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J. Nat. Prod., **1994**, 57 (9), 1275-1278• DOI: 10.1021/np50111a015 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 9, pp. 1275-1278, September 1994

MINOR BIFLAVONOIDS OF *LOPHIRA LANCEOLATA*

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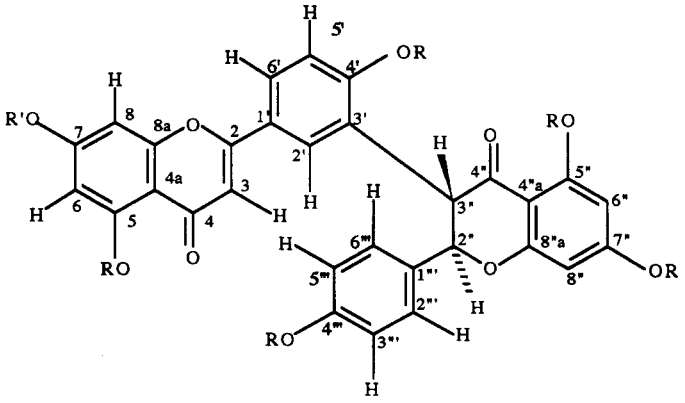
ABSTRACT.—Two new biflavonoids have been isolated in small amounts from the leaves of *Lophira lanceolata* and their structures established from spectroscopic and chemical evidence.

The stem bark and stem heartwood of the Cameroonian medicinal plant, *Lophira lanceolata* Van Tiegh. ex Keay (Ochnaceae), have been under intensive phytochemical investigation leading to the isolation of different types of flavonoids (1–5), some of which show antibacterial and antiviral activity (6,7). We have extended our research to the leaves of this plant from which two new minor biflavonoid constituents, lanceolatins A [**1**] and B [**2**], have been isolated and characterized. This paper deals with the structure elucidation of [**1**] and [**2**].

Lanceolatin A [**1**] was obtained as an amorphous yellow solid, analyzed for $C_{30}H_{20}O_{10}$ (hrms *m/z* $[M]^+$ calcd 540.106, found 540.092). Absorption bands for the following functional groups were noted in the ir spectrum of **1**: phenolic hydroxyl (3302 cm^{-1}), conjugated and chelated carbonyl (1656 cm^{-1}), conjugated double bonds (1629 cm^{-1}), and aromatic rings (1510 cm^{-1}). The eims of **1** showed a molecular peak at *m/z* 540 confirming the molecular formula of $C_{30}H_{20}O_{10}$ and implying 21 unsaturated sites.

From the 1D and 2D ^{1}H-nmr spectra of **1** (Table 1), it was established that 20 protons were located on four benzene rings (one 1,4-disubstituted, one 1,2,4-trisubstituted, and two 1,2,4,6-tetrasubstituted). An aliphatic AB system with trans disposition, a singlet proton at 6.52 ppm, and two equally sharp singlet signals at 12.50 and 13.50 ppm typical of peri hydroxyl groups, were observed.

Evidence that lanceolatin A [**1**] had six hydroxyl groups was established when its complete acetylation furnished a hexaacetate [**3**] ($C_{42}H_{32}O_{16}$, requires 792.169,



1 R=R′=H
2 R=H, R′=Me
3 R=R′=MeCO
4 R=MeCO, R′=Me
5 R=R′=MeCO

TABLE 1. Nmr Data (^{1}H-, 300 MHz and ^{13}C- ,75 MHz, CD_3COCD_3, TMS) for Compounds **1**–**5**.

Position	Compound					
	1	**1**	**2**	**3**	**4**	**5**
	δ_C (ppm) δ_H (ppm) m J (Hz)	^{2-4}J HMBC correlations (^{1}H)	δ_H (ppm) m J (Hz)	δ_H (ppm) m J (Hz)	δ_H (ppm) m J (Hz)	δ_H (ppm) m J (Hz)
2	163.80 s —	H-3, H-2′, H-6′	—	—	—	—
3	102.30 d 6.53 d	H-2′, H-6′	6.53 s	6.51 s	6.53 s	6.57 s
4	182.11 s —	H-3	—	—	—	—
4a	104.28 s —	H-3, H-6, H-8	—	—	—	—
5	162.11 s 12.96 s (OH)	H-6	12.89 s (OH)	—	—	—
6	99.54 d 6.22 d (1.9)	H-8	6.29 d (2.2)	6.56 d (2.3)	6.79 d (2.6)	6.78 d (2.4)
7	163.84 s —	H-6, H-8	3.86 s (MeO)	—	3.90 s	—
8	94.79 d 6.47 d (1.9)	H-6	6.58 d (2.2)	6.82 d (2.3)	6.80 d (2.6)	6.48 d (2.4)
8a	157.33 s —	H-8	—	—	—	—
1′	122.29 s —	H-3, H-2′, H-5′, H-6′	—	—	—	—
2′	131.70 d 7.75 d (2.2)	H-5′, H-6′	7.71 d (2.2)	7.21 d (2.4)	6.59 d (2.4)	7.70 d (2.4)
3′	123.85 s —	H-5′, H-3″	—	—	—	—
4′	158.74 s —	H-5′	—	—	—	—
5′	115.82 d 6.95 d (9.1)	H-6′	6.98 d (8.2)	7.21 d (8.7)	7.16 d (8.6)	7.00 d (8.7)
6′	127.31 d 7.73 dd (9.1, 2.2)	H-2′	7.72 dd (8.2, 2.2)	7.72 dd (8.7, 2.4)	7.71 dd (8.6, 2.4)	7.81 dd (8.7, 2.4)
2″	83.25 d 5.93 d (12.1)	H-2‴, H-6‴	5.91 d (12.0)	6.02 d (12.2)	6.05 d (12.6)	5.91 d (12.4)
3″	55.38 d 4.72 d (12.1)	H-2′	4.68 d (12.0)	6.02 d (12.2)	6.00 d (12.6)	4.61 d (12.4)
4″	196.24 s —	H-2″, H-3″	—	—	—	—
4″a	101.73 s —	H-6″, H-8″	—	—	—	—
5″	166.35 s 12.22 s (OH)	H-6″	12.18 s (OH)	—	—	—
6″	94.81 d 5.99 d (2.2)	H-8″	6.00 d (2.2)	6.43 d (2.0)	6.46 d (2.3)	6.24 d (2.2)
7″	164.80 s —	H-6″, H-8″	—	—	—	—
8″	95.96 d 6.00 d (2.2)	H-6″	6.00 s	6.49 d (2.0)	6.56 d (2.3)	6.24 d (2.2)
8″a	163.07 s —	H-8″	—	—	—	—
1‴	128.06 s —	H-2‴/H-6‴	—	—	—	—
2‴,6‴	129.92 d 7.30 m	H-2″, H-3‴/H-5‴	7.28 m	7.54 m	7.50 m	7.31 m
3‴,5‴	114.81 d 6.73 m	H-2‴	6.73 m	7.10 m	7.05 m	6.80 m
4‴	157.31 s —	H-3‴/H-5‴, H-2‴/H-6‴	—	—	—	—
				2.40 s[b]	3.81 s[a]	3.88 s[a]
				2.33 s[b]	2.39 s[b]	3.81 s (2×)[a]
				2.27 s[b]	2.38 s[b]	3.77 s[a]
				2.22 s[b]	2.27 s[b]	3.75 s[a]
				2.21 s[b]	2.22 s[b]	3.65 s[a]
				1.98 s[b]	2.20 s[b]	

[a]MeO signals.
[b]MeCOO signals.

found 792.181). Compound **3** showed no residual hydroxyl absorption in its ir spectrum, while its ^{1}H-nmr spectrum (Table 1) displayed the six MeCOO signals as sharp singlets at 2.40, 2.33, 2.27, 2.22, 2.21, and 1.98 ppm (each 3H). Confirmation that all six hydroxyl groups in **1** are phenolic came from permethylation with CH_2N_2 which yielded a hexamethyl ether [**5**] ($C_{36}H_{32}O_{10}$, requires 624.199; found 624.211), the ^{1}H-nmr spectrum of which had six singlet signals for six MeO groups at 3.88 (3H), 3.81 (6H), 3.77 (3H), 3.75 (3H), and 3.65 ppm (3H).

A study of long-range couplings in the COSY-LR nmr spectrum of lanceolatin A [**1**] led to two partial structures. Correlation cross-peaks between H-2′ and the H-3″ proton led to the plane structure **1** for lanceolatin A. Assignments of carbon signals were made by comparing the totally decoupled ^{13}C-nmr spectrum with the ^{1}H-^{13}C correlated spectrum as well as with values reported for similar compounds (8,9). Confirmation of signal assignments was obtained from the HMBC nmr spectrum of lanceolatin A [**1**] (Table 1) which also confirmed the carbon skeleton. The large coupling constant (J=12.0 Hz) between H-2″ and H-3″ observed in the ^{1}H-nmr spectrum implies their trans disposition in **1**.

The second minor constituent, lanceolatin B [**2**], obtained as an amorphous yellow solid, is also a biflavonoid, with a molecular formula of $C_{31}H_{22}O_{10}$ obtained from hrms (*m/z* $[M]^+$ requires 554.121; found 554.109). Its ir spectrum was very similar to that of lanceolatin A [**1**] and absorption bands for the following functional groups were present: phenolic hydroxyl (3311 cm^{-1}), conjugated and chelated carbonyl (1661 cm^{-1}), conjugated double bonds (1631 cm^{-1}), and aromatic rings (1515 cm^{-1}). The 1D and 2D ^{1}H-nmr spectra of lanceolatin B [**2**] were also very similar to those of **1**. Observed long-range correlation cross-peaks were the same as for lanceolatin A [**1**]. The presence of a singlet at 3.81 ppm (3H) in the ^{1}H-nmr spectra of lanceolatin B [**2**], assigned to a MeO group, was the main difference between the ^{1}H-nmr spectra of lanceolatins A and B. This suggested that one of the phenolic groups in lanceolatin A was naturally methylated in lanceolatin B, and implied that lanceolatin B must have five phenolic groups.

Confirmation that lanceolatin B [**2**] had five phenolic hydroxyl groups came from acetylation which yielded a penta-acetate derivative [**4**] that analyzed for $C_{41}H_{32}O_{15}$ (hrms *m/z* $[M]^+$ requires 764.174; found 764.166). Its ^{1}H-nmr spectrum had sharp singlet signals for five MeCOO groups at 2.39, 2.38, 2.27, 2.22, and 2.20 ppm.

The lone MeO substituent was placed at C-7 because it was noticed that chemical shift of all protons in lanceolatins A [**1**] and B [**2**] are closely comparable except for H-6 and H-8, for which measured values for both compounds were very different. This was confirmed by correlation cross-peaks observed between the MeO group and either of the protons H-6 and H-8 in the NOESY spectrum of lanceolatin B. All spectral evidence therefore indicated structure **2** for lanceolatin B.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were recorded using KBr discs. Nmr spectra (^{1}H-, 300 MHz and ^{13}C-, 75 MHz) were taken on a Bruker spectrometer using Me_2CO-d_6, with TMS as internal standard. The solvent mixtures used for both cc and tlc were $CHCl_3$-MeOH, 10:1 and 5:1, unless otherwise stated. Si gel of mesh size 0.04–0.063 mm was used for cc and prep. tlc plates were coated with fluorescent (F_{254}) Si gel (thickness 0.25 mm).

PLANT MATERIAL.—Fresh leaves of *Lophira lanceolata* were harvested in Balamba near Bafia in the Center Province of Cameroon. A voucher specimen was deposited in the National Herbarium in Yaoundé.

EXTRACTION AND PURIFICATION.—Sun-dried, ground plant material (5 kg) was extracted with cold MeOH in an iron tank equipped with a mechanical stirrer. The crude extract obtained was concentrated to dryness leaving a dark green residue (250 g) that was re-extracted with Et_2O. The

insoluble fraction (100 g) was first fractionated by gel permeation chromatography over Sephadex LH-20 with MeOH as eluent to give six fractions. Fraction 6 (7 g) was further rechromatographed under the same conditions as before and fractions purified further by cc on Si gel with the solvent mixture $CHCl_3$/MeOH to yield two amorphous yellow solid compounds, lanceolatin A (**1**, 35 mg) and B (**2**, 15 mg).

Lanceolatin A [**1**].—$C_{30}H_{20}O_{10}$; eims *m/z* $[M]^+$ 540; ir (KBr) ν max 3302, 1656, 1629, 1571, 1560, 1510, 1505 cm^{-1}; ^1H nmr (300 MHz, Me_2CO-d_6), see Table 1; ^{13}C nmr (75 MHz, Me_2CO-d_6), see Table 1.

Acetylation of lanceolatin A [**1**].—Lanceolatin A [**1**] (10 mg) was dissolved in a mixture of pyridine (2 ml) and Ac_2O (2 ml). The reaction mixture was maintained at 50° using a H_2O bath for 6 h after which it was concentrated to dryness under vacuum. The resulting crude acetate was first purified by prep. tlc followed by gel permeation cc over Sephadex LH-20 using MeOH as eluent, yielding lanceolatin A hexa-acetate [**3**] (7 mg), as an amorphous solid ($C_{42}H_{32}O_{16}$); eims *m/z* $[M]^+$ 792; ^1H nmr (300 MHz, Me_2CO-d_6), see Table 1.

Permethylation of lanceolatin A [**1**].—Lanceolatin A [**1**] (10 mg) was dissolved in MeOH and methylated with an ethereal solution of CH_2N_2 and the progress of the reaction followed by tlc. The resultant mixture was evaporated to dryness and purified by prep. tlc on Si gel plates with the solvent mixture $CHCl_3$-MeOH (10:1). The major band was recovered and extracted after which it was purified using Sephadex LH-20 in MeOH, yielding lanceolatin A hexamethyl ether [**5**], as an amorphous solid ($C_{36}H_{32}O_{10}$); eims *m/z* $[M]^+$ 624; ^1H nmr (300 MHz, Me_2CO-d_6), see Table 1.

Lanceolatin B [**2**].—$C_{31}H_{22}O_{10}$; eims *m/z* $[M]^+$ 554; ir (KBr) ν max 3302, 1656, 1629, 1571, 1560, 1510, 1505 cm^{-1}; ^1H nmr (300 MHz, Me_2CO-d_6), see Table 1; ^{13}C nmr (75 MHz, Me_2CO-d_6), see Table 1.

Acetylation of lanceolatin B [**2**].—The same procedure as for **1** was applied to lanceolatin B [**2**] (5 mg) and lanceolatin B penta-acetate [**4**], (3 mg) was obtained as an amorphous solid. [**4**] ($C_{41}H_{32}O_{15}$); eims *m/z* $[M]^+$ 764; ^1H nmr (300 MHz, Me_2CO-d_6), see Table 1.

ACKNOWLEDGMENTS

This work was supported by IFS (grant F/1389-2). We thank the botanists Dr. Satabie and Messrs. Mbita and Nole for the identification of plant material. We are also indebted to the Yaoundé University Grants Committee and DAGIC (France) for financial assistance.

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Received 4 January 1994