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4-ARYLCOUMARIN GLYCOSIDES FROM *COUTAREA HEXANDRA**

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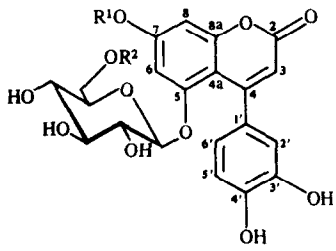
Key Word Index—*Coutarea hexandra*; Rubiaceae; 4-arylcoumarin glycosides; ^{1}H and ^{13}C NMR, FABMS.

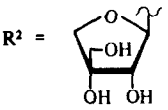
Abstract—The caulis of *Coutarea hexandra* was found to contain four new 4-arylcoumarin glycosides: 5-*O*-(β-D-glucopyranosyl)-7,3′,4′-trihydroxy-4-phenylcoumarin; 5-*O*-(β-D-glucopyranosyl)-7-methoxy-3′,4′-dihydroxy-4-phenylcoumarin; 5-*O*-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl]-7,3′,4′-trihydroxy-4-phenylcoumarin and 5-*O*-[β-D-xylopyranosyl-(1→6)-β-D-glucopyranosyl]-7-methoxy-3′,4′-dihydroxy-4-phenylcoumarin. The structures were assigned by chemical and spectroscopic methods.

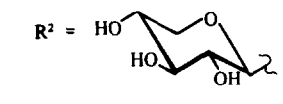
INTRODUCTION

Coutarea hexandra (Jacq.) Schum is widespread in north-eastern Brazil and used in folk-medicine [1–3]. Recently 4-arylcoumarins have been isolated from the benzene extract of caulis of *C. hexandra* [1, 2]. From the stem barks of the same plant, Reher *et al.* [3] have isolated, after acid hydrolysis of a methanolic extract, 7-methoxy-5,2′,5′-trihydroxy-4-phenylcoumarin (**5**). In continuation of our investigation of the active metabolites from the Rubiaceae [4, 5] we describe here the isolation and identification of four new 4-arylcoumarin glycosides (**1–4**) from the caulis of *C. hexandra*.



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





RESULTS AND DISCUSSION

Compounds **1** and **2**, on acid methanolysis, liberated methyl glucoside. The FABMS spectrum of **1**, in negative ion mode, showed a quasi-molecular anion at *m/z* 447 $[M-H]^-$ and a major fragment at *m/z* 285 $[M-H-162]^-$ for the cleavage of a glucose unit. In the FABMS spectrum of **2**, the quasi-molecular anion at *m/z* 461 and a fragment at 299 $[M-H-162]^-$ were observed, which were 14 mass units greater than those of **1** and suggested the presence of an extra methyl group in **2**.

The molecular formulae $C_{21}H_{20}O_{11}$ for **1** and $C_{22}H_{22}O_{11}$ for **2** were deduced by FABMS and DEPT ^{13}C NMR (Table 1). For **1** the ^{1}H NMR spectrum (CD_3OD, Table 2) showed a doublet at δ 4.80 (J = 7.5 Hz) ascribable to an anomeric hydrogen of a β-D-glucopyranose linked to an aromatic ring with an *O*-glycosidic bond. The presence of β-D-glucose in **1** and **2** was confirmed by ^{1}H selective decoupling experiments: irradiating the signal at δ 4.80 in **1** and the H_2O signal at δ 4.90 in **2** (which covered the anomeric hydrogen of glucose), taking away the H-2′ signal at δ 2.62 (*dd*, $J_1 = J_2$ = 7.5 Hz). Also the ^{13}C NMR glucose signals for **1** and **2** were in agreement with literature data (Table 1) [6]. The ^{13}C NMR spectrum of **1**, apart from the sugar signals, exhibited 15 signals, due to a 4-phenylcoumarin skeleton, sorted by ^{13}C and DEPT ^{13}C NMR into six non-oxygenated CH groups (C-3, C-6, C-8, C-2′, C-5′ and C-6′) and nine quaternary carbons, six of which are oxygenated (C-2, C-8a, C-5, C-7, C-3′ and C-4′) (7). The ^{1}H NMR spectrum of **1** in the aromatic region exhibited two signals at δ 6.51 (H-6) and 6.43 (H-8) (each 1H, *d*, J = 2 Hz) indicating the presence of two *meta*-coupled protons on the coumarin ring; a characteristic signal at δ 5.83 (H-3) ascribable to a proton of α-pyrone ring system [1]; and signals at δ 6.80 (H-2′, *d*, J = 2 Hz), 6.79 (H-5′, *d*, J = 7.5 Hz) and 6.69 (H-6′, *dd*, J = 2 and 7.5 Hz) attributable to a 3H ABX system on the 4-phenyl residue.

The ^{1}H NMR spectrum of **2** compared with that of **1** showed small downfield shifts for H-3, H-6 and H-8 (Table 2) and a methoxy signal (δ 3.85, 3H, *s*) which was confirmed also by the ^{13}C NMR signal at 56.5 ppm. In the ^{13}C NMR spectrum of **2** the C-6 and C-8 signals were displaced upfield by 2.8 and 1.1 ppm, respectively, and C-7 was deshielded by 2 ppm, showing the location of methoxy group at C-7 (Table 2).

The substitution pattern of **1** and **2** was confirmed by NOEDS (NOE difference spectra) experiments: in **1** ir-

*Part 10 in the series 'Plant Metabolites', for parts 8 and 9 see refs [4, 5].

Table 1. ^{13}C NMR spectra of compounds **1–4** (in CD_3OD).

Aglycon carbons	DEPT	1	2	3	4
2	C	163.6	163.0	163.5	162.9
3	CH	112.3	113.2	112.3	113.3
4	C	158.3	158.0	158.3	158.1
4a	C	104.5	105.5	105.1	105.8
5	C	158.1	158.0	158.1	158.1
6	CH	98.1	96.7	98.7	96.6
7	C	163.2	165.0	163.3	165.0
8	CH	101.1	100.0	101.1	100.6
8a	C	157.3	157.2	157.3	157.1
1′	C	133.1	132.9	133.2	133.0
2′	CH	117.1	117.0	116.9	117.0
3′	C	145.5	145.5	145.5	145.5
4′	C	146.9	147.0	146.9	147.0
5′	CH	115.9	115.9	115.9	115.9
6′	CH	120.5	120.6	120.7	120.7
–OMe	Me	—	56.5	—	56.6
Sugar carbons*					
G-1″	CH	101.4	101.5	101.6	101.6
G-2″	CH	74.6	74.6	74.6	74.6
G-3″	CH	78.2	78.3	78.1	77.7
G-4″	CH	71.1	71.2	71.4	71.1
G-5″	CH	77.7	77.7	77.7	77.7
G-6″	CH_2	62.5	62.5	68.6	70.3
A-1‴	CH	—	—	111.0	—
A-2‴	CH	—	—	77.2	—
A-3‴	C	—	—	80.5	—
A-4‴	CH_2	—	—	75.1	—
A-5‴	CH_2	—	—	66.0	—
X-1‴	CH	—	—	—	105.5
X-2‴	CH	—	—	—	74.9
X-3‴	CH	—	—	—	77.3
X-4‴	CH	—	—	—	71.4
X-5‴	CH_2	—	—	—	66.8

*A = apiose, G = glucose, X = xylose.

Table 2. ^1H NMR data for compounds **1–4** (CD_3OD)

Proton	1	2	3	4
H-3 (1H, *s*)	5.83	5.90	5.84	5.91
H-6 (1H, *d*, J = 2 Hz)	6.51	6.65	6.50	6.70
H-8 (1H, *d*, J = 2 Hz)	6.43	6.60	6.45	6.65
H-2′ (1H, *d*, J = 2 Hz)	6.80	6.81	6.80	6.80
H-5′ (1H, *d*, J = 7.5 Hz)	6.79	6.81	6.80	6.80
H-6′ (1H, *dd*, J = 2 and 7.5 Hz)	6.69	6.70	6.69	6.71
–OMe (3H, *s*)	—	3.85	—	3.88
G-1″ (1H, *d*, J = 7.5 Hz)	4.80	*	4.72	4.80
G-2″ (1H, *dd*, $J_1 = J_2$ = 7.5 Hz)	2.62	2.62	2.62	2.62
A-1‴ (1H, *d*, J = 2 Hz)	—	—	4.93	—
X-1‴ (1H, *d*, J = 7.5 Hz)	—	—	—	4.25
A-2″′(1H, *d*, J = 2 Hz)	—	—	3.90	—

*The anomeric signal of glucose is submerged by H_2O signal.

radiation at δ 5.83 (H-3) led to the enhancement of the H-2′ (δ 6.80) and H-6′ (δ 6.69) signals indicating a spatial relationship for these protons. These results rule out the presence of a hydroxyl group at C-2′ and confirmed the location of hydroxyl groups at both C-3′ and C-4′. When the anomeric hydrogen signal of glucose (δ 4.80) at C-5 was saturated a NOE was registered for H-6 (δ6.51) while no effect was observed for H-8 resonance (δ6.43). The NOE difference experiments in the spectrum of **2** gave similar results and in addition when the signal for a methoxyl (δ3.85) was saturated, a NOE was registered for both H-6 and H-8.

The location of a glucosyl unit at C-5 in **1** and **2** was confirmed also by the upfield shift of C-2′ (δ2.62, against the usually observed value at δ 3.20–3.40 in CD_3OD) [8]. This shielding effect can be attributed to the spatial orientation of 4-phenyl residue perpendicular to the plane of the coumarin nucleus. Further evidence for the glycosidation at C-5 was derived from the ^{13}C NMR shifts (spectra run in DMSO-d_6–$CDCl_3$ 7:3) of C-5 (−1.0 ppm), C-4a (+3.2 ppm), C-6 (+4.5 ppm) and C-8 (+1.8 ppm) of **2** with respect to the aglycone (**2a**) obtained from acid hydrolysis of **2**.

From these data the structures 5-*O*-(β-D-glucopyranosyl)-7,3′,4′-trihydroxy-4-phenylcoumarin and 5-*O*-(β-D-glucopyranosyl)-7-methoxy-3′,4′-dihydroxy-4-phenylcoumarin were attributed to **1** and **2** respectively.

The ^1H NMR spectrum (Me_2CO-d_6) and the ^{13}C NMR spectrum (DMSO-d_6–$CDCl_3$ 7:3) (see Experimental) of **2a** were superimposable with spectral data reported from Reher [3] for 7-methoxy-5,2′,5′-trihydroxy-4-phenylcoumarin (**5**). In our opinion **5** and **2a** are the same compounds: in fact the results of NOE experiments and the methylenation of **2a** that afforded a C-3′,4′-methylenedioxy derivative **2a** that afforded a C-3′,4′-methylenedioxy derivative **2b**, clearly showed that the hydroxyl groups cannot be at C-2′ and C-5′.

On acid methanolysis, **3** liberated methylapioside and methylglucoside and **4** gave methylxyloside and methylglucoside both in the ratio 1:1. The FAB MS spectrum of **3** showed a quasi-molecular anion at *m/z* 579 $[(M-H)]^-$, a fragment at *m/z* 447 $[(M-H)-132]^-$ indicating the loss of apiosyl unit and a peak at 285 [447 − 162] (aglycone mass) showing the subsequent loss of a glucosyl unit. The FABMS fragmentation pattern of **4** showed peaks at *m/z* 593, 461 and 299, which were shifted 14 mass units relative to **3**.

The molecular formulae $C_{26}H_{28}O_{15}$ for **3** and $C_{27}H_{30}O_{15}$ for **4** were deduced by DEPT ^{13}C NMR and FABMS. The ^1H and ^{13}C NMR aglycone signals of **3** and **4** present small or no modifications with respect to **1** and **2**, respectively. In **3** the presence of a β-D-apiose was deduced by the anomeric hydrogen signal at δ 4.93 (H-1″, *d*, *J* = 2 Hz) and by ^1H selective decoupling experiments: irradiation at δ 4.93 led to the decoupling of H-2″ (δ 3.90, *d*, *J* = 2 Hz). The multiplicity of H-2″ derives from the presence of a quaternary carbon at C-3″, characteristic of apiose structure. The DEPT ^{13}C NMR spectrum of **3** gave 11 carbon signals for the sugar moiety of which three are CH_2 ascribable to C-4″ (75.1 ppm) and C-5″ (66.0 ppm) of apiose and to C-6′ (68.6 ppm of glucose). C-6′ is shifted downfield (β-effect) by 6.1 ppm with respect to C-6′ in **1** and **2** demonstrating the (1→6) linkage between glucose and apiose.

The anomeric carbon signals in **3** were at 111.0 ppm and 101.6 ppm (the latter ascribable to a β-D-glucopyranoside linked to the aglycone as in **1** and **2**, the former to a β-D-apiofuranoside); the other ^{13}C NMR signals for the sugar moiety matched well with a terminal apiose linked to an inner glucose [9].

In **4** the anomeric hydrogen and carbon signals (^1H NMR: δ 4.25, *d*, *J* = 7.5 Hz, H-1″; δ 4.80, *d*, *J* = 7.5 Hz, H-1′; ^{13}C NMR 105.5 ppm, C-1″ and 101.6 ppm, C-1′) and the other ^{13}C NMR sugar signals were in agreement with a β-D-xylopyranosyl unit [6] linked at C-6′ of a β-D-glucopyranosyl unit. From these data the structures 5-*O*-[β-D-apiofuranosyl-(1→6)-β-D-glucopyranosyl)]-7,3′,4′-trihydroxy-4-phenylcoumarin and 5-*O*-[β-D-xylopyranosyl-(1→6)-β-D-glucopyranosyl)]-7-methoxy-3′,4′-dihydroxy-4-phenyl-coumarin were attributed to **3** and **4**, respectively.

EXPERIMENTAL

The FABMS spectra, in negative ion mode, were obtained by dissolving the samples in a glycerol-thioglycerol matrix and placing them on a copper probe tip prior to bombardment with Ar atoms of energy 2–6 kV. The DEPT experiments were performed using polarization transfer pulses of 90 and 135°, respectively, to obtain in the first case only CH groups and in the second positive signals for CH and Me and negative ones for CH_2 groups. Polarization transfer delays were adjusted to an average CH coupling of 135 Hz. The NMR spectra were measured in CD_3OD solutions at 250 MHz (^1H NMR) and at 69.5 MHz (^{13}C NMR) with TMS as int. standard. Determination of NOEDS experiments were performed on samples previously degassed by bubbling Ar through the solution for 40 min.

Biological material. Caulis of *C. hexandra* (Jacq.) Schum. were collected in north-eastern Brazil (Pacatube, Fortaleza) and identified by José Elias de Paula (Universidade Federal de Brasilia).

Isolation. 700 g of air-dried caulis of *C. hexandra* were extracted with MeOH at room temp. The residue (11 g) was chromatographed on Sephadex LH-20 column (80 × 4 cm) with MeOH, in portion of 2 g. 10 ml fractions were collected and analysed by TLC on silica gel in BuOH–HOAc–H_2O 12:3:5. Fractions 18–25 (1.6 g) were further fractionated by HPLC on a C-18 μ-Bondapak column (30 cm × 7.8 mm i.d., flow rate 2.5 ml/min) using MeOH–H_2O (4:6) as eluent to yield pure **3** (63 mg, R_t 8.5 min), **1** (33 mg, R_t 10 min), **4** (25 mg, R_t 13 min) and **2** (73 mg, R_t 17 min). ^1H and ^{13}C NMR (in CD_3OD) are reported in Tables 1, 2.

Acidic methanolysis. Methanolysis of each glycoside (**1–4**) (0.5–1 mg) was achieved as described earlier [10].

Compound **2**. ^{13}C NMR (DMSO-d_6–$CDCl_3$ 7:3): aglycone signals: ppm 162.8 (C-2), 109.0 (C-3), 156.5 (C-4), 101.5 (C-4a), 155.8 (C-5), 97.4 (C-6), 159.6 (C-7), 100.1 (C-8), 155.7 (C-8a), 130.8 (C-1′), 115.3 (C-2′), 143.9 (C-3′), 145.3 (C-4′), 114.4 (C-5′), 118.8 (C-6′); sugar signals G-1/G-6: 97.4, 73.2, 76.0, 69.5, 72.8, 61.2.

Acidic hydrolysis of **2**. A soln of **2** (40 mg) was hydrolysed by refluxing with 0.5 N H_2SO_4 for 1 hr. After cooling the reaction mixture was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with 10% $NaHCO_3$ and H_2O, dried over Na_2SO_4 and concd *in vacuo* and the residue recrys. from $CHCl_3$ to afford **2a** (12 mg).

7-Methoxy-5,3′,4′-trihydroxy-4-phenylcoumarin (**2a**). ^1H NMR (Me_2CO-d_6): δ 3.90 (3H, *s*, -OMe), 5.85 (1H, *s*, H-3), 6.30 (1H, *d*, *J* = 2.5 Hz, H-8), 6.50 (1H, *d*, *J* = 2.5 Hz, H-6), 6.83 (1H, *d*, *J* = 7.5 Hz, H-5′), 6.86 (1H, *d*, *J* = 2.0 Hz, H-2′), 6.75 (1H, *dd*, *J* = 2 and 7.5 Hz, H-6′); ^{13}C NMR (DMSO-d_6–$CDCl_3$ 7:3): ppm 162.8 (C-2), 110.4 (C-3), 157.0 (C-4), 98.2 (C-4a), 156.8 (C-5), 92.9 (C-6),

159.6 (C-7), 98.2 (C-8), 156.0 (C-8a), 130.6 (C-1'), 115.4 (C-2'), 144.0 (C-3'), 145.5 (C-4'), 114.5 (C-5'), 118.8 (C-6'), 55.8 (–OMe).

Methylenation of **2a**. **2a** (12 mg) and dry CsF (40 mg) were shaken and cooled in dry DMF (0.5 ml). CH_2Br_2 was added to the mixture which was stirred at 115° for 15 hr. The cooled mixture was poured into $CHCl_3$ and washed with H_2O. The organic solvent was evapd to dryness and the residue purified by prep. TLC on silica gel with hexane–EtOAc (4:1) to give **2b** (4 mg).

7-Methoxy-5-hydroxy-3',4'-methylenedioxy-4-phenylcoumarin (**2b**). ^{1}H NMR (Me_2CO-d_6):δ 3.90 (–OMe), 5.84 (1H, *s*, H-3), 5.94 (2H, *s*, -O-CH_2-O), 6.31 (1H, *d*, J = 2.5 Hz, H-8), 6.48 (1H, *d*, J = 2.5 Hz, H-6), 6.80–6.90 (3H, *br*, *s* H-2', H-5' and H-6').

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