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THREE 5-METHYLCOUMARINS FROM *CHAPTALIA NUTANS*

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Key Word Index—*Chaptalia nutans*; Asteraceae; 5-methylcoumarin derivatives; nutanocoumarin; 9′-*O*-β-D-glucopyranosyl-nutanocoumarin; 7-*O*-β-D-glucopyranosyl-nutanocoumarin.

Abstract—One new 5-methylcoumarin, along with its two new isomeric glucosides were isolated from the roots of *Chaptalia nutans*. © 1997 Elsevier Science Ltd

INTRODUCTION

Chaptalia nutans (L.) Polak. (Asteraceae, subtribe Mutisiinae) has been used for the treatment of fever, headache, skin diseases, herpes and syphilis in Brazil [1]. Previous chemical investigations reported the isolation and identification of prunasin, parasorbic acid, 5-methyl-3α-hydroxyvalerolactone and 4-*O*-β-glucopyranosyl-5-methylcoumarin from the aerial parts [2–4]. However, the chemical constituents of the roots have not been studied. In this paper we report the isolation and structure elucidation of coumarins from the roots of *Chaptalia nutans*.

RESULTS AND DISCUSSION

Compound **1** was isolated as crystals and assigned the molecular formula $C_{20}H_{24}O_5$ by elemental analysis (Found: C, 69.7; H, 6.9; $C_{20}H_{24}O_5$ requires: C, 69.9; H, 7.0%). The UV spectrum showed absorption maxima at 209 and 320 nm, and the IR spectrum showed absorption bands at 1670, 1626 and 1580 cm^{-1}, suggesting the presence of a coumarin skeleton. The ^1H and ^{13}C NMR spectra (Table 1 and Experimental) showed signals typical of a coumarin moiety, which was identified as 7-hydroxy-5-methylcoumarin from the splitting patterns observed for H-6 and H-8, together with the signals for a phenolic hydroxyl and for an aromatic methyl group. The further 10 signals in the ^{13}C NMR (Table 1) suggested that the 7-hydroxy-5-methylcoumarin moiety was combined with a monoterpene side chain. Coumarins containing monoterpene side chains have been isolated from different genera of the subtribe Mutisiinae [5]. The nature of the monoterpene unit in **1** was deduced by comparison with ^1H NMR spectral data of cycloisobrachycoumarin (**4**) [5]. These spectral data were closely related, except for the presence of the signals assignable to a hydroxymethyl group [δ 3.78 (1H, *dd*,

Table 1. ^{13}C NMR spectral data for compounds **1**–**3** (DMSO-d_6, 75.5 MHz)

C	1	2	3
2	160.7	161.2	160.3
3	104.7	105.0	106.3
4	166.4	166.9	166.6
4a	103.6	104.0	106.1
5	137.3	137.8	137.6
6	114.9	115.3	115.9
7	159.1	159.6	159.5
8	100.2	100.6	101.4
8a	157.3	157.8	157.4
9	21.0	21.1	21.1
1′	15.5	15.8	15.7
2′	88.5	88.8	89.1
3′	45.4	45.6	45.7
4′	38.1	38.1	38.2
5′	22.4	22.7	22.6
6′	125.4	129.5	125.8
7′	135.5	131.9	136.0
8′	21.2	21.5	21.4
9′	59.3	65.8	59.6
10′	19.0	19.1	19.2
1″	—	101.2	100.2
2″	—	73.5	73.4
3″	—	77.1	77.4
4″	—	70.2	69.9
5″	—	77.0	76.8
6″	—	61.2	60.9

Assignments confirmed by ^1H–^{13}C COSY and DEPT experiments.

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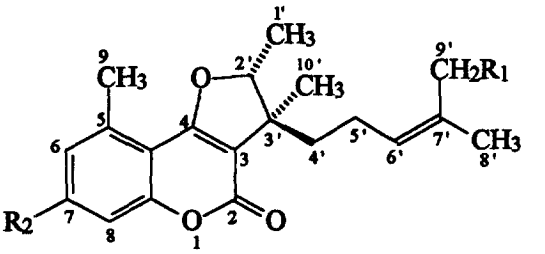
J = 5.2 and 12.3 Hz), 3.87 (1H, *dd*, J = 5.2 and 12.3 Hz)] in **1**, instead of signals due to a C-9′ methyl group. The analysis of the ^{1}H–^{1}H and ^{1}H–^{13}C COSY spectrum confirmed the nature of the monoterpene side chain. The mass spectral data also supported the structure assigned for compound **1**. The base peak at m/z 245 corresponds to a fragment formed by cleavage of C-3′/C-4′ bond. The stereostructure was determined by NOE difference spectroscopy. Irradiation at the frequency of the olefinic proton at δ 5.09, causing an 8.5% enhancement of the H-8′ signal, indicated at *Z*-configured double bond in the side chain. Irradiation of the methyl signal at δ 1.36 (H-1′) resulted in an enhancement of 1.5% for H-10′. No nuclear Overhauser enhancement was observed between H-1′ and H-4′ under the same conditions, indicating that **1** possessed the same C-2′ and C-3′ relative stereochemistries as compound **4**.

Compound **2** was isolated in crystalline form, and showed ion peaks $[M+H]^+$ at m/z 507 and $[M+Na]^+$ at m/z 529 in the positive FAB-mass spectrum, consistent with $C_{26}H_{34}O_{10}$. The ^{1}H and ^{13}C NMR spectrum of **2** were similar to those of **1**, except for the signals of a sugar moiety (one anomeric centre at δ_H 4.10, δ_C 101.2). The sugar moiety was determined as β-D-glucopyranose, based on the chemical shifts of the carbons atoms together with the coupling constant ($J_{1,2}$ = 7.8 Hz) for the anomeric proton. Assignment of the resonances of the protons and carbons of **2** was achieved by a combination of ^{1}H–^{1}H and ^{1}H–^{13}C COSY experiments. The localization of the glucoside moiety of **2** was deduced by comparing the ^{13}C NMR (Table 1) spectral data to those of compound **1**. The signal of C-9′ in **2** was shifted downfield by +6.5 ppm, which indicated that the glucoside unit was linked at this carbon. The relative stereochemistry of **2** was established by NOE difference spectroscopy. Irradiation at the H-6′ frequency enhanced the H-8′ signal in 5.3% and irradiation of the H-1′ resonance enhanced the H-10′ signal in 1.1%. These data indicated that **2** possesses the same C-2′, C-3′, C-6′ and C-7′ relative configuration to those of **1**.

Compound **3** showed ion peaks $[M+H]^+$ at m/z 507 and $[M+Na]^+$ at m/z 529 in the FAB-mass spectrum and was consistent with $C_{26}H_{34}O_{10}$. The ^{1}H and ^{13}C NMR spectra were similar to those of **2** and indicated that the aglycone and sugar moieties were identical in both compounds. The downfield shift of the aromatic protons H-6 and H-8, the fact that the signals for H-6′/C-6′ and H-9′/C-9′ were close to those of **1** rather than **2** and the absence of the proton signal for the phenolic -OH group, required the sugar moiety at C-7. Compound **3** possesses the same relative stereochemistry as in **1** and **2** due to the observation of NOEs (H-6′/H-8′ and H-1′/H-10′).



	R_1	R_2
1	OH	OH
2	OGlu	OH
3	OH	OGlu
4	H	H

EXPERIMENTAL

General. Mps uncorr. ^{1}H NMR (300 MHz) and ^{13}C NMR (75.5 MHz) were recorded in DMSO-d_6 with TMS as int. standard. NOEs experiments were performed using irradiation and acquisition time of 0.6 and 2.500 sec, respectively, on degassed samples at concn of 30 mg ml^{-1}. CC: silica gel 60 (70–230 mesh, Merck). TLC and prep. TLC: Kieselgel 60 F_{254} (0.25 and 1.0 mm thick, Merck). Spots and bands were visualized by UV irradiation (254 and 366 nm).

Plant material. Roots of *C. nutans* were collected in January 1994, in Maringá, Paraná, Brazil. A voucher specimen is deposited at the herbarium of the Universidade Estadual de Maringá (voucher no. 3361).

Extraction and isolation. The air-dried and powdered roots (500 g) were extracted at room temp. with *n*-hexane and further with EtOH. The EtOH extract was concd under red. pres. to give 52.0 g of a residue. Part of this residue (33.8 g) was subjected to CC on silica gel, eluting successively with *n*-hexane (700 ml), *n*-hexane–EtOAc 1:1 (900 ml), EtOAc (500 ml), EtOAc–MeOH 1:1 (1000 ml) and MeOH (1000 ml).

The residue (2.59 g) of the 50% *n*-hexane–EtOAc eluant was rechromatographed on silica gel CC with *n*-hexane, *n*-hexane–$CHCl_3$ (9:1, 8:2, 1:1), $CHCl_3$, $CHCl_3$–MeOH (19:1, 9:1, 4:1, 1:1), affording 33 frs. Frs 12–14 and 15–17, eluted with $CHCl_3$ and $CHCl_3$–MeOH 19:1, respectively, were purified by repeated crystallization to give **1** (830.0 mg).

The residue (2.38 g) obtained from EtOAc eluant was rechromatographed on silica gel with *n*-hexane–EtOAc 1:1, EtOAc, EtOAc–MeOH (19:1, 9:1, 4:1, 1:1) and MeOH, affording 30 frs. Frs 16 and 17, eluted with $CHCl_3$–MeOH 19:1, were purified by repeated prep. TLC (silica gel, $CHCl_3$–MeOH 4:1) to give **2** (270.0 mg) and **3** (121.0 mg).

Nutanocoumarin (**1**): Crystals from Et_2O, mp 203–204°; UV λ_{max}^{MeOH} nm (log ε): 320 (4.24), 209 (4.56); IR ν_{max}^{KBr} cm^{-1}: 3358, 1670, 1626 and 1580. Elemental analysis: $C_{20}H_{24}O_5$ requires C, 69.9; H, 7.0; Found C, 69.7; H, 6.9; EI-MS (probe, 70 eV) m/z (rel. int.): 344 $[M]^+$ (1), 246 (17), 245 (100), 151 (9), 43 (24); ^{1}H NMR (300 MHz, DMSO-d_6): δ 1.14 (3H, *s*, H-10′), 1.36 (3H, *d*, J = 6.6 Hz; H-1′), 1.61 (2H, *m*, H-4′), 1.62 (3H, *s*, H-8′), 1.84 (1H, *m*, H-5′), 2.07 (1H, *m*, H-5′), 2.49 (3H, *s*, H-9), 3.78 (1H, *dd*, J = 5.2 and 12.3 Hz, H-9′),

3.87 (1H, *dd*, J = 5.2 and 12.3 Hz, H-9′), 4.46 (1H, *t*, J = 5.4 Hz, -OH), 4.85 (1H, *q*, J = 6.6 Hz, H-2′), 5.09 (1H, *t*, J = 6.9 Hz, H-6′), 6.55 (1H, *br s*, H-8), 6.56 (1H, *br s*, H-6), 10.48 (1H, *s*, -OH); ^{13}C NMR (75.5 MHz, DMSO-d_6): Table 1.

9′-O-β-D-*Glucopyranosyl-nutanocoumarin* (**2**). Crystals from MeOH, mp 116–118°; $[\alpha]_D^{24}$ −29° (MeOH; *c* 0.69); UV λ_{max}^{MeOH} nm (log ε): 320 (4.12), 209 (4.52); IR ν_{max}^{KBr} cm^{-1}: 3362, 1710, 1625, 1572; FAB-MS *m/z* (rel. int.): 507 $[M+H]^+$ $[C_{26}H_{34}O_{10}+H]^+$ (4), 529 $[M+Na]^+$ (5), 327 (12), 246 (15), 245 (100), 151 (8); ^{1}H NMR (300 MHz, DMSO-d_6): aglycone moiety δ 1.14 (3H, *s*, H-10′), 1.36 (3H, *d*, J = 6.6 Hz, H-1′), 1.64 (3H, *s*, H-8′), 1.66 (2H, *m*, H-4′), 1.87 (1H, *m*, H-5′), 2.05 (1H, *m*, H-5′), 2.50 (3H, *s*, H-9), 3.92 (1H, *d*, J = 11.7 Hz, H-9′), 4.18 (1H, *d*, J = 11.7 Hz, H-9′), 4.45 (1H, *br s*, -OH), 4.87 (1H, *q*, J = 6.6 Hz, H-2′), 4.97 (1H, *br s*, -OH), 5.28 (1H, *t*, J = 6.9 Hz, H-6′), 6.55 (1H, *br s*, H-8), 6.57 (1H, *br s*, H-6), 10.50 (1H, *s*, -OH); glucose moiety: δ 2.90–3.00 (2H, signal patterns unclear due to overlapping, H-2″ and H-5″), 3.02–3.18 (2H, signal patterns unclear due to overlapping, H-3″ and H-4″), 3.60 (1H, *dd*, J = 5.5 and 11.7 Hz, H-6″a), 3.70 (1H, *dd*, J = 2.1 and 11.7 Hz, H-6″b), 4.10 (1H, *d*, J = 7.8 Hz, H-1″).

7-O-β-D-*Glucopyranosyl-nutanocoumarin* (**3**). Crystals from MeOH, mp 94–95°; $[\alpha]_D^{24}$ −69° (MeOH; *c* 0.51); UV λ_{max}^{MeOH} nm (log ε): 318 (4.13), 210 (4.54); IR ν_{max}^{KBr} cm^{-1}: 3346, 1710, 1627, 1565; FEB-MS *m/z* (rel. int.): 507 $[M+H]^+$ $[C_{26}H_{34}O_{10}+H]^+$ (6), 529 $[M+Na]^+$ (1), 327 (20), 246 (15), 245 (100), 151 (12). ^{1}H NMR (300 MHz, DMSO-d_6): aglycone moiety δ 1.16 (3H, *s*, H-10′), 1.38 (3H, *d*, J = 6.6 Hz, H-1′), 1.62 (3H, *s*, H-8′), 1.65 (2H, *m*, H-4′), 1.85 (1H, *m*, H-5′), 2.03 (1H, *m*, H-5′), 2.56 (3H, *s*, H-9), 3.78 (1H, *d*, J = 12.0 Hz, H-9′), 3.87 (1H, *d*, J = 12.3 Hz, H-9′), 4.90 (1H, *q*, J = 6.6 Hz, H-2′), 5.10 (1H, *t*, J = 7.0 Hz, H-6′), 5.39 (1H, *br s*, -OH), 6.81 (1H, *dd*, J = 1.0 and 2.4 Hz, H-6′), 6.89 (1H, *br d*, J = 2.4 Hz, H-8); glucose moiety: δ 3.00–3.40 (4H, signal patterns unclear due to overlapping, H-2″, H-3″, H-4″ and H-5″), 3.50 (1H, *dd*, J = 6.0 and 10.2 Hz, H-6″a), 3.69 (1H, *br d*, J = 10.2 Hz, H-6″b), 4.99 (1H, *d*, J = 7.2 Hz, H-1″).

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