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O-GERANYLATED ISOFLAVONES AND A 3-PHENYLCOUMARIN FROM *MILLETTIA GRIFFONIANA*†

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Key Word Index—*Millettia griffoniana*; Leguminosae; root bark; isoflavones; 3-phenylcoumarin; *O*-geranylated isoflavone.

Abstract—Root bark of *Millettia griffoniana* yielded two new *O*-geranylated isoflavones and a new 3-phenylcoumarin. Their structures were determined on the basis of spectral evidence as 4′-methoxy-7-*O*-[(*E*)-3-methyl-7-hydroxymethyl-2,6-octadienyl]isoflavone, 3′,4′-dihydroxy-7-*O*-[(*E*)-3,7-dimethyl-2,6-octadienyl]isoflavone and 4-hydroxy-5,6,7-trimethoxy-3-(3′,4′-methylenedioxy)phenylcoumarin.

INTRODUCTION

In a previous paper, we reported the isolation and structural elucidation of seven isoflavones and a chalcone from *Millettia griffoniana* [1], a plant that grows in the central part of Cameroon. Further investigation of the chloroform extract of the root bark led to the isolation of two isoflavones and a 4-hydroxy-3-phenylcoumarin. In this paper, we report the structural elucidation of these compounds. To our knowledge, compound **1** is the first example of an isoflavone bearing a hydroxymethyl group on the geranyl side-chain.

RESULTS AND DISCUSSION

Compound **1** was obtained as a yellow powder, m.p. 138–139°C. The molecular formula was determined as $C_{26}H_{28}O_5$ by HREI mass spectroscopy (*m/z* 420.1951 $[M]^+$). It showed characteristic signals for an isoflavone in the ^{1}H-NMR (δ7.84, *s*, H-2) and ^{13}C NMR (δ152.4, C-2) spectra [2]. The ^{13}C NMR, along with the DEPT spectra, revealed the presence of three CH_3, four CH_2, ten CH and nine quaternary carbons, including a carbonyl carbon (δ175.9). The ^{1}H NMR spectrum also showed the presence of a methoxyl group at δ3.77 and an ABX-spin system, with an *ortho*-coupled doublet at δ8.12, a doublet of doublets at δ6.93 and a *meta*-coupled doublet at δ6.70, suggesting that ring-A is unsubstituted at positions 5, 6 and 8. Furthermore, the presence of an AA′BB′-spin system, with doublets at δ6.91 and 7.42 (2H each), indicated that ring-B is substituted at the *para*-position by a methoxyl group. This was further confirmed by the mass spectrum which displayed a peak at *m/z* 132 from RDA cleavage.

The presence of an *O*-geranyl unit in the molecule, in which one geranyl methyl group is substituted by CH_2OH, is deduced from the ^{1}H NMR spectrum which revealed the presence of two methyls at δ1.52 and 1.61, four methylenes, of which two are oxymethylenes (δ4.56, *d*; 4.06, *d*) and two olefinic protons at δ5.20 (*t*) and 5.43 (*t*).

The *Z*-configuration for the double bond, C-6″/C-7″, was assigned on the basis of NOESY experiments. In the NOESY spectra (Fig. 1), a strong interaction is observed, on the one hand, between the methylene protons of CH_2OH (δ4.06) and H-5″ (δ2.08) and, on the other, between the methyl group at δ1.52 and the olefinic proton H-6″ (δ5.20), indicating that the methyl group and the olefinic proton are *cis* to each other. Correlations between the aromatic protons H-6 and H-8 with H-1″ of the geranyl unit are also observed. These data are

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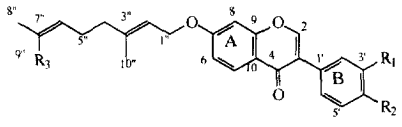
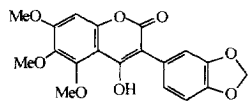
†Part 6 in the series "The *Millettia* of Cameroon". For part 5 see Yankep *et al.* [Yankep, E., Fomum, Z. T. and Dagne, E., *Phytochemistry*, 1997. **46**, 591.].



	R_1	R_2	R_3
1	H	OMe	CH_2OH
2	OH	OH	Me



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consistent with 4′-methoxy-7-*O*-[(*E*)-3-methyl-7-hydroxymethyl-2,6-octadienyl]isoflavone for the structural assignment of **1**.

Compound **2** was also obtained as a yellow powder, m.p. 164–165°C. The molecular formula was deduced as $C_{25}H_{26}O_5$ from its HREI mass spectrum (*m/z* 406.1776 $[M]^+$). The ^1H NMR spectrum showed a typical isoflavone signal at δ7.89 (H-2), an ABX-system at δ8.17 (H-5), 6.82 (H-8) and δ6.91 (H-6) and an ABC-system at δ6.86 (H-5′), 7.08 (H-2′) and δ7.05 (H-6′). The presence of a geranyl unit was evident from the ^1H and ^{13}C NMR spectra (Tables 1 and 2) and its attachment to the oxygen at C-7 was derived from NOESY experiments, which revealed connections between the oxymethylene protons at C-1″ and the aromatic proton at C-6. Thus, compound **2** is identified as 3′,4′-dihydroxy-7-*O*-[(*E*)-3,7-dimethyl-2,6-octadienyl]isoflavone.

Compound **3** was obtained as a yellow powder, m.p. 170–172°C. The molecular formula, $C_{19}H_{16}O_8$, was determined by HREI mass spectroscopy (*m/z* 372.0820 $[M]^+$). Comparison between its ^1H and ^{13}C NMR data with those of thonningine A and B [3], together with the characteristic resonance at δ10.12 for the hydroxyl function at C-4, indicated **3** to be a 4-hydroxy-3-phenylcoumarin-type compound. The ^1H NMR spectrum showed three aromatic protons in an ABC-system for ring-B (δ6.85, *d*; 6.99, *dd* and 7.01, *d*) and one isolated aromatic proton (δ6.68, *s*) for ring-A of a phenylcoumarin. The ^{13}C NMR (Table 2) revealed the presence of three OCH_3, one OCH_2O, four CH and 11 quaternary carbons, including an ester carbonyl carbon (δ162.6). Thus, the structure of compound **3** is deduced to be 4-hydroxy-5,6,7-trimethoxy-3-(3′,4′-methylenedioxy)phenylcoumarin.

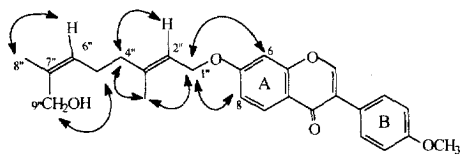

Fig. 1. 2D H-H COSY and 2D NOESY correlations for compound **1**.

EXPERIMENTAL

General

UV: MeOH. IR: KBr. ^1H NMR and ^{13}C NMR (Bruker AMXR 300, 300 and 75 MHz in $CDCl_3$, respectively). EIMS: 70 eV. CC: silica gel 60 F_{254} (230–400 mesh, Merck). TLC: silica gel 60 F_{254} (Merck). Prep. TLC: silica gel 60 $F_{254+366}$ self made plates (Merck); spots and bands were viewed by UV light (254 and 366 nm). TLC and prep. TLC solvent system I: petrol–EtOAc (3:2).

Plant material

The root bark of *M. griffoniana* was collected from Onguesse province, in the central part of Cameroon, in 1994. A voucher specimen is deposited at the National Herbarium, Yaounde, Cameroon.

Extraction and isolation

Powdered dried root bark (20 kg) was successively extracted with *n*-hexane (24 h; 3 × 20 l) and chloroform (24 h; 3 × 20 l) yielding 710 and 550 g, respectively. The concd $CHCl_3$ extract (50 g) was successively washed with petrol and EtOAc. The EtOAc-sol. part was further purified by CC on silica gel eluting with petrol–EtOAc gradients, fol-

Table 1. ^1H NMR spectral data for compounds **1–3** in $CDCl_3$

H	1	2	3
2	7.84 *s*	7.89 *s*	–
5	8.12 *d* (8.9)	8.17 *d* (8.7)	–
6	6.93 *dd* (8.9, 2.3)	6.91 *dd* (8.7, 2.2)	–
8	6.70 *d* (2.3)	6.82 *d* (2.2)	6.68 *s*
2′	7.42 *d* (8.7)	7.08 *brs*	7.01 *d* (1.7)
3′	6.91 *d* (8.7)	–	–
5′	6.91 *d* (8.7)	6.86 *d* (8.0)	6.85 *d* (8.4)
6′	7.42 *d* (8.7)	7.05 *dd* (8.0, 2.0)	6.99 *dd* (8.4, 1.7)
1″	4.56 *d* (6.5)	4.60 *d* (6.5)	–
2″	5.43 *t* (6.3)	5.47 *t* (6.5)	–
4″	2.15 *m*	2.08 *m*	–
5″	2.08 *m*	2.08 *m*	–
6″	5.20 *t* (6.7)	5.08 *t* (6.9)	–
8″	1.52 *s*	1.59 *s*	–
9″	4.06 *d*	1.72 *s*	–
10″	1.61 *s*	1.67 *s*	–
OCH_2O	–	–	5.95 *s*
4-OH	–	–	10.12 *s*
5-OMe	–	–	4.16 *s*
6-OMe	–	–	3.86 *s*
7-OMe	–	–	3.92 *s*
4′-OMe	3.77 *s*	–	–

Coupling constants (*J* in Hz) in parentheses.

Table 2. ^{13}C NMR spectral data for compounds **1–3** in $CDCl_3$

C	1	2	3
2	152.4	154.8	160.9
3	124.9	126.3	101.2
4	175.9	178.0	162.6
5	128.2	128.5	146.9
6	115.4	116.5	137.6
7	163.1	164.7	147.3
8	101.3	103.3	96.7
9	157.9	159.7	156.9
10	118.4	118.2	103.9
1′	124.2	125.8	124.6
2′	130.5	114.6	111.1
3′	114.4	148.1	149.9
4′	159.6	149.9	149.0
5′	114.4	117.5	108.1
6′	130.5	121.4	124.3
1″	65.8	66.9	–
2″	119.3	121.5	–
3″	142.1	141.8	–
4″	39.9	40.6	–
5″	26.2	27.4	–
6″	127.7	125.0	–
7″	135.1	132.6	–
8″	17.2	17.8	–
9″	61.9	16.7	–
10″	21.6	25.9	–
OCH_2O	–	–	101.0
5-OMe	–	–	62.9
6-OMe	–	–	61.4
7-OMe	–	–	56.4
4′-OMe	55.8	–	–

Signal assignments are based on ^1H–^{13}C COSY and DEPT spectra.

lowed by prep. TLC using system **I**, resulting in the isolation of **1** (20 mg), **2** (30 mg) and **3** (11 mg).

4′-Methoxy-7-O[(E)-3-methyl-7-hydroxymethyl-2,6-octadienyl]isoflavone (**1**)

Yellow powder, m.p. 138–139°C. $[\alpha]_D^{23}$ −3.67° ($CHCl_3$; *c* 0.055). UV λ_{max} nm: 249, 298. IR ν_{max} cm^{-1}: 3436, 2924, 1631, 1514, 1444, 1384, 1292, 1250. ^1H NMR (Table 1) and ^{13}C NMR (Table 2). EIMS *m/z* (rel. int.): 420 $[M]^+$ (20), 372 (10), 282 (16), 269 (21), 268 (100), 267 (7) 132 (7), 107 (7), 93 (11); HR-EIMS *m/z*: 420.1951 $[M]^+$ (calcd for $C_{26}H_{28}O_5$: 420.1937).

3′,4′-Dihydroxy-7-O-[(E)-3,7-dimethyl-2,6-octadienyl]-isoflavone (**2**)

Yellow powder, m.p. 164–165°C. $[\alpha]_D^{23}$ −7.66° ($CHCl_3$; *c* 0.044). UV λ_{max} nm: 202, 248, 292. IR ν_{max} cm^{-1}: 3435, 2925, 1624, 1509, 1384, 1281, 1192. ^1H NMR (Table 1) and ^{13}C NMR (Table 2). EIMS *m/z* (rel. int.): 406 $[M]^+$ (9), 337 (5), 326 (11), 282 (31), 271 (19), 270 (100); HR-EIMS *m/z*: 406.1776 $[M]^+$ (calcd for $C_{25}H_{26}O_5$: 406.1780).

4-Hydroxy-5,6,7-trimethoxy-3-(3′,4′-methylenedioxy)phenylcoumarin (**3**)

Yellow powder, m.p. 170–172°C. $[\alpha]_D^{23}$ −12.58° ($CHCl_3$; *c* 0.027). UV λ_{max} nm: 215, 329. IR ν_{max} cm^{-1}: 3430, 3256, 2924, 1710, 1639, 1613, 1573, 1501, 1458, 1399, 1284. ^1H NMR (Table 1) and ^{13}C NMR (Table 2). EIMS *m/z* (rel. int.): 372 $[M]^+$ (100), 357 (3), 211 (25), 210 (18), 195 (9), 171 (5), 162 (9); HR-EIMS *m/z*: 372.0820 $[M]^+$ (calcd for $C_{19}H_{16}O_8$: 372.0845).

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