Two New Flavone Glycosides from *Paullinia pinnata*

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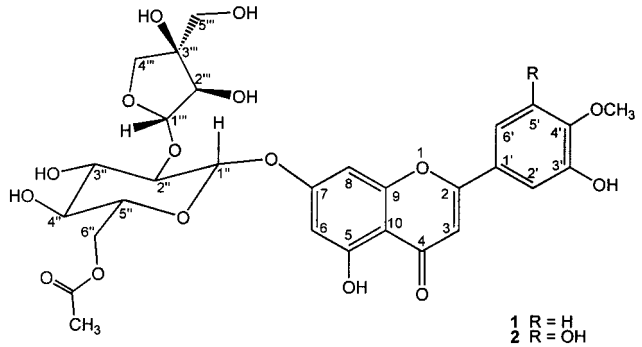
Phytochemical investigation of the leaves of *Paullinia pinnata* L. (Sapindaceae) resulted in the isolation of the two new flavone glycosides characterized as diosmetin-7-*O*-(2″-*O*-*β*-D-apiofuranosyl-6″-acetyl-*β*-D-glucopyranoside) (**1**) and tricetin-4′-*O*-methyl-7-*O*-(2″-*O*-*β*-D-apiofuranosyl-6″-acetyl-*β*-D-glucopyranoside) (**2**).

Paullinia pinnata L. (Sapindaceae) is an African woody vine widely used in traditional medicine for the treatment of human malaria.[1] Previous phytochemical and pharmacological investigations have shown the presence of triterpene saponins and cardiotonic catechol tannins in *P. pinnata* collected from West Africa.[2,3] On the other hand, numerous reports on a related species, *P. cupana*, commonly known as guarana, appear in the current literature and focus mainly on the caffeine and essential oil content of the seeds.[4] Seeds of other *Paullinia* species, such as *P. meliaefolia* and *P. elegans*, have been studied only for their lipid and fatty acid contents.[5,6] Phytochemical investigation of the aerial parts of *P. pinnata* has resulted in the isolation of two new flavonoid glycosides (**1** and **2**), the structure elucidation of which will be discussed herein.



Flavone glycoside **1** was obtained from an EtOAc fraction of the total extract. The ^{1}H NMR spectrum of **1** showed signals of two distinct regions, an upfield region from *δ* 3.2 to 5.4 indicative of sugars and a downfield aromatic region characteristic of a flavonoid aglycon. The ^{1}H signal at *δ* 12.87 suggested a hydroxyl group at C-5. This was supported by the UV bathochromic shift resulting from the addition of $AlCl_3$ shift reagent and which was unchanged after addition of HCl.[7] The ^{1}H signals at *δ* 2.02 (3H, s) and 3.86 (3H, s) suggested an acetyl moiety and a methoxy group, respectively. The ^{13}C NMR and DEPT 135 spectra showed 29 carbon signals for two methyls, three methylenes, 13 methines, and 11 quaternaries. Three of the 29 carbons belonged to methoxy (*δ* 55.9) and acetoxy substituents (*δ* 20.6, 170.3). The presence of three methylenes at *δ* 63.4, 64.3, and 74.1 among the remaining 11 carbons indicated that the flavonoid aglycon was linked to a hexose and a pentose, and that the pentose was most probably apiose. On reviewing the literature, the ^{1}H and ^{13}C NMR data of the aglycon matched those reported for 5,7,3′-trihydroxy-4′-methoxyflavone (diosmetin),[8,9] which was confirmed by 2D NMR (COSY and HMBC). For the sugar moiety, ^{13}C chemical shifts were in accord with a 2-*β*-D-apiosyl-6-acetyl-*β*-D-glucoside. The apiosyl anomeric carbon C-1‴ showed a signal at *δ* 108.9, along with the signals of one quaternary carbon at *δ* 79.4 (C-3‴), an oxymethine at *δ* 76.2 (C-2‴), and two oxymethylenes at *δ* 74.1 (C-4‴) and 64.3 (C-5‴).[10] For the inner sugar, ^{13}C chemical shifts were in accord with those reported for 6-acetyl glucoside,[11] with a downfield shift of the C-6″ by ca. 2 ppm and an upfield shift of the C-5″ by ca. 3 ppm from normally expected values. HMBC correlations between the H-6″ protons (*δ* 4.06 and 4.36) and the acetyl carbonyl carbon (*δ* 170.3) established C-6″ as the position of acetylation. Also, 3-bond correlations between the apiosyl anomeric proton H-1‴ (*δ* 5.36) and the glucosyl carbon C-2″ (*δ* 75.7), as well as correlations between the glucosyl proton H-2″ (*δ* 3.57) and the apiosyl anomeric carbon C-1‴ (*δ* 108.8), established the linked position between the two sugar moieties. The large coupling constant between H-1″ and H-2″ (7.1 Hz) was typical of a *β*-glucosidic linkage to the flavonoid aglycon, which is in line with observed data for flavone glucosides.[12,13] The glycosidation position was unambiguously determined by 3-bond correlation between the glucosyl anomeric proton H-1″ (*δ* 5.22) and ring A C-7 (*δ* 162.6) using gradient-selected HMBC.[14] Finally, acid hydrolysis of the glycoside followed by TLC analysis showed that D-glucose was one of the two sugars, in addition to D-apiose. The structure of **1** was thus established as diosmetin-7-*O*-(2″-*O*-*β*-D-apiofuranosyl-6″-acetyl-*β*-D-glucopyranoside).

The flavone glycoside (**2**) was obtained from the same EtOAc fraction as **1**. The ^{1}H NMR spectrum of **2** was almost identical with that of **1** in the upfield region from *δ* 2.02 to 5.36, indicating the same identity and pattern of sugar substitution. The aromatic region, however, showed fewer signals than previously observed for **1**. The doublets at *δ* 6.43 and 6.68 were assigned to H-6 and H-8, respectively, and indicated that ring A had the same 5,7-dioxygenated pattern as **1**. The ^{1}H signal at *δ* 6.99 (2H, s) was assigned to H-2′ and H-6′ and suggested a 3′,4′,5′ trisubstitution with identical groups at C-3′ and C-5′. A literature survey of the ^{13}C chemical shifts on model flavonoids revealed that the flavonoid aglycon was 5,7,3′,5′-trihydroxy-4′-methoxy-

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flavone (4′-*O*-methyltricetin).[15] COSY and HMBC correlations for **2** were almost identical with those of **1** and indicated that the only difference between the two compounds was the hydroxylation of **2** at C-5′. Mass spectral data were in accord with the proposed structure for **2** (*m*/*z* 652) with 16 mass units in excess of **1**. Thus, **2** was assigned as tricetin-4′-*O*-methyl-7-*O*-(2″-*O*-*β*-D-apiofuranosyl-6″-acetyl-*β*-D-glucopyranoside).

The isolated flavonoid glycosides **1** and **2** are reported here for the first time. Further phytochemical and biological investigation of this herb is currently being pursued in order to validate its use in folk medicine.

Experimental Section

General Experimental Procedures. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. The IR spectra were recorded with an ATI Mattson Genesis Series FT−IR spectrophotometer. UV spectra were obtained with a Hitachi U2000 dual beam spectrophotometer. The ^{1}H and ^{13}C NMR spectra were obtained in DMSO-d_6 on a Bruker Avance DRX-400 FT spectrometer operating at 400 and 100 MHz, respectively. Gradient HMBC experiments were conducted on a Bruker Avance DRX-500 FT spectrometer operating at 500 MHz. HRESIMS was conducted on a Bruker BioApex 3.0 at the University of Mississippi. TLC analyses were carried out on precoated Si 250F (Merck) Si gel plates. The developing systems used were as follows: A (for glycosides), 0.1% HCO_2H−CH_3CN (65:35, v/v) and B (for sugars), EtOAc−*n*-BuOH−H_2O (20:70:10, v/v). Visualization of plates was performed using visible light, UV light (365 nm), natural product−poly(ethylene glycol) (NP/PEG),[16] and 50% H_2SO_4 spray reagents. For column chromatography, the adsorbent used was normal-phase (63−200 mesh, Merck) and reversed-phase C_{18} Si gel (Baker).

Plant Material. The leaves of *P. pinnata* L. were collected in April 1997, in the Northwest Province of Cameroon. A voucher specimen, verified by Dr. Clare Wirmum of Medicinal Foods and Plants Research Center, Bamenda, Cameroon, is deposited at the Heifer Project International Herbarium, Bamenda, Cameroon.

Extraction and Isolation. The air-dried leaves of *P. pinnata* (355 g) were crushed to fine powder in a waring blender and macerated three times with 1.5 L 95% EtOH at room temperature for 72 h. The extract was filtered and the filtrate reduced in vacuo to a greenish residue that was dissolved in 150 mL of MeOH and partitioned between H_2O and hexane (50:100, v/v). The aqueous portion was further extracted with EtOAc (5 × 100 mL). The EtOAc portion was concentrated under vacuum to yield 18.9 g of a brown powder. The EtOAc extract (8.0 g) was chromatographed on a Si gel column using a gradient of 0−100% MeOH in $CHCl_3$ (400-mL fractions). A total of 68 fractions was collected and pooled to 14 major fractions based on TLC analysis. Fraction 7, eluted with 20% MeOH, yielded a yellow residue (886 mg). The yellow residue (200 mg) was rechromatographed on a C_{18} column (40 g, 54 × 2 cm) eluting with increasing gradient of 40−100% MeOH in H_2O. A total of 20 fractions was collected and pooled to three major fractions (F1, F2, F3) based on TLC analysis. F2 was dissolved in 20% aqueous MeOH, passed through a C_{18} Waters Sep-pak cartridge, and concentrated under vacuum to yield **1** (56.4 mg) and **2** (29.3 mg).

Acid Hydrolysis of 1. Glycoside **1** (3 mg) was refluxed in 2 N HCl (0.5 mL) for 2 h. The aglycon was extracted with EtOAc (0.3 mL × 3). After separating the organic layer, the aqueous phase was neutralized with $NaHCO_3$ and lyophilized. The lyophilized residue was dissolved in pyridine (0.2 mL) and analyzed by TLC in solvent B.

Diosmetin-7-*O*-(2″-*O*-*β*-D-apiofuranosyl-6″-acetyl-*β*-D-glucopyranoside) (1): pale yellow amorphous powder, mp 221−223 °C; R_f 0.47 (system A); UV (MeOH) λ_{max} (log ϵ) 252 (4.28), 267 (4.27), 339 (4.32) nm, (MeOH−$AlCl_3$) 275 (4.31), 356 (4.22), 384 (4.21) nm, unchanged in the presence of HCl; IR (KBr) ν_{max} 3444, 2925, 2825, 1744, 1655, 1614 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 637.1788 (calcd for $C_{29}H_{33}O_{16}$ [M + H]$^+$ 637.1768).

Table 1. ^{1}H and ^{13}C NMR[a] Data of **1** and **2** in DMSO-d_6 at 400 and 100 MHz, Respectively

	1		**2**	
carbon	δ C	δ H (m, *J* Hz)	δ C	δ H (m, *J* Hz)
2	164.2 s		164.2 s	
3	103.9 d	6.80 (s)	104.7 d	6.69 (s)
4	182.0 s		182.1 s	
5	161.2 s		161.3 s	
6	99.5 d	6.40 (d, 2.0)	99.5 d	6.43 (d, 2.0)
7	162.6 s		162.7 s	
8	94.8 d	6.70 (d, 2.0)	94.8 d	6.68 (d, 2.0)
9	157.0 s		157.0 s	
10	105.6 s		105.7 s	
5-O*H*		12.87 (s)		12.89 (s)
1′	122.9 s		125.7 s	
2′	113.2 d	7.40 (d, 2.1)	106.0 d	6.99 (s)
3′	146.9 s		151.3 s	
4′	151.4 s		139.2 s	
5′	112.2 d	7.08 (d, 8.5)	151.3 s	
6′	118.9 d	7.55 (dd, 8.5, 2.1)	106.0 d	6.99 (s)
O*C*H$_3$	55.9 q	3.86 (s)	60.0 q	3.78 (s)
1″	97.9 d	5.22 (d, 7.1)	97.9 d	5.23 (d, 7.1)
2″	75.7 d	3.57 (m)	75.7 d	3.56 (m)
3″	76.6 d	3.53 (m)	76.6 d	3.54 (m)
4″	70.1 d	3.20 (dd, 9.0, 9.0)	70.1 d	3.20 (dd, 9.0, 9.0)
5″	73.8 d	3.77[b]	73.8 d	3.78[b]
6″	63.4 t	4.06 (dd, 11.3, 6.9)	63.4 t	4.06 (dd, 11.4, 7.0)
		4.36 (dd, 11.3, 1.4)		4.34 (dd, 11.4, 1.5)
*C*H$_3$CO	20.6 q	2.02 (s)	20.7 t	2.01 (s)
CH$_3$*C*O	170.3 s		170.3 s	
1‴	108.9 d	5.36 (br s)	108.8 d	5.35 (s)
2‴	76.2 d	3.76[b]	76.2 d	3.78[b]
3‴	79.4 s		79.4 s	
4‴	74.1 t	3.69 (d, 9.3)	74.1 t	3.67 (d, 9.3)
		3.95 (d, 9.3)		3.93 (d, 9.3)
5‴	64.3 t	3.34 (s)	64.3 t	3.32 (s)

[a] ^{13}C multiplicities were determined by DEPT 135 experiment. [b] A multiplet of overlapping signals in the range 3.53−3.57 and 3.75−3.78 ppm, respectively.

Tricetin-4′-*O*-methyl-7-*O*-(2″-*O*-*β*-D-apiofuranosyl-6″-acetyl-*β*-D-glucopyranoside) (2): pale yellow amorphous powder, mp 230−232 °C; R_f 0.40 (system A); UV (MeOH) λ_{max} (log ϵ) 267 (4.34), 334 (4.32) nm, (MeOH−$AlCl_3$) 275 (4.30), 344 (4.16), 385 (4.13) nm, unchanged in the presence of HCl; IR (KBr) ν_{max} 3426, 2924, 2854, 1727, 1657, 1617 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HRESIMS *m*/*z* 653.1729 (calcd for $C_{29}H_{33}O_{17}$ [M + H]$^+$ 653.1718).

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References and Notes

(1) Chhabra, S. C.; Mahunnah, R. L. A.; Mshiu, E. N. *Ethnopharmacology* **1991**, *33*, 143−157.
(2) Kerharo, J.; Adam, J. G. *La Pharmacopee Senegalaise Tradionelle*; Vigot: Paris, 1974.
(3) Bowden, K. *J. Pharmacol.* **1962**, *18*, 173−174.
(4) Benoni, H.; Dallakian, P.; Taraz, K. *Z. Lebensm.-Unters. Forsch.* **1996**, *203*, 95−98.
(5) Spitzer, V. *J. High Resolut. Chromatogr.* **1995**, *18*, 413−416.
(6) Spitzer, V. *Phytochemistry* **1996**, *42*, 1357−1360.
(7) Mabry, T. J.; Markham, K. R.; Thomas, M. B. In *The Systematic Identification of Flavonoids*; Springer-Verlag: New York, 1970; pp 51−52.
(8) Ramesh, P.; Nair, A. G. R.; Subramanian, S. *Curr. Sci.* **1979**, *48*, 67.
(9) Timmermann, B. N.; Mues, R.; Mabry, T. J.; Powell, A. M. *Phytochemistry* **1979**, *18*, 1855.
(10) Hamburger, M.; Gupta, M.; Hostettmann, K. *Phytochemistry* **1985**, *24*, 2689−2692.

(11) Markham, K. R.; Ternai, B.; Stanley, R.; Geiger, H.; Mabry, T. J. *Tetrahedron* **1978**, *34*, 1389−1397.
(12) Mabry, T. J.; Markham, K. R.; Thomas, M. B. In *The Systematic Identification of Flavonoids*; Springer-Verlag: New York, 1970; pp 268−269.
(13) Harborne, J. B.; Williams, C. A. In *The Flavonoids: Advances in Research*; Harborne, J. B., Mabry, T. J., Eds.; Chapman and Hall: London, 1982; p 265.
(14) Wilker, W.; Leibfritz, D.; Kerssebaum, R.; Bermel, W. *Magn. Reson. Chem.* **1993**, *31*, 287−292.
(15) Gaydou, E. M.; Bianchini, J. P. *Bull. Soc. Chim. Fr.* **1978**, 43.
(16) Wagner, H.; Bladt, S. *Plant Drug Analysis*; Springer: Berlin, 1996; p 362.

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