Two New Triterpenoids from *Lawsonia alba*

Bina S. Siddiqui, Muhammad Nadeem Kardar, and Shazia Khan

H. E. J. Research Institute of Chemistry, University of Karachi-75270, Pakistan

Reprint requests to Prof. B. S. Siddiqui. E-mail: bina@khi.comsats.net.pk

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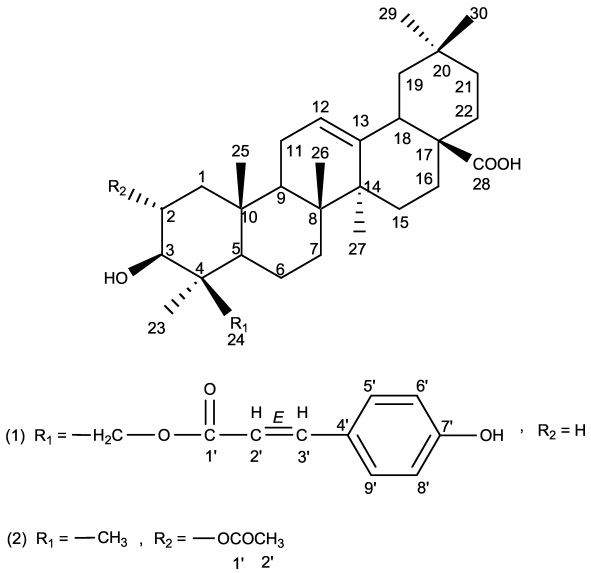
Two new triterpenoids, lawsowaseem and lawsoshamim, have been isolated from *Lawsonia inermis* and their structures established as 3β-hydroxy-24-*p*-*E*-coumaroyloxy-olean-12-en-28-oic acid, and 2-acetoxy-3β-hydroxy-olean-12-en-28-oic acid on the basis of spectral evidences, particularly 2D NMR studies.

Key words: *Lawsonia inermis* Linn. (syn. *Lawsonia alba* Lam. Lythraceae), Lawsowaseem, Lawsoshamim

Introduction

Lawsonia is a monotypic genus represented by *Lawsonia inermis* Linn. (syn. *Lawsonia alba* Lam. Lythraceae), a native of North Africa and South-West 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 colouring 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 for the 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 earlier studies on the chemical constituents of the aerial parts of *L. alba*, four new constituents Lawsonin, lawsonic acid, lawsonicin and lawsonadeem were reported [5, 6]. A continuation of these investigations has resulted in the isolation and structure elucidation of two further compounds named as lawsowaseem (**1**) and lawsoshamim (**2**). The structures of the new constituents **1** and **2** have been elucidated through 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. These studies form the basis of present communication.



Results and Discussion

Compound **1** did not show the molecular ion peak in the EI-MS, however, the FAB (-ve) mass spectrum showed the molecular ion peak at *m/z* 617 $(M\text{-}1)^+$, and the exact mass measurement at *m/z* 617.8615 using FAB (-ve) mode accounted for the elemental composition $C_{39}H_{53}O_6$. The molecular formula was further supported through exact measurement of various mass fragment ions (Fig. 1) and ^{13}C NMR spectroscopy (Broad Band, and DEPT). It showed IR absorptions at 3500 – 2600 br. (OH and COOH), 1738 – 1680 (acid and ester carbonyls) and 1600 – 1380 cm^{-1} (four peaks, aromatic ring); and UV maximum at 203 and 282 nm. The ^{1}H NMR spectrum contained the signals due to six tertiary methyl groups ($\delta = 0.84$,

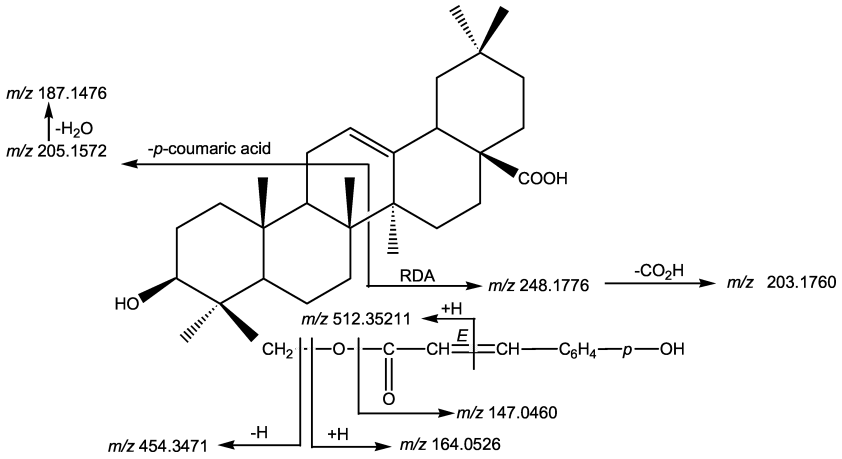


Fig. 1. Diagnostic mass spectral fragmentation of **1**.

0.88, 0.90, 0.94 1.00 and 1.03), which indicated its triterpenoid skeleton. These along with a double doublet at $\delta = 2.80$ ($J = 13.6$, 4.0 Hz, H-18) suggested that it belongs to oleanane series [7]. One of the hydroxyl groups indicated by the ^{1}H NMR spectrum was placed at C-3 on biogentic grounds. Its β disposition was evident from the chemical shift ($\delta = 3.33$) and coupling constants (*dd*, $J = 9.6$ and 4.4 Hz, H-3) [8]. The ^{1}H NMR spectrum further displayed resonance for an olefinic proton at $\delta = 5.26$ (t, $J = 3.3$ Hz, H-12). The characteristic retro-Diels-Alder fragmentation of **1** at *(m/z* 248.1776; $C_{16}H_{24}O_2$ and 203.1760; $C_{15}H_{23}$) along with the characteristic ^{13}C NMR chemical shifts of C-12 and C-13 at $\delta = 122.5$ and 144.8 respectively suggested that **1** has a double bond at C-12 and a carboxylic group at C-17 [9]. The relative abundance of the fragments at *m/z* 248 (48%) and 203 (100%) in the ElMS further supported the C-17 position of the carboxyl function [7]. A notable observation was two sets of AB doublets at $\delta = 7.60$ (1H, $J = 15.9$ Hz, H-3'), 6.30 (1H, $J = 15.9$ Hz, H-2'), 7.42 (2H, $J = 8.5$ Hz, H-5' and H-9') and 6.82 (2H, $J = 8.5$ Hz, H-6' and H-8'). The chemical shifts and coupling constants of these doublets indicated the presence of α *p-E*-coumaroyloxy substituent which was also supported by the ^{13}C NMR chemical shifts (Table 1) and high resolution mass spectrum which showed significant fragments at *m/z* 147.0460 ($C_9H_7O_2$) and 164.0526 ($C_9H_8O_3$) along with an ion at *m/z* 454.3471 ($C_{30}H_{46}O_3$) resulting from the loss of *p*-coumaric acid from the molecular ion peak (Fig. 1). Furthermore, two methylene protons were present at $\delta = 4.52$ (d, $J = 12.1$ Hz, H-24a) and $\delta = 4.35$ (d, $J = 12.1$ Hz, H-24b), which permitted the placement of the ester function at C-24 since in case of its equaterial orientation (C-23) these appear at $\delta = 3.8$ [8, 10, 11]. In the light of these data the structure of (**1**) has been arrived at as 3β-hydroxy-24-*p*-*E*-coumaroyloxyolean-12-en-28-oic acid.

Compound **2** did not show the molecular ion peak in the E1-MS, however the FD and FAB (-ve) mass spectrum showed the molecular ion peak at *m/z* 514 and 513 $(M\text{-}1)^+$ respectively, and the exact mass measurement at *m/z* 513.7519 using FAB (-ve) mode accounted for the elemental composition $C_{32}H_{49}O_5$. The molecular formula was further supported through ^{13}C NMR spectroscopy (Broad Band, and DEPT). It showed IR absorptions at 3500 (br. OH), 1730 – 1699 cm^{-1} (acid and ester carbonyls). The ^{1}H NMR spectrum showed the presence of seven methyl singlets ($\delta = 0.710.83$, 0.88, 0.90, 1.00, 1.03 and 1.10) which indicated its triterpenoid skeleton. The ^{1}H NMR spectrum further displayed resonance for an olefinic proton at $\delta = 5.24$ (t, $J = 3.3$ Hz, H-12). These data along with a double doublet at $\delta = 2.80$ ($J = 13.6$, 4.0 Hz, H-18) suggested that **2** belongs to the oleanane series of triterpenoids with the carboxylic group located at C-17 [7]. The retro-Diels-Alder fragmentation of **2** (*m/z* 248 and 203) as well as the characteristic ^{13}C NMR chemical shifts of C-12 and C-13 at $\delta = 122.7$ and 143.6 respectively further revealed that other substituents (hydroxyl and ester groups) are in ring A/B. The hydroxyl group was placed at C-3 on biogenetic grounds. Its β-disposition was evident from the chemical shift and coupling constant of H-3 ($\delta = 3.17$, d, $J = 11.2$ Hz). The multiplicity of H-3 further showed that C-2 is also substituted. Hence an acetoxy substituent indicated by NMR (δ_H 2.05, 3H, s, $\delta_{C=O}$ 171.5, δ_{CH3} 21.3) was placed at

Table 1. ^{13}CNMR spectral data of **1** and **2** (400 MHz, $CHCl_3$).

Carbon atoms	**1** δ [ppm]	Type	**2** δ [ppm]	Type
1	38.7	CH_2	43.7	CH_2
2	27.2	CH_2	73.3	CH
3	78.1	CH	80.9	CH
4	38.8	C	39.8	C
5	55.3	CH	55.2	CH
6	18.3	CH_2	18.2	CH_2
7	32.7	CH_2	32.4	CH_2
8	39.5	C	39.4	C
9	47.7	CH	47.6	CH
10	37.1	C	38.4	C
11	23.6	CH_2	22.9	CH
12	122.5	CH	122.7	CH
13	144.8	C	143.6	C
14	42.1	C	41.6	C
15	28.1	CH_2	27.6	CH_2
16	23.6	CH_2	23.5	CH_2
17	46.6	C	46.5	C
18	42.2	CH	41.0	CH
19	45.4	CH_2	45.9	CH_2
20	30.7	C	30.6	C
21	33.7	CH_2	33.8	CH_2
22	32.4	CH_2	32.5	CH_2
23	28.2	CH_3	28.5	CH_3
24	68.3	CH_2	16.3	CH_3
25	15.7	CH_3	17.1	CH_3
26	16.9	CH_3	16.6	CH_3
27	23.7	CH_3	25.9	CH_3
28	180.6	C	181.6	C
29	33.1	CH_3	33.0	CH_3
30	23.6	CH_3	23.5	CH_3
1’	167.2	C	171.5	C
2’	115.4	CH	21.3	CH_3
3’	144.6	CH	–	–
4’	132.7	C	–	–
5’	129.9	CH	–	–
6’	116.2	CH	–	–
7’	160.2	C	–	–
8’	116.2	CH	–	–
9’	129.9	CH	–	–

C-2 (δc 73.3) with α disposition which was supported by a six-line patteren of H-2 at $\delta = 4.92$ (ddd, $J = 11.2$, 11.2, 4.4 Hz). These observations led to arrive at the structure of **2** as *2*-acetoxy-3β-hydroxy-olean-12-en-28-oic acid.

Experimental Section

General

The NMR spectra were recorded on Bruker AMX-400 instrument with TMS as int. standard. MS were obtained on Finnigan MAT 311A instrument. IR spectra were recorded on JASCO IR A-1 spectrophotometer, whereas UV spectra were recorded on Hitachi U-3200 spectrophotometer. Optical rotations were measured on JASCO DIP-360 digital polarimeter. The petrol used was of the boiling range 60 – 80 °C.

Plant material

The aerial parts (40 kg) of *Lawsonia alba* 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 at the Herbarium of the same University.

Isolation

The plant material (40 kg) was extracted with dichloromethane (×5) at room temperature and the marc left was repeatedly (×5) extracted with methanol also at room temperature. The solvent from both these extracts was removed separately under reduced pressure. The gummy residue left on removal of the solvent at reduced pressure from the dichloromethane extract was treated with treated with Et_2O to yield Et_2O soluble and Et_2O insoluble fractions. Et_2O soluble fraction was subjected to column chromatography over silica gel GF_{254}. The column was eluted with P.E. P.E.-EtOAc and EtOAc in order of increasing polarity. The fractions were combined on the basis of TLC to give 20 fractions. Out of these, fractions No. 4 (5.0 mg, P.E-E.A, 9.5:0.5 eluate), and No. 5 (10.0 mg, P.E-E.A, 9:1 eluate), were pure showing single spots on TLC. Their spectral studies revealed them to be new constituents and they were named as lawsowaseem (**1**) and lawsoshamim (**2**) respectively.

*3β-Hydroxy-24-p-E-coumaroyloxy-olean-12-en-28-oic (**1**)*

Amorphous powder, $[\alpha]_D^{27} + 0.045$ (MeOH, c, 0.46), UV (MeOH) $\lambda_{max} = 203$, 282 nm. IR ($CDCl_3$) $\tilde{\nu}_{max} = 3500 - 2600$, 1738 – 1680, 1600 – 1380 cm^{-1}. ^{1}H NMR ($CHCl_3$, 400 MHz): δ 0.84, 0.88, 0.90, 0.94, 1.00 and 1.03 (3H, s, 6 X CH_3), 2.80 (1H, dd, $J = 13.6$, 4.0 H-18), 3.33 (1H, dd, $J = 9.6$, 4.4 Hz, H-3), 4.35 (1H, d, $J = 12.1$, H-24b), 4.52 (1H, d, $J = 12.1$, H-24a), 5.26 (1H, $J = 3.3$, H-12), 6.30 (1H, d, $J = 15.9$, H-2’), 6.82 (1H, d, $J = 8.5$, H-6’ and H-8’), 7.42 (1H, d, $J = 8.5$, H-5’ and H-9’), 7.60 (1H, d, $J = 15.9$, H-3’). HRMS *m/z*: 454.3471 ($C_{30}H_{46}O_3$; calculated for $C_{30}H_{46}O_3$ 454.3446), 248.1776 ($C_{16}H_{24}O_2$), 205.1572 ($C_{14}H_{21}O$), 203.1760 ($C_{15}H_{23}$), 164.0526 ($C_9H_8O_3$), 147.0460 ($C_9H_7O_2$). EIMS *m/z*: (rel.int %) = 454.0 (M^+-164) (8.0), 408.5 (14.0), 393.4 (2.9), 248.2 (100), 204.2 (40.3), 203.7 (85.2), 164.2 (4.4), 147.2 (21.6), 133.2 (39.5). ^{13}C NMR (75 MHz): Table 1.

*2-Acetoxy-3β-hydroxy-olean-12-en-28-oic acid (**2**)*

Amorphus powder; $[\alpha]_D^{27} + 3.8$ (MeOH, c, 0.03). UV (MeOH) $\lambda_{max} = 206.8$, 195.6 nm. IR ($CDCl_3$) $\tilde{\nu}_{max} = 3500$, 2941.2, 2868.0, 1699.2, 1460, 1253.6, 1029 cm^{-1}. ^{1}H NMR

($CHCl_3$, 400 MHz): 0.71 (3H, s, H-25), 0.83 (3H, s, H-26), 0.88 (3H, s, H-29), 0.90 (3H, s, H-30), 1.00 (3H,s, H-24), 1.03 (3H, s, H-23), 1.10 (3H, s, H-27), 2.05 (3H, s, H-2'), 2.80 (1H, dd, $J = 13.6$, 4.0 H-18), 3.17 (1H, d, $J = 11.2$ Hz, H-3), 4.92 (1H, ddd, $J = 11.2$, 11.2, 4.4 Hz, H-2), 5.24 (1H, t, $J = 3.3$ Hz, H-12). EIMS *m/z* (rel. int %) 408.6 (M^+-106) (1.3), 248.0 (100), 202.0 (87.8), 189.0 (18.7), 132.9 (27.2). ^{13}C NMR (75 MHz): Table 1.

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