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LANCEOLINS A AND B: NITRILE GLYCOSIDE ESTERS FROM *LOPHIRA LANCEOLATA*

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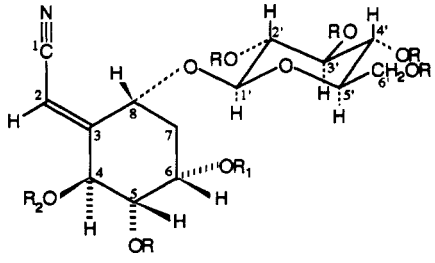
ABSTRACT.—From the stem heart-wood of *Lophira lanceolata*, two new nitrile glycoside esters, lanceolins A and B, have been isolated. Both structures were established from spectroscopic and chemical evidence.

Lophira lanceolata Van Tiegh. ex Keay (Ochnaceae), commonly known as "kwet" by the Tikars and "mbantou" by the Bamouns, is widely distributed in the woody savannahs of Cameroon. Extracts of its stem bark found to show antiviral properties have been extensively studied to identify the chemical content. Several new bi- and tetraflavonoids, some of which are potential antitumor agents, have also been reported (1–7). We have extended our investigations to the stem heart-wood of this plant. From an Me_2CO extract, two new nitrile glycoside esters, lanceolins A and B, were obtained and their structural elucidation is described in this report.



1 R=R_1=H, R_2=Bz
2 R=R_2=H, R_1=Bz
3 R=R_1=Ac, R_2=Bz

Lanceolin A [**1**], was obtained as an amorphous white solid and analyzed for $C_{21}H_{25}O_{10}N$ by hrms, implying a molecular mass of 451 and hence 10 unsaturated sites for this compound. Its ir spectrum showed absorption bands typical of one or more OH groups (3320 cm^{-1}), a CN functionality (2215 cm^{-1}), a conjugated ester carbonyl (1718 cm^{-1}), a conjugated double bond (1623 cm^{-1}) and an aromatic ring (1602 and 1501 cm^{-1}). When boiled with HCl, **1** yielded HCN and a sugar that was identified as glucose, thus implying that it is a nitrile glucoside.

Complete acetylation (Ac_2O/pyridine) of **1** gave an amorphous solid **3**, with the molecular formula $C_{36}H_{37}O_{16}N$ being established from hrms, and whose ir spectrum showed no residual OH absorption, but had intense absorption bands for the CN function (2285 cm^{-1}) and acetate carbonyls (1748 cm^{-1}). From the 1D ^{1}H-nmr spectrum of **3**, sharp singlet signals for six acetate groups at δ 2.07, 2.03, 2.02, 2.01, 1.99, and 1.94 ppm were observed, clearly implying that lanceolin A had six OH groups, all transformed by acetylation to give **3**.

From 1D and 2D 300 MHz ^{1}H-nmr spectra of **1**, the anomeric sugar proton appeared as a doublet at 4.47 ppm (J=7.9 Hz), while signals of the other sugar protons were found between 3.22 and 3.85 ppm (Table 1). Other substituents include a benzoyl group (δ 8.12 [2H, ortho], 7.51 [2H, meta], and 7.62 [1H, para]) and a trihydroxy methylene cyclo-

TABLE 1. Nmr Data (^{13}C-, 75 MHz, and ^{1}H-, 300 MHz) for Compounds **1–4**.[a]

Position	Compound												
	1[b]				**2**[b]				**3**[b]				**4**[c]
	δ_C (ppm)	δ_H (ppm)	m	*J* (Hz)	δ_C (ppm)	δ_H (ppm)	m	*J* (Hz)	δ_C (ppm)[d]	δ_H (ppm)	m	*J* (Hz)	δ_C (ppm)
1	116.4 s	—			116.2 s	—			117.3 s				118.0 s
2	94.9 d	5.87	d	2.2	97.4 d	5.82	d	2.1	94.9 d	5.85	d	2.2	98.6 d
3	163.7 s	—			159.0 s	—			166.4 s				157.1 s
4	67.9 d	5.20	dd	10.2, 2.2	69.3 d	5.18	dd	9.9, 2.1	71.5 d	6.42	dd	10.3, 2.2	127.7 d
5	76.6 d	4.87	dd	10.2, 3.2	74.7 d	3.65	dd	9.9, 3.2	76.0 d	5.16	dd	10.3, 3.7	140.6 d
6	65.7 d	4.38	ddd	3.2, 3.2, 3.2	69.0 d	5.49	ddd	3.2, 3.2, 3.2	72.6 d	5.46	ddd	3.1, 3.7, 3.4	65.3 d
7	33.9 t	2.56	ddd	15.9, 3.2, 3.0	33.4 t	2.56	ddd	15.7, 2.8, 3.2	33.7 t	2.52	ddd	16.0, 3.1, 3.0	36.0 t
		2.04	ddd	15.9, 3.2, 3.2		2.05	ddd	15.7, 3.2, 2.9		2.27	ddd	16.0, 3.7, 3.4	
8	73.3 d	5.14	dd	3.2, 3.0	77.7 d	4.96	dd	3.2, 2.9	77.1 d	5.03	dd	3.7, 3.0	72.5 d
1′	100.9 d	4.47	d	7.9	102.5 d	4.35	d	7.9	104.3 d	5.06	d	10.0	101.5 d
2′	69.9 d	3.22	dd	7.9, 7.9	72.1 d	3.10	dd	8.3, 8.3	71.8 d	5.02	dd	9.2, 8.6	71.7 d
3′	73.9 d	3.37	m		73.4 d	3.37	dd	8.6, 8.0	74.7 d	5.33	dd	9.6, 9.2	74.4 d
4′	76.9 d	3.31	m		69.3 d	3.32	dd	9.9, 8.3	78.0 d	5.05	dd	10.0, 9.6	78.0 d
5′	77.8 d	3.34	m		73.0 d	3.26	dd	9.6, 2.1	78.1 d	3.92	ddd	10.0, 4.9, 2.4	78.1 d
6′	60.9 t	3.85	dd	12.0, 2.3	62.5 t	3.87	dd	11.8, 2.5	63.2 t	4.22	dd	12.2, 2.4	63.1 t
		3.62	m			3.63	dd	11.8, 6.0		4.04	dd	12.2, 4.9	
1″	165.2 s	—			170.7 s	—			166.5 s	—			—
2″	130.0 s	—			130.4 s	—			129.2 s	—			—
3″, 7″	129.5 d	8.12	m		130.3 d	8.13	m		130.7 d	7.96	m		—
4″, 6″	128.6 d	7.51	m		129.6 d	7.47	m		129.1 d	7.51	m		—
5″	133.3 d	7.62	m		134.4 d	7.60	m		133.6 d	7.65	m		—
AcOO										2.07 s, 2.03 s, 2.02 s, 2.01 s, 1.99 s, 1.94 s			

[a]Spectra are referenced to TMS.
[b]Spectrum run in $(CD_3)_2CO$.
[c]Spectrum run in DMSO-d_6.
[d]Data for carbonyl signals are presented in the text.

hexane partial structure with a vinyl nitrile substituent. From the HMBC nmr spectrum of **1**, correlations were noticed between the H-1′ of glucose and the C-8 carbon of the methylene cyclohexane residue, establishing that the glucosidic bond is between C-1′ and C-8. It also displayed correlations between the benzoyl carbonyl and the H-4 proton in the methylene cyclohexane residue leading to structure **1** for lanceolin A, and to **3**, for its totally acetylated derivative.

The relative stereochemistry of the protons of the cyclohexane ring of **1** was deduced from their mutual coupling constants. A large coupling constant of 10.2 Hz found between H-4 and H-5 is indicative of their *trans*-diaxial disposition in a chair conformation of the cyclohexane ring, while the small value of 2.3 Hz observed between H-5 and H-6 implies that they are cis and that H-6 is equatorial. This was confirmed by the fact that H-6 shows two weak couplings (3.2 and 3.2 Hz) with both methylene protons, H-7_a and H-7_b. Because H-8 equally shows weak couplings (3.2 and 3.0 Hz) with H-7_a and H-7_b, it follows that H-8 also is equatorial.

Lanceolin B [**2**], was shown to be an isomer of **1**, since both have the same molecular formula $C_{21}H_{25}O_{10}N$ from hrms. Their ir spectra were very similar, both showing absorptions for OH (3218 cm^{-1}), CN (2212 cm^{-1}), conjugated aromatic ester carbonyl (1715 cm^{-1}), and aromatic functions, and their nmr spectra (1D and 2D) presented signals for the same proton systems, but with some chemical shift differences (Table 1). Consequently, in **2**, signals defining partial structures of glucosyl, benzoyl, and vinyl cyano methylene cyclohexyl groups were identified as in **1**. A careful examination of the HMBC nmr spectrum of lanceolin B showed that in the methylene cyclohexane residue, its H-8 proton had correlations with the C-1 carbon of glucose while its C-6 carbon was correlated with the benzoyl carbonyl leading to structure **2** for lanceolin B. Analysis of the coupling constants of the protons of the methylene cyclohexane ring in **2** reveals the same stereochemistry as in **1**.

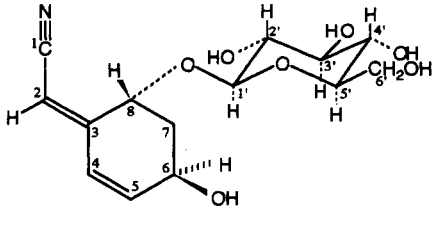
Further confirmation that **1** and **2** possess the same carbon skeleton came from a comparative ^{13}C-nmr study of menisdaurin [**4**], lanceolin A [**1**], its acetate [**3**], and lanceolin B [**2**]. Chemical shifts of corresponding protons in **1**, **2**, and **3** are approximately the same while the main difference observed between either **1** or **2** and **4**, concerns the C-4 and C-5 carbons, which accommodate a double bond in **4**, and vicinal dihydroxy groups in **1** and **2**. In **3**, carbons of the methyl groups of the six acetate functions appear at 21.1, 20.9, 20.6, 20.6, 20.6, and 20.5 ppm, with the carbonyls affording signals at δ 183.8, 170.2, 170.1, 169.8, 169.4, and 165.6 ppm (Table 1).

Biosynthetically, it is possible that **1** and **2** may have been derived from **4** by a natural trans dihydroxylation at C-4 and C-5 followed by subsequent esterification by benzoic acid either with the C-4 hydroxyl to give lanceolin A [**1**] or with the C-6 hydroxyl to give lanceolin B [**2**].

Murakami *et al.* (8) have recently described four similar compounds, namely, lophirosides A, B, C and D, isolated from the MeOH extract of the stem bark of the sister species, *Lophira alata*. These compounds differ from **1** and **2** in that they are diesters, while lanceolins A and B are monoesters.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps are uncorrected and were taken on a Kofler apparatus. Nmr spectra (^1H and ^{13}C) were deter-



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mined on a Bruker 300.13 MHz spectrometer with samples dissolved in Me_2CO-d_6. Fabms were obtained using a VG MMZ-AB-HF spectrometer.

PLANT MATERIAL.—*Lophira lanceolata* stem wood was harvested from Balamba in central Cameroon in February 1990, and a voucher specimen was deposited in the National Herbarium at Yaoundé, Cameroon.

EXTRACTION AND ISOLATION.—The stem wood of *Lophira lanceolata* was first chopped into small pieces, air-dried, and finally reduced to a fine powder (12 kg) that was extracted mechanically with cold Me_2CO. Evaporation of solvent gave a brownish gum (180 g) that was chromatographed over Si gel (500 g, 0.2–0.5 mm) with a gradient mixture of CH_2Cl_2 and MeOH. The fractions collected were grouped into four major fractions F_1, F_2, F_3, and F_4 after tlc analysis on Si gel plates [eluent: CH_2Cl_2-MeOH (10:1) and (5:1)].

Fraction F_4 (4 g) was the major fraction and served as starting material for further separation. This fraction was separated into six smaller fractions (F_{4a}, F_{4b}, F_{4c}, F_{4d}, F_{4e}, and F_{4f}) by gel permeation chromatography over Sephadex LH-20. A repeat of this procedure on F_{4b} gave three further subfractions (F_{4b1}, F_{4b2}, and F_{4b3}). The middle fraction F_{4b2} was further fractionated into F_{4b2a}, F_{4b2b}, F_{4b2c}, and F_{4b2d} using Sephadex LH20 (MeOH). Repeated prep. tlc of fraction F_{4b2a} (1.8 g) on Si gel plates with the eluent mixture of *n*-BuOH-AcOH-H_2O-EtOAc (3:1:1:10) gave two pure compounds, lanceolins A [**1**], (25 mg) and B [**2**] (15 mg).

LANCEOLIN A [**1**].—$C_{21}H_{25}O_{10}N$, white amorphous solid; hrfabms *m/z* $[M-H]^-$ calcd 450.1380, found 450.1362; ir ν max (KBr) 3320, 2215, 1718, 1623, 1602, and 1505 cm^{-1}; ^1H nmr (300 MHz) and ^{13}C nmr (75 MHz), see Table 1.

ACETYLATION OF LANCEOLIN A [**1**].—Lanceolin A (10 mg) was dissolved in pyridine (2 ml) in a 5-ml round-bottomed flask, and Ac_2O (5 ml) was added. The flask was well corked and left in the oven at 50° for four h, after which the solvent was removed under vacuum and the powder obtained purified over Sephadex LH-20 with MeOH as eluent to give lanceolin A hexa-acetate [**3**], $C_{26}H_{37}O_{16}N$, 6 mg; ir ν max (KBr) 2865, 2285, 1715, 1748, 1625 cm^{-1}; hrfabms *m/z* $(M-H)^-$ calcd 618.2001, found 618.2012, ^1H nmr (300 MHz) and ^{13}C nmr (75 MHz), see Table 1.

LANCEOLIN B [**2**].—$C_{21}H_{25}O_{10}N$, white amorphous solid; hrfabms *m/z* $(M-H)^-$ calcd 450.1380, found 450.1372; ir ν max (KBr) 3218, 2212, 1715, 1621, 1601, and 1500 cm^{-1}; ^1H nmr (300 MHz) and ^{13}C nmr (75 MHz), see Table 1; cims *m/z* 452 $(M+H)^+$, eims (110°, 70 eV), *m/z* 289 (100), 271 (18), 198 (32), 185 (23), 180 (65), 167 (36), 138 (10), 120 (12), 105 (37).

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