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Triflavonoids of *Ochna calodendron*

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Abstract

Phytochemical investigation of the dichloromethane extract of *Ochna calodendron* leaves resulted in the isolation and identification of three known isoflavones, irilone, 3′-methoxyirilone and prunetin, one cyanoglucoside, menisdaurin, in addition to two new triflavonoid constituents, namely caloflavans A and B. The structures of the new compounds were established by spectroscopic methods (EI–MS, FAB–MS, ^{1}H NMR, ^{13}C NMR, HMBC and HMQC). © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Ochna calodendron; Ochnaceae; Leaves; Isoflavones; Cyanoglucoside; Triflavonoids; Caloflavan A, B

1. Introduction

In the course of our research directed toward the isolation and identification of bioactive constituents from economic and medicinal Cameroonian plants of the Ochnaceae (Bouquet, 1969), we previously reported the characterization of the biflavonoids, calodenone (Messanga et al., 1992), calodenin A and lophirone K (Messanga et al., 1994), and calodenin C (Messanga et al., 1998) from the stem bark of *Ochna calodendron*. As part of our chemical search for other minor constituents of this species, we now report the results of the investigation of the leaves, leading to the isolation of four known compounds in addition to two new triflavonoid pigments, caloflavans A (**1**) and B (**2**), the structures of which are described herein.

2. Results and discussion

The coarsely powdered leaves of *O. calodendron* were extracted exhaustively with dicloromethane at room temperature in a tank equipped with a mechanical stirrer. The extract obtained after removal of the solvent was repetitively fractionated by CC over silica gel and filtration over Sephadex LH-20 to afford three isoflavonoids, irilone (Dhar and Kalla, 1973), 3′-methoxyirilone (Arisawa et al., 1973) and prunetin (Mizuno et al., 1990), a cyanoglucoside, menisdaurine (Takahashi et al., 1998) and two novel (**1** and **2**) triflavonoid derivatives.

The (+) FAB mass spectrum of caloflavan A (**1**) showed $[M+H]^+$ at *m*/*z* 787 consistent with the molecular formula $C_{45}H_{38}O_{13}$. The ^{13}C NMR spectrum (Table 1) exhibited resonances for 45 carbon atoms, among which 36 aromatic carbons (nine of them bearing an oxygen atom and 19 a proton), seven aliphatic methines (four of which were oxymethines), one methylene group at δ_C 31.0 and one carbonyl of a ketone at δ_C 206.6. Assignments were deduced from the ^{13}C–^{1}H COSY spectrum. The ^{1}H NMR spectrum displayed 19 aromatic protons bound to sp$_2$-type carbon atoms. From detailed analysis of ^{1}H–^{1}H COSY NMR experiment, they were distributed into three 1,4-disubstituted (Ai type), two 1,2,4-trisubstituted (Bi type) and one pentasubstituted (B3) aromatic rings, whereas the aliphatic protons formed two spin systems: (i) $-C_{c2}H(O\text{-})–C_{\alpha 2}H(C_{\beta 2}H)–C_{\alpha 1}H–C_{\beta 1}H(O\text{-})-$ and (ii)$-C_{c3}H_2–C_{\alpha 3}H(O\text{-})–C_{\beta 3}H(O\text{-})-$ (Fig. 1).

Long-range connectivities observed in the HMBC spectrum enabled the connection between the substructures and with the aromatic rings as shown in structure **1** (Table 1). Carbon atoms of the βi type generally showed correlations with αi methines and aromatic protons at 2- and 6-positions on Ai rings, whereas carbon

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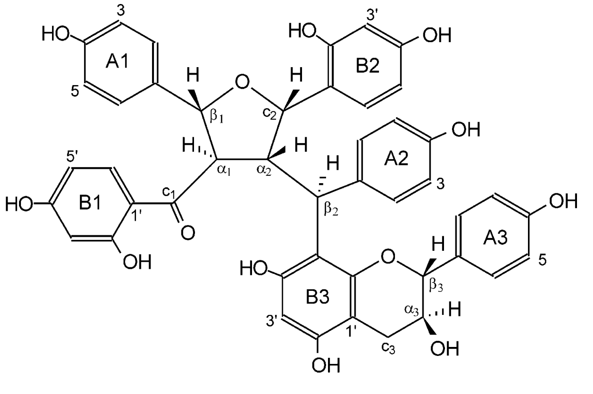

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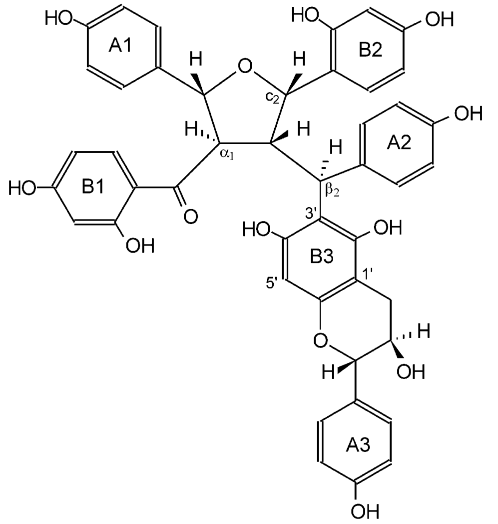

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atoms of ci types were correlated to the proton at 6′-position on the relative aromatic Bi rings. The carbonyl carbon atom (δ_C 206.6) which showed cross peaks with protons at δ_H 3.87 (α_1), 5.26 (β_1) and with the aromatic proton at 6′-position on B1 ring at δ_H 6.65, was assigned at c_1. Long-range connectivities between the proton at δ_H 4.98 (c_2) and the vicinal carbon at δ_C 52.9 (α_2) and also with the carbons at δ_C 86.3 (β_1) and 46.7 (β_2) suggest a five-membered heterocyclic ring system. The proton at δ_H 4.59 (β_2) was correlated to the carbon atoms at δ_C 88.2 (c_2) and also to the carbons at δ_C 154.2, 110.3 and 156.3 all located at 4′, 5′ and 6′-positions respectively on B3 ring. In addition, the latter carbon (δ_C 156.3, C-6′, rB3) showed cross correlation peaks with the two protons of the methylene group at δ_H 2.72 and 2.18 (c_3), and also with the oxymethine at δ_H 3.87 (β_3) leading to **1** for caloflavan A, which thus results from a condensation of three flavonoid units (U1U3) in such a way as depicted above where the substructures U1 + U2 (i) and U3 (ii) corresponding to a biflavonyl and a catechin moieties respectively are clearly defined.

The relative configuration of each substructure was deduced from coupling constant values and mainly from NOE measurements. The large value of the couplings between H-α_2 and H-β_2 ($^3J_{H-H}=11.7$ Hz) and between H-β_3 and H-α_3 ($^3J_{H-H}=9.5$ Hz) indicated these two to be in a *trans*-diaxial relative disposition. The ROESY spectrum showed strong correlation spots between spin systems belonging to the heterocyclic rings giving information on their relative disposition (Fig. 1), which was further confirmed from NOE difference measurements. In addition, NOE data between heterocyclic and aromatic protons allowed us to verify the assignment of the aromatic rings. The proton at α_1 (δ_H 3.87) showed strong NOEs with the protons—2, 6 of A1-ring (6%) in agreement with their *cis*-relative position on the five-membered heterocyclic ring. This agreed with the NOEs measured between H-α_2 (δ_H 4.54) and protons at c_2 (5%), β_1 (2%) and aromatic H-6′ (B1 ring, 2%), which are on the other side of the ring. No proof has been obtained on the relative stereochemistry of substructure (U1, U2) as compared to substructure (U3). In consideration of the preceding results, the relative stereochemistry shown in **1** was proposed for caloflavan A.

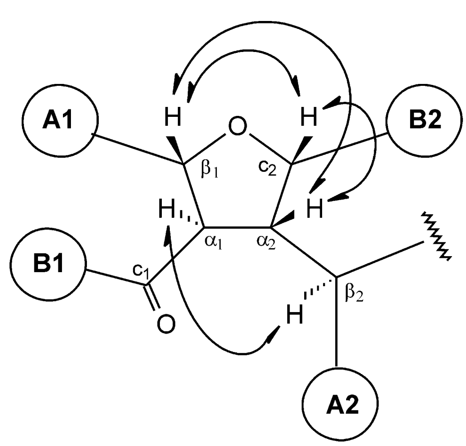

Fig. 1. Selected ROESY correlations for the five-membered heterocyclic ring system in compound **1**.

The (+) FAB mass spectrum of caloflavan B (**2**) showed a $[M+H]^+$ at m/z 787 in agreement with the molecular formula $C_{45}H_{38}O_{13}$, showing it was an isomer of caloflavan A. Its ^{1}H NMR spectrum was very similar to that of caloflavan A, displaying 19 aromatic protons and eight protons bound to sp$_3$-type carbon atoms. From detailed analysis of ^{1}H–^{1}H COSY NMR experiment, they were distributed into three 1,4-disubstituted, two 1,2,4-trisubstituted and one pentasubstituted (B3) aromatic ring, whereas the aliphatic protons formed two spin systems similar to those already observed for **1**: (i)–CH(O-)–CH(CH)–CH–CH(O-)–, as H-α_2 (δ_H 4.62) showed coupling with H-β_2 (δ_H 4.72), H-c_2 (δ_H 5.16) and H-α_1 (δ_H 3.82), and this latter with H-β_1 (δ_H 4.89), and (ii) –CH$_2$–CH(O-)–CH(O-)– as H-α_3 (δ_H 3.74) showed couplings with H$_2$-$c_3c_{3'}$ (δ_H 2.76 and 2.07) and H-β_3 (δ_H 4.29). These partial structures were further connected on the basis of the ^{1}H-detected heteronuclear multiple

Table 1
NMR spectral data (^1H, 300 MHz and ^{13}C, 75 MHz, CD_3OD, TMS) for compounds **1** and **2**

No. C	Ring	**1**				**2**		
		δ_C	$\delta_H m$	J(Hz)	HMBC correlations (^1H)	δ_C	$\delta_H m$	J (Hz)
Isombamichalcone moiety								
1	A1	133.3	–		H-β_1, H-α_1	132.2	–	
2,6	A1	128.4	7.17 *d*	8.4	H-β_1	128.7	7.14 *d*	8.5
4	A1	158.9	–		H-2/H-6 (A1), H-3/H-5 (A1)	158.3	–	
3,5	A1	116.3	6.74 *d*	8.4		116.2	6.67 *d*	8.5
β-1		86.3	5.26 *d*	5.8	H-α_1, H-2/H-6 (A1), H-α_2, H-c_2	85.9	4.89 *d*	6.6
α-1		62.3	3.87 *m*	–	H-β_1, H-c_2	62.0	3.82 *m*	–
c-1		206.6	–		H-α_1, H-β_1, H-6′ (B1)	205.2	–	
1′	B1	114.0	–		H-α_1, H-3′ (B1), H-5′ (B1)	114.1	–	
2′	B1	166.6				166.4		
3′	B1	103.3	6.02 *d*	2.4	H-5′ (B1)	103.3	6.08 *d*	2.2
4′	B1	167.1	–		H-6′ (B1)	165.9	–	
5′	B1	108.9	5.85 *dl*	8.8	H-3′ (B1)	109.5	5.86 *dd*	2.1; 8.9
6′	B1	130.6	6.65 *d*	8.9		133.8	6.65 *d*	9.0
1	A2	135.5			H-β_2	135.4		
2,6	A2	131.8	6.84 *d*	8.3	H-β_2	131.6	6.61 *d*	8.6
4	A2	155.7	–		H-2/H-6 (A2)	153.9		
3,5	A2	114.8	6.05 *d*	8.5		114.6	6.32 *d*	8.6
c-2		88.2	4.98 *d*	6.9	H-β_1, H-α_2, H-β_2, H-6′ (B2)	83.3	5.16 *d*	6.7
α-2		52.9	4.54 *m*	–	H-β_1, H-β_2, H-c_2	51.2	4.62 *m*	–
β-2		46.7	4.59 *d*	11.7	H-c_2, H-α_2, H-α_1, H-2/H-6 (A2)	46.5	4.72 *d*	11.9
1′	B2	11.9.1	–		H-c_2, H-3′ (B2), H-5′ (B2)	120.1	–	
2′	B2	157.8			H-c_2	157.7		
3′	B2	103.7	6.31 *dl*	2.0	H-5′ (B2)	103.4	6.13 *d*	2.2
4′	B2	158.9	–		H-6′ (B2)	158.6	–	
5′	B2	106.6	5.98 *dl*	8.2	H-3′ (B2)	107.3	6.09 *dl*	8.6
6′	B2	132.6	6.32 *d*	8.4	H-c_2	130.9	7.19 *d*	8.8
Afzelechin moiety								
1	A3	130.9	–	–	H-α_3, H-β_3, H-3/H-5 (A3)	130.9	–	
2,6	A3	130.7	7.35 *d*	8.4	H-β_3	131.0	7.31 *d*	8.5
4	A3	158.3	–		H-2/H-6 (A3)	158.4	–	
3,5	A3	115.9	6.95 *d*	8.4		115.9	6.92 *d*	8.5
β-3		88.1	3.87 *d*	9.5	H-α_3, H-c_3, H-2/H-6 (A3)	82.8	4.29 *d*	9.5
α-3		68.8	3.26 *m*	–	H-β_3, H_2-c_3	67.8	3.74 *m*	–
c-3		31.0	2.72 *dd*	15.6; 6.0	H-β_3	30.3	2.76 *dd*	15.7; 5.7
			2.18 *dd*	15.6; 9.1			2.07 *dd*	15.7; 10.2
1′	B3	102.3	–		H-α_3, H_2-c_3	101.8		
2′	B3	155.4	–		H-3′ (B3)	155.3		
3′	B3	96.0	6.00 *s*	–		111.1		
4′	B3	154.2	–		H-β_2, H-3′ (B3)	154.3		
5′	B3	110.3	–		H-β_2, H-3′ (B3)	96.2	5.74 *s*	
6′	B3	156.3	–		H-β_2, H-β_3, H_2-c_3	156.4		

bond connectivity (HMBC) spectrum. The aromatic carbon at δ_C 156.4 (B3 ring) showed cross correlation peaks with the proton H-β_3, the two methylene protons on c_3 position and the singlet at δ_H 5.74 on B3 ring, leading to the structure of caloflavan B as shown in **2**. When compared to the corresponding substructures of caloflavan A, the relative stereochemistry of the heterocyclic rings as shown by the NOE difference measurements was found to be the same for both the substructure (U1, U2) and (U3). All these results strongly suggested that caloflavan B (**2**) was an isomer of caloflavan A (**1**). These two compounds may both arise, from a biosynthetic point of view, from the condensation of the biflavonoid isombamichalcone previously isolated from the stem bark of *Lophira lanceolata* (Ochnaceae) (Ghogomu et al., 1989) with afzelechin either on C-6 or C-8 positions (A ring) leading to caloflavan A (**1**) and B (**2**) respectively.

3. Experimental procedure

3.1. General

$[\alpha]_D$ values were measured on a Perkin-Elmer 141 polarimeter. NMR spectra were recorded on a Brücker AC 300 (^1H, 300 MHz; ^{13}C, 75 MHz; CD_3OD) with the CHD_2OD (δ 3.313) signal as internal reference. Positive

FAB mass spectra were obtained on a ZAB–HF mass spectrometer.

3.2. Plant material

Leaves of *Ochna calodendron* Gilg. and Mildbr. were harvested in Ngoumbou (Cameroon) in March 1997. A voucher specimen (No. HNC 1484-Oc) is deposited in the National Herbarium in Yaoundé.

3.3. Extraction and isolation

The coarsely pulverized air-dried plant material (4 kg) was extracted with CH_2Cl_2 at room temperature. The extract (36 g) afforded after evaporation of the solvent was subjected to silica gel CC with a gradient mixture of CH_2Cl_2–MeOH, starting from pure CH_2Cl_2 to yield five CC fractions (F-1: 4.8 g, F-2: 6.7 g, F-3: 2.9 g, F-4: 3.3 g and F-5: 13.6 g). Chromatography of the fraction F-2 over a silica gel (300 g) column with hexane–EtOAc gradient yielded β-sitosterol (733 mg). Fraction F-3 was further fractionated by CC (silica gel, 200 g) into three parts (F-3a: 0.4 g, F-3b: 0.7 g, F-3c: 1.3 g). Fractionation of F-3b over silica gel (50 g) column with H_2CCl_2–MeOH (50:1) afforded β-sitosterol (15 mg), irilone (87 mg), 3′-methoxyirilone (72 mg) and prunetin (123 mg). Fraction F-5 was chromatographed over Sephadex LH-20 (MeOH) to yield four parts: (F-5a: 1.2 g, F-5b: 4.8 g, F-5c: 0.5 g, F-5d: 5.2 g). Gel filtration of F-5b with MeOH gave menisdaurin (1.7 g) and a fraction containing mainly β-sitosterol-*O*-β-D-glucopyranoside. F-5d was subjected to repeated chromatography over Sephadex LH-20 (MeOH) and preparative TLC (silica gel, CH_2Cl_2–MeOH, 4:1) to yield two compounds caloflavan A (**1**, 4 mg) and caloflavan B (7 mg) as glassy solids.

3.3.1. Caloflavan A (1)

$C_{45}H_{38}O_{13}$, amorphous solid; $[\alpha]_D^{28}$ +31° (MeOH; *c* 0.015); ^1H- and ^{13}C NMR data, see Table 1; (+) HRFABMS $[M+H]^+$ 787.1531 (calc. 787.1528).

3.3.2. Caloflavan B (2)

$C_{45}H_{38}O_{13}$, amorphous solid; $[\alpha]_D^{28}$ +28° (MeOH; *c* 0.037); ^1H- and ^{13}C NMR data, see Table 1; (+) HRFABMS $[M+H]^+$ 787.1546 (calc. 787.1528).

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