A New *C*-Glucosylflavone from *Sorindeia juglandifolia*

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Phytochemical investigation of the leaves of *Sorindeia juglandifolia* A. Rich. led to the isolation and identification of a new *C*-glucosylflavone, 2'',6''-di-*O*-acetyl-7-*O*-methyl vitexin (**1**), together with seven known compounds, 2''-*O*-acetyl-7-*O*-methyl vitexin (**2**), mearnsitrin (**3**), robustaflavone (**4**), 3-*O*-galloyl catechin (**5**), tachioside (2-methoxy-benzene-1,4-diol-1-*O*-*β*-glucopyranoside) (**6**), 3*β*-*O*-D-glucopyranosyl-*β*-stigmasterol (**7**), and methyl gallate (**8**). The structures of **1** and the known compounds were established by IR, UV, MS, 1D, and 2D NMR spectra and by comparison with those of related compounds.

Key words: *Sorindeia juglandifolia*, Anacardiaceae, 2'',6''-Di-*O*-acetyl-7-*O*-methyl Vitexin, Chemotaxonomy

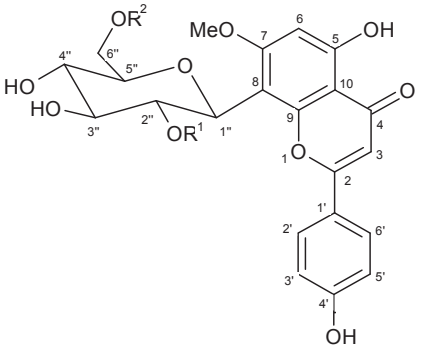
Introduction

Sorindeia juglandifolia A. Rich. (Anacardiaceae) is a tree or flowering shrub growing up to 5 m in height which is widely distributed in tropical Africa. In traditional folk medicine, the plant is used for the treatment of liver diseases, and a decoction of its leaves is used as gargle for mouth sores in children (Berhaut, 1971). Recently, the fruit extracts have been indicated to contain 2,3,6-trihydroxy benzoic acid and 2,3,6-trihydroxy (methyl) benzoate exhibiting potent antiplasmodial activities *in vitro* and *in vivo* (Kamkumo *et al.*, 2012). As part of our continuing chemical investigations of Cameroon medicinal plants (Pegnyemb *et al.*, 2005; Ngo Mbing *et al.*, 2009; Ndongo *et al.*, 2010), a new *C*-glucosylflavone, 2'',6''-di-*O*-acetyl-7-*O*-methyl vitexin (**1**), along with seven known compounds, 2''-*O*-acetyl-7-*O*-methyl vitexin (**2**), mearnsitrin (**3**), robustaflavone (**4**), 3-*O*-galloyl catechin (**5**), tachioside (2-methoxy-benzene-1,4-diol-1-*O*-*β*-glucopyranoside) (**6**), 3*β*-*O*-D-glucopyranosyl-*β*-stigmasterol (**7**), and methyl gallate (**8**), were isolated from the leaves of *S. juglandifolia* (Fig. 1). We report here the isolation and structure elucidation of compound **1**.

Results and Discussion

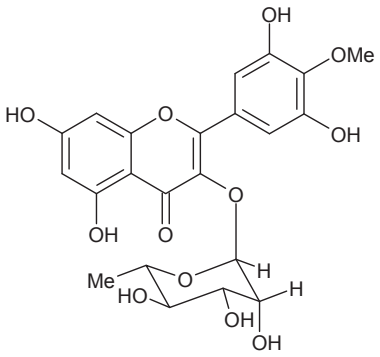
Structure elucidation

Compound **1** was obtained as an amorphous yellow solid, whose IR absorption bands were indicative of hydroxy (3418 cm^{-1}) and carbonyl groups (1720 cm^{-1}) and an aromatic ring (1607 cm^{-1}). The compound gave a positive Shinoda test (Shinoda, 1928), characteristic of flavonoids. The UV spectra identified **1** to bear two free hydroxy groups at positions 5 and 4' (Mabry *et al.*, 1970). The molecular formula $C_{26}H_{26}O_{12}$ was determined from the pseudo-molecular ion peaks of $[M+H]^+$ and $[M+Na]^+$, obtained by HR-ESI-MS. The ^1H NMR spectrum of **1** (Table I) showed a singlet at δ_H 13.37 ppm corresponding to a chelated hydroxy group at C-5 of the flavone aglycone, and a doublet at δ_H 4.94 ppm (J = 10.1 Hz) suggesting an anomeric proton of a *β*-linked sugar (Monties *et*

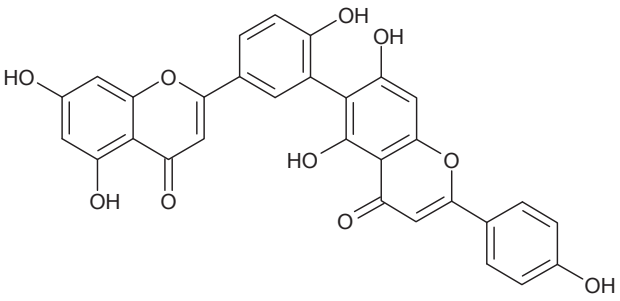


$R^1 = R^2$ = acetyl: 2",6"-Di-*O*-acetyl-7-*O*-methyl vitexin (**1**)

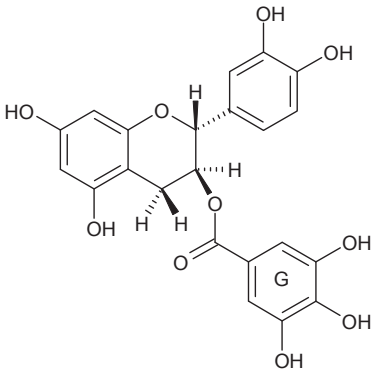
R^1 = acetyl, R^2 = H: 2"-*O*-Acetyl-7-*O*-methyl vitexin (**2**)



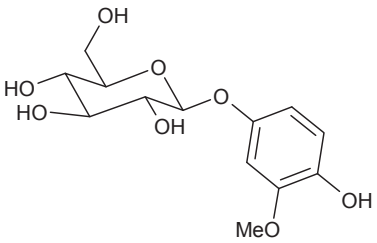
Mearnsitrin (**3**)



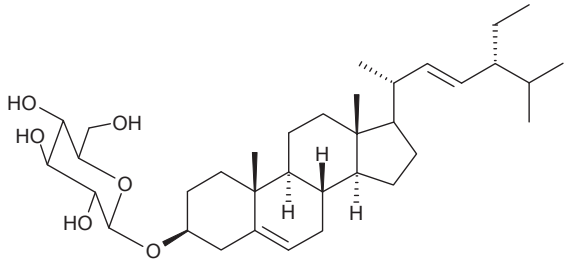
Robustaflavone (**4**)



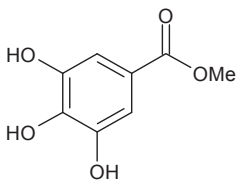
3-*O*-Galloyl catechin (**5**)



Tachioside (**6**)



3*β*-*O*-D-Glucopyranosyl-*β*-stigmasterol (**7**)



Methyl gallate (**8**)

Fig. 1. Chemical structures of compounds **1**–**8**.

Table I. ^{1}H (500 MHz, DMSO-d_6) and ^{13}C (125 MHz, DMSO-d_6) NMR data of compounds **1** and **2**.

No.	**1**		**2**	
	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)
2	–	163.9	–	164.2
3	6.86 (s)	102.4	6.84 (s)	102.3
4	–	181.9	–	182.0
5-OH/5-C	13.37 (s)	161.6	13.37 (s)	161.5
6	6.51 (s)	94.7	6.50 (s)	94.7
7	–	162.6	–	162.6
8	–	102.9	–	103.4
9	–	155.4	–	155.4
10	–	104.2	–	104.2
1’	–	121.2	–	121.3
2’/6’	8.04 (d; J = 8.9 Hz)	128.5	8.10 (d; J = 8.9 Hz)	129.0
3’/5’	6.95 (d; J = 8.9 Hz)	115.8	6.93 (d; J = 8.9 Hz)	115.7
4’	–	161.2	–	161.1
1”	4.94 (d; J = 10.1 Hz)	70.7	4.88 (d; J = 10.6 Hz)	70.6
2”	5.32 (t; J = 9.2 Hz)	72.1	5.28 (t; J = 9.3 Hz)	72.4
3”	3.59 (m)	75.2	3.53 (m)	75.5
4”	3.58 (m)	70.2	3.51 (m)	70.3
5”	3.51 (m)	78.3	3.34 (m)	82.0
6”	4.39 (dd; J = 17.4, 12.4 Hz) 4.10 (dd; J = 5.7, 17.4 Hz)	63.6	3.81 (dd; J = 16.9, 11.2 Hz) 3.59 (dd; J = 5.0, 16.9 Hz)	60.9
7-OCH_3	3.91 (s)	56.6	3.91 (s)	56.6
2”-O-*C*O-CH_3	–	168.9	–	168.9
6”-O-*C*O-CH_3	–	170.0	–	–
2”-O-CO-*C*H_3	1.65 (s)	20.3	1.68 (s)	20.3
6”-O-CO-*C*H_3	1.85 (s)	20.6	–	–

al., 1976). A 3H singlet at δ_H 3.91 ppm indicated a methoxy group at an aromatic ring. AA’BB’ resonances at δ_H 8.04 (2H, d, J = 8.9 Hz) and 6.95 ppm (2H, d, J = 8.9 Hz) indicated a *p*-disubstituted B-ring. The ^{13}C NMR spectrum (Table I) revealed 24 carbon signals, which suggested the presence of a flavonoid moiety, a sugar residue, a methoxy and – with respect to signals at $\delta_{C/H}$ 170.0/1.85 ppm and 168.9/1.65 ppm – two acetoxy groups in **1**. Six carbon signals between δ_C 63.6 and 78.3 ppm were assigned to a sugar residue, suggesting that **1** is a flavone *C*-glycoside. The sugar moiety was determined to be *β*-glucose from analysis of ^{1}H and ^{13}C NMR data. Compared with the resonances of the known compound **2** (Bombardelli *et al.*, 1974; Ngo Mbing *et al.*, 2009; Ndongo *et al.*, 2010), the data suggested the presence of the vitexin skeleton moiety in **1**. The signals of C-5” (δ_C 78.3 ppm) and C-6” (δ_C 63.6 ppm) of the sugar moiety showed a downfield shift of $\Delta\delta$ 3.7 ppm and an upfield shift of $\Delta\delta$ 2.7 ppm, respectively, compared with the corresponding data (δ_C 82.0 ppm, 60.9 ppm) of **2**; the other shifts remained nearly unchanged. These data revealed that one of the two acetate groups is attached to the C-6” position of the sugar moiety. This was further established by HMBC experiments (Fig. 2), where long-range correlations between the diastereotopic protons at δ_H 4.39 ppm (H-6”a) and 4.10 ppm (H-6”b) and the ester carbonyl carbon atom at δ_C 170.0 ppm (COO-6”) were observed. Although the second acetate group did not show an HMBC cross-signal with

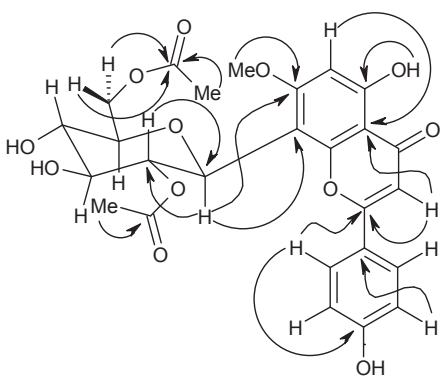


Fig. 2. HMBC correlations for compound **1**.

the sugar moiety or the aglycone as in **2**, it was confirmed to be also at C-2'' by comparing the data with those of the latter and with those of vitexin (Lin *et al*., 1999). The carbon signal of C-2'' (δ_C 72.1 ppm) showed a downfield shift of only $\Delta\delta$ 0.3 ppm in comparison with the corresponding signal (δ_C 72.4 ppm) of **2**, but an upfield shift of $\Delta\delta$ 1.2 ppm with respect to vitexin (δ_C 70.9 ppm).

Key correlations were observed between the anomeric proton at δ_H 4.94 ppm and carbon atom signals at δ_C 102.9 ppm (C-8), 155.4 ppm (C-9), and 162.6 ppm (C-7), indicating that the sugar moiety is attached at C-8 of the flavone aglycone. The methoxy singlet at δ_H 3.91 ppm coupled with the carbon signal at δ_C 162.6 ppm (C-7), establishing the position of OMe at C-7 of the flavone unit. From the foregoing observations, compound **1** was characterized as 2'',6''-di-*O*-acetyl-7-*O*-methyl vitexin (Fig. 1).

Chemotaxonomical importance

The family Anacardiaceae is distinguished from the related families by the presence of intrastaminal discs, resin ducts, unilocular ovaries, and drupaceous fruits. This family includes a number of economically important plants, which yield edible fruits, gums, resins, tan-dyes, and wood. It is also known for its toxic long-chain aliphatic phenolic compounds such as anacardol, anacardic acid, urushol, and hydrourushol, which possess irritant and allergenic properties (Umadevi *et al*., 1988).

Earlier chemical reports from the family showed phenolic compounds as major constituents: mangiferin from the root bark of *Mangifera*; quercetin, myricetin, and apigenin glycosides from the leaves of *Rhus* spp., robustaflavone from seeds of *Rhus* (Lin and Chen, 1974), biflavones and bichalcones from *Rhus* (Chen and Lin, 1975; Masesane *et al*., 2000; Mdee *et al*., 2003), *Myracrodruon* (Bandeira *et al*., 2003) and *Semecarpus* (Rao *et al*., 1973; Murthy, 1992), fustin and fisetin from heartwood of *Rhus* spp. (Chen *et al*., 1974), and 2,3,6-trihydroxy benzoic acid and 2,3,6-trihydroxy (methyl) benzoate from *Sorindeia* (Kamkumo *et al*., 2012). The results showed that the *Sorindeia* genus is also a source of phenolic compounds such as flavonoids which have been reported from other species within the family Anacardiaceae.

Experimental

General experimental procedures

UV/Vis spectra were recorded on a Perkin-Elmer (Billerica, MA, USA) Lambda 15 UV/Vis spectrometer. IR spectra were obtained from a Perkin-Elmer 1600 Series FTIR spectrometer. ^{1}H NMR spectra were recorded on Varian (Agilent Technologies Inc., Santa Clara, CA, USA) Unity 300 (300.542 MHz), Bruker (Bruker Daltonics, Bremen, Germany) AMX 300 (300.145 MHz), and Varian Inova 500 (499.8 MHz) instruments. ^{13}C NMR spectra were recorded on Varian Unity 300 (75.5 MHz) and Varian Inova 500 (125.7 MHz) instruments. Electrospray-ionization mass spectrometry (ESI-MS) and high-resolution mass spectrometry (HR-ESI-MS) were performed on a micrOTOF orthogonal accelerated time-of-flight mass spectrometer (Bruker Daltonics, Bremen, Germany), as well as on an Apex IV 7 Tesla Fourier-transform ion cyclotron resonance mass spectrometer (Bruker Daltonics, Billerica, MA, USA). Thin-layer chromatography (TLC) was carried out on pre-coated silica gel sheets of Polygram SIL G/UV254 (Macherey-Nagel, Düren, Germany). Size-exclusion chromatography was performed on Sephadex LH-20 (lipophilic Sephadex; Amersham Biosciences purchased from Sigma-Aldrich Chemie, Steinheim, Germany).

Plant material

The leaves of *Sorindeia juglandifolia* A. Rich. were collected at Mont-Kalla in the central region of Cameroon in May 2008 and identified botanically by Victor Nana (National Herbarium, Yaoundé, Cameroon). A voucher specimen (35032/HNC) was deposited in the National Herbarium in Yaoundé, Cameroon.

Extraction and isolation

The air-dried and powdered leaves of *Sorindeia juglandifolia* (1.5 kg) were percolated for 48 h with MeOH at room temperature, and afterwards the solution was filtered and concentrated under vacuum. The crude MeOH extract (158.7 g) was suspended in water and partitioned with *n*-hexane, ethyl acetate, and *n*-butanol successively in the same volume seven times to give 14.4, 28.6, and 14.3 g extract residues, respectively. The residual aqueous layer was finally evaporated and freeze-dried

to obtain 58.4 g of residue. The EtOAc extract (28.6 g) was subjected to column chromatography (CC) on silica gel (CH_2Cl_2/MeOH gradient of increasing polarity) to give five fractions, T_1–T_5, on the basis of TLC composition. Fraction T_2 (2.1 g) was subjected to repeated CC on silica gel eluted with CH_2Cl_2/MeOH (10:1) and on Sephadex LH-20 eluted with MeOH to yield 5 mg 2''-*O*-acetyl-7-*O*-methyl vitexin (**2**) and 6 mg 3*β*-*O*-D-glucopyranosyl-*β*-stigmasterol (**7**). Fraction T_3 (2.9 g) was firstly chromatographed on a silica gel column (CH_2Cl_2/MeOH, 10:1 and 5:1) to give subfractions T_{3A} and T_{3B}, which were further purified using the same procedure as before to yield 8 mg mearnsitrin (**3**) and 10 mg robustaflavone (**4**). Fraction T_5 (3.6 g) was purified by CC with CH_2Cl_2/MeOH (10:1, 8:1, and 5:1) to yield 3 mg 3-*O*-galloyl catechin (**5**), 4.5 mg 2'',6''-di-*O*-acetyl-7-*O*-methyl vitexin (**1**), and 19 mg methyl gallate (**8**). Further purification of fraction T_1 was conducted by repeated silica gel column chromatography and preparative TLC to give 8 mg tachioside (**6**).

2'',6''-Di-O-acetyl-7-O-methyl vitexin (**1**): Yellow amorphous solid. – R_f = 0.20 (CH_2Cl_2/5% MeOH). – $[\alpha]_D^{20}$ –61° (*c* 0.5, MeOH). – UV (MeOH): λ_{max} (log ε) = 228 (4.64), 256 (4.52), 269 (4.01), 293 (3.90), 332 (3.25) nm. – IR (KBr): ν_{max} = 3418, 1720, 1642, 1607, 1580, 1500, 1440, 1360, 1280, 1240, 1210, 1110, 1080, 840 cm^{-1}. – ^1H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (125 MHz, DMSO-d_6): see Table I. – HR-ESI-MS: *m/z* = 531.1493 $[M+H]^+$ (calcd. for $C_{26}H_{27}O_{12}$, 531.1497), 553.1313 $[M+Na]^+$ (calcd. for $C_{26}H_{26}O_{12}Na$, 553.1317).

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