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Short Report

Chemical Constituents of *Cordia piauhiensis* – Boraginaceae

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Uma nova saponina triterpênica monodesmosídica, caracterizada como ácido 3β-*O*-α-L-ramnopiranosil-(1→2)-β-D-glicopiranosil pomólico, foi isolada de *Cordia piauhiensis* Fresen (Boraginaceae). Sua estrutura foi determinada através de extensiva análise de métodos de RMN, incluindo os experimentos ^{1}H, ^{1}H-COSY, HMQC, HMBC e NOESY. Os triterpenóides ácido quinóvico, ácido cinchólico, ácido 3β-*O*-6-deoxi-β-D-glicopiranosídeo cinchólico e 3β-*O*-β-D-glicopiranosídeo quinóvico foram também isolados.

A new monodesmoside triterpenoid saponin characterized as 3β-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl pomolic acid, was isolated from *Cordia piauhiensis* Fresen (Boraginaceae). Its structure was determined by extensive NMR analysis, including ^{1}H, ^{1}H-COSY, HMQC, HMBC, and NOESY experiments. In addition, four known triterpenoids: quinovic acid, cincholic acid, cincholic 3β-*O*-6-deoxy-β-D-glucopyranoside acid and quinovic 3β-*O*-β-D-glucopyranoside acid were also isolated.

Keywords: *Cordia piauhiensis*, Boraginaceae, triterpenes, saponins

Introduction

The genus *Cordia* (Boraginaceae), a known source of benzoquinones,[1] naphthoquinones,[2] hydroquinones, cromenes,[3] triterpenes,[4] sesquiterpenes,[5] polyphenols,[6] and flavonoids[7] comprises about 250 species distributed throughout the New World.[8] Many compounds originally isolated from *Cordia* species have been reported as presenting several biological activities such as antifungal, larvicidal, anti-inflammatory and anti-androgenic.[2,4,6,7]

As part of our current interest in *Cordia* species from Northeastern Brazil flora we have investigated *C. piauhiensis* Fresen (syn.: *Cordia rufescens* A. DC.), an endemic shrub distributed in the South, Southeast and Northeast regions of Brazil.[8] In a previous paper we reported the isolation and characterization of a triterpenoid bidesmoside saponin from the stems of this plant.[9] Continuing with our phytochemical research we now report the isolation and structural elucidation of 3β-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl pomolic acid (**1**), a novel monodesmoside saponin, along with four triterpenoids and other known compounds.

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Results and Discussion

Compound **1** was isolated as an amorphous solid, mp 218-220 °C. The ESIMS (negative ion mode) showed a molecular ion peak at *m/z* 779 ([M]$^-$), consistent with a molecular formula $C_{42}H_{68}O_{12}$, which was confirmed by its ^{1}H and ^{13}C NMR spectral data (Tables 1). Its IR spectrum showed strong absorption bands at ν_{max} 3428 (hydroxyl) and 1695 cm^{-1} (carboxyl). The ^{13}C NMR BB and DEPT spectra of **1** (Table 1) indicated 42 carbon atoms, related to 8 methyls, 10 methylenes, 16 methines and 8 non-hydrogenated carbons. Besides the 30 carbons assigned to the aglycone, 12 carbons were assigned to two sugar units. The ^{13}C NMR spectra demonstrated the presence of

Table 1. ^{1}H (300 MHz) and ^{13}C NMR (75 MHz) assignments for the aglycone moiety of **1** by DEPT, HMQC and HMBC

Atom	δ_C	δ_H	$^2J_{CH}$	$^3J_{CH}$
1	40.2	1.05		3H-25
2	26.8	1.85		
3	90.5	3.18 (dd, 12.3, 4.3)		H-1', 3H-23, 3H-24
4	40.4	-	3H-23, 3H-24	
5	57.5	0.83		3H-23, 3H-24, 3H-25
6	19.6	1.97		
7	34.4	1.23		3H-26
8	41.2	-	3H-26	3H-27
9	48.7	1.67	3H-25, 3H-26	
10	38.0	-	3H-25	
11	24.8	1.98		
12	129.6	5.28 (t)		
13	140.1	-	H-18	
14	42.7	-	3H-27	3H-26
15	29.8	1.03		3H-27
16	27.3	1.65		
17	49.2	-	H-18	
18	55.2	2.52 (br s)		3H-29
19	73.9	-	3H-29	3H-30
20	43.2	1.37	3H-30	3H-29
21	27.4	1.70		
22	39.1	1.72		
23	28.7	1.06 (s)		3H-24
24	17.3	0.86 (s)		3H-23
25	16.2	0.95 (s)		
26	17.7	0.79 (s)		
27	25.0	1.33 (s)		
28	182.6	-		
29	27.3	1.19 (s)		
30	16.8	0.93 (d, 6.8)		

Experiments in CD_3OD; Chemical shifts (δ) in ppm; Coupling constants (J) in Hz.

a trisubstituted double bond (δ 129.6 and 140.1), a carboxylic acid group (δ 182.6), an oxymethine carbon (δ 90.5), an oxygenated non-hydrogenated carbon (δ 73.9) and seven methyl groups between δ 16.2 and 28.7, consistent with an aglycone, which was established to be the 3β,19α-dihydroxyurs-12-en-28-oic acid, also known as pomolic acid, after appropriate NMR and mass spectrometry (negative ion peak at *m/z* 471) analysis, and comparison with literature data.[10] The two sugar units were evidenced by two anomeric carbon signals at δ 105.7 and 102.0 correlated with the proton signals at δ 4.41 (d, J 7.2 Hz) and 5.36 (d, J 1.3 Hz), respectively, in the HMQC spectrum. To determine the identity of each monosaccharide interglycoside linkage, as well as to establish the linkage of the disaccharide chain to the aglycone, ^{1}H and ^{13}C NMR assignments were unambiguously made by a combination of several 2D experiments such as ^{1}H, ^{1}H-COSY, HMQC, HMBC and NOESY. In the ^{1}H NMR spectrum of **1** the presence of a glucose unit was supported by the anomeric proton signal at δ 4.41 and two double doublets for the oxymethylene protons at δ 3.66 and 3.83. Similarly, the presence of a rhamnose unit was readily supported by the characteristic methyl doublet at δ 1.21 and by the anomeric proton signal at δ 5.36.[11] The anomeric configurations for the sugar moieties were defined as β for the glucose and α for the rhamnose from their coupling constants of 7.2 and 1.3 Hz, respectively and by comparison with data from literature.[11] The linkage between the two sugar units was established from the HMBC correlation between the signals of the anomeric proton of the rhamnose at δ 5.36 and the carbon signal of the glucose at δ 79.1 (C-2). The attachment of the disaccharide chain was particularly determined based on the relevant correlation between C-1' (δ 105.7) and H-3 (δ 3.18) in the HMBC spectrum, and by NOE correlation between H-1'(δ 4.41) and H-3 (δ 3.18), observed in the NOESY experiment as shows in Figure 1. This also was confirmed by the conspicuous deshielding of C-3 (δ 90.5) as well as by analogy with the NMR data of the known saponins.[9,12] Thus, the structure of **1** was determined as the 3β-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl pomolic acid.

The known triterpenoids were identified by spectroscopic analysis and comparison with published data

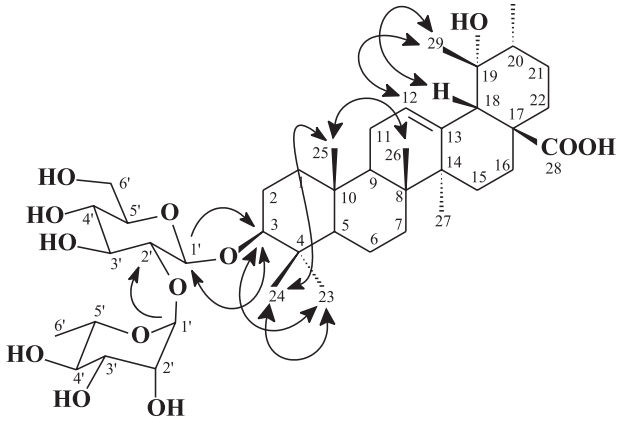
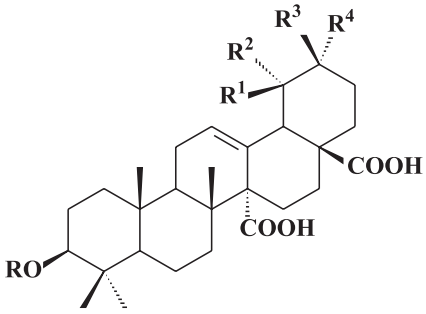


Figure 1. Selected HMBC (single arrows, →) and NOESY (double arrows, ↔) correlations for **1**.

to be quinovic acid (**3**) and cincholic acid (**4**),[13] obtained as a 3:1 mixture, respectively, cincholic 3*β*-*O*-6-deoxy-*β*-D-glucopyranoside acid (**5**)[13] and quinovic 3*β*-*O*-*β*-D-gluycopyranoside acid (**6**).[14] Mannitol[15] (**2**) and *β*-sitosterol-*β*-D-glucoside (**7**)[16] were also isolated.



3 R = R^2 = R^3 = H, R^1 = R^4 = Me

4 R = R^1 = R^2 = H, R^3 = R^4 = Me

5 R = 6-deoxy-β-D-glucosyl, R^2 = R^3 = H, R^3 = R^4 = Me

6 R = β-D-glucosyl, R^2 = R^3 = H, R^1 = R^4 = Me

Experimental

General experimental procedures

Melting points were determined using a digital Mettler Toledo FP90 apparatus. The optical rotations were measured on a Perkin-Elmer 341 digital polarimeter. IR spectra (KBr pellets) were recorded using a Perkin-Elmer FT-IR 1000 spectrometer. ESIMS were measured on a Micromass Quatro LC instrument. NMR spectra were recorded on a Bruker Avance DRX-500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) or DPX-300 (300 MHz for ^{1}H and 75 MHz for ^{13}C) spectrometers using pyridine-d_5, CD_3OD or D_2O as solvents. Chemical shifts, given on the δ scale, were referenced to internal DSS for D_2O solution, and to the residual undeuterated portion of the deuterated organic solvent, for proton (pyridine, δ_H 8.74, 7.58, 7.22; CD_3OD, δ_H 2.31), and the center peak of the deuterated solvent (pyridine, δ_C 150.35, 135.91, 123.87; CD_3OD, δ_C 49.15). Column chromatography was run using silica gel 60 (70 - 230 mesh, Vetec) and Sephadex LH-20 (Pharmacia). TLC was performed on precoated silica gel poliester sheets (kieselgel 60 F_{254}, 0.20 mm, Merck). Saponins were detected by spraying with vanillin/perchloric acid/EtOH solution followed by heating at 120 °C, while the sugar was detected by spraying the orcinol reagent.

Plant material

Cordia piauhiensis was collected in August 1999, from Barreiro Grande – Crato County, State of Ceará, and identified by Prof. Edson Paula Nunes. A herborized specimen (# 29.104) has been stored at the Herbario Prisco Bezerra (EAC) of the Departamento de Biologia, Universidade Federal do Ceará.

Extraction and isolation

Air-dried and powdered roots (2.8 Kg) and stems (1.7 Kg) were individually extracted exhaustively with EtOH at room temperature. After evaporation of the solvents under reduced pressure the crude extracts were obtained. Upon concentration of the EtOH extract from roots a precipitate was obtained, which was filtered and washed successively with acetone and EtOH to yield **2** (15 g). The remaining EtOH liquors were evaporated (198 g) and coarsely fractionated over silica gel by elution with n-hexane, $CHCl_3$ followed by EtOAc and finally MeOH. The

Table 2. ^{1}H (300 MHz) and ^{13}C NMR (75 MHz) assignments for the sugar moieties of **1** by DEPT, HMQC and HMBC

Atom	δ_C	δ_H	Atom	δ_C	δ_H
Glc 1'	105.7	4.41 (d, 7.2)	Rha 1"	102.0	5.36 (d, 1.3)
2'	79.1	3.41 (t, 7.3)	2"	73.8	3.96 (br s)
3'	79.6	3.47 (t, 8.8)	3"	72.3	3.75 (dd, 9.5, 3.3)
4'	72.1	3.30	4"	74.1	3.39 (t, 9.5)
5'	77.7	3.20 (m)	5"	70.1	3.99 (m)
6'	62.9	3.83 (d, 11.6), 3.66 (dd, 11.6, 5.1)	6"	18.1	1.21 (d, 6.2)

Experiments in CD_3OD; Chemical shifts (d) in ppm; Coupling constants (*J*) in Hz.

EtOAc fraction (27 g) was subjected to silica gel CC eluting with n-hexane-EtOAc (8:2, 6:4, 4:6, 2:8 and 0:10) followed by EtOAc-MeOH gradients (8:2, 5:5 and 0:10). A precipitate was obtained from a n-hexane-EtOAc (4:6) subfraction, which was filtered and recrystallized from MeOH to yield a binary mixture of **3** and **4** (102 mg). The EtOAc subfraction was further chromatographed over silica gel to give **5** (95 mg) and **6** (450 mg) by elution with n-hexane-EtOAc (1:9) and EtOAc, respectively. Similarly, the EtOH extract of the stems (103 g) was fractionated over silica using $CHCl_3$, EtOAc, acetone and MeOH as eluent. The EtOAc fraction (8.1 g) was chromatographed over silica gel eluting with CH_2Cl_2, $CHCl_3$-EtOAc gradients (8:2, 5:5, 3:7 and 0:10) and MeOH. The subfraction $CHCl_3$ yielded a precipitate which was purified by recrystallization in MeOH to yield **3** (21 mg). Likewise compound **7** (32 mg) was isolated from subfraction $CHCl_3$-EtOAc (3:7). The acetone fraction (39.7 g) was dissolved in H_2O-MeOH (8:2) and partitioned with EtOAc and *n*-BuOH. The EtOAc fraction (10.9 g) was subjected to silica gel CC eluting with EtOAc-MeOH (3:7, 5:5 and 0:10). Subfraction EtOAc-MeOH (3:7, 590 mg) was chromatographed twice over Sephadex LH-20 eluting with MeOH to afford **1** (43 mg).

*3β-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl pomolic acid (**1**)*

White amorphous solid; mp 218-220 °C; $[\alpha]_D^{20}$ - 0.44° (*c* 0.05, MeOH); IR (KBr) ν_{max}/cm^{-1} : 3428, 2932, 2883, 1695, 1653, 1457, 1131, 1050; ^1H (300 MHz) and ^{13}C NMR (75 MHz) data, see Table 1.

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