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NEW PHENOLIC GLUCOSIDES FROM *LAWSONIA INERMIS*

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ABSTRACT.—The isolation and structure determination of novel phenolic glucosides, lawsoniaside [**1**] and lalioside [**5**], from *Lawsonia inermis* are described. Evidence from spectroscopic data and chemical transformations showed **1** and **5** to be 1,2,4-trihydroxynaphthalene-1,4-di-β-D-glucopyranoside and 2,3,4,6-tetrahydroxyacetophenone-2-β-D-glucopyranoside, respectively.

Lawsonia inermis ("lali" in Hausa) (Lythraceae) is a shrub that is semicultivated in the savannah region of West Africa (1). Lali leaves are commonly used as cosmetics for staining hands and feet in northern Nigeria and as an ingredient in local medicinal preparations in the Middle East and Asia. The aqueous extract of the leaves of *L. inermis* has been shown to possess reproducible activity in vitro against a wide variety of microorganisms (2,3). The chemistry of the constituents of *L. inermis* has been of interest for at least 50 years, and the occurrence of β-sitosterol glucoside (4), flavonoids (4,5), quinoids (6,7), naphthalene derivatives (7,8), gallic acid (7), coumarins (9), and xanthones (4,10) in *Lawsonia* leaves has been reported. We have examined extracts of the leaves of *L. inermis* collected at Bayero University, Nigeria, and found luteolin (4) and four known glucosides (4,5,8) as well as two novel phenolic glucosides, lawsoniaside [**1**], and lalioside [**5**]. In this communication, we report the isolation and structure determination of the new compounds.

The EtOH extract of the dried leaves of *L. inermis* was redissolved in aqueous MeOH and extracted successively with *n*-hexane, CCl_4, and $CHCl_3$. Separation of the polar compounds was achieved through initial chromatography of the aqueous MeOH extract on highly porous polymer HP-20 (Mitubishi Kasei), eluting with a mixture of H_2O and EtOH and further chromatographies on Sephadex LH-20 and Si gel. This procedure gave the two new phenolic glucosides, lawsoniaside [**1**] and lalioside [**5**], along with previously reported luteolin 7-glucoside (5), luteolin 3′-glucoside (5), and 1,2-dihydroxy-4-glucosyloxy-naphthalene (8). The $CHCl_3$ and CCl_4 extracts gave luteolin (4) and β-sitosterol glucoside (4), respectively.

Lawsoniaside [**1**] had mp 263–264°, $[\alpha]_D$ −51.2° (c = 0.49, DMSO) and was assigned the molecular formula $C_{22}H_{28}O_{13}\cdot\frac{1}{2}H_2O$ on the basis of elemental analyses and fabms. The ir spectral data indicated the presence of hydroxy groups (ν max 3650–3050 cm^{-1}) and an aromatic ring (ν max 1640 and 1610 cm^{-1}). The ^1H nmr of **1** (DMSO-d_6 + D_2O) showed two broad doublets at δ 8.18 (1H, J = 7.5 Hz) and 8.33 (1H, J = 8.5 Hz), two doublets of double doublets at δ 7.30 (1H, J = 8.5, 7.5, and 1 Hz) and 7.45 (1H, J = 8.5, 7.5, and 1 Hz), and a singlet at δ 6.87 (1H, s) in addition to the signals due to the sugar moiety. The ^{13}C-nmr spectrum (Table 1) established the presence of two hexopyranose units and displayed five doublets and five singlets between δ 100 and 160 due to sp^2 carbons in the off-resonance spectrum. The uv of **1** showed λ max (MeOH) at 233, 286, 296, and 336 nm, which is very similar to that of 1,2-dihydroxy-4-*O*-glucosyloxy-naphthalene (8). These data suggested a 1,2,4-trisubstituted naphthalene structure of lawsoniaside. Compound **1** liberated 1-*O*-methyl glucoside on acid methanolysis and gave the nonaacetate **2** on acetylation. The ^1H-nmr spectrum of **2** displayed one phenolic acetoxy peak at δ 2.36 and eight alcoholic acetoxy peaks, suggesting a diglucoside structure for **1**. Methylation of lawsoniaside with

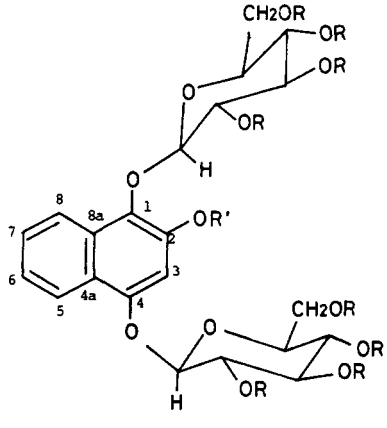
TABLE 1. ^{13}C-nmr Chemical Shifts (δ)[a] of Lawsoniaside [**1**].

Carbon		Carbon	
1,2,4-trihydroxynaphthalene		Glucose	
1	150.7	1	101.4, 102.8
2	145.9	2	73.3 73.9
3	106.7	3	77.0[b], 77.1[b]
4	120.3	4	69.5 69.7
4a	129.8	5	76.1[b], 76.4[b]
5	121.9[c]	6	60.7 (×2)
6	121.5[c]		
7	122.4		
8	126.6		
8a	132.7		

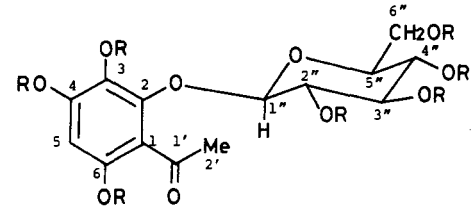
[a]The spectrum was taken in $(CD_3)_2SO$.
[b,c]May be reversed.

ethereal CH_2N_2 gave the monomethyl ether **3**, mp 274–276°, from which the octaacetate **4** was obtained on acetylation. Proton nOe experiments on the monomethyl ether **3** showed a 9.1% enhancement of the signal at δ 7.19 (1H, s) when the singlet at δ 3.89 (3H, s, OMe) was irradiated. This result is consistent with the 1,4-diglucoside structure proposed for lawsoniaside [**1**]. The ^{1}H-nmr coupling constants of anomeric protons at δ 4.52 (1H, d, J=7.5 Hz) and 4.92 (1H, d, J=7.5 Hz) suggested β-glycosidic linkages. Lawsoniaside [**1**] was, thus, identified as 1,2,4-trihydroxynaphthalene-1,4-di-β-D-glucopyranoside on the basis of all available data.

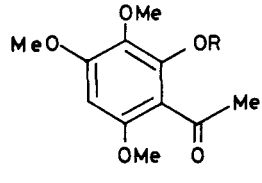
Lalioside [**5**], $[\alpha]_D$ +62.5° (c=0.8, MeOH) is an hygroscopic and air sensitive amorphous powder. Fabms of **5** gave molecular ion peaks at m/z 369 $[M+Na]^+$ and 385 $[M+K]^+$ from which the molecular weight of 346 for lalioside was deduced. The uv spectrum of **5** displayed λ max (MeOH) at 238, 283, and 344.5 nm, which shifted to 255, 327, and 410 nm on addition of $AlCl_3$. The ir spectrum showed strong hydroxy



1 R=R′=H
2 R=R′=Ac
3 R=H, R′=Me
4 R=Ac, R′=Me



5 R=H
6 R=Ac



7 R=H
8 R=Ac

absorption at 3650–3050 cm^{-1}, bands at 1590 and 1510 cm^{-1} due to an aromatic ring, and a band at 1630 cm^{-1}, which is characteristic of carbonyl absorptions in ortho hydroxy aryl ketones. The ^{1}H-nmr spectrum (DMSO-d_6) of **5** showed a typical peak for the Ac group (δ 2.64, 3H, s), a pentasubstituted benzene ring (δ 6.14, 1H, s), and three phenolic hydroxy groups at 8.60, 10.36, and 12.63 in addition to the peaks for the sugar moiety. The ^{13}C-nmr spectrum firmly established the presence of a hexopyranose group and confirmed a pentasubstituted benzene ring structure for **5**.

Acid hydrolysis of **5** gave glucose. The stereochemistry of the glycosidic linkage was determined as β, judged from the coupling constant (J = 7.5 Hz) of the anomeric proton signal at δ 4.81. Acetylation of **5** afforded the heptaacetate derivative **6** whose ^{1}H-nmr spectrum contained typical peaks for three phenolic and four alcoholic acetoxy groups. Taken together, lalioside [**5**] has a structure in which two hydroxy groups and a glucosyloxy group are substituted on an ortho hydroxy acetophenone skeleton. In order to establish the positions of substituents relative to the methyl ketone group, lalioside [**5**] was converted to **7** through methylation of **5** and methanolysis of the methylated product. The ^{1}H-nmr spectrum ($CDCl_3$) of **7** contained three methoxy signals at δ 3.81, 3.90, and 3.94 and one phenolic hydroxy group at δ 13.80. A hydrogen-bonded carbonyl absorption at 1620 cm^{-1}, typical for ortho hydroxy aryl ketones, in the ir of **7** indicated that the sugar substituent is ortho to the Ac group. The ir and ^{1}H-nmr data for **7** are in perfect agreement with those observed for authentic 2-hydroxy-3,4,6-trimethoxyacetophenone [**7**] (11). These data suggested a 2,3,4,6-tetrahydroxyacetophenone-2-β-D-glucopyranoside structure for **5**.

EXPERIMENTAL

GENERAL PROCEDURES.—Melting points are uncorrected. ^{1}H nmr 200 MHz, ^{13}C nmr 50.1 MHz. TMS as internal standard; fabms: gun high voltage, 3.0 kV; matrix, thioglycerol; eims: 70 eV; gc: column, 1.5% OV-1 2m, carrier gas, N_2, 50 ml/min; cc: Mitubishi Kasei HP-20, Si gel 60 and Sephadex LH-20; tlc: Si gel 60 F245 (Merck) precoated plates (0.25 mm in thickness).

PLANT MATERIAL.—The plant material was collected in Kano, Nigeria, in August 1985 and identified as *L. inermis* by Dr. S.A. Ghazanfar and Mr. Ali Garko. A voucher specimen was deposited in the herbarium of Bayero University, Kano, Nigeria.

EXTRACTION AND ISOLATION.—Dried, milled leaves of *L. inermis* (1 kg) were thoroughly extracted with EtOH (4 liters). The extract was evaporated in vacuo to give 177 g of material that was redissolved in 90% MeOH (300 ml). The solution was extracted with *n*-hexane (300 ml × 3), and the *n*-hexane layer was washed with 90% MeOH (200 ml), dried, and concentrated in vacuo to give Fraction I (19 g). H_2O (62.5 ml) was added to the combined 90% MeOH layer, and the solution was extracted with CCl_4 (300 ml × 3). The CCl_4 layer was washed with 80% MeOH, dried, and concentrated in vacuo to give Fraction II (5 g). H_2O (164 ml) was added to the combined 80% MeOH layer, and the solution was extracted with $CHCl_3$ (500 ml × 3). The $CHCl_3$ layer was dried and concentrated in vacuo to give Fraction III (13 g). The 65% MeOH layer was evaporated in vacuo to give Fraction IV (113 g).

An aliquot (5.32 g) of Fraction IV was chromatographed on HP-20 (4 × 48 cm) column using H_2O/EtOH mixtures containing increasing amount of EtOH as eluent. The column was washed with H_2O (2 liters), 20% EtOH (1 liter), 50% EtOH (1 liter), and EtOH (2 liters), collecting 500 ml fractions.

Fractions 6 and 7 gave a residue (945 mg) which was further chromatographed on a column of Sephadex LH-20 (2.5 × 87 cm) using MeOH as eluent, collecting 7-ml fractions. Fractions 55–59 gave a residue (119 mg) from which lawsoniaside [**1**] (58 mg) was obtained as colorless needles after recrystallization from MeOH. Fractions 60–64 contained lalioside [**5**] (306 mg).

Fraction 8 (1.54 g) was chromatographed on a Si gel (Merck, PF_{254}; 90 g) column using $CHCl_3$/MeOH/H_2O mixtures as eluent. The column was washed with $CHCl_3$-MeOH-H_2O (85:15:1) (1 liter), $CHCl_3$-MeOH-H_2O (80:20:1) (500 ml) and $CHCl_3$-MeOH-H_2O (75:25:1) (1 liter) in that order, collecting 7-ml fractions. Fractions 65–80 (571 mg) were further chromatographed on Sephadex LH-20 using MeOH as eluent to give 1,2-dihydroxy-4-glucosyloxynaphthalene (374 mg). Fractions 81–109 (149 mg) and 141–200 (240 mg) were separately purified by cc on Sephadex LH-20, using MeOH as eluent, to give luteolin 3′-*O*-glucoside (47 mg) and luteolin 7-*O*-glucoside (21 mg), respectively.

An aliquot (3 g) of Fraction II was chromatographed on a Si gel column using $CHCl_3$/MeOH as eluent. $CHCl_3$-MeOH (9:1) eluted sitosterol-β-D-glucoside (74 mg) as colorless needles.

An aliquot (7.35 g) of Fraction III was chromatographed on a Si gel column using $CHCl_3$/MeOH as eluent. $CHCl_3$-MeOH (97:3) eluted 140 mg of material from which luteolin (13 mg) was recovered after repeated chromatography and recrystallization from MeOH.

PHYSICAL DATA OF THE NEW COMPOUNDS.—Lawsoniaside [**1**], mp 263–264°, $[\alpha]^{21}D$ −51.2° (c = 0.49, DMSO); uv λ max (MeOH) nm (ε) 233 (120000), 286 (14300), 296 (13800), and 336 (7530); ir ν max (KBr) cm^{-1} 3650–3050, 1640, 1610, 1470, 1370, 1280, 1205, 1075, 995, 900, 840; ^1H nmr (DMSO-d_6 + D_2O) δ 4.52 (1H, d, J = 7.5 Hz, anomeric-H), 4.92 (1H, d, J = 7.5 Hz, anomeric-H), 6.87 (1H, s, 3-H), 7.30 (1H, ddd, J = 8.5, 7.5, and 1 Hz, 6-H), 7.45 (1H, ddd, J = 8.5, 7.5, and 1 Hz, 7-H), 8.18 (1H, br d, J = 7.5 Hz, 5-H), 8.33 (1H, br d, J = 8.5 Hz, 8-H); ^{13}C nmr see Table 1; fabms *m/z*: $[M + Na]^+$ (+NaI) 523, $[M + K]^+$ (+KI) 538. *Anal.* calcd for $C_{22}H_{28}O_{13} \cdot \frac{1}{2} H_2O$: C 51.86, H 5.74; found C 51.49, H 5.53.

Lalioside [**5**], an amorphous powder, $[\alpha]^{23}D$ +62.5° (c = 0.8, MeOH); uv λ (MeOH) nm (ε) 238 (10210), 283 (12430), 344.5 (4930); λ max (MeOH + $AlCl_3$) nm (ε) 255 (8110), 327 (22380), and 410 (5190); ir ν max (KBr) cm^{-1} 3650–3050, 1630, 1590, 1510, 1370, 1280, 1255, 1070 (br), 1035, 965, 910, 840; ^1H nmr (CD_3OD) δ 2.72 (3H, s), 4.93 (1H, d, J = 7.5 Hz), 6.16 (1H, s); δ (DMSO-d_6) 2.64 (3H, s, 2′-H), 4.81 (1H, d, J = 7.5 Hz, anomeric-H), 6.14 (1H, s, 5-H), 8.60 and 10.36 (each 1H, br s, OH × 2), 12.63 (1H, s, 6-OH); ^{13}C nmr (CD_3OD) δ 32.9 (C-2′), 62.5 (C-6″), 71.2 (C-4″), 75.5 (C-2″), 78.0 and 78.6 (C-3″ and/or C-5″), 100.8 (C-1″), 106.4 (C-5), 110.5, 132.0, 146.5, 155.4, and 158.5 (C-2, 3, 4 or 6), 205.6 (C-1′); fabms *m/z*: $[M + Na]^+$ (+NaI) 369, $[M + K]^+$ (+KI) 385.

METHANOLYSIS OF LAWSONIASIDE [**1**].—Lawsoniaside (1.2 mg) was dissolved in MeOH 1.7 N HCl (1 ml), and the solution was refluxed for 2 h. After cooling, the mixture was evaporated in vacuo, and the residue was trimethylsilylated with a mixture (1 ml) consisting of pyridine, hexamethyldisilazane, and trimethylchlorosilane (10:2:1, v/v/v) at 100° for 10 min. The conditions for glc analysis are as follows: column temperature, 180°; injection temperature, 200°. This sample (r_t 8.65 min) was identified with an authentic sample obtained from methyl-1-*O*-glucoside.

LAWSONIASIDE NONAACETATE [**2**].—Acetylation of lawsoniaside [**1**] (10 mg) with Ac_2O and pyridine gave the nonaacetate **2** (15 mg) as an amorphous powder. Ir ν max ($CHCl_3$) cm^{-1} 1760, 1750, 1630, 1605, 1375, 1240, (br), 1070, 1045, 905; ^1H nmr ($CDCl_3$) δ 2.00 (3H, s, OAc), 2.01 (3H, s, OAc), 2.05 (6H, s, 2 × OAc), 2.06 (6H, s, 2 × OAc), 2.08 (3H, s, OAc), 2.14 (3H, s, OAc), 2.36 (3H, s, OAc), 5.01 (1H, d, J = 8 Hz, anomeric-H), 6.81 (1H, s, 3-H), 7.52 (2H, m, 6-H, and 7-H), and 8.09 (2H, m, 5-H and 8-H); fabms *m/z* $[M + Na]^+$ (+NaI) 901, $[M + K]^+$ (+KI) 917.

LAWSONIASIDE MONOMETHYL ETHER [**3**].—Lawsoniaside **1** (25.6 mg) was dissolved in MeOH (50 ml) and treated with ethereal CH_2N_2 until the yellow color persisted. The reaction was left for 1 h, treated with a few drops of HOAc, and evaporated in vacuo. The residue was recrystallized from MeOH to give **3** (11 mg) as colorless needles, mp 274–276°. Ir ν max (KBr) cm^{-1} 3650–3050, 1635, 1605, 1510, 1470, 1380, 1360, 1285, 1080, 925, 900, 850; ^1H nmr (DMSO-d_6) δ 3.89 (3H, OMe), 7.19 (1H, s, 3-H), 7.34 (1H, br dd, J = 8 and 8 Hz, 6-H), 7.46 (1H, br dd, J = 8 and 8 Hz, 7-H), 8.21 (1H, br d, J = 8 Hz, 5-H), and 8.27 (1H, br d, J = 8 Hz, 8-H); fabms *m/z* $[M + Na]^+$ (+NaI) 537, $[M + K]^+$ (+KI) 553.

LAWSONIASIDE MONOMETHYL ETHER OCTAACETATE [**4**].—Lawsoniaside monomethyl ether **3** (5 mg) was acetylated in the usual manner to give the octaacetate **4** (7 mg) as an amorphous powder. Ir ν max ($CHCl_3$) cm^{-1} 1760, 1630, 1605, 1370, 1240 (br), 1070, 1045, 910; ^1H nmr ($CDCl_3$) δ 1.92 (3H, OAc), 2.02 (3H, s, OAc), 2.05 (6H, s, 2 × OAc), 2.07 (12H, s, 4 × OAc), 3.94 (3H, s, OMe), 6.98 (1H, s, 3-H), 7.32–7.50 (2H, 6-H and 7-H), 7.93 (1H, br d, J = 8 Hz, 5-H), 8.13 (1H, br d, J = 8 Hz, 8-H); fabms *m/z* $[M + Na]^+$ (+NaI) 873, $[M + K]^+$ (+KI) 889.

LALIOSIDE HEPTAACETATE [**6**].—Lalioside **5** (20 mg) was acetylated with Ac_2O and pyridine in the usual manner to give the heptaacetate **6** (24 mg) as an amorphous powder. Ir ν max ($CHCl_3$) cm^{-1} 1760, 1710, 1610, 1475, 1430, 1370, 1230 (br), 1180, 1140, 1070, 1040; ^1H nmr ($CDCl_3$) δ 2.007, 2.011, 2.08, 2.09, 2.22, 2.28, 2.33, and 2.48 (each 3H, s, 7-OAc + 2′-H), 3.64 (1H, m, 5″-H), 3.98 (1H, dd, J = 14.5 and 2 Hz, 6″-H), 4.25 (1H, dd, J = 14.5 and 4.5 Hz, 6″-H), 6.96 (1H, s, 5-H); fabms *m/z* $[M + Na]^+$ (+NaI) 613, $[M + K]^+$ (+KI) 679.

HYDROLYSIS OF LALIOSIDE [**5**].—Lalioside [**5**] (2.8 mg) was dissolved in 2% H_2SO_4 (5 ml), and the solution was refluxed for 3 h. After cooling, the mixture was neutralized with Amberlite IRA-410 (OH form), and the ion exchange resin was filtered off. The filtrate was concentrated in vacuo, trimethylsilylated as before and analyzed on gc (column temperature 170°, detection temperature 190°). Glucose (r_t 12.9 and 19.1 min) was identified with an authentic sample.

2-HYDROXY-3,4,6-TRIMETHOXYACETOPHENONE [7].—Lalioside [**5**] (83 mg) was dissolved in MeOH (10 ml) and treated with ethereal CH_2N_2 as described for **3**. To a solution of the residue dissolved in anhydrous Me_2CO (40 ml) was added anhydrous K_2CO_3 (400 mg) and MeI (1 ml). The mixture was refluxed for 4 days, filtered, and evaporated. The residue was dissolved in methanolic HCl (2 M, 6 ml) and refluxed for 2 h. The solvent was removed in vacuo, and the residue was dissolved in $CHCl_3$ (20 ml), washed with saturated NaCl, dried, and evaporated in vacuo to give crystalline residue (13.7 mg). Uv λ max (MeOH) nm (ε) 231 (9522), 287.5 (15750), 328.5 (3276); λ max (MeOH + $AlCl_3$) nm (ε) 313 (27140), 381 (3809); ir ν max ($CHCl_3$) cm^{-1} 1620, 1600, 1470, 1440, 1420, 1295, 1285, 1130, 995, 840; ^1H nmr ($CDCl_3$) δ 2.62 (3H, s, Ac), 3.81, 3.90, and 3.94 (each 3H, s, OMe_3), 5.97 (1H, s), 13.80 (1H, s, OH); eims *m/z* $[M]^+$ 226.0829. Calcd for $C_{11}H_{14}O_5$, 226.0839. A sample of this product (2.4 mg) gave the monoacetate **8** (2.1 mg) on acetylation. Ir ν max ($CHCl_3$) cm^{-1} 1760, 1680, 1610, 1500, 1470, 1410, 1340, 1260, 1195, 1105; ^1H nmr ($CDCl_3$) δ 2.29 and 2.47 (each 3H, s, 2 × Ac), 3.77, 3.86, and 3.92 (each 3H, s, 3 × OMe), 6.40 (1H, s); eims *m/z* $[M]^+$ 268.0965. Calcd for $C_{13}H_{16}O_6$, 268.0947. The methanolysis product was identified with an authentic sample of 2-hydroxy-3,4,6-trimethoxyacetophenone [7] by comparisons of ir and ^1H-nmr spectra.

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