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Note

A new 5-deoxyflavone glycoside from the aerial parts of *Calea clausseniana*

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Abstract—A new 5-deoxyflavone glycoside, identified as 7-*O*-(α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl)-3′,4′,7-trihydroxyflavone (**1**), was isolated from the aerial parts of *Calea clausseniana*. Its structure was determined by spectral analysis.

Keywords: Calea clausseniana; Asteraceae; 5-Deoxyflavone glycoside; NMR analysis

Calea clausseniana Baker (Asteraceae), one of the 110 species of the genus *Calea*, occurs mainly in Mexico, Central and South America.[1] In continuation of the phytochemical investigation of the genus *Calea*, we now report the isolation of a new 5-deoxyflavone glycoside from the ethanol extract of the aerial parts of the plant, which has been characterized as 7-*O*-(α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl)-3′,4′,7-trihydroxyflavone (**1**) together with known 3-*O*-(α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranosyl)-quercetin.[2,3] These compounds showed no in vitro trypanocidal activity against the trypomastigote forms of *Trypanosoma cruzi*.

Fractionation of the ethanol extract from the dried aerial parts of *C. clausseniana* by Sephadex LH-20, polyvinylpyrrolidone (PVP) and HPLC yielded the 5-deoxyflavone glycoside (**1**) and the flavonol glycoside, quercetin-3-*O*-α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranoside. The ^{1}H and ^{13}C NMR data (Table 1) indicated that **1** was a flavonoid with an unusual substitution pattern. This preliminary observation was confirmed by extensive analysis of both one- and two-dimensional ^{1}H and ^{13}C NMR spectra. The aromatic region of the ^{1}H NMR spectrum of **1** contained three signals at δ 6.89 (dd, $J = 8.3$ and 2.0 Hz, δ_C 113.5 by HMQC), 7.10 (d, $J = 2.0$ Hz, δ_C 99.4), and 7.72 (d, $J = 8.3$ Hz, δ_C 125.8), suggesting the presence of a monosubstituted ring A and three signals at δ 6.84 (d, $J = 8.6$ Hz, δ_C 116.4), 7.30 (dd, $J = 8.6$ and 2.0 Hz, δ_C 125.1) and 7.44 (d, $J = 2.0$ Hz, δ_C 118.5), consistent with the signals of a 3′,4′-disubstituted ring B. On the basis of the ^{1}H–^{1}H COSY spectrum, these proton signals were assigned in rings A and B, respectively. Likewise, a final signal resonance in the aromatic region at δ 6.72 p (1H, s, δ_C 113.4) was assigned to H-3, typical of a flavone. Thus, the aglycone component of **1** was confirmed to be a 3′,4′,7-trihydroxyflavone.

The ^{1}H NMR spectrum of **1** revealed the presence of two one-proton doublets at δ 5.33 ($J = 7.0$ Hz) and 5.14 ($J = 1.5$ Hz), representative of two anomeric protons, together with a methyl doublet δ 1.20 ($J = 6.0$ Hz), that in the HMBC spectrum were correlated with carbon signals at δ 98.0, 110.8 and 18.4. The other sugar signals were overlapped in the region between δ 3.00 and 3.80.

The β-configuration for the D-glucopyranosyl unit was deduced from ^{1}H NMR data; the corresponding $J_{H1''-H2''}$ coupling constant of 7.0 Hz was characteristic of a β-linkage. Taking into account the magnitude of the coupling constant ($^{1}J_{C\text{-}1'''-H\text{-}1'''}$ 173.6 Hz) in the native molecule, it was inferred that the rhamnosyl residue had a α-anomeric configuration.[4]

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Table 1. ^{1}H and ^{13}C NMR data of **1** in DMSO-d_6[a]

Position	δ_H (mult, J in Hz)	δ_C	HMBC
2		145.7	H-3
3	6.72 s	113.4	
4		181.8	H-5
5	7.72 d (8.3)	125.8	
6	6.89 dd (2.0; 8.3)	113.5	
7		164.5	H-5 and H-1″
8	7.10 d (2.0)	99.4	
9		167.3	H-5
10		115.8	
1′		123.5	H-3
2′	7.44 d (2.0)	118.5	
3′		145.9	
4′		148.8	
5′	6.84 d (8.6)	116.4	
6′	7.30 dd (2.0; 8.6)	125.1	
1″	5.33 d (7.0)	98.0	
2″		76.6	H-1‴
3″		77.3	
4″		69.9	
5″		77.5	
6″		60.8	
1‴	5.14 d (1.5)	100.8	
2‴		70.8	
3‴		70.7	
4‴		72.2	
5‴		68.7	
6‴	1.20 d (6.0)	18.4	

[a] Assignments were confirmed by COSY, HMQC and HMBC.

HMBC correlations were observed between H-1 (δ 5.33) of the glucose and carbon resonance at δ 164.5 (C-7). These data revealed the linkage between glucose and C-7 of the aglycone. The position of the inter-glycosidic linkage was confirmed by observing the correlations between H-1 (δ 5.14) of the rhamnose and the carbon resonance C-2 of glucose at δ 76.6, in the HMBC spectrum. These data were also compared with ^{13}C NMR spectral data of the 7-*O*-(β-D-glucopyranosyl)-3′,4′,7′-trihydroxyflavone.[5] The C-2 glucose carbon signal in compound **1** was observed at δ 76.6, shifted upfield by δ 3.1 ppm due to glucosylation, of this position by L-rhamnopyranose, while that of 7-*O*-(β-D-glucopyranosyl)-3′,4′,7′-trihydroxyflavone appeared at δ 73.5 (both in DMSO-d_6).

Based upon all of the above evidence, the structure of **1** was elucidated as 7-*O*-(α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl)-3′,4′,7-trihydroxyflavone (Fig. 1).

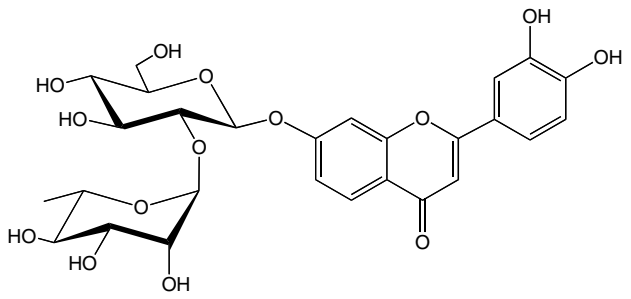

Figure 1. Structure of compound **1**.

The structure of the other compound isolated, 3-*O*-(α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranosyl)-quercetin, was established by comparison of its spectral data with literature values.[6]

1. Experimental

1.1. General methods

^{1}H (400 MHz) and ^{13}C and DEPT 135 NMR (100 MHz) spectra were recorded on a Bruker DRX 400 spectrometer in DMSO-d_6 using TMS as an internal standard. Chemical shifts are reported in δ (ppm) and coupling constants (J values) are in Hz. 2D NMR experiments (^{1}H–^{1}H COSY, ^{13}C–^{1}H HMQC and ^{13}C–^{1}H HMBC) were performed using a Bruker DRX 500 spectrometer. HPLC separation was performed with a Shimadzu LC-6A system equipped with a Model SPD-6AV UV–vis detector. The IR spectrum was recorded on a Nicolet Protégé 460 spectrophotometer. The UV spectrum was obtained on a Hitachi U-3501 spectrophotometer.

1.2. Plant material

The aerial parts of this plant were collected in November 1997, in Minas Gerais, BR-050, Km 131, Brazil, and were identified by Professor Edward E. Schilling and Professor Jimi N. Nakajima, Department of Botany, University of Tennessee and Department of Biology, Universidade Federal de Uberlândia-MG, respectively. A voucher specimen (SPFR 04702) was deposited in the Herbarium of the Department of Biology, FFCLRP/USP, Ribeirão Preto, Brazil.

1.3. Extraction and isolation

Dried and powdered aerial parts of *C. clausseniana* (333 g) were exhaustively extracted with dichloromethane and ethanol at rt to give 5.0 and 17.7 g of crude extracts, respectively. A portion of the ethanol extract (2.0 g) was purified by chromatography on a Sephadex LH-20 column using MeOH as eluent to give 40 fractions. Fractions 17-21 (144 mg) were again purified by chromatography on polyvinylpyrrolidone (PVP) to yield A (46 mg), which was submitted to HPLC (ODS column 20 × 250 mm, MeOH–water, 4:6, flow rate 9 mL × min^{-1}) to give 6 mg of 3-*O*-(α-L-rhamnopyranosyl-(1→6)-β-D-galactopyranosyl)-quercetin and 7 mg of 7-*O*-(α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl)-3′,4′,7-trihydroxyflavone (**1**). The dichloromethane extract was purified by chromatography on a column packed with silica gel 60 H (Merck). Elution with *n*-hexane, *n*-hexane/ethyl acetate (crescent gradient), ethyl acetate/MeOH (crescent gradient) and MeOH afforded a mixture of β-sitosteryl and stigmasteryl glucopyranosides (30 mg).

1.4. 7-*O*-(α-L-Rhamnopyranosyl-(1→2)-β-D-glucopyranosyl)-3′,4′,7-trihydroxyflavone (1)

Brown gum; UV (MeOH): $\lambda_{max} = 255$, 274, 330, 401 nm; IR (MeOH film): $\nu_{max} = 3378$, 2922, 1608, 1521, 1493, 1446, 1381, 1273, 1131, 1071, 814 cm^{-1}; ^{1}H and ^{13}C NMR (400 and 100 MHz), see Table 1.

1.5. Trypanocidal activity[3]

The two flavonoids were evaluated for their in vitro trypanocidal activity against the trypomastigote forms of *Trypanosoma cruzi* by using infected blood without any addition, infected blood containing DMSO in equivalent amounts as the sample, and infected blood containing gentian violet at a concentration of 250 μg/mL were used as negative and positive controls, respectively. The bioassays were performed in triplicate. Neither compounds displayed trypanocidal effects against the trypomastigote forms of *Trypanosoma cruzi*.

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