Isolation and structural elucidation of a new pentaflavonoid from *Ochna calodendron*

Bernard Blaise Messanga,*[a] Samuel Fon Kimbu,[a] Beibam Luc Sondengam[a] and Bernard Bodo[b]

[a] *Organic Chemistry Department, Faculty of Science, University of Yaoundé I, B.P. 812, Yaoundé, Cameroon. E-mail: messbb@uycdc.uninet.cm*
[b] *Laboratoire de Chimie des Substances Naturelles (CNRS ESA 8041), Muséum National d'Histoire Naturelle, 63 rue Buffon, 75005 Paris, France*

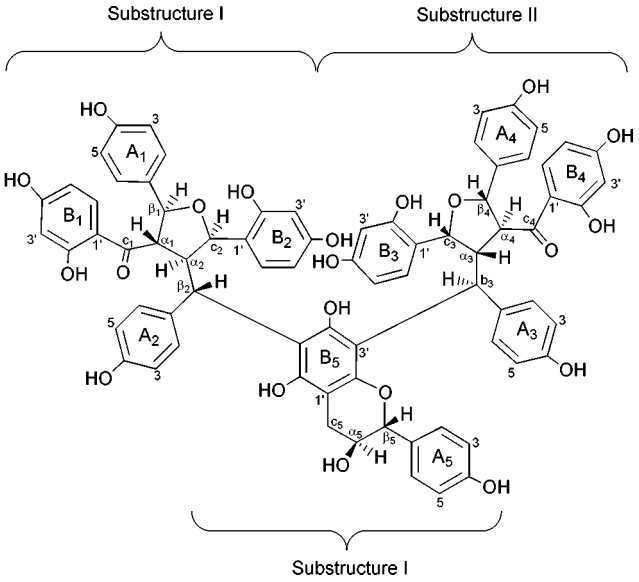
Received (in Montpellier, France) 26th January 2001, Accepted 15th May 2001
First published as an Advance Article on the web 13th July 2001

A new pentaflavonoid, ochnachalcone, was isolated from the stem bark of *Ochna calodendron* and its structure elucidated by spectroscopic means including HMQC, HMBC and ROESY experiments.

The bark of *Ochna calodendron* Gilgiana and Mildbraedii (Ochnaceae), indigenous to Central Africa (Gabon, Congo Brazzaville, Equatorial Guinea, Republic of Central Africa and Cameroon), has been used in traditional medicine as a remedy for the treatment of toothache, liver infections and dysentery.[1] *O. calodendron* is also a rich source of biflavonoids and isoflavonoids. Previous phytochemical studies have reported the isolation of chalcone dimers such as calodenone, lophirone K and calodenins A, B and D, as well as the known isoflavonoids irilone and 3′- and 4′-methoxyirilone.[2–5] The renewed interest in these classes of compounds[6–8] prompted us to undertake a new thorough chemical investigation of this plant. Fractionation of the more polar portion of the ethyl acetate crude extract led to the isolation of a new pentameric chalcone, ochnachalcone (**1**) along with two further compounds described for the first time from this plant, namely (+)-catechin and quercitrin. We report herein the structural elucidation of the novel pentaflavonoid **1**.

Results and discussion

The new compound **1** was obtained as an amorphous yellow solid and showed a pseudomolecular $[M + H]^+$ ion at *m/z* 1299 in its (positive) FAB mass spectrum, consistent with the molecular formula $C_{75}H_{62}O_{21}$. Its ^1H NMR spectrum displayed signals for 32 aromatic protons, 12 methines and one methylene Table 1. From detailed analysis of a ^1H–^1H COSY NMR experiment, the aromatic protons were identified as belonging to five 1,4-disubstituted and four 1,2,4-trisubstituted aromatic rings. The aliphatic protons formed three spin systems: (i) $C_{\beta1}H(OR_1)$–$C_{\alpha1}H$–$C_{\alpha2}H(C_{\beta2}HR_2)$–$C_{c2}H(OR_3)$, because H-$\alpha_2$ (δ_H 4.69) was correlated to H-c_2 (δ_H 5.41) and H-β_2 (δ_H 4.98), whereas H-α_1 (δ_H 3.85) showed couplings with H-β_1 (δ_H 4.97) and H-α_2; (ii) $C_{c3}H(OR_4)$–$C_{\alpha3}H(C_{\beta3}HR_5)$–$C_{\alpha4}H$–$C_{\beta4}H(OR_6)$, because H-$\alpha_3$ (δ_H 4.47) showed couplings with H-c_3 (δ_H 5.17), H-β_3 (δ_H 4.95) and H-α_4 (δ_H 3.76), whereas H-β_4 (δ_H 4.77) was correlated to H-α_4; (iii) $C_{c5}H_2$–$C_{\alpha5}H(OR_7)$–$C_{\beta5}H(OR_8)$, because H-α_5 (δ_H 3.07) showed couplings with H_2-c_5, -$c_{5'H}$ 1.23 and 1.79) and H-β_5 (δ_H 3.56). These spin systems were further extended by long-range COSY correlations into three substructures I, II, III. Long-range connectivities were observed between H-β_1 and H-2, 6 (δ_H 7.26, A_1 ring), H-α_1 and H-6′ (δ_H 5.95, B_1 ring), H-β_2 and H-2, -6 (δ_H 7.27, A_2 ring) and between H-c_2 and both H-β_1 and H-6′ (δ_H 7.89, B_2 ring) related to substructure I, between H-c_3 and H-6′ (δ_H 7.25, B_3 ring), H-β_3 and H-2, -6 (δ_H 7.08, A_3 ring) and H-α_4 and H-6′ (δ_H 5.99, B_4 ring) for substructure II and finally between H-β_5 and H-2, -6 (δ_H 7.25, A_5 ring) related to substructure III.



The ^{13}C–^1H long–range connectivities observed in the HMBC spectrum enabled us to verify the connectivity between the aliphatic protons and the aromatic rings. Carbon atoms of the β_i type generally showed correlations with α_i methines and aromatic protons at 2 and 6 on A_i rings, whereas carbon atoms of the c_i type were connected with the proton at 6′ on the relative aromatic B_i rings (Table 2). The carbonyl carbon atom (δ_C 207.1), which showed cross peaks with H-β_1, H-α-$_1$ and with the aromatic proton H-6′ at 5.95 ppm (B_1 ring), was assigned as c_1, whereas the second carbonyl carbon atom at δ_C 206.8, showing cross correlation peaks with protons at δ_H 4.77 (β_4), 3.76 (α_4) and with the aromatic proton H-6′ at 5.99 (B_4 ring), was assigned as c_4. In addition, the cross correlation peaks observed between H-β_2 (substructure I) and the carbon atoms C_6, C_5 and C_4 on the so far unidentified B_5 ring, between H-β_3 (substructure II) and the carbon atoms C_2, C_3 and C_4 (B_5 ring) and between the carbon atom C_2 (δ_C 153.0, B_5 ring) and the two methylene protons at δ_H 1.23 and 1.79 (substructure III) enabled us to connect substructure I, II and III as shown in **1**.

The relative configuration of each substructure was deduced

 DOI: 10.1039/b100437i

Table 1 ^{13}C and ^{1}H NMR data for compound **1** (CD_3OD, TMS)

Ochnachalcone (**1**)											
C	Ring	δ_c	δ_H	Mult.	J/Hz	C	Ring	δ_C	δ_H	Mult.	J/Hz
Isombamichacone moieties											
1	B_2	120.2	—			1	B_3	119.0	—		
2	B_2	158.5	—			2	B_3	158.4	—		
3	B_2	103.5	6.29	d	2.4	3	B_3	103.4	6.17	d	2.4
4	B_2	159.2	—			4	B_3	158.3	—		
5	B_2	107.2	6.36	dd	2.4, 8.4	5	B_3	107.2	6.14	dd	2.4, 8.2
6	B_2	131.4	7.89	d	8.5	6	B_3	130.8	7.25	m	—
c_2		85.1	5.41	d	4.1	c_3		84.7	5.17	d	2.8
α_2		52.9	4.69	m	—	α_3		50.3	4.47	m	—
β_2		48.0	4.98	d	13.4	β_3		47.7	4.95	d	13.1
1	A_2	135.9				1	A_3	135.7	—		
2	A_2	131.4	7.27	m	—	2	A_3	131.3	7.08	d	8.6
3	A_2	115.6	6.54	d	8.6	3	A_3	115.5	6.52	d	8.6
4	A_2	155.7	—			4	A_3	155.7	—		
5	A_2	115.6	6.54	d	8.6	5	A_3	115.5	6.52	d	8.6
6	A_2	131.4	7.27	m	—	6	A_3	131.3	7.08	d	8.6
1	A_1	132.4	—			1	A_4	131.9	—		
2	A_1	129.2	7.26	m		2	A_4	129.0	7.25	m	—
3	A_1	116.3	6.80	d	8.7	3	A_4	116.2	6.76	d	8.6
4	A_1	158.3	—			4	A_4	158.1	—		
5	A_1	116.3	6.80	d	8.7	5	A_4	116.2	6.76	d	8.6
6	A_1	129.2	7.26	m	—	6	A_4	129.0	7.25	m	—
β_1		86.3	4.97	d	7.8	β_4		86.5	4.77	d	8.4
α_1		61.0	3.85	m		α_4		60.7	3.76	dd	5.6, 8.3
c_1		207.1	—			c_4		206.8	—		
1	B_1	114.3	—			1	B_4	114.0	—		
2	B_1	165.9	—			2	B_4	166.3	—		
3	B_1	102.4	5.95	d	2.4	3	B_4	102.9	5.98	d	2.4
4	B_1	166.1	—			4	B_4	166.4	—		
5	B_1	109.5	4.34	dd	2.4, 8.9	5	B_4	109.4	4.63	dd	2.4, 8.9
6	B_1	134.0	5.95	d	8.8	6	B_4	134.0	5.99	d	8.5
Afzelechin moiety											
1	B_5	103.4	—			1	A_5	131.3	—		
2	B_5	153.0	—			2	A_5	131.4	7.25	m	
3	B_5	111.9	—			3	A_5	115.7	6.91	d	8.6
4	B_5	153.7	—			4	A_5	158.9	—		
5	B_5	114.1	—			5	A_5	115.7	6.91	d	8.6
6	B_5	152.1	—			6	A_5	131.4	7.25	m	—
β_1		82.3	3.56	d	9.7						
α_1		67.2	3.07	m	—						
c_1		30.8	1.79	dd	6.4, 15.2						
			1.23	dd	10.3, 15.5						

from coupling constant values and mainly from NOE measurements. The large values of the coupling constants between H-α_2 and H-β_2 ($^3J_{H-H}$ = 13.4 Hz), between H-α_3 and H-β_3 ($^3J_{H-H}$ = 13.1 Hz) and between H-α_5 and H-β_5 ($^3J_{H-H}$ = 9.5 Hz) indicated these three pairs of protons to have an antiperiplanar relationship. The ROESY spectrum showed strong correlation spots between spin systems belonging to the heterocyclic rings, giving information on their relative disposition. Strong correlation peaks were observed between H-α_2 and both H-c_2 and H-β_1 and between H-α_3 and both H-c_3 and H-β_4 indicating a *cis* relative disposition on the heterocyclic rings to which they are located. Further NOE difference measurements confirmed these results as well as assignments that arose from the observation of strong NOEs between H-β_1 and H-2, -6 (A_1 ring) (11%), H-c_2 and H-6′ (B_2 ring) (10%), H-α_1 and H-6′ (B_1 ring) (6%), H-β_2 and H-2, -6 (A_2 ring) (12%), H-c_3 and H-6′ (B_3 ring) (12%), H-β_4 and H-2, -6 (A_4 ring) (9%), H-β_3 and H-2, -6 (A_3 ring) (13%), H-α_4 and H-6′ (B_4 ring) (7%) and finally between H-β_5 and H-2, -6 (A_5 ring) (10%).

The relative stereochemistry of the substructure I and II furan rings was confirmed from strong NOEs between H-c_2 and both H-β_1 (6%) and H-α_2 (5%) but not with H-α_1 and H-β_2 for substructure I, and between H-c_3 and both H-β_4 (5%) and H-α_3 (5%) but not with H-α_4 and H-β_3 related to substructure II, suggesting that these spin systems were in *cis* relative dispositions in the corresponding substructures.

Substructure III is identical to afzelechin, while substructures I and II are identical to isombamichalcone,[9] a biflavonoid previously described from the stem bark of *Lophira lanceolata* and which has a biosynthesis involving the condensation of two chalcone units. It is reasonable to assume that asymmetric centres in substructures I and II have the same absolute configurations. This hypothesis leads to the representation of the relative configuration of substructure I with respect to substructure II as shown in **1**, the absolute configuration of the complete molecule not being determined. Structure **1** is thus proposed for ochnachalcone. To the best of our knowledge, this report is the first describing the characterization of a pentaflavonoid from a member of the Ochnaceae family.

Experimental

General

Column chromatography (CC) was carried out on silica gel 60 (Fluka, 230–400 mesh), silanized silica 60 gel (RP-2) from Merck using a H_2O–MeOH gradient, and on Sephadex LH-20 (Pharmacia). Elutions were checked by TLC (silica 60

Table 2 ^{1}H–^{13}C long-range correlations (●) observed in the HMBC spectrum of ochnachalcone (**1**)

^{1}H / ^{13}C	α_1	β_1	A_1[a]	B_1[b]	α_2	β_2	c_2	A_2[a]	B_2[b]	α_3	β_3	c_3	A_3[a]	B_3[b]	α_4	β_4	A_4[a]	B_4[c]	α_5	β_5	c_5	A_5[a]
α_1		●			●		●															
β_1	●		●		●		●															
c_1	●	●		●	●																	
A_1 (1, 2/6)	●	●																				
B_1 (1, 2/6)	●																					
α_2	●	●				●	●															
β_2					●		●	●														
c_2	●	●			●	●			●													
A_2 (1, 2/6)					●	●																
B_2 (1, 2/6)					●		●															
α_3											●	●										
β_3										●		●	●									
c_3										●	●			●	●	●						
A_3 (1, 2/6)										●	●											
B_3 (1, 2/6)										●		●										
α_4										●	●	●				●						
β_4										●		●			●		●	●				
c_4															●	●						
A_4 (1, 2/6)															●	●						
B_4 (1, 2/6)															●							
β_5																				●	●	
β_5																			●		●	●
c_5																			●	●		
A_5 (1, 2/6)																			●	●		
B_5																			●	●	●	

[a] Refers to protons H-2, 6. [b] Refers to proton H-2. [c] Refers to proton H-6.

F_{254}, Merck), using CH_2Cl_2–MeOH (5 : 1) as the solvent system. TLC was visualized under UV (254 nm) and by spraying with an ethanolic vanillin–sulfuric acid solution, followed by heating. The $[\alpha]_D$ value was measured on a Perkin–Elmer 141 polarimeter, and IR spectra were recorded on a Nicolet Impact 400D spectrometer. NMR spectra were recorded on a Bruker AC 300 spectrometer (^{1}H, 300 MHz; ^{13}C, 75 MHz; CD_3OD) with the CHD_2OD (δ 3.313) signal as internal reference. Long-range ^{1}H–^{13}C COSY NMR spectra were obtained with $J = 7$ Hz. Positive FAB mass spectra were obtained on a ZAB-HF mass spectrometer.

Plant material

Stem bark of *Ochna calodendron* Gilg. and Mildbr. was harvested in Ngoumbou (Cameroon) in April 1997. A voucher is deposited in the National Herbarium in Yaoundé (Cameroon).

Extraction and isolation

Sun-dried, ground plant material (30 kg) was extracted with cool MeOH in a tank equipped with a mechanical stirrer. The crude extract obtained was concentrated to dryness giving a dark brown residue (2.5 kg), which was re-extracted with EtOAc. Further evaporation of the solvent from the soluble fraction gave a brown solid (600 g), a part of which (140 g) was fractionated by CC on silica gel with a gradient mixture of CH_2Cl_2–MeOH, starting from pure CH_2Cl_2, to yield 8 fractions (E_1–E_8). Fraction E_6 (8.5 g) containing the pentaflavonoid was further chromatographed on an RP-2 column. Elution with a H_2O–MeOH gradient, starting with pure water, gave 12 fractions. The flavonoid-enriched fraction (2.78 g) was further purified on a Sephadex LH-20 column eluted with pure MeOH, to yield three fractions (I–III). Fraction I (0.57 g) was re-chromatographed on an RP-2 column eluting with a mixture of H_2O–MeOH (6 : 4) to afford (+)-catechin and quercitrin. Fraction III (0.41 g) was purified by repeated chromatography over Sephadex LH-20 (MeOH) to yield ochnachalcone (**1**), which was further purified on an RP-2 column (H_2O–MeOH, 70 : 30) to give pure **1** (8 mg).

Ochnachalcone (1). $C_{75}H_{62}O_{21}$, amorphous yellow solid; $[\alpha]_D^{28}$ + 63 (MeOH; *c* 0.025). IR (KBr): ν/cm^{-1} 3434, 2926, 1738, 1665, 1598, 1450, 1376, 1254, 1214, 956, 833. HRFAB-MS: $[M + H]^+$ 1299.2244 (calc. for $C_{75}H_{62}O_{21}$: 1299.2238). (+) FAB-MS: *m/z* (rel. int) 1299 (2) $[M + H]^+$, 1011 (8), 789 (4), 492 (7), 365 (7), 339 (26), 243 (43), 201 (85), 153 (10), 137 (4), 121 (100), 119 (29), 107 (4), 98 (23). For ^{1}H and ^{13}C NMR data, see Table 1.

Acknowledgements

This work was financially supported by grant no. F/2924-1 from the International Foundation for Science awarded to B. B. M. We also thank Mr Alain Blond (Laboratoire de Chimie des Substances Naturelles, CNRS ESA 8041, Muséum National d'Histoire Naturelle, Paris) for the NMR analysis and Professor Raphaël Ghogomu Tih (Département de Chimie Organique, Faculté des Sciences, Université de Yaoundé I, Yaoundé) for his constructive advice.

References

1 A. Bouquet, *Féticheurs et Médecines Traditionnelles du Congo* (*Brazzaville*), O.R.S.T.O.M., Paris, 1969, p. 178.
2 B. B. Messanga, R. T. Ghogomu, B. L. Sondengam, M. T. Martin and B. Bodo, *J. Nat. Prod.*, 1992, **55**, 245.
3 B. B. Messanga, R. T. Ghogomu, B. L. Sondengam, M. T. Martin and B. Bodo, *Phytochemistry*, 1994, **35**, 791.
4 B. B. Messanga, R. T. Ghogomu, B. L. Sondengam, M. T. Martin, A. Blond, J. P. Brouard and B. Bodo, *Planta Med.*, 1998, **64**, 760.
5 B. B. Messanga, R. T. Ghogomu, B. L. Sondengam, M. T. Martin and B. Bodo, *Fitoterapia*, 1998, **LXIX**, 439.
6 A. Murakami, H. Ohigashi, M. Jisaka, M. Hirota, R. Irie and K. Koshimizu, *Cancer Lett.* (*Shannon, Irel.*), 1991, **58**, 101.
7 A. Murakami, S. Tanaka, H. Ohigashi, M. Hirota, R. Irie, N. Takeda, A. Tatematsu and K. Koshimizu, *Biosci., Biotechnol., Biochem.*, 1991, **55**, 1151.
8 J. D. Felicio, E. Gonçalez, M. M. Braggio, L. Costantino, A. Albasini and A. P. Lins, *Planta Med.*, 1995, **61**, 217.
9 R. T. Ghogomu, B. L. Sondengam, M. T. Martin and B. Bodo, *Phytochemistry*, 1990, **29**, 2289.