

Isoflavonoids from Seeds of *Milletia griffoniana* (Bail), 15*

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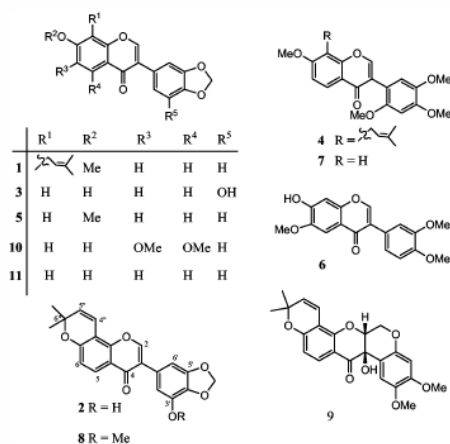
Dedicated to Professor Ermias Dagne on the occasion of his 60th birthday

Three new isoflavonoids, griffonianone F, G and H were isolated from the seeds of *Milletia griffoniana*, along with the known prebarbigerone, pseudobaptigenin, pseudobaptigenin methyl ether, tephrosin, dipteryxine, odoranthine, 7,4',5'-tetramethoxyisoflavone and iso-jamaicin. Their structures were assigned on the basis of spectroscopic data and chemical transformations.

Key words: *Milletia griffoniana*, Leguminosae, Papilionoideae, Isoflavones

Introduction

The genus *Milletia* (subfamily: Papilionoideae, family: Leguminosae) consists of ca. 100 species of climbers and trees, and many of its members have been shown to possess insecticidal properties [2]. Previous work on various plant parts of *M. griffoniana* has resulted in the isolation of prenylated isoflavones: griffonianone A, B, C [3], and D [4], geranylated isoflavones and 3-phenylcoumarin [5, 6], and recently we have characterized antiparasitic isoflavonoids from the hexane extract of seeds of *Milletia griffoniana* Bail [1]. We now report the isolation and structural elucidation of three new isoflavonoids, griffonianone F (1), G (2), and H (3) along with eight known compounds, namely: 7,2',4',5'-tetramethoxy-8-prenylisoflavone (prebarbigerone, 4) [7], pseudobaptigenin methyl ether (5) [8], 7-hydroxy-6,3',4'-trimethoxyisoflavone (odoranthin, 6) [9], 7,2',4',5'-tetramethoxyisoflavone (7) [10], iso-jamaicin (8) [11], tephrosin (9) [12], 7-hydroxy-3',4'-methylenedioxyisoflavone (dipteryxine, 10), [9] and (pseudobaptigenin, 11) [13] from the acetone extract of the same part of the plant. The known compounds were identified by compari-



son of their spectroscopic data with those in the literature.

Results and Discussion

Compound 1 was obtained as a white powder, m.p 79–80 °C. The molecular formula, C₂₂H₂₀O₅, was obtained by HREIMS (*m/z* 364.1024, M⁺).

* Part 15 of the series *Milletia* of Cameroon. For part 14, see ref [1].

Table 1. ^1H , ^{13}C NMR and HMBC data of griffonianone F (1) in CDCl_3 and griffonianone H (3) in DMSO-d_6 .

Position	$^1\text{H NMR}^*$	Griffonianone F (1)			$^1\text{H NMR}^*$	Griffonianone H (3)		
		$^{13}\text{C NMR}$	HMBC**	$^2J_{\text{CH}}$		$^{13}\text{C NMR}$	HMBC**	$^2J_{\text{CH}}$
2	7.98 s	152.9	C-3	C-1', C-4, C-9	8.33 s	152.4	C-2	C-1', C-9
3	—	124.5	—	—	—	127.7	—	—
4	—	176.7	—	—	—	174.9	—	—
5	8.19 d (8.8)	125.6	C-6, C-10	C-4, C-7, C-9	7.98 d (8.7)	128.0	C-6, C-10	C-4, C-7
6	7.02 d (8.8)	114.3	C-5, C-7	C-9, C-10	6.99 dd (8.7, 1.6)	115.0	C-5, C-7	C-8, C-10
7	—	161.3	—	—	—	162.9	—	—
8	—	118.9	—	—	—	100.5	—	—
9	—	155.9	—	—	—	157.8	—	—
10	—	109.5	—	—	—	116.9	—	—
1'	—	126.3	—	—	—	125.5	—	—
2'	7.11 d (1.6)	110.2	C-1', C-3'	C-4', C-6'	6.92 d (1.5)	102.1	C-3', C-1''	C-4', C-6'
3'	—	147.9	—	—	—	148.5	—	—
4'	—	148.1	—	—	—	137.7	—	—
5'	6.86 d (8.8)	108.7	C-4', C-6'	C-1', C-3'	—	144.7	—	—
6'	6.95 dd (1.6, 8.8)	109.2	C-1', C-5'	C-3, C-2', C-4'	6.88 d (1.5)	110.3	C-1', C-5'	C-2', C-4'
1''	3.55 d (6.7)	22.5	C-2'', C-8	C-7, C-9, C-3''	—	—	—	—
2''	5.24 t (6.7)	121.8	C-1'', C-3''	C-8, C-4'', C-5''	—	—	—	—
3''	—	132.8	—	—	—	—	—	—
4''	1.71 s	26.1	C-3''	C-2'', C-5''	—	—	—	—
5''	1.84 s	18.2	C-3''	C-2'', C-4''	—	—	—	—
OCH ₃	3.96 s	56.5	—	C-7	—	—	—	—
O-CH ₂ -O	5.99 s	101.5	—	C-3', C-4'	6.05 s	101.4	—	C-3', C-4'

* Coupling constants in Hz are given in parentheses; ** HMBC correlations are shown from protons to carbons.

The isoflavone nature was deduced from its IR (ν 1630 cm^{-1}), UV/vis (λ_{max} 249 and 295 nm) and NMR ($\delta_{\text{H}} = 7.98$, s, H-2, and $\delta_{\text{C}} = 152.9$, C-2) [14] spectra. The ^{13}C NMR (Table 1) along with the DEPT spectra, revealed the presence of three methyls, two methylenes, seven methines and ten quaternary carbons, including a carbonyl at $\delta_{\text{C}} = 176.7$. A vinyl proton triplet at $\delta_{\text{H}} = 5.24$ ($^2J = 6.7$ Hz), a two protons doublet at $\delta_{\text{H}} = 3.55$ ($^2J = 6.7$ Hz) and two methyl signals at $\delta_{\text{H}} = 1.71$ and 1.84 suggested the presence of a 3,3-dimethylallyl group. The ^1H NMR spectrum also showed signals assignable to one methoxyl group at $\delta = 3.96$ and a methylenedioxy substituent at $\delta_{\text{H}} = 5.99$. The presence of one set of deshielded *ortho*-coupled doublet at $\delta = 8.19$ and 7.02, ($^2J = 8.7$ Hz), indicated that the isoflavone nucleus lacks substitution at C-5 and C-6 positions. The placement of the dimethylallyl moiety and the methoxyl group on ring A was possible from the presence in the mass spectrum of a fragment with m/z 218. The methylenedioxy substituent is, therefore, on ring B. The aromatic region also showed an ABM spin-coupled system at $\delta = 6.86$ (1H, d, 8.8 Hz) 6.95 (1H, dd, 1.6 Hz and 8.8 Hz) and $\delta = 7.11$ ppm (1H, d, 1.6 Hz), which, together with the ion fragment observed at m/z 146, resulting from the

RDA fission, allowed us to conclude that ring C is substituted only with a methylenedioxy group at positions 3' and 4'. Additional evidence in support of the above substitution pattern came from the HMBC spectrum (Table 1), which revealed among others, correlations between the oxygenated carbon signal at $\delta_{\text{C}} = 161.3$ (C-7) and the resonance of the two methylene protons at $\delta_{\text{H}} = 3.55$ (H-1'') on the one hand, and with the aromatic proton (H-5) on the other, and between proton at $\delta = 7.11$ (H-2') and carbons at $\delta = 120.4$ (C-1'') and 147.9 (C-3'). The foregoing analysis led to the definition of the structure of 1 as 7-methoxy-8-(3,3-dimethylallyl)-3',4'-methylenedioxyisoflavone, a novel compound for which the trivial name griffonianone F is proposed.

Compound 2 was obtained as a white powder m. p. 210–211 °C. The molecular formula $\text{C}_{21}\text{H}_{16}\text{O}_6$ was suggested from HREIMS (m/z 364.1023). It reacted positively with FeCl_3 , and methylation of its phenolic hydroxy group gave a mono-methyl ether clearly giving the partial structure $\text{C}_{21}\text{H}_{15}\text{O}_5(\text{OH})$. The presence in its ^1H NMR spectrum (Table 2) of a singlet for H-2 at $\delta_{\text{H}} = 8.25$ and two relatively deshielded *ortho*-coupled doublets at $\delta = 7.98$ and 6.91 ($^2J = 8.7$ Hz) indicated an isoflavone nucleus oxygenated at C-7 and

Table 2. ^1H , ^{13}C NMR and HMBC data of griffonianone G in acetone- d_6 (2).

Positions	^1H NMR*	Griffonianone G (2)		
		^{13}C NMR	HMBC**	
			$^2J_{\text{CH}}$	$^3J_{\text{CH}}$
2	8.25 s	153.1	C-3	C-1', C-4, C-9
3	—	127.5	—	—
4	—	175.5	—	—
5	7.98 d (8.7)	126.6	C-6, C-10	C-4, C-7, C-9
6	6.91 d (8.7)	115.3	C-5, C-7	C-8, C-10
7	—	150.7	—	—
8	—	110.6	—	—
9	—	158.4	—	—
10	—	118.4	—	—
1'	—	125.5	—	—
2'	6.73 d (1.5)	102.1	C-1', C-3'	C-3, C-4', C-6'
3'	—	149.4	—	—
4'	—	135.9	—	—
5'	—	141.2	C-4', C-6'	C-1', C-3'
6'	6.82 d (1.5)	112.6	C-1', C-5'	C-3, C-2', C-4'
4''	6.80 d (10.0)	114.8	C-5'', C-8	C-7, C-9, C-6''
5''	5.93 d (10.0)	131.3	C-4'', C-6''	C-8, C-6''Me
6''	—	79.0	—	—
CH ₃	1.50 s	27.7	C-6''	C-5''
O-CH ₂ -O	5.99 s	101.9	—	C-4', C-5'

* Coupling constants in Hz are given in parentheses; ** HMBC correlations are shown from protons to carbons.

lacking substituents at C-5 and C-6 positions. The ^1H NMR further showed an AB-system ($^2J = 10$ Hz) at $\delta = 5.93$ and 6.80 which, together with the six-proton singlet at $\delta = 1.58$ ppm, suggested the presence of a 2,2-dimethylpyranyl ring. In the aromatic region, the *meta*-spin coupled system at $\delta = 6.73$ (*d*, 1.5 Hz) together with the proton singlet at $\delta = 5.93$ and the significant ion fragment observed at m/z 162, clearly established piperonyl nature of ring B and the presence of OH at position 3'. In its ^{13}C NMR (Table 2), the presence of signals at $\delta_{\text{C}} = 114.8$ and 131.3 coupled with significant ion fragment observed at m/z 187 indicated that the *gem*-dimethylpyranyl ring was fused angularly on ring A [15]. Compound 2 was identified as 3'-hydroxy-4',5'-methylenedioxy-6''-dimethylpyrano[2'',3'';7,8]isoflavone and the trivial name, griffonianone G, is suggested for it. Further supporting evidence for this assignment was obtained from the chemical conversion by methylation of griffonianone G to the known isojamaicin (8) obtained from the same extract.

Compound 3, obtained as a whitish powder, (m. p. 230–232 °C), was assigned the molecular formula C₁₆H₁₀O₆ from HREIMS (m/z 298.2470 M⁺). It reacted positively with FeCl₃ and was identified as an isoflavone through its IR (ν 1637 cm⁻¹) UV/vis (λ_{max}

245, 249 and 302 nm), ^1H NMR (8.33, *s*, H-2) and ^{13}C NMR spectra. The DEPT spectrum revealed signals for 16 different carbon atoms corresponding to one methylene, six methines and nine quaternary carbons including carbonyl carbon at $\delta = 174.9$. As in compound 1, the piperonyl nature of ring B was evident from its EIMS (m/z 162, RDA fission of ring C), its ^1H NMR spectrum which showed a 2H methylenedioxy proton signal at $\delta = 6.04$ and a *meta*-spin coupled system at $\delta = 6.88$ and 6.92 (1H each, *d*, 1.5 Hz) and its ^{13}C NMR spectrum which indicated a methylenedioxy carbon signal at $\delta_{\text{C}} = 101.4$ and two *meta*-oriented aromatic carbon signals at $\delta_{\text{C}} = 102.1$ and 110.3 (Table 1). The confirmation of the above assignment for griffonianone H was possible from HMBC experiment showing long range correlation between protons H-8, with two oxygenated carbon at $\delta = 162.9$ (C-7) and 157.7 ppm (C-9). Thus griffonianone H is identified as 7,3'-dihydroxy-4',5'-methylenedioxyisoflavone.

Isojamaicin (8) was obtained as white powder (m. p. 205–206 °C) and gave a negative FeCl₃ test. The HREIMS revealed a molecular ion peak at m/z 378.1204, consistent with the molecular formula C₂₂H₁₈O₆. The ^1H NMR spectrum was similar with that reported in the literature [11]. The structure assignment is confirmed by the ^{13}C and 2D-NMR spectral data.

Experimental Section

General

Melting points were determined using a Reichert microscope apparatus and are uncorrected. UV spectra were taken in MeOH. IR spectra were taken in KBr disks. EIMS were obtained at 70 eV ionization energy. NMR spectra were taken on a Bruker DMX 300 spectrometer (^1H 300 MHz and ^{13}C 75 MHz) in CDCl₃ solution using TMS as internal standard, unless otherwise stated. Si gel 60F₂₅₄ (230–400 mesh, Merck) and 60 F₂₅₄ (Merck) were used for column chromatography with petrol containing increasing amounts of EtOAc as eluent. Spots were viewed by UV illumination at 254 and 366 nm.

Plant material

The seeds of *Milletia griffoniana* Bail. were collected in November 2001 at Onguesse, in the central province of Cameroon. A voucher specimen (No. 32315/SRF/HNC) identified by Mr. Nana has been deposited at National Herbarium, Yaounde, Cameroon.

Extraction and isolation

The dried and powdered seeds of *M. griffoniana* (2 kg) were extracted with *n*-hexane and acetone (for each: 24 h, 3 × 20 l) at room temperature yielding 460 g *n*-hexane and 150 g acetone extracts. The acetone extract was subjected to flash column chromatography over Si gel with gradients of EtOAc in petrol. A total of 130 fractions of ca. 500 ml each were collected and combined on the basis of their TLC analysis leading to four major series: A (75 g, petrol-EtOAc 9:1), B (45 g, petrol-EtOAc 4:1), C (15 g, petrol-EtOAc 1:1) and D (10 g, EtOAc). Series A were a mixture of oil and series B shown by TLC (Liebermann-Buchard reagent) to contain triterpenoids. These two series were not investigated. Further purification of series C and D was achieved by column chromatography, PTLC or gel permeation through Sephadex LH20. Series C afforded **1** (30 mg), **4** (50 mg), **5** (25 mg), **6** (60 mg), **7** (50 mg) and **8** (45 mg) while series D afforded **9** (25 mg), **2** (40 mg), **10** (70 mg) and **3** (7 mg).

Griffonianone F (1)

White powder. – M. p. 79–80 °C. – $R_f = 0.70$ [toluene / petrol / ethyl acetate (3:3:4)]. – UV/vis (MeOH) $\lambda_{max} = 294, 244$ nm. – IR (KBr) $\nu = 2950, 1630, 1610, 1460, 1300, 1275, 1245, \text{ and } 724$ cm⁻¹. – ¹H and ¹³C NMR (see Table 1). – MS (EI, 70 eV): m/z (%) = 365 (100) [M+H]⁺, 350 (41), 339 (22), 218 (44), 146 (10), 187 (15). – HRMS (EI, 70 eV): $M^+ / z = 364.1024$ (calcd. for C₂₂H₂₀O₅ 364.1311).

Griffonianone G (2)

White powder (30 mg). – M. p. 210–211 °C. – $R_f = 0.76$ [toluene/hexane/ethyl acetate (3:3:4)]. – UV/vis (MeOH): $\lambda_{max} = 249$ and 295 nm. – IR (KBr): $\nu = 3330, 2950, 1645, 1590, 1470, 1400, 1275, 1245, 1110, 983, 863, 810$ and 724 cm⁻¹. – ¹H and ¹³C NMR (see Table 2). – MS (EI, 70 eV): m/z (%) = 365 (100) [M+H]⁺, 350 (44), 322 (21), 187 (43), 162 (10). – HRMS (EI, 70 eV): $M^+ / z = 364.1023$ (calcd. for C₂₁H₁₆O₆ 364.0947).

Griffonianone H (3)

Whitish powder. – M. p. 230–232 °C. – $R_f = 0.69$ [toluene/petrol/ethyl acetate (3:3:4)]. – UV/vis (MeOH): $\lambda_{max} = 244, 249, \text{ and } 300$ nm. – IR (KBr): $\nu = 3400, 1650, 1620, 1530, 1470, 1310, 1275, 1, 1110, 983, 863, 810$ and 724 cm⁻¹. – ¹H and ¹³C NMR (see Table 1). – MS (EI, 70 eV): m/z (%) = 299 (100) [M+H]⁺, 270 (42), 136 (43), 162 (10). – HRMS (EI, 70 eV): $M^+ / z = 298.2470$ (calcd. for C₁₆H₁₀O₆, 298.0477).

Isojamaicin (8)

White powder. – M. p. 205–206 °C. – UV/vis (MeOH): $\lambda_{max} = 249, 295$ nm. – IR (KBr): $\nu = 2950, 1650, 1620, 1530, 1470, 1310, 1275, 1245, 1110, 983, 863, 810$ and 724 cm⁻¹. – ¹H (300 MHz, CDCl₃): $\delta = 1.50$ (6H, s, 6''-Me₂), 3.94 (3H, s, OCH₃), 5.99 (2H, s, OCH₂O), 5.93 (1H, d, ³J = 1.5 Hz, H-6''), 6.80 (1H, d, ²J = 10 Hz, H-4''), 6.87 (1H, d, ³J = 1.5 Hz, H-2'), 6.90 (1H, d, ²J = 8.7 Hz, H-6), 7.95 (1H, s, H-2), 8.06 (1H, d, ²J = 8.7 Hz, H-5). – ¹³C {¹H}-NMR (75 MHz, CDCl₃): $\delta = 27.7$ (6''-Me₂), 58.3 (OCH₃), 79.0 (C-2''), 101.9 (OCH₂O), 102.1 (C-2'), 110.6 (C-8), 112.6 (C-6'), 114.8 (C-3''), 115.3 (C-6), 118.4 (C-10), 125.5 (C-1'), 126.6 (C-5), 127.5 (C-3), 131.3 (C-4''), 135.9 (C-4'), 141.2 (C-5'), 149.4 (C-3'), 150.7 (C-7), 153.1 (C-2), 158.4 (C-9), 175.5 (C-4). – MS (EI 70 eV): m/z (%) = 379 (100) [M+H]⁺, 364 (44), 350 (22), 187 (44), 176 (10). – HRMS (EI 70 eV): $M^+ / z = 378.1204$ (calcd. for C₂₂H₁₈O₆, 378.1103).

Methylation of Griffonianone G

Compound **2** (20 mg) was methylated with Me₂SO₄-K₂CO₃ in Me₂CO to give the methylether (isojamaicin (**8**)), m. p. 205–206 °C, identical in all respects to a natural sample.

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