Journal of Natural Products
Vol. 52, No. 2, pp. 284-288, Mar-Apr 1989

LOPHIRONES D AND E: TWO NEW CLEAVED BIFLAVONOIDS FROM *LOPHIRA LANCEOLATA*

R. GHOGOMU TIH, B.L. SONDENGAM,

Département de Chimie Organique, Université de Yaoundé, B.P. 812, Yaoundé, Cameroun

M.T. MARTIN, and B. BODO*

Laboratoire de Chimie, UA 401 CNRS, Muséum National d'Histoire Naturelle, 63 rue Buffon, 75231 Paris Cedex 05, France

ABSTRACT.—The isolation of lophirones D and E, new biflavonoids from the stem bark of *Lophira lanceolata*, is reported. Their structures were determined on the basis of ^{1}H-nmr, ^{13}C-nmr, ms, and ir spectral data.

Lophira lanceolata Van Tiegh. ex Keay (Ochnaceae) is a highly branched tree widely distributed in the woody savannas of tropical Africa, where the natives use various parts (leaves, roots, wood, bark) to treat different health problems. Prominent medical uses include treatment of toothache (Cameroon), liver infections (Togo), female sterility, dysentery, and cough (Nigeria). Recent phytochemical studies on related members of the Ochnaceae family describe isolation of several new unusual biflavonoids (1–6).

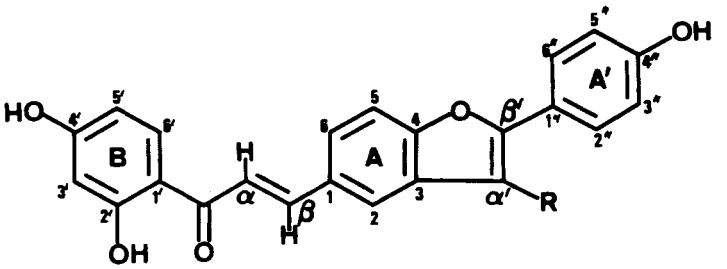
Spurred by its clinical applications, we have examined the stem bark of *L. lanceolata* and reported the structures of three isomeric biflavonoids: lophirones A, B, and C (5,6). Further purification of the same extract has led to the isolation of two new constituents together with known lophirones A, B, and C. The two new compounds are cleaved flavonoids named lophirones D and E, for which structures **1** and **2** have been assigned from spectral and chemical evidence.

RESULTS AND DISCUSSION

The Me_2CO extract of air-dried, ground stem bark of *L. lanceolata* afforded a crude mixture of biflavonoids that was fractionated into three fractions (F_1, F_2, and F_3) by cc. Lophirones A, C, and E were purified by repeated cc over Si gel while preparative tlc was used to separate lophirone B from lophirone D.

Lophirone D was obtained as yellow crystals (mp 270–276° dec) from Me_2CO. Ms measurements established a molecular formula of $C_{24}H_{16}O_6$ as the molecular ion was at *m/z* 400 in eims and confirmed by cims with $[M+H]^+$ ion at *m/z* 401. Its ir spectrum displayed intense absorption bands at 3342 (OH), 1660 (sh), 1623, and 1605 (conjugated, chelated C=O) and at 1505 cm^{-1} (C=C and Ar).

Complete methylation with MeI/K_2CO_3 in dry Me_2CO afforded a trimethyl ether ($C_{27}H_{22}O_6$): its ^{1}H-nmr spectrum had three singlet signals at δ 3.943 (3H), 3.887



1 R=CHO
2 R=H

(3H), and 3.872 ppm (3H) corresponding to three methoxyl groups. As no residual hydroxyl absorption was seen in the ir spectrum, lophirone D has three hydroxyl functions.

The ^{13}C-nmr spectrum of lophirone D had signals for all the 24 carbon atoms in the molecular formula, including signals for two carbonyl groups (δ 191.19 and 186.09 ppm), ten quaternary sp^2 carbons with five substituted by an oxygen atom, and twelve tertiary sp^2 carbons (Table 1).

TABLE 1. Nmr Data of Lophirones D and E (Me_2CO-d_6, TMS, ^{1}H 250.13 MHz, ^{13}C 62.8 MHz).

Carbon	Compound					
	1			2		
	δ_C	δ_H	J(Hz)	δ_C	δ_H	J(Hz)
1′	113.00 s			114.34 s		
2′	165.05[a] s			167.69[a] s		
3′	102.50 d	6.399 d	2.4	104.00 d	6.394 d	2.4
4′	165.53[a] s			167.66[a] s		
5′	108.14 d	6.519 dd	8.9,2.4	109.80 d	6.504 dd	8.9,2.4
6′	132.07 d	8.217 d	8.9	133.49 d	8.197 d	8.9
C=O	191.19 s			193.14 s		
α	120.98 d	7.924 d	14.6	120.52 d	7.967 d	15.1
β	143.42 d	7.979 d	14.6	145.75 d	8.002 d	15.1
1	126.10 s			131.82[b] s		
2	122.10 d	8.590 d	1.6	122.53 d	8.096 d	1.6
3	131.45 s			131.63[b] s		
4	154.04 s			157.34 s		
5	111.64 d	7.626 d	8.6	112.30 d	7.631 dd	8.6,0.8
6	126.34 d	7.981 dd	8.6,1.6	125.63 d	7.823 dd	8.6,1.6
CHO	186.09 d	10.373 s				
α′	114.00 s			100.08 d	7.152 d	0.8
β′	165.65[a] s			159.17[c] s		
1″	118.25 s			122.85 s		
2″	130.76 d	7.940 m		127.65 d	7.823 m	
3″	116.11 d	7.128 m		116.77 d	6.991 m	
4″	160.67 s			159.75[c] s		
5″	116.11 d	7.128 m		116.77 d	6.991 m	
6″	130.76 d	7.940 m		127.65 d	7.823 m	
OH		13.340 s			13.500 s	

[a,b,c]Values in the same column with the same superscript may be interchanged.

The ^{1}H-nmr spectrum of lophirone D had certain signals very similar to those found in the spectrum of lophirones B and C. The spin systems involved were obtained from the 2D COSY spectrum leading to the definition of two trisubstituted ortho-para benzene rings (A and B), one para-disubstituted benzene ring (A′), a *trans* double bond (J = 14.5 Hz) conjugated to a carbonyl group, and an aldehyde proton singlet at δ 10.373 ppm, which did not collapse on addition of D_2O. Another sharp singlet (1H) at δ 13.340 ppm was assigned to a hydroxyl proton strongly chelated to a peri carbonyl group.

Because the H-6′ proton (ring B) showed long range coupling (2D COSY LR) with H-α of the double bond, and the H-2,H-6 protons (ring A) with H-β, a chalcone residue substituted in the 3-position was established. This chalcone moiety gave rise to signals that were similar to those observed in the ^{1}H-nmr spectra of lophirones B and C.

Of the six oxygen atoms in the molecular formula, three occur in hydroxyl functions and two in carbonyl groups. The remaining oxygen and carbon atoms can only be used to make a furan ring fused with benzene ring A that has the aldehyde and para-disubstituted benzene ring (A′) as substituents. The low value of the $^5J_{H2\text{-}H6}$ coupling

constant (1.6 Hz) was indicative of the presence of a benzofuran ring. Long-range coupling observed between the H-2 proton (ring A) and the aldehyde proton implied that ring A′ is fixed onto the carbon adjacent to the heterocyclic oxygen, leading to structure **1** for lophirone D.

The obtained ^{13}C-nmr spectra were in agreement with the proposed structure **1**; complete assignment of signals was made after studying the totally decoupled, *J*-modulated ^{13}C–^{1}H-coupling-correlated spectra of lophirone D, as well as comparing observed data with those of related compounds (6).

Major fragmentation pathways observed in the ei mass spectrum are also consistent with the proposed structure. An intense $[M-H]^+$ ion at *m/z* 399 was due to the loss of a hydrogen radical from the aldehyde group. The base peak appeared at *m/z* 264 resulting from an RDA rearrangement while other abundant fragments were found at *m/z* 264, 251, 236, 163, and 137 (Scheme 1).

Lophirone E formed yellow crystals from Me_2CO (mp 266–268°) and gave a molecular ion $[M]^+$ at *m/z* 372, 28 amu lower than **1**, in agreement with the molecular formula $C_{23}H_{16}O_5$. Its ir spectrum was very similar to that of **1** but showed only one chelated and conjugated carbonyl absorption at 1639 cm^{-1}.

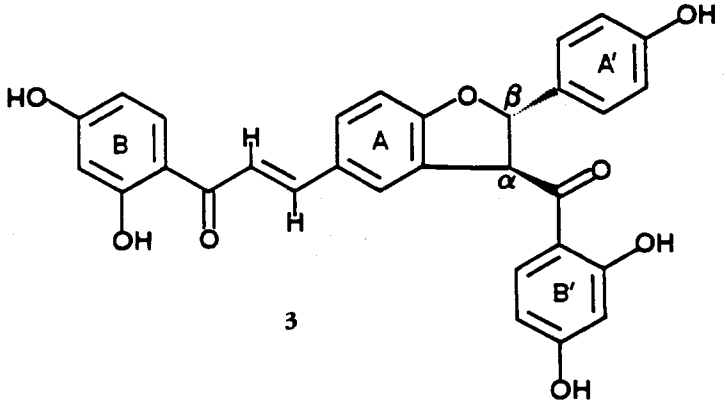
Close resemblance was found between the ^{13}C-nmr spectra of lophirones E and D (Table 1). Although all 23 sp^2 carbon atoms required by the molecular formula of lophirone E were present, its spectrum lacked the aldehyde carbonyl signal. The *J*-modulated nmr spectrum of lophirone E enabled the distinction of 9 quaternary, 13 tertiary, and one conjugated carbonyl carbon atoms.

Its ^{1}H-nmr spectrum was also similar to that of **1**; by using 2D COSY techniques, all proton spin systems involved were defined and found to be exactly the same as in **1** but with differences in chemical shift of certain protons. The ring-B′ protons appeared more shielded in lophirone E while the aldehyde proton singlet at 10.373 ppm in **1** was replaced by an ethylenic proton doublet ($J = 0.8$ Hz) at 7.152 ppm due to a ^{5}J coupling with the H-5 proton of ring A, showing that the aldehyde substituent in **1** had been replaced by a proton (H-α′) implying structure **2** for lophirone E.

The ^{13}C-nmr spectrum of lophirone E is compatible with structure **2**. The C-α′ carbon atom gives an upfield doublet signal at δ 100.08 ppm.

More evidence for structure **2** came from the electronic impact spectrum of lophirone E, which had major fragmentation pathways similar to those found in **1**. Even though the base peak appeared at *m/z* 137 in the eims of **2**, abundant ions at *m/z* 236, 223, 163, and 121 corresponded to those earlier obtained for **1** (Scheme 1).

Lophirones D and E may be considered as biflavonoids formed by condensation of two chalcone moieties as found in lophirone C [**3**]. Dehydrogenation of the C-α′–C-β′



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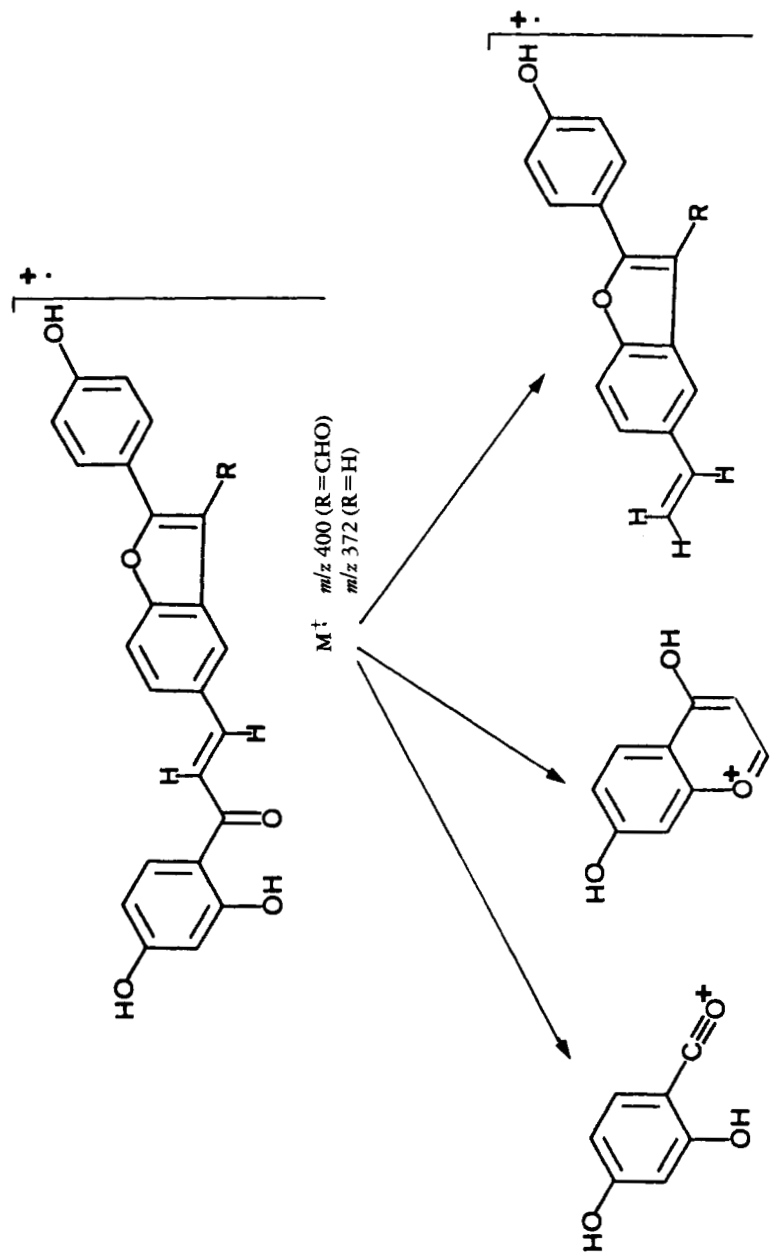

SCHEME 1. Main fragmentations from the ei mass spectra of lophirones D [1] (R=CHO) and E [2] (R=H).

bond of this precursor followed by the elimination of either a phenyl or a benzoyl ring give rise to **1** or **2**, respectively.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were taken on a Kofler microscope and are uncorrected. Nmr studies were performed in TMS/Me_2CO-d_6 on a WM 250 Bruker, eims on a Thomson-Houston THN 208 mass spectrometer, and cims 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, unless stated otherwise, and the granulometry of SiO_2 was 0.04–0.063 mm for cc and Si gel plates F_{254} 0.25 mm in thickness for preparative tlc.

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

EXTRACTION AND PURIFICATION.—Air-dried stem bark of *L. lanceolata* 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 reextracted with EtOAc. The soluble fraction was concentrated to give a dark brown gum (21 g) which was fractionated over Sephadex LH 20 column (200 g, MeOH) into 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 sub-fractions: F_{2a}, F_{2b}, F_{2c}, F_{2d}, and F_{2e}. Sub-fraction F_{2a} was purified by another cc [Si gel, CH_2Cl_2-MeOH (10:1)] to give lophirone E (30 mg) while F_{2b} was first fractionated into F_{2b1} and F_{2b2} by cc [CH_2Cl_2-EtOAc (9:1)] of increasing polarity. Purification of F_{2b1} as above gave pure lophirone C (230 mg) while preparative tlc [Si gel, CH_2Cl_2-MeOH (10:1)] of unseparated fractions gave lophirones B (100 mg) and D (60 mg). Sub-fraction F_{2b2}, after repeated cc under the same conditions as above, gave lophirone A (186 mg), along with two other compounds whose structures are under investigation.

Lophirone D [**1**].—Compound **1** ($C_{24}H_{16}O_6$): mp 270–276° (Me_2CO); ir (KBr paste) ν cm^{-1} 3342, 1660 (sh), 1623, 1605, 1505, 1439, 1373, 1279, 1218, 1134, 1058, 1020, 846, 805; eims (70 eV, 200°) *m/z* (%) 401 (24), $[M]^+$ 400 (91), 399 (69), 382 (13), 264 (100), 263 (44), 251 (31), 238 (17), 236 (26), 223 (19), 207 (14), 164 (14), 163 (93), 137 (74), 121 (16), 120 (73).

Methylation of lophirone D [**1**].—Compound **1** (13 mg) was dissolved in anhydrous Me_2CO in the presence of excess dry K_2CO_3 with stirring for 10 min. Then excess MeI was added and stirring continued for 24 h, after which it was filtered and the filtrate concentrated to give a crude powdery methyl ether (7 mg) that was purified by preparative tlc on Si gel plates [solvent hexane-EtOAc (1:1)] and crystallized from Me_2CO: mp 190.5–191.5°; eims (70 eV, 200°) *m/z* (%) $[M]^+$ 442 (19), 427 (5), 414 (17), 372 (4), 292 (10), 266 (9), 265 (21), 205 (12), 190 (10), 178 (15), 177 (17), 165 (100), 151 (35), 135 (40), 122 (29), 107 (21); ^1H nmr (250.13 MHz, Me_2CO-d_6) δ ppm 10.271 (1H, s, -*CH*O), 8.412 (1H, s, H-2), 8.010 (2H, m, H-2″,6″), 7.838 (1H, d, J = 9.3 Hz, H-6), 7.792 (1H, d, J = 9.3 Hz, H-5), 7.692 (1H, d, J = 15.7 Hz, H-β), 7.654 (1H, d, J = 8.6 Hz, H-6′), 7.592 (1H, d, J = 15.7 Hz, H-α), 7.201 (2H, m, H-3″,5″), 6.717 (1H, d, J = 2.0 Hz, H-3′), 6.672 (1H, dd, J = 8.6 and 2.0 Hz, H-5′), 3.943, 3.887, and 3.872 (each 3H, s, 3-OMe).

Lophirone E [**2**].—Compound **2** ($C_{23}H_{16}O_5$): mp 266–268° (Me_2CO); ir (KBr paste) ν cm^{-1} 3440, 3292, 1639, 1602, 1565, 1509, 1434, 1290, 1271, 1231, 1141, 1117, 1030, 974, 846, 826, 795, 782; eims (70 eV, 200°) *m/z* (%) 373 (2), $[M]^+$ 372 (12), 237 (5), 236 (54), 223 (24), 210 (20), 163 (59), 152 (27), 151 (24), 137 (100), 121 (57).

ACKNOWLEDGMENTS

We thank Dr. M. Satabie (National Herbarium), Messrs. Nole and Mbita for collection and identification of plant material, the Dr. J.P. Brouard for mass spectra. We are grateful to the Yaounde University Grants Committee for financial assistance.

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Received 6 September 1988