), 3H-26 (11.2)
H-9	H-5 (1.5), H_a-1 (3), Hα-7 (25),
H-13	3H-26 (15), Hβ-11 (12.6), Hβ-15 (25), 3H-28 (16)
H-18	3H-27 (30), Hα-12 (2.8), Hα-16 (1.5)
3H-23	H_a-1 (6.0), H-5 (12.5), H_b-24 (15)
Ha-24	3H-25 (22), Hβ-6 (3.0)
3H-25	3H-26 (5.5), H-24 (10.1), Hβ-11, H_a-1 (7.8)
3H-26	3H-25 (5), H-13 (8.8), Hβ-11 (31)
Ha-29	3H-30 (21), H-18 (1.9)



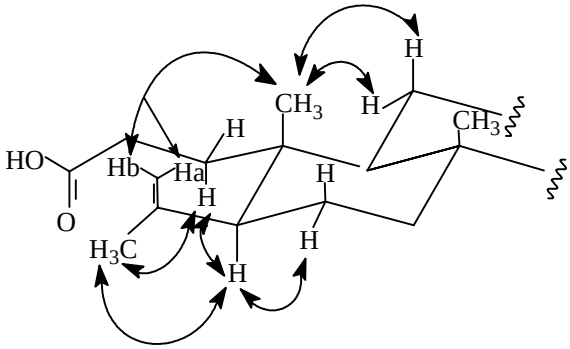


Figure 1. Selected NOESY correlations for 1.

tra of the triterpene **1** resembled very closely those of lupeol[9] and lupene[10]. Prominent differences in the ^{1}H and ^{13}C values between lupeol and **1** were only observed in the A-ring region; the value of δ 78.8 attributed to C-3 in lupeol[9,11] was inexistent in the molecule of **1**. However, the signal at δ 179.91 could be attributable to C-3, if we consider that the modification in the A-ring of the compound in discussion is related to the breaking of the C-3/C-4 bond (*seco*-lupane-A ring). These results allowed us to deduce that compound **1** possesses the same structure as canaric acid, previously described in the literature[6,7].

Interpretation of ^{1}H-^{1}H COSY, HMQC, HMBC, NOE and NOESY data for **1** led to the unambiguous elucidation of the structure. The relative stereochemistry of **1** was assigned on the basis of coupling constants (Table 1) and NOESY data (Fig. 1). The α-axial orientation of H-5 was confirmed by a strong NOE between H-5 and the hydrogens assigned as H-1, H-6α, H-9α, H-7α and 3H-23, and the lack of an NOE between it and the olefinic hydrogens assigned as H-24. Also consistent with the axial orientation of H-5 was a strong correlation observed with the axial methyl hydrogens 3H-25 and olefinic hydrogens H-24. Strong NOE correlations between 3H-25 and H-11β, between 3H-26 and H-7β and H-13β, between 3H-27 and H-12α, H-16α and H-18α, between 3H-28 and H-19β were consistent with *trans* B/C/D ring junctions with *seco*-ring A in a diequatorial conformation and ring D in a half boat conformation. Other correlations observed in the NOESY and NOE difference spectra are depicted in Table 2 and with the aid of COSY spectra and coupling constant values the relative stereochemistry of the *seco*-lupane triterpene **1** was established and shown to be identical to dihydrocanaric acid isolated from *Hoya naumanii*[12].

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