Indian Journal of Chemistry
Vol. 36B, March 1997, pp. 252-256

Lawnermis acid: A new anticomplementary triterpenoid from *Lawsonia inermis* seeds†

Geeta Handa*, Aruna Kapil, Shalini Sharma & Jagdev Singh
Regional Research Laboratory, Jammu-Tawi 180 001, India

Received 26 January 1996; revised 23 August 1996

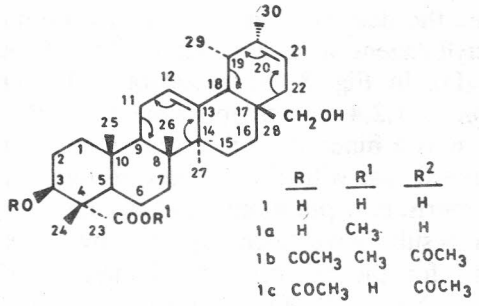
Bioassay guided fractionation of the methanolic extract of the defatted *Lawsonia inermis* seeds has led to the isolation of two new triterpenoids lawnermis acid **1** and its methyl ester **1a**. Their structures have been established as 3β, 28β-dihydroxy-ursa-12, 20-dien-23α-oic acid **1** and its methyl ester **1a** on the basis of chemical and spectra evidence. Compound **1** shows significant anticomplementary activity *in vitro* tests. It inhibits the immunohaemolysis of antibody coated sheep erythrocytes by pooled guinea pig serum.

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[1] in India and Middle East countries as a cosmetic for colouring palms of hands and dyeing of hair for personal adornment. They are also used as a prophylactic in the form of paste or decoction for the skin inflammation. The essential oil obtained from the flowers finds use in perfumery due to its β-ionone content.

The leaves have been reported to contain various compounds[2,3] like coumarins, flavonoids, gallic acid, naphthoquinones, naphthalene derivatives, lupane type triterpenoids, aliphatic constituents, phenolic glycosides and xanthones. Recently, reports have appeared on the chemical constituents of the roots[3] and bark[4-6]. However, no report on the chemical constituents of the seeds except for its analysis[1] for fatty acids, waxes and colouring matter content and also for biological activity is available in the literature. Keeping this in view a systematic study on the non-phenolic constituents of the seeds of *Lawsonia inermis* has been carried. The bioassay guided fractionation has led to the isolation of a new compound, lawnermis acid characterised as 3β, 28β-dihydroxy-ursa-12, 20-dien-23α-oic acid **1** and its methyl ester **1a** which are reported in this paper.

Compounds **1** and **1a** were isolated by column



	R	R^1	R^2
1	H	H	H
1a	H	CH_3	H
1b	$COCH_3$	CH_3	$COCH_3$
1c	$COCH_3$	H	$COCH_3$

chromatography followed by flash chromatography and preparative TLC. Support for the structure of the compound **1** occurring as a free carboxylic acid (IR: 1692 cm^{-1}) and its carboxymethyl ester **1a** is evident from the M^+ peak at m/z 484 for **1a** and identical TLC and spectral data with the compounds obtained by deacetylation of LI-5Ac and LI-5AcMe. Further confirmation of the structure of **1** and **1a** having a basic α-amyrin skeleton similar to quinovic acid[7] was obtained by conversion to the diacetate derivative **1c** and carboxymethyl diacetate derivative **1b** respectively.

Formation of the diacetate **1b** the acetoxyl protons of which appeared in ^1H NMR at δ 1.91 and δ 2.10 (s, 2 × OAc) accounted for the presence of two hydroxyl groups in **1**. β-Configuration of the C_3-OH group was supported by the appearance of the peak at δ 4.35 (H-3, d, J = 11 Hz) as reported by Kohma and Ogura[8]. The appearance of the peak at δ 2.2 (d, J = 11 Hz) indicated H-18 belonging to the ursane series[7,9]. Other signals appeared at δ 3.5 for $-OCH_3$, 3.72, 3.78 (AB_q, J = 11 Hz for $C_{28}.CH_2OH$), 1.61 for vinylic methyl[9], 5.29 and δ 5.30 for the protons of C_{12}-C_{13}

†RRL Communication No. 2196.

and C_{20}-C_{21} double bonds and 0.83, 0.85, 0.95, 1.18 for the remaining methyl groups. The ^1H NMR spectral data of **1a** further lent support to structure of **1**.

Support for the isolated nature of the double bond at 1610 cm^{-1} and the absence of the gem-dimethyl doublet at 1385, 1380 cm^{-1} indicating substitution of either C-23 or C-24 was obtained from the IR spectra of all the compounds **1, 1a, 1b** and **1c**.

In the ^{13}C NMR of **1b** (Table I) C_3-OH appeared at δ 81.0, C_{20}-C_{21} double bond carbons at 136, 120, C_{28}-CH_2-OH at 68.0, C_{23}-OCH_3 at 51.0, the two acetate carbonyls at 168.5 and 168.6 and the C-23 carbonyl at δ 181.5. The α-nature of the C-23 was in agreement with the ^{13}C NMR chemical shift for C-24 at δ 14.0 as reported for quinovic acid[7]. The rest of the ^{13}C NMR peaks for **1b** and **1a** were in agreement with the proposed structure.

Table I—^{13}C NMR spectral assignments of compounds **1a** and **1b**

Carbon No.	Compounds	
	LI-5AcMe **1b**	LI-5Me **1a**
1	42.0	42.0
2	30.5	30.5
3	81.0	80.5
4	53.5	52.0
5	56.5	57.0
6	21.5	22.0
7	35.0	34.5
8	39.0*	39.0*
9	46.0*	46.0*
10	40.0*	40.0*
11	24.0*	24.5*
12	123.0	123.0
13	149.1	141.5
14	45.0	45.5
15	32.0	33.0
16	27.5	27.5
17	45.0	43.5
18	44.0	44.0*
19	29.0	29.0
20	136.0	139.0
21	120.0	120.0
22	22.5	23.5*
23	181.5	182.0
24	14.0	14.0
25	15.0	15.0
26	16.0	16.0
27	16.5	16.5
28	68.0	61.0
29	19.0	19.5
30	31.5	
OAC	168.6 168.5	
OCH_3	51.5	52.0

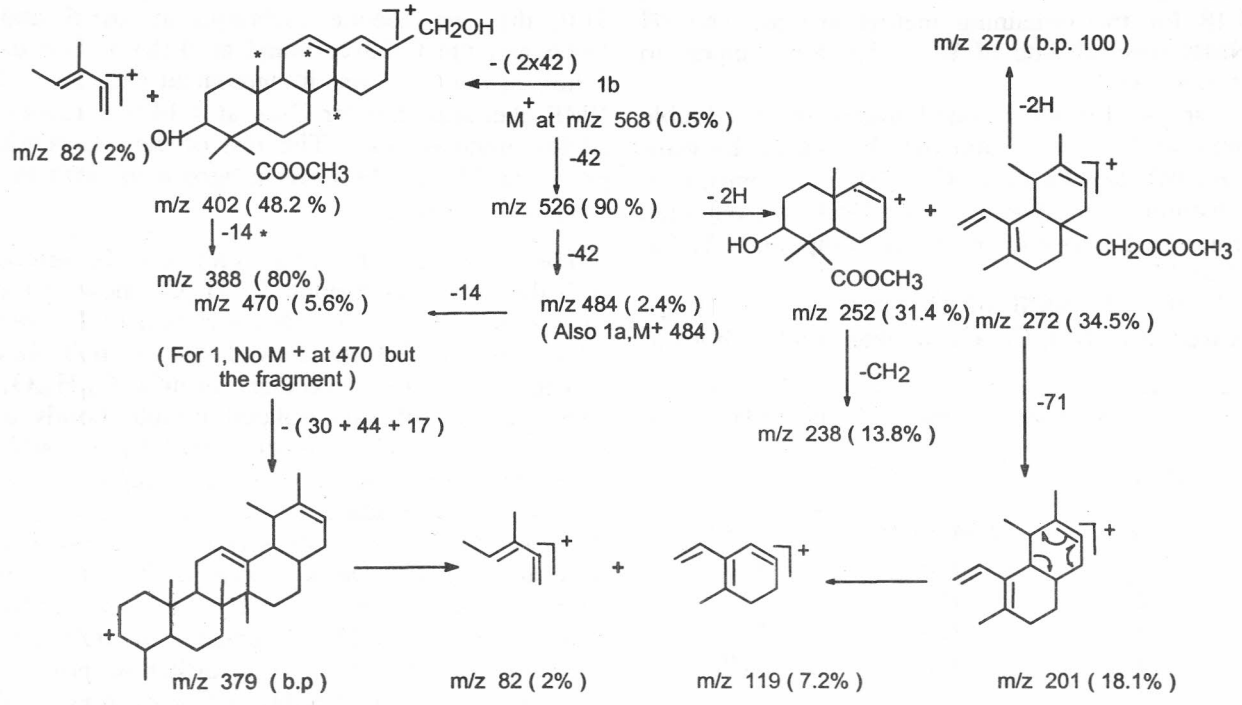
Spectra were recorded in $CDCl_3$ using TMS as internal standard.
*Assignments interchangeable within the columns.

The mass spectrum of the compound **1b** agreed with the molecular formula $C_{35}H_{52}O_6$ showing the M^+ at m/z 568 (0.5%). Deacetylation of **1b** with KOH gave **1a** which showed M^+ at m/z 484 agreeing with the molecular formula $C_{31}H_{48}O_4$. The presence of two isolated double bonds at C_{12}-C_{13} and C_{20}-C_{21} was supported by a double retro-Diels Alder decomposition of rings 'C' and 'E' (Scheme I) leading to the fragments at m/z 402 (48.2%) indicating presence of $-CH_2OH$ at position-28 and the peak at m/z 82 (2%) supported the vinylic methyl at C-20[9] and the double bond at C_{20}-C_{21}. The fragments at m/z 252 (34.1%) and 238 (13.8%) lent further support for the presence of $-COOCH_3$ at C-4 in ring-A and those at m/z 272 (34.5%) and 201 (18.1%) for the substitution of C-28 by $-CH_2OH$. All the combined evidence together led to the establishment of structure **1** as 3β, 28β-dihydroxy-ursa-12, 20-dien-23α-oic acid and **1a** its methyl ester.

Biological assay

The complement system plays an important role in the host defence system, inflammations or allergic reactions. Since complement is one of the major mediators of inflammatory response[13,14], it is possible that inhibition of complement activity would be expected to inhibit inflammatory moldels involving complement activation. The earlier findings for boswellic acids and oleanolic acid possessing antiinflammatory and anticomplementary activities and as inhibitor of C_3-convertase of classical complement pathway[15,16] give support to the mechanism for inhibiting inflammatory reaction indicating thereby the key role of complement in inflammation[17].

Keeping this in view the column chromatographed fractions of *L. inermis* were screened for their anti-complementary activity as compared with aspirin which served as standard (Table II). The marked action of the compound as well as that of the fraction yielding these compounds was found to be 100 μg (100 μg/0.1 mL diluent). A maximum inhibitory effect of 91% on haemolytic activity of complement system towards antibody coated sheep erythrocytes induced by freshly



Scheme I

Table II—Effect of compounds on classical complement pathway

Compd	Anticomplementary activity	
	E_{413}/60 min (Mean ± SD)	Inhibition (%)
Control	1.347 ± 0.004	—
1a	1.333 ± 0.003	1.03
1	0.118 ± 0.005	91.23
Aspirin	0.450 ± 0.010	66.59

The data are the mean ± SD of triplicate determinations from three experiments.

pooled guinea pig serum was observed in compound **1**. Thus anticomplementary activity compounds can be used in the therapy of inflammations and other disorders associated with complement activation.

Experimental Section

IR spectra were recorded on a PC-16 FT Perkin-Elmer or Hitachi 270-30 spectrometer, ^1H NMR spectra on a PMX 60-S1 and Bruker AC-P400 spectrometer with TMS as internal standard, ^{13}C NMR spectra on a FTNMR-FX-90Q multinuclear machine and GC-MS spectra on a Shimadzo GP 2000 spectrometer. For bioassay Perkin-Elmer UV/VIS spectrophotometer Lambda 3β and for flash chromatography Eyela EF-10 flash chromatography were used.

Plant material. The seeds of *Lawsonia inermis* were identified and supplied by Dr T N Srivastava of the Botany Division of the Laboratory.

Extraction and isolation. The air-dried coarsely powdered and defatted *L. inermis* seeds (10 kg) on extraction with methanol in a soxhlet yielded an extract (1.8 kg) showing about 68% activity. The extract was treated with water (6L) and *n*-βuOH (6L) to give an inactive $CHCl_3$ extract (10 g) and 90% active *n*-βuOH extract (90 gm). The *n*-βuOH extract was treated with 3% Na_2CO_3 solution (4.5 L) and extracted with $CHCl_3$ to give an unsaponifiable fraction (168 g) exhibiting about 90% activity. The saponified aqueous alkaline extract was acidified with dil. HCl and extracted with $CHCl_3$ to yield a saponified fraction (320 g).

A part of the unsaponifiable fraction (40 g) was charged over a column (90 × 5.5 cm) of silica gel (230-400 mesh: 100 g and 60-120 mesh: 350 g) and eluted with petrol (60-80°), C_6H_6, EtOAc and their mixtures collecting in all 300 fractions of 250 mL each. Fraction 1-10 yielded a compound LI-1 (m.p. 78-80°), 31-40 (petrol: 90-C_6H_6: 10), LI-2 (m.p. 65-70°), 201-207 (C_6H_6: 95—EtOAc:

5), LI-3 (m.p. 220-266), 208-215 (C_6H_6: 45: EtOAc: 5), LI-4 (m.p. 160-70°) and 261-95 (C_6H_6: 80—EtOAc: 20), LI-5 (m.p. 190-260°: 2.5 g a mixture of three compounds). The residue LI-5 (2.0 g) on flash chromatography over silica gel (200 g: 230-400 mesh) collecting in all 70 fractions each of 10 mL using hexane and hexane: EtOAc mixture yielded on evaporation from fractions 57-59 (hexane-EtOAc 60:40) a dark brown viscous residue (180 mg) which was a mixture of two compounds. This on preparative TLC (C_6H_6-$CHCl_3$-MeOH 70:10:1) yielded LI-5A (**1**, 60 mg) which showed 90% biological activity. IR (KBr): 3420 (−OH), 2936, 2870, 1696 (−COOH), 1610 (double bond), 1392, 1385, 1280, 1186, 1056 and 1040 cm^{-1}; no M^+ peak at m/z 470 but other peaks at m/z 380, 379 (b.p.), 378, 119,82, 58 and the other inactive compounds LI-5B, (**1a**, 40 mg); IR (KBr): 3418 (−OH), 2936, 2870, 1725, 1610 (double bond), 1466 (ether linkage), 1390, 1385, 1280, 1185, 1058 and 1040 cm^{-1}; M^+ at m/z 484 and other peaks at m/z 470, 426, 412, 398, 388, 252, 235, 119, 101, 83, 82 (b.p.) 58 and 43.

Acetylation of LI-5. The compound LI-5 (500 mg) was treated with acetic anhydride (4.0 mL) and pyridine (2 mL). The mixture was kept at room temperature for 24 hr, then heated on a water-bath for 1 hr, and worked up in usual manner yielding the acetate LI-5Ac, m.p. 200-40°, a mixture of two compounds **1b** and **1c**.

Methylation of LI-5Ac. The acetyl derivative LI-5Ac (200 mg) obtained above was methylated with diazomethane and the reaction mixture was worked up as usual to yield a compound LI-5AcMe ester **1b** which crystallised from EtOAc-MeOH, m.p. 145-50°, $[\alpha]_D^{28}+0.003$ (0.3 $CHCl_3$); IR (KBr): 2934, 2850, 1730, 1610, 1450, 1368, 1200 and 1054 cm^{-1}; ^{1}HNMR ($CDCl_3$ 400 MHz): δ 0.6 (3H, s), 0.83 (3H, d), 0.85 (3H, s), 0.95 (3H, s), 1.18 (3H, s), 1.63 (3H, s), 1.91 and 2.10 (2×OAc, s), 2.2 (1H, br d, J=11 Hz), 3.40 (3H, s), 3.72, 3.78 (2H, AB_q, J=11 Hz), 4.35 (1H, d, J=11 Hz), 5.24 and 5.30 (2H, br, d); ^{13}C NMR (Table I); MS: (eV-70): M^+ at m/z 568 (0.5%) and other peaks appeared at m/z 526 (90%), 484 (2.4), 470 (5.6), 454 (13.6), 402 (48.2%), 379 (0.4), 272 (34.2), 271 (86.6), 270 (b.p. 100%), 268 (68.5), 254 (15.2), 252 (34.1), 251 (10.7), 250 (16.4), 238 (13.8), 235 (95.5), 233 (56.4), 219 (61.2), 217 (32.1), 203 (42.4), 201 (19.1), 187 (33.0), 185 (20.5), 167 (2.0), 166 (4.0), 165 (3.0), 121 (0.3), 119 (7.2), 118 (7.0), 83 (6.0), 82 (2.0), 71 (1.5), 59 (0.8), 58 (30.0), 44 (7.9) and 43 (99.0).

Deacetylation of LI-5AcMe (**1b**) to LI-5B (**1a**): The methylated acetate LI-5AcMe (**1b**) obtained above was deacetylated by refluxing on a water bath with 4% methanolic KOH and the reaction mixture was worked up as usual to yield the methylated derivative, LI-5Me **1a** $[\alpha]_D^{26}+33$ ($CHCl_3$, c, 0.6); IR (KBr): 3420 (−OH), 2932, 2860, 1730 (ester), 1610 (double bond), 1465, 1460, 1380, 1360, 1264, 1240, 1200, 1040, 1030, 800 and 760 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.80 (s×2×CH_3), 1.18, 1.20, 1.25 (3×CH_3), 1.60 (s, CH_3), 2.20 (1H, d, J=11 Hz), 3.5 (s, OCH_3), 4.08, 4.15 (24, AB_q, J=11Hz), 4.4 (d, 1H, J=11 Hz), 5.1, 5.2 (d, 2H). This product was found to be identical (TLC, IR, ^{13}C NMR) with the compound LI-5 (**1a**).

Deacetylation of LI-5Ac. Deacetylation of LI-5Ac (100 mg) was conducted as above giving a mixture of 2 spots on TLC corresponding to LI-5A (**1**) and LI-5B (**1a**). The IR spectrum of the mixture exhibited a band at 1696 cm^{-1} due to the carboxylic group of the compound **1**.

Bioassay. The *in vitro* haemolysing effect of the compounds **1** and **1a** (Table II) vis the classical pathway was determined by Hart method[11,12]. The veronal saline buffer (*p*H 7.3) was used as diluent in complement assay and fresh pooled guinea pig serum as a source of complement. Sensitized sheep erythrocytes were incubated with complement incubated fractions. Simultaneously standard drug like aspirin as well as controls were also run. The degree of haemolysis produced by released haemoglobin in the supernatant after sedimentation of the remaining erythrocytes was determined spectrophotometrically at 413 nm.

References

1 *The Wealth of India: Raw materials*, Vol. 6 (Council of Scientific & Industrial Research, New Delhi), **1962**, p. 47.
2 Takeda Y & Fatope M O, *J Nat Prod*, 51, **188**, 725.
3 Gupta S, Ali M, Alam M S, Niwa M & Sakai T, *Phytochemistry*, 31, **1992**, 2558.
4 Gupta S, Ali M & Alam M S, *Phytochemistry*, 33, **1993**, 723.
5 Chakrabarty T, Podder G & Pyrek J S, *Phytochemistry*, 21, **1982**, 1854.
6 Gupta S, Ali M & Alam M S, *Indian J Chem*, 31B, **1992**, 705.
7 Miana G A & A L Hazimi H M G, *Phytochemistry*, 26, **1987**, 285.
8 Kojima H & Ogura H, *Phytochemistry*, 28, **1989**, 1703.
9 Khan M S Y, Javed K, Khan M H, Shamsi M A & Siddiqui A A, *Phytochemistry*, 31, **1991**, 1989.
10 Hota R K & Bapuji M, *Phytochemistry*, 35, **1994**, 1073.
11 Hart LAt, Enckevort van P H, Dijk Van H, Silva de KTD & Labadie R P, *J Ethnopharmacology*, 23, **1988**, 61.
12 Hart LAt, BergVan Den A J L, Kuis L, Dijk Van H & Labadie R P, *Planta Med*, 55, **1989**, 509.

13 Dias da Silva W & Lepow I H, *J Exp Med*, 125, **1967**, 921.
14 Goldstein I M, Inflammation, basic principles & clinical correlates, edited by J I Gallin, I M Goldstein & R Synderman (Raven Press Ltd), pp. 55-74.
15 Kapil A, Moza N, *Int J Immunopharmacol*, 14, **1992**, 1139.
16 Kapil A, Sharma S, *J Pharmac Pharmacol*, 46, **1994**, 922.
17 Sell S, *Immunology, immunopathology and immunity*, 3rd Edn, edited by R Harper and M D Hagerstown, **1980**, p. 242.