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STRUCTURES OF LOPHIRONES I AND J, MINOR CLEAVED CHALCONE DIMERS OF *LOPHIRA LANCEOLATA*

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ABSTRACT.—Two minor cleaved biflavonoids, lophirones I [**2**] and J [**3**], have been isolated from the stem bark of *Lophira lanceolata*; their structures were determined from spectroscopic and chemical evidence.

Previous phytochemical studies on the stem bark of *Lophira lanceolata* Van Tiegh. ex Kaey (Ochnaceae) afforded several interesting biflavonoids peculiar to the Ochnaceae (1–8). In a continuation of our investigation on this extract, we have isolated two new minor constituents, lophirones I and J, to which structures **2** and **3**, respectively, have been assigned. In this paper we report their structural elucidation using spectroscopic and chemical evidence.

The total Me_2CO extract of the stem bark was fractionated into soluble and insoluble fractions by re-extraction with EtOAc. The soluble portion contained the crude mixture of biflavonoids and was further partitioned into three portions (F_1–F_3) by cc over Si gel. The major fraction (F_2) was purified by repeated cc to give two pure compounds, one identified as the earlier isolated lophirone E [**1**] (1) and the other a new biflavonoid which was named lophirone I [**2**]. Further purification of unseparated subfractions by cc, coupled with preparative tlc, furnished three additional known compounds, isombamichalcone (6) and lophirones C and D, and a second new biflavonoid, lophirone J [**3**].

Lophirone I, obtained as an amorphous yellow compound and analyzed for $C_{23}H_{16}O_5$ by hrms, is an isomer of lophirone E [**1**], with a molecular mass of 372, confirmed from eims. Its ir spectrum showed intense absorption for the same functional groups as lophirone E, at ν cm^{-1} 3112 (OH), 1664 (conjugated C=O), and at 1602, 1507 (C=C and Ar). The ^{1}H-nmr spectrum of lophirone I (Table 1) showed the signals of all the protons defining rings A, B, A′, and D as found in the spectrum of lophirone E with relatively minor chemical shift differences of corresponding protons. Important differences observed between the two spectra included the signals of the protons of the chelated OH group (δ 13.5) and the trans double bond (δ_1 7.067; δ_2 8.002), found in the spectrum of lophirone E [**1**] but absent in that of lophirone I. Instead, the ^{1}H-nmr spectrum of lophirone I had signals for an ABX system of three aliphatic protons ($\delta_{\alpha 1}$ 3.131; $\delta_{\alpha 2}$ 2.768 and δ_β 5.643) suggesting that the chalcone function in lophirone E [**1**] has been replaced by a chalcone function in lophirone I leading to structure **2**. This implied that lophirone I must have only two residual OH groups, both of which are non-chelated, and thus would be easily methylated with CH_2N_2.

Permethylation of lophirone I with excess ethereal CH_2N_2 gave **3**, of molecular formula $C_{25}H_{20}O_5$ obtained from hrms, which showed a molecular peak at *m/z* 400 in eims. Its ir spectrum showed no residual OH absorption, and its ^{1}H-nmr spectrum had two sharp singlet signals (δ_1 3.398 and δ_2 3.413, 3H each) assigned to two MeO groups, thus confirming the presence of only two non-chelated

TABLE 1. ^{1}H-nmr Data of Lophirones E [**1**], I [**2**], and J [**3**] (Me_2CO-d_6, TMS, 300 MHz).

Carbon	Compound						ring
	1		**2**		**3**		
	ppm m	*J* (Hz)	ppm m	*J* (Hz)	ppm m	*J* (Hz)	
C-3′ . . .	6.394 d	2.4	6.454 d	2.3	6.589 dd	8.7; 2.3	B
C-5′ . . .	6.504 dd	8.9; 2.4	6.580 dd	2.3; 8.7	6.465 d	2.3	B
C-6′ . . .	8.187 d	8.9	7.738 d	8.7	7.738 d	8.7	B
C-α . . .	7.967 d	15.1	α_1 3.121 dd	13.0; 16.7	3.125 dd	12.9; 16.7	
			α_2 2.768 dd	2.9; 16.7	2.764 dd	2.9; 16.7	
C-β . . .	8.002 d	15.1	5.643 dd	2.9; 13.0	5.659 dd	12.9; 2.9	
C-2 . . .	8.096 d	1.6	7.136 d	1.8	8.122 d	1.8	A
C-5 . . .	7.631 dd	8.6; 0.8	7.583 d	8.6	7.569 d	8.4	A
C-6 . . .	7.823 dd	8.6; 1.6	7.485 dd	8.6; 1.8	7.524 dd	8.4; 1.8	A
C-α′ . . .	7.152	0.8	7.795 s		5.696 s		D
C-2″ . . .	7.823 m		7.810 m		7.759 m		A′
C-3″ . . .	6.991 m		6.965 m		7.015 m		A′
C-5″ . . .	6.991 m		6.965 m		7.015 m		A′
C-6″ . . .	7.823 m		7.810 m		7.759 m		A′
OH . . .	13.500 s				(OMe) 3.413 s		
					(OMe) 3.398 s		

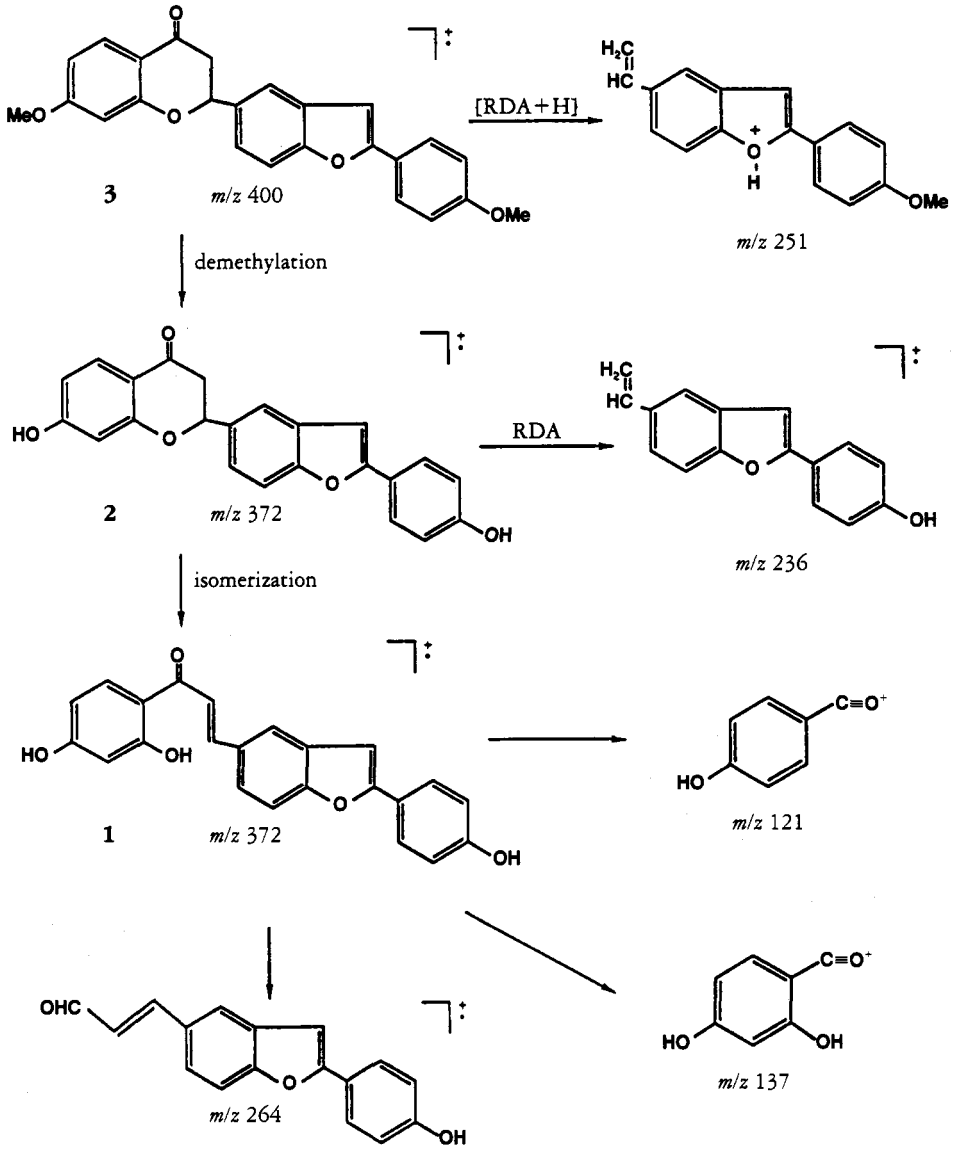
OH groups in lophirone [**2**], both transformed by methylation to MeO groups in the permethylated derivative.

Further confirmation of the presence of the flavanone ring came from eims analysis of **2** for which an RDA transposition clearly explained the presence of the intense peak at *m/z* 236. Other important ions include those at *m/z* 121 and *m/z* 137 (Scheme 1). Chemical evidence came from the cyclization of lophirone E [**1**] in acid medium, which gave a compound identical to **2** (R_f, ir, nmr, ms).

The second minor constituent, lophirone J [**3**], whose molecular formula was analyzed as $C_{25}H_{20}O_5$ using hrms, was obtained as an amorphous yellow compound. Cims showed $[M+H]^+$ ion at *m/z* 401, confirming a molecular mass of 400. Its ir spectrum was very similar to that of lophirone I [**2**], in that it also had intense absorption bands at 1655 cm^{-1} (conjugated C=O), 1607 and 1507 (C=C and Ar) but lacked absorptions typical of the OH group.

The ^{1}H-nmr spectrum (Table 1) of lophirone J [**3**] was very similar to that of lophirone I [**2**], and signals for protons defining the rings A, B, C, D, and A′ were observed. In addition, two sharp singlet signals (each 3H) at δ 3.414 and 3.398, assigned to two MeO groups, were observed. These results suggested that lophirones I [**2**] and J [**3**] have the same carbon skeleton but differ only in their substituent groups. Lophirone I [**2**] has two OH groups, at 4′ (ring B) and at 4″ (ring A′), while lophirone J [**3**] has two MeO groups at the same positions as in structure **2**. Chemical evidence came from the methylation of lophirone I [**2**], which led to a compound identical to lophirone J [**3**] (R_f, ms, ir, nmr). The ^{13}C signal of the flavanone carbonyl appeared as a singlet at 190.20 ppm, while signals for C-α and C-β (ring D) were observed at δ 45.23 and 81.03 as a triplet and a doublet, respectively. Signals at δ 53.45 and 53.36 confirmed the presence of two MeO groups in **3**. The eims of **3** had an intense rearrangement peak at *m/z* 251. Structures of other abundant ions observed at *m/z* 149 and 264 were easily derived (Scheme 1).

Biosynthetically, lophirone I [**2**] would be expected to be derived from lophirone E [**1**] by cyclization of the chalcone function, while lophirone J [**3**]



SCHEME 1. Major fragments in ms of **2** and **3**.

would be obtained from lophirone I [**2**] by natural methylation of the OH groups.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr studies were performed in Me_2CO-d_6 solution on a Bruker WM 300 instrument using TMS as internal standard, while eims were made on a Thomson-Houston THM 208 mass spectrometer. Cims was carried out on a Nermag sidar $V_{3.0}$ instrument with NH_3 as ionizing gas. The solvent used for both cc and tlc was CH_2Cl_2-MeOH (10:1), and Si gel for cc had the mesh size 0.04–0.063 mm. Si gel plates (F_{254}) used for preparative tlc had thickness 0.25 mm.

PLANT MATERIAL.—*L. lanceolata* was harvested at Foumban, Cameroon, in 1987, and a voucher specimen was deposited at the National Herbarium, Yaounde, Cameroon.

EXTRACTION AND PURIFICATION.—Air-dried stem bark was ground to give a fine powder (5 kg) which was extracted with cold Me_2CO in a tank equipped with a mechanical stirrer. After filtration and removal of solvent, the resultant gum was re-extracted with EtOAc. The soluble portion was concentrated to give a dark brown gum (21 g)

which was fractionated over a Sephadex LH20 column (200 g, MeOH) into fractions F_1 (0.2 g), F_2 (18 g), and F_3 (1.8 g).

Fraction F_2 contained the mixture of biflavonoids and was further fractionated by cc [Si gel, CH_2Cl_2-MeOH (10:1)] into five subfractions: F_2a, F_2b, F_2c, F_2d, F_2e. Subfraction F_2a was purified by another cc on Si gel with the same solvent system as before to give lophirone E [**1**] (30 mg) and lophirone J [**3**] (5 mg). Purification of subfraction F_2b as above gave a mixture of biflavonoids which was separated by preparative tlc to give lophirone I [**2**] (5 mg).

Lophirone I [**2**].—$C_{23}H_{16}O_5$; fab-hrms $[M+H]^+$ 373.1072 (calcd 373.1066); $[\alpha]^{20}D$ +2.3 (c=0.48, Me_2CO), ir (KBr paste) ν cm^{-1} 3112, 1664, 1612, 1507, 1468, 1279, 1267, 163 (21), 152 (26), 137 (68), 121 (44); ^1H nmr see Table 1; eims (110°, 70 eV) *m/z* (%) $[M]^+$ 372 (100), 265 (9), 236 (57), 163 (21), 152 (26), 137 (68), 121 (44), 110 (74) (Scheme 1).

Cyclization of lophirone E [**1**].—Lophirone E (10 mg) was dissolved in HCOOH (5 ml), and the resulting solution was heated under reflux in an H_2O bath for 30 min, after which the reaction medium was poured into ice-cold H_2O. The precipitate obtained was filtered, dried, and purified on preparative tlc plates of Si gel using CH_2Cl_2-MeOH (10:1) to give the cyclized product (8 mg), identified as lophirone I [**2**] (R_f, ir, ms, and nmr data).

Methylation of lophirone I [**2**].—Lophirone I [**2**] (5 mg) was dissolved in MeOH (3 ml), and an ethereal solution of CH_2N_2 was added in small portions after which the medium was allowed to stand at room temperature until disappearance of the yellow color of CH_2N_2. When no further reagent was consumed, the medium was concentrated giving a dimethyl ether (3 mg) identical to lophirone J [**3**] in all physical data (R_f, ir, ms, nmr).

Lophirone J [**3**].—$C_{25}H_{20}O_5$; fab-hrms $[M+H]^+$ 401.1382 (calcd 401.1379); $[\alpha]^{20}D$ −1.4 (c=0.42, Me_2CO); ir (KBr paste) ν cm^{-1} 3296, 1655, 1607, 1608, 1457, 1284, 1175, 840, 813; eims (70 eV, 110°) *m/z* (%) $[M]^+$ 400 (100), 372 (6), 279 (6), 264 (94), 251 (48), 163 (18), 149 (12), 137 (24), 110 (12), 94 (19) (Scheme 1); ^{13}C nmr (62.8 MHz, Me_2CO-d_6) ppm 45.23 (t, C-d), 53.36 (q, OMe), 53.45 (q, OMe), 81.03 (d, C-β), 101.01 (d, C-α′), 103.49 (d, C-3′), 111.37 (d, C-5), 111.56 (d, C-5′), 113.18 (S, C-1′), 116.49 (d, C-3″), 121.68 (d, C-2), 122.23 (s, C-1″), 123.60 (d, C-6), 129.46 (s, C-3), 129.90 (s, C-1), 130.23 (d, C-2″, -6″), 135.33 (d, C-6″), 155.36 (s, C-4), 159.50 (s, C-4″), 164.46 (s, C-4″), 165.46 (s, C-2′), 190.21 (s, C=O).

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