A New Benzoylglucoside and a New Prenylated Isoflavone from *Lophira lanceolata*

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A new benzoylglucoside, lanceoloside A (**1**), and a new prenylated isoflavone, lanceolone (**2**), have been isolated from the air-dried leaves of *Lophira lanceolata*. Both structures were established from spectroscopic and chemical evidence.

The stem bark of *Lophira lanceolata* Van. Tiegh ex Keay (Ochnaceae), a tree widely distributed in the woody savannas of tropical Africa and exploited for several medicinal uses,[1] is known for the presence of several biflavonoids and tetraflavonoids.[2−6] We recently reported the structures of lanceolatins A and B, two minor biflavonoids from the methanolic extract of the leaves.[7] Further investigations have led to the isolation of two new compounds, namely, lanceoloside A (**1**) and lanceolone (**2**). Their structures were determined on the basis of chemical and spectroscopic data.

The Et_2O-insoluble part of the MeOH extract of the air-dried leaves of *Lophira lanceolata* was a dark green gum. It was fractionated into six portions by gel permeation chromatography over Sephadex LH20. The sixth fraction (LLF_F) was purified by repeated column chromatography to give lanceoloside A (**1**) and lanceolone (**2**). Compound **1** ($C_{19}H_{20}O_9$) was isolated as an amorphous, pale yellow solid and had absorption bands at 3325 cm^{-1} (hydroxyl), 1632 cm^{-1} (conjugated carbonyl), and 1540 cm^{-1} (aromatic) in its IR spectrum. Its ^{13}C NMR spectrum had signals for 19 carbon atoms among which were one ketone carbonyl, eight aromatic methines, four quaternary sp^2 carbons (three bearing an oxygen), and six sp^3 methines (each carrying an oxygen substituent).

Evidence that lanceoloside A (**1**) had five hydroxyl groups came from acetylation (Ac_2O/pyridine), which gave a white amorphous solid **3** ($C_{29}H_{30}O_{14}$). Its ^1H NMR spectrum had five sharp singlets at δ 2.31, 2.26, 2.05, 2.03, and 2.02 (each 3H) assigned to five acetyl groups.

From 1D and 2D ^1H NMR (300 MHz) spectra of lanceoloside A (**1**), the anomeric sugar proton appeared as a doublet at δ 4.80 (J = 7.3 Hz), while signals of the other sugar protons were found between δ 3.55 and 4.85. Other substituents included a *p*-hydroxybenzoyl group [δ 7.92 (2H, ortho); 6.94 (2H, meta)] and a *p*-hydroxyphenoxy group [6.66 (2H, ortho); 6.95 (2H, meta)]. Further confirmation of these data came from acid hydrolysis of **1**, which yielded *p*-hydroxybenzoic acid, *p*-hydroxyphenol, and a sugar that was identified as glucose. From the HMBC spectrum of lanceoloside A (**1**), correlations were noticed between the H-1 proton of glucose and the C-1′ carbon of the *para*-hydroxyphenol residue, establishing that the glucosidic bond is between C-1 and C-1′. It also displayed correlations between the benzoyl carbonyl and the methylene protons of the glucose residue leading to structure **1** for lanceoloside A, and to **3**, for its totally acetylated derivative.

The second constituent, lanceolone (**2**), was obtained as an amorphous yellow compound and analyzed for $C_{21}H_{18}O_5$ by HRMS, indicating 13 unsaturated sites for this compound. It became green with ethanolic ferric chloride and pink with Na-Mg/HCl. Compound **2** showed UV absorption maxima (MeOH) at 282 and 330-nm characteristic of isoflavone. Absence of a shift on addition of NaOAc or $AlCl_3$ in the UV spectrum indicated the absence of free OH groups at C-5 and C-7.

Acetylation (Ac_2O−pyridine) of **2** gave an amorphous solid (**4**), with the molecular formula $C_{23}H_{20}O_6$ being established from HRMS, and whose IR spectrum showed no residual OH absorption. From the ^1H NMR spectrum of **4** a sharp singlet for an acetate group at δ 2.29 (3H) was observed, implying that lanceolone (**2**) had only one free OH group that was transformed by acetylation to give **4**.

The ^1H NMR spectrum of **2** showed a six-proton singlet at δ 1.47 assignable to a *gem*-dimethyl group and two AB doublets at δ 5.56 and 6.70 that were assignable to H-10 and H-9 protons, respectively, suggesting the presence of a 2,2-dimethylpyrane ring. A singlet at δ 7.77 (1H) was assignable to the H-2 proton of an isoflavone. Protons due to the methoxyl group at δ 3.91 (3H, s) and to a *para*-disubstituted phenyl group at δ 7.38 (2H, m) and 6.84 (2H, m) were also found in the ^1H NMR spectrum of **2**. Lanceolone monoacetate (**4**) showed marked downfield shifts of H-3′,5′ and H-2′,6′ signals (B-ring), consistent with a 4′-hydroxyl group in structure **2**, implying that the 2,2-dimethylchromene ring and the lone MeO group were attached to the A-ring. A negative Gibbs test[8] and the presence of an aromatic proton appearing as a singlet at δ 6.30 assigned to H-6, established attachment of the 2,2-dimethylchromene ring to C-7 and C-8, and the MeO group at C-5.

A clue to the orientation of the dimethylchromene ring came from the HMBC spectrum of **4** in which correla-

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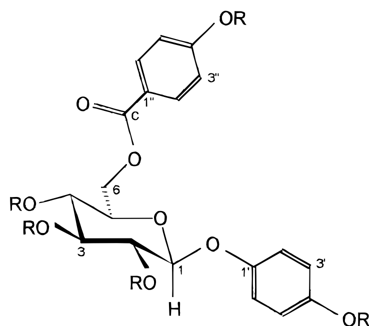

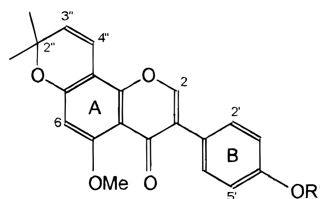

tions through two or three bonds were noticed between H-6 (δ_H 6.31) of the A-ring and both C-7 (δ_C 157.8) and C-5 (δ_C 161.2) and between H-4″ (δ_H 6.70) of the dimethylchromene ring and both C-7 and C-8a (δ_C 153.2), suggesting that the dimethylchromene ring had an angular orientation in **2**. Long-range coupling through an ethereal oxygen atom was observed between the three protons of the MeO group (δ_H 3.91) and C-5, confirming the location of the MeO group at C-5 in **2**.

Thus, the structure **2** was established as 5-methoxy-4′-hydroxy-2″,2″-dimethylpyrano(3″,4″,7,8)isoflavone. Further confirmation of this structure came from the EIMS analysis of lanceolone for which a retro-Diels–Alder transposition clearly explained the presence of peaks at *m/z* 232 (due to a fragment from ring A having one OMe group and a 2,2-dimethylpyran ring) and at *m/z* 118 (due to a fragment from ring B having one OH group).

Experimental Section

General Experimental Procedures. IR spectra were obtained using KBr disks. NMR spectra (^{1}H, 300 MHz and ^{13}C, 75 MHz) were recorded with Me$_2$CO-d_6 as solvent on a Bruker spectrometer with TMS as internal standard. For HMBC spectra, the delay was 70 μs (J_{CH} = 7 Hz). The solvent used for both column chromatography and TLC was $CHCl_3$–MeOH (10:1 and 5:1) unless stated otherwise. Preparative TLC plates were coated with fluorescent (F_{254}) Si gel, thickness 0.25 mm. Column chromatography was conducted on either Merck Si gel or Sephadex LH20.

Plant Material. Leaves of *Lophira lanceolata* Van. Tiegh. ex Keay. were harvested in Balamba, Cameroon, in 1991. A voucher specimen was deposited at the National Herbarium in Yaoundé, Cameroon.

Extraction and Purification. Air-dried leaves of *L. lanceolata* (5 kg) were pulverized and extracted with cold MeOH in an iron tank equipped with a mechanical stirrer. Removal of solvent gave a dark green gum (250 g), which was washed with Et_2O. The insoluble fraction (100 g) was then divided into six fractions by gel permeation chromatography over Sephadex LH20 with MeOH as the solvent.

Fractions 3, 4, and 6 were further purified by column chromatography and finally by repeated preparative TLC eluted using a mixture of $CHCl_3$–MeOH (10:1) to give β-sitosterol-β-D-glucoside, (+) epicatechin, lophirone A, and **1** (30 mg) and **2** (21 mg).

Lanceoloside A (1): obtained as an amorphous, pale yellow solid; IR (KBr paste) ν_{max} 3325, 1632, 1560, 1540, 940, and 860 cm^{-1}; ^{1}H NMR (Me$_2$CO-d_6; 300 MHz) δ 4.80 (1H, d, J = 7.3 Hz, H-1), 3.56 (1H, m, H-2), 3.60 (1H, m, H-3), 3.58 (1H, m, H-4), 3.80 (1H, m, H-5), 4.34 (1H, dd, J = 9.5, 2.3 Hz, H-6a), 4.70 (1H, dd, J = 9.5, 2.3 Hz, H-6b), 6.66 (2H, m, H-2′, H-6′), 6.95 (2H, m, H-3′, H-5′), 7.92 (2H, m, H-2″, H-6″), 6.94 (2H, m, H-3″, H-5″); ^{13}C NMR (Me$_2$CO-d_6; 75 MHz) δ 103.1 (d, C-1), 74.6 (d, C-2), 77.8 (d, C-3), 71.5 (d, C-4), 75.0 (d, C-5), 64.7 (t, C-6), 151.9 (s, C-1′), 116.3 (d, C-2′, C-6′), 118.9 (d, C-3′, C-5′), 153.5 (s, C-4′), 196.4 (s, C–c), 116.8 (s, C-1″), 132.5 (d, C-2″, C-6″), 116.0 (d, C-3″, C-5″), 162.8 (s, C-4″); EIMS *m/z* 392 [M$^+$] (100), 252 (20), 195 (41), 180 (84), 132 (18), 95 (28), 89 (12); HREIMS *m/z* 392.1546 (calcd for $C_{19}H_{20}O_9$, 392.1534).

Hydrolysis of 1. Lanceoloside A (15 mg) was dissolved in 7% H_2SO_4 and refluxed on an H_2O bath at 100 °C for 4 h. The reaction mixture was diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was evaporated to dryness and purified by preparative TLC over Si gel with $CHCl_3$–MeOH (9:1) as eluent. Two aglycons were isolated and identified as *p*-hydroxybenzoic acid and *p*-hydroxyphenol through direct comparison with authentics samples (TLC, MP, IR).

Acetylated Derivative 3. Lanceoloside A (**1**) (5 mg) was dissolved in pyridine (2.5 mL), and Ac_2O (2.5 mL) was added. The mixture was left overnight at 50 °C in a H_2O bath. Distilled H_2O (100 mL) was added, and the compound that precipitated was dried and purified over Sephadex LH20 to give **3** (3 mg): ^{1}H NMR (300 MHz; Me$_2$CO-d_6) δ 5.28 (1H, d, J = 7.3 Hz, H-1), 3.95 (1H, m, H-2), 3.98 (1H, m, H-3), 3.96 (1H, m, H-4), 4.35 (1H, m, H-5), 4.50 (1H, dd, J = 9.5, 2.3 Hz, H-6a), 5.02 (1H, dd, J = 9.5, 2.3 Hz, H-6b), 6.91 (2H, m, H-2′, H-6′), 7.20 (2H, m, H-3′, H-5′), 8.04 (2H, m, H-2″, H-6″), 7.20 (2H, m, H-3″, H-5″), 2.31 (3H, s, $COCH_3$), 2.26 (3H, s, $COCH_3$), 2.05 (3H, s, $COCH_3$), 2.03 (3H, s, $COCH_3$), 2.02 (3H, s, $COCH_3$); HREIMS *m/z* 602.2302 (calcd for $C_{29}H_{30}O_{14}$, 602.2298).

Lanceolone (2): was obtained as an amorphous yellow compound; IR (KBr paste) ν_{max} 3315, 2966, 2815, 1630, 1618, 1510 and 1225 cm^{-1}; ^{1}H NMR (Me$_2$CO-d_6; 300 MHz) δ 7.77 (1H, s, H-2), 6.30 (1H, s, H-6), 7.38 (2H, m, H-2′, H-6′), 6.84 (2H, m, H-3′, 5′), 5.56 (1H, d, J = 10.1 Hz, H-3″), 6.70 (1H, d, J = 10.1 Hz, H-4″), 1.47 (6H, s, 2 × CH_3), 3.91 (3H, s, OCH_3); ^{13}C NMR (Me$_2$CO-d_6; 75 MHz) δ 150.5 (d, C-2), 123.3 (s, C-3), 175.5 (s, C-4), 109.7 (s, C-4a), 161.5 (s, C-5), 97.1 (d, C-6), 157.2 (s, C-7), 102.4 (s, C-8), 154.2 (s, C-8a), 126.0 (s, C-1′), 130.8 (d, C-2′, C-6′), 115.4 (d, C-3′, C-5′), 157.8 (s, C-4′), 78.4 (s, C-2″), 128.3 (d, C-3″), 115.2 (d, C-4″), 28.1 (q, CH_3), 29.0 (q, CH_3), 56.4 (q, CH_3O); EIMS *m/z* 350 [M$^+$] (96), 311 (25), 281 (33), 250 (47), 232 (100), 215 (10), 180 (28), 118 (52), 101 (18), 86 (29); HREIMS *m/z* 350.1402 (calcd for $C_{21}H_{18}O_5$, 350.1399).

Acetylation of Lanceolone (2). The same procedure as for **1** was applied to lanceolone (10 mg), and **4** (7 mg) was obtained as an amorphous solid: IR (KBr paste) ν_{max} 2228, 1742, 1395, 1375, 850 and 740 cm^{-1}; ^{1}H NMR (Me_2CO-d_6; 300 MHz) δ 7.79 (1H, s, H-2), 6.31 (1H, s, H-6), 7.53 (2H, m, H-2′, H-6′), 7.10 (2H, m, H-3′, 5′), 5.57 (1H, d, J = 10.0 Hz, H-3″), 6.70 (1H, d, J = 10.0 Hz, H-4″), 1.47 (6H, s, 2 × CH_3), 3.91 (3H, s, OCH_3), 2.29 (3H, s, $COCH_3$); ^{13}C NMR (Me_2CO-d_6; 75 MHz) δ 150.3 (d, C-2), 125.3 (s, C-3), 174.4 (s, C-4), 109.6 (s, C-4a), 161.2 (s, C-5), 96.6 (d, C-6), 157.8 (s, C-7), 102.4 (s, C-8), 153.2 (s, C-8a), 129.6 (s, C-1′), 130.3 (d, C-2′, C-6′), 121.3 (d, C-3′, C-5′), 150.4 (s, C-4′), 78.1 (s, C-2″), 127.4 (d, C-3″), 114.9 (d, C-4″), 28.2 (q, 2 × CH_3), 55.9 (q, CH_3O), 165.9 (3H, s, CH_3COO); HREIMS m/z 392.1715 (calcd for $C_{23}H_{20}O_6$, 392.1723).

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