Pergamon

PII: S0031-9422(98)00277-5

Phytochemistry Vol. 49, No. 8, pp. 2525–2528, 1998

Printed in Great Britain
0031-9422/98/$ - see front matter

PRENYLISOFLAVANONES FROM *GEOFFROEA DECORTICANS*

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(*Received* 10 *February* 1998)

Key Word Index—*Geoffroea decorticans*; Leguminosae; stembark; isoflavanone; (3*R*)-5,7,2′,3′-tetrahydroxy-4′-methoxy-5′-prenylisoflavanone; (3*R*)-7,2′,3′-trihydroxy-4′-methoxy-5′-prenylisoflavanone; (3*S*)-3,7,2′,3′-tetrahydroxy-4′-methoxy-5′-prenylisoflavanone.

Abstract—Three new isoflavanones, named (3*R*)-5,7,2′,3′-tetrahydroxy-4′-methoxy-5′-prenylisoflavanone. (3*R*)-7,2′,3′-trihydroxy-4′-methoxy-5′-prenylisoflavanone and (3*S*)-3,7,2′,3′-tetrahydroxy-4′-methoxy-5′-prenylisoflavanone were isolated from the stembark of *Geoffroea decorticans*. The structures were confirmed by means of spectral analysis including 2D-NMR techniques.

INTRODUCTION

The small tree *Geoffroea decorticans* (Hook. & Arn.) Burkart (Leguminosae) [1], is one of five species of the genus *Geoffroea* which is distributed through South America [2]. In the Bolivian Chaco region, it is commonly known as "Quimiri" or "Kumbaru" and currently used as a remedy for dysentery [1]. Little is known about this genus and only the presence of non-specific phytohaemagglutinins has been reported [3].

In the present study we report the isolation and identification of three new isoflavanones (**1**–**3**), as well as lupeol and lupenone from the stembark of *G. decorticans*. The isoflavanones are biosynthetic intermediates between isoflavones and pterocarpan or isoflavan phytoalexins, and they often accumulate when leguminous plants are challenged by fungi or abiotic agents [4].

RESULTS AND DISCUSSION

Fractionation of the ethanolic extract of *Geoffroea decorticans* (see Experimental) yielded three isoflavanoids. Compound **1** [$C_{21}H_{22}O_7$], showed in its mass spectrum a parent peak at *m/z* 386 (53%), and two fragment ions at *m/z* 153 and 234 originating from a retro-Diels-Alder cleavage of ring C. The ion peak at *m/z* 153 (100%) suggested the presence of two hydroxyl groups in ring A. On the other hand, the ion fragment *m/z* 234 arising from ring B suggested that this ring had one methoxyl, one prenyl and two hydroxyl substituents. Peaks at *m/z* 69 and 55 confirmed the presence of a prenyl group. The weak relative intensity of the fragment M-55 (4%) was as a result of the loss of a neutral fragment C_4H_7 from a prenyl group, such a loss being characteristic of compounds having a prenyl group adjacent to methoxyl group(s) [5]. The IR spectrum displayed absorption bands due to the presence of hydroxyl group(s) (3378 cm^{-1}), and a carbonyl group (1636 cm^{-1}). The ^1H NMR spectrum (500 MHz, CD_3OD) (Table 1) revealed the presence of: (i) three signals of an ABX system due to the C-2 (δ 4.44 *dd* and 4.60 *dd*) and C-3 protons (δ 4.18 *dd*) of an isoflavanone skeleton; (ii) four signals [δ 1.65 (3H, *s*), 1.69 (3H, *s*), 3.22 (2H, *d*, J = 7.4 Hz), 5.20 (1H, *t*, J = 7.4 Hz)] indicating the presence of one prenyl group: (iii) three signals in the aromatic region corresponding to the B-ring proton H-6′ (δ 6.36, *s*), and two protons with a *meta* coupling in ring A [δ 5.92 (*d*, 2.0 Hz) and 5.89 (*d*, J = 2.0 Hz)] assignable to H-6 and H-8 respectively; (iv) finally, a singlet at δ 3.76 was ascribed to

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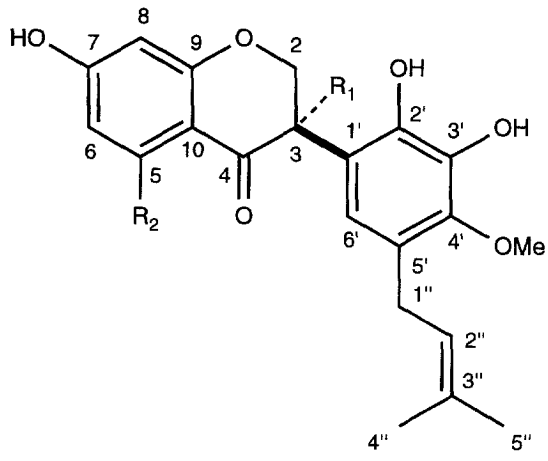

1 $R_1 = H, R_2 = OH$
2 $R_1 = R_2 = H$
3 $R_1 = OH, R_2 = H$

the methoxyl group of position 4', in agreement with the HMBC experiment [6]; thus, the long-range correlation of C-4' with H-1", and H-6', as well as with the protons of the C-4' methoxyl group were observed. The H-6' proton was assigned by its long-range coupling with C-1" and C-3, showing that the prenyl group was attached at C-5'. Furthermore, the benzylic coupling observed between H-6' and H-1" in the COSY experiment was in agreement with the proposed assignment.

The ^{13}C NMR spectrum ($CDCl_3/CD_3OD$) of **1**, (Table 2), was consistent with a prenylisoflavanone structure. The most prominent signals were: (i) the presence of a prenyl substituent, which was confirmed by five resonances at δ 27.5 (CH_2), 122.7 (CH), 132.0 (C), 17.3 (CH_3) and 25.2 (CH_3). The chemical shift of the methylene carbon indicated that one of the *ortho* positions adjacent to the prenyl group was replaced by an oxygenated substituent and the other by a hydrogen atom [7]; (ii) the chemical shift of the carbonyl resonance (C-4) at δ 196.9 was indicative of an hydroxyl substituent in position 5 [8]; (iii) the deshielding of the methoxyl group (δ 60.2) indicated that it was *ortho*-disubstituted, acquiring an out-of-plane conformation instead of the planar disposition adopted when it is *ortho*-monosubstituted or *ortho*-nonsubstituted [9]; (iv) the presence of hydroxyl group substituents at both C-2' and C-3' was established by the deshielding effect on the quaternary carbons (δ 142.0 and 137.6, respectively). The signal at δ 142.0 was assigned to C-2' by its long-range coupling with H-3 as well as with H-6'. Thus, compound **1** is 5,7,2',3'-tetrahydroxy-4'-methoxy-5'-prenylisoflavanone. Its absolute configuration was established as (3*R*) on the basis of a positive Cotton Effect at 347 nm in its CD spectrum [10].

Table 1. ^1H NMR, HMQC and HMBC data for compound **1** (*J* given in Hz in parentheses)

H	δ	HMQC	HMBC
2a	4.44*dd* (11.0, 5.2)	69.8 *t*	C-3, C-4, C-9, C-1'
2b	4.60*dd* (11.0, 9.4)	69.8 *t*	C-4, C-9,
3	4.18*dd* (9.4, 5.2)	46.4 *d*	C-4, C-2'
6	5.92 *d* (2.0)	96.1 *d*	C-10
8	5.90 *d* (2.0)	95.0 *d*	C-9
6'	6.36 *s*	119.8 *d*	C-3, C-2', C-4', C-1"
1"	3.23 *d* (7.4) (2H)	27.5 *t*	C-4', C-5', C-6', C-2", C-3"
2"	5.20 *brt* (7.4, <1)	122.7 *d*	
4"	1.66 *s*	17.3 *q*	C-2", C-3", C-5"
5"	1.70 *s*	25.2 *q*	C-2", C-3", C-4"
OMe	3.75 *s*	60.2 *q*	C-4'

Table 2. ^{13}C NMR chemical shifts for compounds **1**–**3**.Solvents: **1**: $CDCl_3/CD_3OD$, **2**: CD_3COCD_3, **3**: CD_3OD.

C	1	2	3
2	69.8 *t*	71.4 *t*	74.9 *t*
3	46.4 *d*	48.0 *d*	75.8 *s*
4	196.9 *s*	191.4 *s*	192.0 *s*
5	164.0 *s*	130.1 *d*	130.9 *d*
6	96.1 *d*	111.3 *d*	112.0 *d*
7	166.4 *s*	165.2 *s*	166.5 *s*
8	95.0 *d*	103.4 *d*	103.5 *d*
9	163.2 *s*	164.7 *s*	164.9 *s*
10	102.3 *s*	115.5 *s*	114.0 *s*
1'	117.2 *s*	119.4 *s*	121.9 *s*
2'	142.0 *s*	143.5 *s*	143.3 *s*
3'	137.6 *s*	138.8 *s*	139.4 *s*
4'	145.5 *s*	146.4 *s*	147.7 *s*
5'	126.0 *s*	126.2 *s*	126.4 *s*
6'	119.8 *d*	120.6 *d*	118.6 *d*
1"	27.5 *t*	28.5 *t*	28.8 *t*
2"	122.7 *d*	124.3 *d*	124.5 *d*
3"	132.0 *s*	132.1 *s*	132.8 *s*
4"	17.3 *q*	17.7 *q*	17.8 *q*
5"	25.2 *q*	25.8 *q*	25.9 *q*
OMe	60.2 *q*	60.8 *q*	60.9 *q*

Compound **2**, $[C_{21}H_{22}O_6]$, showed in its mass spectrum a molecular ion peak at *m/z* 370 (56%) and two fragment ions at *m/z* 137 and 234. The most significant difference compared to compound **1**, was the fragment at *m/z* 137 (100%) which was indicative of the presence of a single hydroxyl group in ring A. The ^1H NMR spectrum (500 MHz, CD_3OD) though similar to that of **1**, showed four downfield signals corresponding to the proton H-6' (δ 6.31 *s*), and three protons of ring A [δ 7.75 (*d*, J = 8.5 Hz), 6.50 (*dd*, J = 8.5, 2.0 Hz) and 6.32 (*d*, J = 2.0 Hz)] establishing an *ortho*, *ortho-meta*, *meta* coupling system due to the protons H-5, H-6 and H-8, respectively. The proton H-5 is deshielded by its *peri* interaction with the carbonyl group. The chemical shift of the carbonyl group at C-4 (191.4 ppm) corroborated the absence of an hydroxyl substituent at C-5 position [8]. Thus, compound **2** is 7,2',3'-trihydroxy-4'-methoxy-5'-prenylisoflavanone. A positive Cotton Effect at 340 nm in its CD spectrum again indicates a (3*R*) configuration [10].

Table 3. ^{1}H NMR, HMQC and HMBC data for compound **2** (*J* given in Hz in parentheses)

H	δ	HMQC	HMBC
2a	4.46 *dd* (11.0, 5.0)	71.4 *t*	C-3, C-4, C-9, C-1′
2b	4.62 *dd* (11.0, 9.5)	71.4 *t*	C-3, C-4, C-9, C-1′
3	4.11 *dd* (9.5, 5.0)	48.0 *d*	C-2, C-4, C-1′, C-2′, C-6′
5	7.75 *d* (8.5)	130.1 *d*	C-4, C-9
6	6.50 *dd* (8.5, 2.0)	111.3 *d*	C-8, C-10
8	6.32 *d* (2.0)	103.4 *d*	C-6, C-7, C-9, C-10,
6′	6.31 *s*	120.6 *d*	C-3, C-2′, C-4′, C-1″
1″	3.19 *d* (6.7)	28.5 *t*	C-4′, C-5′, C-6′, C-2″, C-3″
2″	5.16 *brt* (6.7, < 1)	124.3 *d*	C-1″, C-4″, C-5″
4″	1.62 *s*	17.7 *q*	C-2″, C-3″, C-5″
5″	1.66 *s*	25.8 *q*	C-2″, C-3″, C-4″
OMe	3.71 *s*	60.8 *q*	C-4′

In the mass spectrum of compound **3** [$C_{21}H_{22}O_7$] a weak parent peak at *m/z* 386 (6%) was detected, as well as prominent fragments at *m/z* 137 (100%) and 250 (4%) caused by a retro-Diels-Alder cleavage. The base peak at *m/z* 137 resulted from the A-ring fragment and clearly showed that this moiety possessed one hydroxyl group. On the other hand, the fragment ion at *m/z* 250 suggested that there was one methoxyl, one prenyl and three hydroxyl group substituents in the other moiety. The ^{1}H NMR spectrum (500 MHz, CD_3OD) was very similar to that of **2**; the most important difference being the AB system at δ 4.88 *d* and 4.21 *d* (J_{AB} = 12.0 Hz) assignable to the two C-2 protons, indicating that position 3 was substituted. This was confirmed, in the ^{13}C NMR spectrum of compound **3** (Table 2) in which the signals of C-2, C-3 and C-1′ are shifted by +3.2, +27.4 and +3.0 ppm (relative to those of compound **2**), thus corroborating the presence of the hydroxyl group at C-3. Thus, compound **3** is 3,7,2′,3′-tetrahydroxy-4′-methoxy-5′-prenylisoflavanone. A negative Cotton Effect at 331 nm in its CD spectrum indicates that its absolute configuration is (3*S*).

The isoflavanoids pervilleanone and its 3′-*O*-demethyl derivative, from *Millettia pervilleana*, have the same substitution pattern as compounds **2** and **3** [10]. The isolation of prenylated isoflavanones lacking an hydroxyl group at C-5 is very rare in nature, and their presence in several genera of leguminous plants, like *Sophora*, is of intrageneric chemosystematic value [11].

EXPERIMENTAL

General. Mps were uncorr. IR spectra were measured in KBr discs or as dry films. EIMS at 70 eV. ^{1}H, ^{13}C NMR, DEPT, NOE, COSY, HMQC, and HMBC spectra were recorded in a Bruker 360 and in a Varian VXR 500, using the solvent specified. Chemical shifts were reported in δ units (ppm) and coupling constants (*J*) in Hz. Silica gel Merck (60–200 mesh) and silica gel 60 G (mean particle size 15 μm) were used for CC and VLC, respectively, and silica gel 60 F_{254} Merck (0.25 mm and 1 mm) for analyt. and prep. TLC. Spots on chromatograms were detected under UV light (254 and 365 nm) and by spraying with 10% H_2SO_4 followed by heating.

Plant material. The stembark of *G. decorticans* was collected near Brecha (Santa Cruz, Bolivia), in November 1995. The plant material was identified by Rossy de Michel and a voucher specimen is on deposit in the Herbario Nacional de Bolivia, La Paz.

Extraction and isolation

Dried stembark of *Geoffroea decorticans* (1.5 kg) was crushed and macerated with light petrol (40–60°) at room temperature for 3 days. The extract was evapd under red. pres., and the residue (6.3 g) was chromatographed by CC affording lupeol (300 mg) and lupenone (9 mg). After this, the plant material was extracted with EtOH at room temperature affording 16.3 g of crude extract which was partitioned with CH_2Cl_2. The CH_2Cl_2 extract (2.6 g) was chromatographed by VLC on silica gel eluting with light petrol, increasing the polarity with EtOAc giving isoflavanones **1** (70 mg), **2** (120 mg) and **3** (20 mg). Final purification was carried out by prep. TLC using light petrol–EtOAc (3:2) as eluent.

(3R)-5,7,2′,3′-tetrahydroxy-4′-methoxy-5′-prenylisoflavanone (**1**). mp 256–258°C; $[\alpha]_D$ +3° (MeOH, *c* 0.43); IR ν_{max} cm^{-1}: 3378, 2923, 1636, 1466, 1376, 1274, 1162, 1087, 1027. EI-MS *m/z* (rel. int.) 386 (47) [M$^+$], 331 (4), 260 (24), 245 (20), 234 (8), 154(35), 153 (100), 77 (15), 69 (35), 55 (15). For ^{1}H and ^{13}C NMR see Tables 1 and 2.

(3R)-7,2′,3′-trihydroxy-4′-methoxy-5′-prenylisoflavanone (**2**). mp 152–154°C; $[\alpha]_D$ +6° (MeOH, *c* 0.56); IR ν_{max} cm^{-1}: 3357, 2916, 1601, 1504, 1467, 1372, 1274, 1162, 1084, 1026, 836. EI-MS *m/z* (rel. int.) 370 (56) [M$^+$], 315 (5), 234 (13), 219 (12), 179 (10), 178 (12), 137 (100), 69 (28), 55 (15). For ^{1}H and ^{13}C NMR see Tables 3 and 2, respectively.

(3S)-3,7,2′,3′-tetrahydroxy-4′-methoxy-5′-prenylisoflavanone (**3**). mp 214–216°C; $[\alpha]_D$ +9.3° (MeOH, *c* 1.19); IR ν_{max} cm^{-1}: 3372, 1602, 1468,

Table 4. ^{1}H NMR, HMQC and HMBC data for compound **3** (*J* given in Hz in parentheses)

H	δ	HMQC	HMBC
2a	4.21 *d* (12.0)	74.9 *t*	C-3, C-4, C-9, C-1′
2b	4.88 *d* (12.0)	74.9 *t*	C-4, C-9, C-1′
5	7.80 *d* (8.6)	130.9 *d*	C-4, C-7, C-9
6	6.55 *dd* (8.6, 2.0)	112.0 *d*	C-8, C-10
8	6.34 *d* (2.0)	103.5 *d*	C-6, C-9, C-10
6′	6.77 *s*	118.6 *d*	C-3, C-2′, C-4′, C-1″
1″	3.25 *d* (7.4)	28.8 *t*	C-4′, C-5′, C-6′, C-2″, C-3″
2″	5.22 *t* (7.4)	124.5 *d*	C-4″, C-5″
4″	1.68 *s*	17.8 *q*	C-2″, C-3″, C-5″
5″	1.71 *s*	25.9 *q*	C-2″, C-3″, C-4″
OMe	3.76 *s*	60.9 *q*	C-4′

1374, 1277, 1162, 1091. EI-MS *m/z* (rel. int.) 386 (6) [M^+],. 369 (13), 368 (47), 313 (12), 299 (13), 250 (4), 138 (12), 137 (100), 121 (12), 53 (11). For ^1H and ^{13}C NMR see Tables 4 and 2, respectively.

Lupeol and *lupenone* were identified by comparison of their chromatographic and spectroscopic data ($[\alpha]_D$, IR, MS, ^1H and ^{13}C NMR) with those available in the literature [12].

Acknowledgements—The authors are grateful to the Guarani communities of the Izozog (Bolivia) as well as M. Sauvain (ORSTOM) for their collaboration during the ethnobotanic work. Part of this work was financially supported by CIRIT-CICYT (project QFN95-4711) and the Comissionat per Universitats i Recerca de la Generalitat de Catalunya. The authors are also grateful for the financial support provided by the Programa de Cooperación Científica con Iberoamérica.

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