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STRUCTURE OF LOPHIRONES B AND C, BIFLAVONOIDS FROM THE BARK OF *LOPHIRA LANCEOLATA*

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Key Word Index—*Lophira lanceolata*; Ochnaceae; biflavonoids.

Abstract—Two new isomeric biflavonoids, lophirone B and lophirone C have been isolated from the stem bark of *Lophira lanceolata*. Their structures were determined by the use of chemical and spectroscopic methods including 2D NMR techniques.

INTRODUCTION

In continuation of our phytochemical investigation of the medicinal plants from Cameroon, we analysed the bioactives constituents of *Lophira lanceolata* Van Tiegh ex Keay (Ochnaceae), a tree which is widely distributed in the woody savanas of tropical Africa and is used in folk medicine [1]. We previously reported the structural elucidation of a rearranged biflavonoid isolated from the bark of this tree [2] and we now describe the isolation and structural elucidation of two new biflavonoids from the same source.

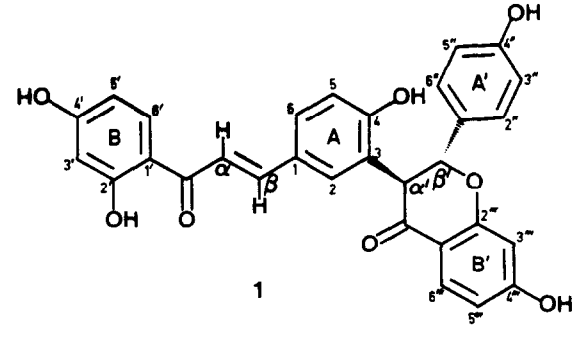
RESULTS AND DISCUSSION

The crude acetone extract of the air-dried finely ground stem bark was re-extracted with ethyl acetate and the soluble fraction further purified first by repeated column chromatography on silica gel and then by preparative thin layer chromatography (TLC). Lophirones B and C were isolated alongside earlier reported lophirone A [2]. Both compounds were obtained as yellow microcrystalline solids from acetone.

When analysed by CIMS lophirones A, B and C gave each a pseudo-molecular ion $[M+H]^+$ at *m/z* 511 in agreement with the molecular formula $C_{30}H_{22}O_8$. The ^{13}CNMR spectra of lophirones B and C both showed signals for all the 30 carbon atoms of the molecular formula: two carbonyls, 24 sp^2 carbon atoms, six of which bearing an oxygen atom and forming four aromatic rings, a disubstituted double bond and two aliphatic methines (Table 1). The spectra of these two flavonoids differed mainly by the δ_c of one of the carbonyl groups which was at δ191.37 in **1** but at δ201.36 in **2**.

The examination of the ^1H 1D and 2D COSY NMR spectra indicated that lophirones B and C had the following elements of structure: a *p*-disubstituted A′ ring, *o,p*-trisubstituted A, B and B′ rings and a disubstituted double bond conjugated to a carbonyl group (Table 1). In addition, the spectra showed two vicinal sp^3 methine protons at δ4.531 and 5.933 for lophirone B and at δ5.465 and 6.204 for lophirone C.

Permethylation of lophirone B (MeI, K_2CO_3) afforded a pentamethyl ether **3**, $C_{35}H_{32}O_8$ and as the IR spectrum of **3** showed no residual hydroxyl absorption, lophirone B must bear five phenolic hydroxyls. The remaining oxygen atom and unsaturation site must thus be assigned to a heterocyclic ring.



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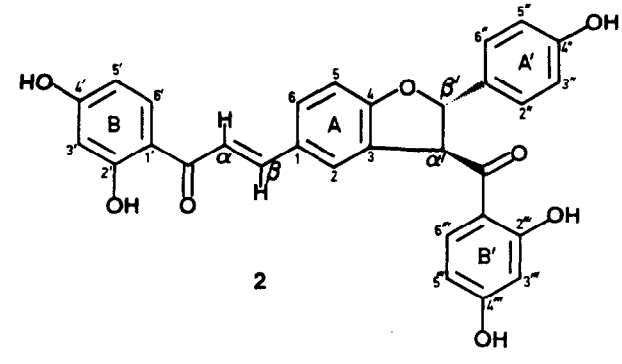

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Table 1. NMR data of lophirones B and C (Me_2CO-d_6, TMS int. ref., ^1H: 250.13 MHz, ^{13}C: 62.8 MHz)

C	Lophirone B: 1				Lophirone C: 2				3	
	δ_C (*mult.*)	δ_H (*mult.*)		*J* (Hz)	δ_C (*mult.*)	δ_H (*mult.*)		(*J* (Hz)	δ_H (*mult.*)	*J* (Hz)
1′	114.54[a]	*s*			114.69[a]	*s*				
2′	167.48	*s*			167.66	*s*				
3′	103.80	*d*	6.353	*d* 2.2	103.91	*d*	6.350	*d* 2.4	6.556	*d* 2.4
4′	165.58	*s*			166.68[b]	*s*				
5′	108.80	*d*	6.432	*dd* 9.0, 2.2	108.81	*d*	6.433	*dd* 9.0, 2.4	6.700	*dd* 8.8, 2.4
6′	133.10	*d*	8.001	*d* 9.0	133.19	*d*	7.972	*d* 9.0	7.838	*d* 8.8
C=O	192.80[b]	*s*			192.80	*s*				
α	118.42	*d*	7.648	*d* 15.4	119.29	*d*	7.684	*d* 15.4	7.360	*d* 15.8
β	145.13	*d*	7.710	*d* 15.4	144.78	*d*	7.794	*d* 15.4	7.433	*d* 15.8
1	124.83	*s*			129.34	*s*				
2	133.83	*d*	7.519	*d* 2.1	126.94	*d*	7.599	*d* 1.8	7.367	*d* 2.2
3	130.28	*s*			132.13	*s*				
4	158.53[c]	*s*			158.86	*s*				
5	116.70	*d*	6.830	*d* 8.1	111.02	*d*	6.987	*d* 8.4	6.944	*d* 8.6
6	130.43	*d*	7.500	*dd* 8.1, 2.1	132.13	*d*	7.809	*dd* 8.4, 1.8	7.504	*dd* 8.6, 2.2
1‴	115.20[a]	*s*			113.78[a]	*s*				
2‴	164.44	*s*			165.65[b]	*s*				
3‴	103.70	*d*	6.454	*d* 2.2	104.05	*d*	6.414	*d* 2.4	6.649	*d* 2.3
4‴	165.37	*s*			163.21	*s*				
5‴	111.44	*d*	6.629	*dd* 8.6, 2.2	109.69	*d*	6.552	*dd* 9.0, 2.4	6.608	*dd* 8.7, 2.3
6‴	130.07	*d*	7.805	*d* 8.6	134.69	*d*	7.965	*d* 9.0	7.618	*d* 8.7
C=O	191.37[b]	*s*			201.36	*s*				
α′	55.93	*d*	4.531	*d* 12.2	57.62	*d*	5.465	*d* 6.8	4.555	*d* 12.0
β′	83.90	*d*	5.933	*d* 12.2	88.63	*d*	6.204	*d* 6.8	5.893	*d* 12.0
1″	127.57	*s*			129.34	*s*				
2″	129.82	*d*	7.305	*m*	128.67	*d*	7.312	*m*	7.361	*m*
3″	115.80	*d*	6.727	*m*	116.54	*d*	6.864	*m*	6.864	*m*
4″	158.84[c]	*s*			158.86	*s*				
5″	115.80	*d*	6.727	*m*	116.54	*d*	6.864	*m*	6.864	*m*
6″	129.82	*d*	7.305	*m*	128.67	*d*	7.312	*m*	7.361	*m*
OH			13.620	*s*			13.517	*s*	OMe 3.921	
							12.540	*s*	3.893	
									3.878	
									3.780	
									3.716	

[a–c] May be reversed within the same column.

Long range ^1H–^1H couplings observed on the 2D COSY LR spectrum of lophirone B showed coupling between: the methine proton at δ5.933 (–O–CH <, β′) and the protons H-2″,6″ of ring A′, the ethylenic proton α to the carbonyl group and H-6′ proton (ring B) and the ethylenic proton β and H-2 (ring A). Obtained coupling information led to the two substructures: a trihydroxy 2′,4′,4-chalcone alkylated at position-3 and a dihydroxyflavanone alkylated at the carbon atom α to the carbonyl group. Both units can only be linked one way leading to structure **1** for lophirone B. The large value (12.2 Hz) of the ^3J coupling constant between protons H-α′ and H-β′ indicated that they have a relative *trans* di-axial stereochemistry.

The isomeric lophirone C showed similar structural features: from the comparison of its NMR data to that of **1** the presence of a 4,2′,4′-trihydroxychalcone moiety alkylated at position-3 (ring A) was deduced (Table 1). The H-2 proton on the A ring showed a long range coupling with a methine proton (H-α′) adjacent to a carbonyl group. This proton was in turn coupled with the oxymethine proton at δ6.204 (H-β′) which finally showed long range coupling with the aromatic protons of the *p*-disubstituted A′ ring. As lophirone C had two strongly chelated hydroxyl groups (δ13.517 and 12.540) the remaining dihydroxyphenyl ring (B′) must be linked to the carbonyl.

Complete acetylation of lophirone C with acetic anhydride/pyridine afforded a pentacetate derivative, (CIMS: *m/z* 721) indicative of five phenolic hydroxyls, as expected. Assignments of the ^{13}C NMR spectrum of lophirone C, accomplished by using heteronuclear ^1H–^{13}C 2D

COSY, were in good agreement with the proposed structure (Table 1). The high value of the $\delta_{C=O}$ at 201.36 suggested that this conjugated carbonyl group was not included in a ring as was the homologue carbonyl in the structure of lophirone B. The low value (1.8 Hz) of the $^4J_{H2-H6}$ coupling constant between *meta* protons 2 and 6 (ring A) was indicative of a fused ring system. The ^3J coupling constant between protons H-α' and H-β' (6.8 Hz) did not afford information on their relative configuration as similar values were observed [3–5] for *cis* and *trans* isomeric dihydrobenzofuran derivatives. Inspection of NMR NOE measurements showed high values between H-α' and H-6''' (23%) and between H-β' and H-(2'' + 6'') (6%). Lower NOE values (from 3 to 5%) were observed between H-α' and H-β', H-(2'' + 6'') and H-2. Therefore these four protons must be almost equidistant from H-α'. This set of results indicated a *trans* configuration of H-α' and H-β' and led to structure **2** for lophirone C.

From a biosynthetic point of view these two new biflavonoids can arise from a direct condensation (C-α→C-3) of the 2',4',4-trihydroxy chalcone on itself to form a dimer which can cyclise to give either a chromanone ring as in **1** or a dihydrofuran ring as in **2**.

EXPERIMENTAL

Mps: uncorr. NMR studies were performed in TMS–acetone-d_6. Solvent used for both column (CC) and thin layer (TLC) chromatography was CH_2Cl_2–MeOH mixt. unless, otherwise stated and the granulometry of silica gel 60 was 0.04–0.063 mm for CC and silica gel plates F_{254} 0.25 mm in thickness for prep. TLC. *Lophira lanceolata* Van Tiegh ex Keay was collected at Foumban (Cameroon) in 1987. A voucher specimen was deposited at the National Herbarium (Yaounde, Cameroon).

Well-dried and ground *Lophira lanceolata* stem bark (5 kg) was extracted with cold Me_2CO at room temp. for 24 hr with stirring. The concd filtrate gave a dark brown gum (96 g) which was re-extracted with EtOAc. The soluble fraction was concd to give a gum (21 g) which was fractionated on a Sephadex LH 20 column (eluent MeOH). The main fraction (18 g) was further purified by repeated silica gel CC (CH_2Cl_2 with increasing proportion of MeOH) and then by prep. TLC (CH_2Cl_2–MeOH: 10:1).

Lophirone B (**1**). $C_{30}H_{22}O_8$, yellow crystals, mp 251–253°, dec. (acetone), $[\alpha]_D^{25}$ +7° (Me_2CO; *c* 0.4), CIMS (NH_3) *m/z*: 511 $[M+H]^+$, 493 $[M-H_2O+H]^+$. EIMS (110°, 70 eV) *m/z* (%): 492 (1.1, $[M-H_2O]^+$), 400 (2.4), 399 (2.2), 388 (2.0), 374 (1.4), 373 (1.1), 355 (1.4), 264 (1.5), 263 (1.3), 252 (1.4), 239 (1.4), 226 (1.0), 205 (0.7), 163 (3.9), 137 (20.2), 110 (100), 94 (43.2), 82 (28.4), 81 (40.6).

Lophirone B pentamethylether (**3**), $C_{35}H_{32}O_8$. Lophirone B, (13 mg), was dissolved in dry Me_2CO in the presence of excess dry K_2CO_3 with stirring for 10 min. Then excess Me was added and stirring continued for 24 hr, after which it was filtered and the concd filtrate purified by CC on silica gel (solvent hexane: EtOAc, 1:1) to give lophirone B pentamethylether **3** (8 mg). CIMS (NH_3) *m/z*: 598 $(M+NH_4)^+$, 581 $(M+H)^+$.

Lophirone C (**2**). $C_{30}H_{22}O_8$, yellow crystals, mp 191–193°, dec. (Me_2CO), $[\alpha]_D^{25}$ −16.3° (Me_2CO; *c* 0.5), CIMS (NH_3) *m/z*: 528 $[M+NH_4]^+$, 511 $[M+H]^+$, 493 $[M-H_2O+H]^+$. EIMS (110°, 70 eV) *m/z* (%): 510 (0.2, M^+), 508 (0.3), 492 (2.2, $[M-H_2O]^+$), 463 (0.3), 401 (3.1), 400 (8.8), 399 (8.0), 388 (6.2), 387 (5.5), 374 (8.1), 373 (8.2), 355 (5.4), 263 (10.5), 252 (5.1), 251 (5.8), 238 (6.4), 236 (7.1), 225 (5.4), 223 (4.2), 207 (4.6), 205 (4.0), 163 (11.3), 137 (100), 110 (45.1), 94 (11.7), 81 (38.8).

Lophirone C penta-acetate (**4**). Lophirone C (10 mg) was dissolved in a Ac_2O–pyridine (1:1) mixture and kept at room temp. for 6 hr, poured into H_2O and extracted with Et_2O. The organic layer washed with H_2O was dried over Na_2SO_4 and evapd. The residue was chromatographed on silica gel plates (solvent hexane–EtOAc: 1:1) to yield **4** (5 mg). CIMS (NH_3) *m/z*: 738 $[M+NH_4]^+$, 721 $[M+H]^+$. EIMS (110°, 70 eV) *m/z* (%): 720 (1.0, M^+), 678 (6.4), 660 (21.5), 636 (12.4), 619 (19.0), 618 (18.5), 592 (7.0), 576 (21.2), 533 (6.8), 457 (8.5), 442 (8.9), 441 (8.4), 416 (10.6), 400 (9.2), 399 (9.7), 373 (6.3), 355 (9.6), 261 (3.7), 236 (6.8), 179 (39.3), 163 (7.2), 137 (100), 121 (34.8). RMN (200 MHz) δ (ppm): 2.13 (s), 2.14 (s), 2.19 (s), 2.22 (s) and 2.25 (s), 3H each, 5 OAc.

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