Two New and a Known Compound from *Lawsonia inermis*

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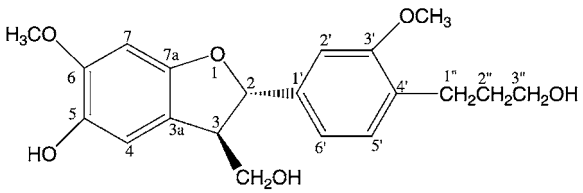
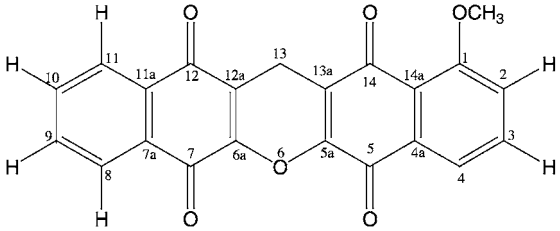
A new obtusafuran derivative, lawsonicin (**1**), and a new naphthaquinone, lawsonadeem (**2**), along with a known constituent, vomifoliol (**3**), were isolated from the aerial parts of *Lawsonia alba* and characterized by chemical transformation and spectroscopic experiments, including 2D-NMR techniques.

1. Introduction. – *Lawsonia* is a monotypic genus represented by *Lawsonia inermis* LINN. (syn. *Lawsonia alba* LAM. Lythraceae), a native of North Africa and Southwest Asia, widely cultivated as an ornamental hedge and dye plant. The leaves of *L. inermis* have long been used in India and Middle East countries as a cosmetic for coloring palms of hands and dyeing of hair for personal adornment [1]. The leaves are also used as a prophylactic in the form of paste or decoction against skin inflammation [2]. The essential oil obtained from the flowers finds use in perfumery due to its β-ionone content. The plant has been reported to contain various compounds like coumarins, flavonoids, gallic acid, naphthalene derivatives, lupane-type triterpenoids [3], aliphatic constituents, phenolic glycosides, and xanthones [4]. As a result of studies on the chemical constituents of the aerial parts of *L. alba*, two new constituents, lawsonin and lawsonic acid, were reported earlier [5]. A continuation of these investigations resulted in the isolation and structure elucidation of three further compounds, *i.e.*, of the two new constituents named lawsonicin (**1**) and lawsonadeem (**2**) and of the known constituent vomifoliol (**3**). The structures of the new constituents **1** and **2** were elucidated by spectral studies including 1D ^{1}H- and ^{13}C-NMR (broad band and DEPT) and 2D NMR (COSY-45, NOESY, *J*-resolved, HMQC, and HMBC) analysis. The known compound **3** was identified by comparison of its spectral data with those reported in [6]. These studies form the basis of the present communication.

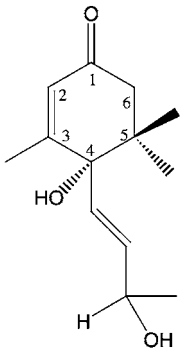
2. Results and Discussion. – The EI-MS and HR-EI-MS of lawsonicin (**1**) showed the molecular-ion peak at *m*/*z* 360.1565 accounting for the elemental composition $C_{20}H_{24}O_6$ suggesting nine unsaturations in the molecule. The UV absorption maximum at 282.2 nm was suggestive of its aromatic nature. A detailed NMR-data analysis (see *Table 1*) revealed that **1** is a dihydrobenzofuran derivative resulting from an interesting biosynthetic pathway [7]. The spectral features led to the assignment of 2,3-dihydro-5-hydroxy-3-(hydroxymethyl)-2-[4-(3-hydroxypropyl)-3-methoxy)phenyl]-6-methoxy-1-benzofuran (**1**) to the structure of lawsonicin, which was finally confirmed by acetylation of **1** to **1a** (Ac_2O/pyridine).



1 lawsonicin



2 lawsonadeem



3 vomifoliol

The ^{1}H-NMR spectrum of **1** showed the presence of two one-proton *s* at δ 6.56 (H–C(4)) and 6.60 (H–C(7)) having connectivities with C-atoms at δ 116.5 (C(4)) and 112.8 (C(7)) in the HMQC plot. The NMR data revealed the presence of another benzene ring linked to the benzofuran moiety at C(2) by the presence of two one-proton *d* at δ 6.85 ($J = 1.9$ Hz, H–C(2′)) and 6.72 ($J = 8.1$ Hz, H–C(5′)) along with a one-proton *dd* at δ 6.77 ($J = 8.1$, 1.9 Hz, H–C(6′)). The C-atoms connected to these protons were observed at δ 108.9 (C(2′)), 114.5 (C(5′)), and 119.5 (C(6′)) in the HMQC spectrum. Beside these, the ^{1}H-NMR spectrum had signals for a hydroxypropyl chain manifested by two *t* at δ 2.54 ($J = 7.5$ Hz, 2 H–C(1″)) and 3.53 ($J = 6.5$ Hz, 2 H–C(3″)) and a *tt* at δ 1.70 ($J = 7.5$, 6.5 Hz, 2 H–C(2″)). Their connected C-atoms were observed at δ 32.0, 61.5, and 34.5, respectively, in the HMQC plot. Moreover, two *s* at δ 3.75 and 3.77 for two MeO groups and a *m* at δ 3.72–3.76 for two nonequivalent hydroxymethylene protons were present. The position of the MeO groups was established by NOESY interactions. Thus, H–C(7) had connectivity with the MeO at δ 3.77, and H–C(4) was

Table 1. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$) *of Compounds* **1** *and* **1a**. δ in ppm, J in Hz.

	1		**1a**
	δ(c)	δ(H)	δ(H)
H–C(2)	88.0	5.41 (*d*, J = 7.0)	5.49 (*d*, J = 7.0)
H–C(3)	53.2	3.45 (*ddd*, J = 8.0, 7.0, 5.0)	3.73 (*ddd*, J = 8.0, 7.0, 5.0)
C(3a)	127.9	–	–
H–C(4)	116.5	6.56 (*s*)	6.63 (*s*)
C(5)	144.2	–	–
C(6)	146.7	–	–
H–C(7)	112.8	6.60 (*s*)	6.61 (*s*)
C(7a)	146.5	–	–
CH_2–C(3)	63.6	3.72–3.76 (*m*)	4.44 (*dd*, J = 11.5, 5.0, H_a); 4.26 (*dd*, J = 11.5, 8.0, H_b)
C(1′)	135.5	–	2.63 (*t*, J = 8.0)
H–C(2′)	108.9	6.85 (*d*, J = 1.9)	6.99 (*d*, J = 2.0)
C(3′)	145.7	–	–
C(4′)	133.1	–	–
H–C(5′)	114.5	6.72 (*d*, J = 8.1)	6.97 (*d*, J = 8.1)
H–C(6′)	119.5	6.77 (*dd*, J = 8.1, 1.9)	6.93 (*dd*, J = 8.1, 1.9)
2 H–C(1″)	32.0	2.54 (*t*, J = 7.50)	2.63 (*t*, J = 8.0)
2 H–C(2″)	34.5	1.70 (*tt*, J = 7.5, 6.5)	1.9 (*tt*, J = 8.0, 6.5)
2 H–C(3″)	61.5	3.53 (*t*, J = 6.5)	4.07 (*t*, J = 6.50)
MeO	55.9	3.75 (*s*)	3.78 (*s*)
MeO	53.7	3.77 (*s*)	3.87 (*s*)
3 MeCOO	–	–	2.28 (*s*), 2.05 (*s*), 2.02 (*s*)

connected to H–C(3). Moreover, H–C(2′) had connectivity with MeO at δ 3.75, H–C(5′) with 2 H–C(1″) and H–C(2) with both H–C(2′) and H–C(6′). These data left a hydroxymethyl moiety to be located, which could be accommodated at C(3) of the dihydrofuran moiety as H–C(3) appeared as a *ddd* at δ 3.45 (J = 8.0, 7.0, 5.0 Hz; δ(c) 53.2) and H–C(2) resonated at δ 5.41 as a *d* (J = 7.0 Hz). This arrangement was supported by COSY interactions of H–C(2) with H–C(3) and of H–C(3) with both protons of CH_2OH.

The ^{1}H-NMR of the acetyl derivative **1a** showed three three-proton *s* at δ 2.28, 2.05, and 2.02, thus confirming the presence of three OH groups in **1**. The relative configuration as drawn in the structure was established by the NOE values (*Table 2*) observed for various protons of the acetyl derivative **1a**. Thus, a higher

Table 2. *NOEs of* **1a**

Protons irradiated	Protons enhanced	% Enhancement
H–C(2)	H–C(4)	0.7
	H–C(7)	0.7
	H_a of CH_2–C(3)	5.4
	H_b of CH_2C(3)	2.3
	H–C(2′), H–C(6′)	7.8
H–C(3)	H–C(4)	very weak
H–C(4)	H_a of CH_2–C(3)	1.5
	H_b of CH_2–C(3)	3.0
H_b of CH_2–C(3)	H_a of CH_2–C(3)	14.7
	H–C(2′), H–C(6′)	very weak
2 H–C(2″)	2 H–C(3″)	12.5
MeO (3.78)	H–C(2′)	26.0
MeO (3.87)	H–C(7)	22.6
MeCOO–C(5)	H–C(4)	6.2

NOE was observed between H–C(2) and H_a of CH_2–C(3) (5.4%) and between H–C(2) and H–C(2′), H–C(6′) (7.8%), while irradiation of H–C(2) or H–C(3) did not enhance the signal of each other. These observations are in analogy with those reported earlier for a similar *trans* relationship of H–C(2) and H–C(3) [8]. The NOE experiments further supported the exact assignment of the signals of the two MeO groups.

The HR-EI-MS of lawsonadeem (**2**) showed the molecular-ion peak at *m*/*z* 372.3234 corresponding to the formula $C_{22}H_{12}O_6$. The UV absorption maxima at 253.0 and 287.5 nm were suggestive of an aromatic system, whereas the IR spectrum demonstrated the presence of conjugated carbonyl groups (1685, 1653 cm^{-1}) and an aromatic moiety (1600–1400 cm^{-1}). The NMR data of lawsonadeem (*Table 3*) were consistent with the structure 1-methoxy-13*H* dibenzo[*b*,*i*]xanthene-5,7,12,14-tetrone.

Table 3. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$) *of Compound* **2**. δ in ppm, *J* in Hz.

	δ(C)	δ(H)
C(1)	161.8	
H–C(2)	124.7	7.64 (*d*, *J* = 7.9)
H–C(3)	130.8	7.60 (*dd*, *J* = 7.9, 7.0)
H–C(4)	124.1	7.91 (*d*, *J* = 7.0)
C(4a)	124.3	–
C(5)	184.2[a])	–
C(5a)	151.5[b])	–
C(6a)	150.9[b])	–
C(7)	183.5[a])	–
C(7a)	128.6	–
H–C(8)	126.4	8.08 (*d*, *J* = 8.0)
H–C(9)	136.4	7.77 (*t*, *J* = 8.0)
H–C(10)	134.1	7.69 (*t*, *J* = 8.0)
H–C(11)	126.1	8.05 (*d*, *J* = 8.0)
C(11a)	132.5	–
C(12)	187.7[c])	–
C(12a)	146.5[d])	–
H_a–C(13)	54.6	3.83 (*d*, *J* = 17.0)
H_b–C(13)	–	3.10 (*d*, *J* = 17.0)
C(13a)	146.5[d])	–
C(14)	186.5[c])	–
C(14a)	114.9	–
MeO	54.6	3.82 (*s*)

[a])–[d]) Values are interchangeable.

The ^{1}H-NMR spectrum of **2** showed four *d* at δ 8.08 (*J* = 8.0 Hz, H–C(8)), 8.05 (*J* = 8.0 Hz, H–C(11)), 7.91 (*J* = 7.0 Hz, H–C(4)), and 7.64 (*J* = 7.9 Hz, H–C(2)), two *t* at δ 7.77 (*J* = 8.0 Hz, H–C(9)) and 7.69 (*J* = 8.0 Hz, H–C(10)), and one *dd* at δ 7.60 (*dd*, *J* = 7.9, 7.0 Hz, H–C(3)). These protons had their connectivities with C-atoms at δ 126.4 (C(8)), 126.1 (C(11)), 124.1 (C(4)), 124.7 (C(2)), 136.4 (C(9)), 134.1 (C(10)), and 130.8 (C(3)), respectively, in the HMQC spectrum. A three-proton downfield *s* at δ 3.82 (δ(c) 54.6) suggested a MeO group which could be placed at C(1) as it showed interaction with H–C(2) in the NOESY plot.

The HR-EI-MS of vomifoliol (**3**) showed the molecular-ion peak at *m*/*z* 224.2959 corresponding to the formula $C_{13}H_{20}O_3$. The UV spectrum exhibited an absorption maximum at 237.4 nm and the IR spectrum important bands at 3350 (OH) and 1680 cm^{-1} (α,β-unsaturated carbonyl group). The molecular formula suggested four

unsaturations in the molecule, two of which were justified by the α,β-unsaturated carbonyl system indicated by the IR spectrum and clearly manifested by the NMR spectra (*Table 4*). The spectral data were compatible with the structure of vomifoliol for **3**. Vomifoliol has earlier been reported from *Rauwolfia vomitoria* [6], but assignments based on 2D NMR data are described for the first time. A compound with the same structure was also reported from *Podocarpus blumei* with the name blumenol [9]. The spectral data of the compound isolated in the present work match those of reported earlier [6][9].

Table 4. *^{1}H- and ^{13}C-NMR Data* ($CDCl_3$) *of Compound* **3**. δ in ppm, J in Hz.

	δ(c)	δ(H)
C(1)	197.8	–
H–C(2)	126.9	5.62 (*s*)
C(3)	162.5	–
C(4)	79.0	–
C(5)	41.0	–
H_a–C(6)	49.7	2.96 (*d*, J = 12.0)
H_b–C(6)	–	2.09 (*d*, J = 12.0)
H–C(1′)	129.0	5.82 (*d*, J = 16.0)
H–C(2′)	135.8	5.75 (*dd*, J = 16.0, 5.0)
H–C(3′)	68.0	4.40 (*dq*, J = 7.0, 5.0)
Me(4′)	24.0	1.30 (*d*, J = 7.0)
Me_β–C(5)	23.7	0.90 (*s*)
Me_α–C(5)	22.8	0.95 (*s*)
Me–C(3)	18.8	1.80 (*s*)

Thus the ^{1}H-NMR spectrum of **3** showed a one-proton *s* at δ 5.62 (H–C(2); δ(c) 126.9, HMQC) and a three-proton *s* at δ 1.80 (Me–C(3); δ(c) 18.8, HMQC) for a vinylic Me group located at C(β) of the α,β-unsaturated carbonyl system. Additionally, the ^{1}H-NMR spectrum had signals at δ 5.82 (*d*, J = 16.0 Hz, H–C(1′); δ(c) 129.0, HMQC) and 5.75 (*dd*, J = 16.0, 5.0 Hz, H–C(2′); δ(c) 135.8, HMQC) representing a C=C bond in an open chain with (*E*) configuration. These data and the lack of any further olefinic functionality suggested that the fourth unsaturation should be justified by a ring moiety. Furthermore, the ^{1}H-NMR spectrum had two three-proton signals at δ 0.90 and 0.95 connected with C-atoms at δ 23.7 and 22.8, respectively, in the HMQC plot, and a three-proton *d* at δ 1.30 (J = 7.0 Hz, Me(4′)) having a connected C-atom at δ 24.0 in the HMQC plot. A one-proton *dq* was observed at δ 4.40 (J = 5.0, 7.0 Hz, H–C(3′); δ(c) 68.0, HMQC). The COSY interactions of H–C(1′) with H–C(2′), H–C(2′) with H–C(3′), and H–C(3′) with Me(4′) allowed to formulate the side chain as depicted in the structure. These observations along with the two one-proton *d* at δ 2.96 H_a–C(6) and 2.09 H_b–C(6), each with a coupling constant of 12.0 Hz, suggested a nonequivalent CH_2 group adjacent to the carbonyl group and connected to a tetrasubstituted C-atom.

Experimental Part

General. The petroleum ether used was of the boiling range 60–80°. CC = column chromatography. Optical rotations: *Jasco DIP-360* digital polarimeter. UV Spectra: MeOH solns.; *Hitachi-U-3200* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: $CHCl_3$ solns.; *Jasco IR-A-1* spectrophotometer; in cm^{-1}. NMR Spectra: $CDCl_3$ solns.; *Bruker AMX-500* instrument, at 500 (^{1}H) or 125 MHz (^{13}C); δ in ppm rel. to $SiMe_4$, J in Hz. MS: *Finnigan MAT-311A* instrument; in *m*/*z* (rel. %).

Plant Material. The aerial parts (40 kg) of *Lawsonia inermis* were collected from the region of University of Karachi, Pakistan, in Oct. 1998. The plant was identified by Dr. *Surayya Khatoon*, University of Karachi, and a voucher specimen (No. 67503) has been deposited in the Herbarium of the same university. The plant material

(40 kg) was extracted with CH_2Cl_2 (5 ×) at r.t. and the marc left was repeatedly (5 ×) extracted with MeOH also at r.t. The solvent from both these extracts was evaporated separately. The gummy residue from the CH_2Cl_2 extract was treated with petroleum ether to give petroleum ether soluble and insoluble fractions. The latter fraction was treated with Et_2O to yield Et_2O-soluble and Et_2O-insoluble fractions. The Et_2O-insoluble fraction was divided into AcOEt-soluble and AcOEt-insoluble fractions. The latter fraction was subjected to vacuum-liquid chromatography (VLC; silica gel GF_{254}, $CHCl_3$, $CHCl_3$/MeOH, then MeOH): 15 fractions. Of these, *Fr. 10* (730 mg) was further separated by CC (silica gel GF_{254}, $CHCl_3$, $CHCl_3$/MeOH, then MeOH). Of the obtained CC fractions, *Fr. 26* (15 mg; $CHCl_3$/MeOH 9.5 :0.5 eluate) and *30* (20 mg; $CHCl_3$/MeOH 9 :1 eluate) were pure compounds (by TLC); *i.e.*, *lawsonicin* (**1**) and *lawsonadeem* (**2**), resp. The VLC *Fr. 5* was also subjected to CC (silica gel GF_{254}, $CHCl_3$, $CHCl_3$/MeOH, then MeOH). The fraction eluted with $CHCl_3$/MeOH 9 :1) yielded the pure constituent *vomifoliol* (**3**).

Lawsonicin (=*2,3-Dihydro-5-hydroxy-3-(hydroxymethyl)-2-[4-(3-hydroxypropyl)-3-methoxyphenyl]-6-methoxy-1-benzofuran*; **1**). Amorphous powder (15 mg). UV (MeOH): 282.2 (2.87). IR ($CHCl_3$): 3650 (OH), 2900 (C−H), 1600–1450 (arom.) 1350 (C−O). ^{1}H- and ^{13}C-NMR: *Table 1*. HR-EI-MS: 360.1565 (20, M^+). EI-MS: 360 (20, M^+), 342 (100), 138 (40).

Acetylation of **1**. To a soln. of **1** (10 mg) in pyridine, Ac_2O (1.0 ml) was added and the mixture left at r.t. The mixture was poured over crushed ice and extracted with AcOEt. After usual workup of the AcOEt phase and purification by TLC (silica gel, $CHCl_3$/MeOH 9.5 :0.5), *lawsonicin triacetate* (**1a**) was obtained. Amorphous powder (6.0 mg). ^{1}H-NMR: *Table 1*. NOE: *Table 2*. EI-MS: 486 (13, M^+), 426 (17), 309 (5), 283 (6), 249 (8), 189 (11), 137 (41).

Lawsonadeem (=*1-Methoxy-13*H-*dibenzo*[b,i]*xanthene-5,7,12,14-tetrone*; **2**). Amorphous powder (10 mg). UV (MeOH): 253.0 (3.12), 287.5 (2.67). IR ($CHCl_3$): 3350 (OH), 2900 (C−H), 1685, 1653 (conj. C=O), 1600–1400 (arom.), 1350 (C−O). ^{1}H- and ^{13}C-NMR: *Table 3*. HR-EI-MS: 372.3234 (30, M^+). EI-MS: 372 (20, M^+), 328 (100), 384 (61), 189 (32), 162 (29), 150 (12), 132 (19), 109 (10).

Vomifoliol (=*(4*S*)-4-Hydroxy-4-[(1*E*,3*R*)-3-hydroxybut-1-enyl]-3,5,5-trimethylcyclohex-2-en-1-one*; **3**). Amorphous powder (15 mg). UV (MeOH): 237. IR ($CHCl_3$): 3350 (OH), 2900 (C−H), 1680 (C=O), 1350 (C−O). ^{1}H- and ^{13}C-NMR: *Table 4*. HR-EI-MS: 224.2959 (30, $M^{+\cdot}$). EI-MS: 224 (1, M^+), 168 (30), 150 (27), 135 (28), 124 (100), 107 (22).

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