NOTE

Isolation and Identification of Triterpenoids from Flowers of *Lawsonia inermis*

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A new oleanane-type triterpenoid, 2 α , 3 β -dihydroxyolean-18-en-28-oic acid (**3**) along with five known triterpenoids, β -sitosterol (**1**), oleanolic acid (**2**), crataegolic acid (**4**), alphitolic acid (**5**) and colosolic acid (**6**) were isolated from the flowers of *Lawsonia inermis*. Their structures were elucidated by spectroscopic analyses.

Keywords: *Lawsonia inermis*, Oleanane-type triterpenoid, Lupane-type triterpenoid, Steroidal.

Lawsonia inermis is a much branched glabrous shrub or small tree, cultivated for its leaves although stem bark, roots, flowers and seeds have also been used in traditional medicine¹. The plant is reported to contain flavonoids, tannins and phenolic compounds, alkaloids, terpenoids, quinones, coumarins, xanthenes and fatty acids²⁻⁴. The plant has been reported to have analgesic, hypoglycemic, hepatoprotective, immunostimulant, antiinflammatory, antibacterial, antimicrobial, antifungal, antiviral, antiparasitic, antitrypanosomal, antidermatophytic, antioxidant, antifertility, tuberculostatic and anticancer properties^{2,5-8}. It is now considered as a valuable source of unique natural products for development of medicines against various diseases and also for the development of industrial products.

The flowers of *Lawsonia inermis* were collected in 2010 from India. The following instruments were used to obtain physical data: JEOL spectrometer (600 MHz for ¹H NMR, 150 MHz for ¹³C NMR) and ESI-MS spectra that recorded with Agilent 6510 Q-TOF LC/MS apparatus. The following instrumental conditions were used for column chromatography: Diaion HP-20, Chromatorex ODS DM1020T and Sephadex LH-20. Shimadzu LC-6AD liquid chromatograph; Shimadzu RID-10A refractive index detector; Preparative column: YMC-Pack ODS-A (250 $\times$ 20 mm i.d., S-5 μ m, 12 nm).

Extraction and Isolation: The flowers (2 Kg) of *Lawsonia inermis* was extracted three times with MeOH under reflux for 3 h. Evaporation of the solvent under reduced pressure provided the MeOH extract (636.5 g, 31.83 %). Part of the

MeOH extract (200 g) was partitioned into an EtOAc-H₂O (1:1, v/v) mixture to yield an EtOAc-soluble fraction (85.6 g, 13.62 %) and an aqueous fraction; Most of the EtOAc-soluble fraction (80.5 g) from the flower was subjected to ordinary-phase silica gel column chromatography {Hexane-EtOAc [(10: 1, v/v) $\rightarrow$ (2: 1, v/v) $\rightarrow$ (1: 2, v/v)] $\rightarrow$ CHCl₃-MeOH [(10: 1, v/v)] $\rightarrow$ CHCl₃-MeOH-H₂O [(65: 35: 10, v/v/v, lower layer)] $\rightarrow$ MeOH} to give 16 fractions. Fr. 11 (5.03 g) was subjected to ordinary-phase silica gel column chromatography {hexane-CHCl₃ [(75: 25, v/v) $\rightarrow$ (50: 50, v/v) $\rightarrow$ (25: 75, v/v)] $\rightarrow$ CHCl₃ $\rightarrow$ CHCl₃-MeOH [(90: 10, v/v) $\rightarrow$ (80: 20, v/v) $\rightarrow$ (50: 50, v/v)] $\rightarrow$ MeOH} to give 6 fractions {fr. 11-1 (10.7 mg), fr. 11-2 (2.1 mg), fr. 11-3 (9.8 mg), fr. 11-4 (78.7 mg), fr. 11-5 (154.5 mg), fr. 11-6 (206.7 mg)}. Fr. 11-1 (10.7 mg) was purified by HPLC [MeOH, HPLC column: YMC-Pack ODS-A (250 $\times$ 20 mm i.d., S-5 μ m, 12 nm)] to give β -sitosterol (**1**, 5.3 mg). Fr. 11-3 (9.8 mg) was purified by HPLC [MeOH, HPLC column: YMC-Pack ODS-A (250 $\times$ 20 mm i.d., S-5 μ m, 12 nm)] to give oleanolic acid (**2**, 6.5 mg). Fr. 11-5 (154.5 mg) was purified by HPLC [MeOH: H₂O (90: 10), HPLC column: YMC-Pack ODS-A (250 $\times$ 20 mm i.d., S-5 μ m, 12 nm)] to give 2 α ,3 β -dihydroxyolean-18-en-28-oic acid (**3**, 2.7 mg), crataegolic acid (**4**, 135.3 mg), alphitolic acid (**5**, 13.9 mg). Fr. 11-6 (206.7 mg) was purified by HPLC [MeOH: H₂O (90: 10), HPLC column: YMC-Pack ODS-A (250 $\times$ 20 mm i.d., S-5 μ m, 12 nm)] to give colosolic acid (**6**, 94.1 mg), crataegolic acid (**4**, 112.6 mg).

In a continuing search for bioactive or new secondary metabolites from traditional medicinal plants, we investigated

methanol extracts of the flowers of *L. inermis*. We report here on the isolation and structure elucidation of a new oleanane-type triterpenoid (**3**). The structure of compound **3** was elucidated on the basis of detailed spectroscopic analyses and by comparison with reported data. In addition, the known triterpenoids, β -sitosterol (**1**)⁹, oleanolic acid (**2**)¹⁰, crataegolic acid (**4**)¹¹, alphitolic acid (**5**)¹² and colosolic acid (**6**)¹³ were isolated (Fig. 1).

Fig. 1. structures of isolated triterpenoids **1-6**.

Compounds **3** were isolated as amorphous powders, it showed positive optical rotation ($[\alpha]_D^{30} = +18.82^\circ$ in MeOH). The molecular formula were determined to be $C_{30}H_{48}O_4$ on the basis of a quasi-molecular ion at m/z 472 $[M]^+$ by HR-ESI-MS. The IR spectrum showed strong absorption bands at 3388 and 1727 cm^{-1} , typical for the hydroxyl and carbonyl group, respectively. Detailed analyses of 1H , ^{13}C NMR spectra of **3** (Table-1) and comparison with 3β -acetoxy-olean-18-en-28-oic, morolic acid acetate¹⁴, particularly the ^{13}C of double bond (δ 136.7, C-18; δ 133.5, C-19), showed that the basic skeletons were similar, compound **3** was formulated as olean-18-ene triterpenoid. Another important structure unit (δ 2.10, H-1 β ; δ 3.71, H-2; δ 2.99, H-3) was assembled using DQF and HMBC experience (Fig. 2). The 1H NMR spectrum of **3** showed a signal at δ_H 3.71 (1H, m) and δ_H 2.99 (1H, d, $J = 8.94$) that suggested the presence of two OH at C-2 and C-3, as a result of the DQF correlation between H-1/H-2 and H-2/H-3 and HMBC cross-peaks from H-3 to C-2 and H-23/24 to C-3. Additionally, comparison of spectral data of **3** with $2\alpha, 3\beta$ -dihydroxyolean-28-oic acid, revealed that **3** differed from $2\alpha, 3\beta$ -dihydroxyolean-28-oic acid¹⁵ only in the absence of Gao *et al.*¹² double bond, so that, the structure of **3** was formulated as $2\alpha, 3\beta$ -dihydroxyolean-18-en-28-oic acid. The relative configuration of **3** was determined through the NOESY correlations (Fig. 2). between H-1 β (δ_H 2.10, m)/H-2(δ_H 3.71, m), H-2(δ_H 3.71, m)/H₃-24 β (δ_H 0.8, s), H-3 (δ_H 2.99, d, $J = 8.94$)/H₃-23 α (δ_H 1.01, s). Thus, the structure of **3** was established as $2\alpha, 3\beta$ -dihydroxyolean-18-en-28-oic acid.

Fig. 2. Key 2D NMR correlation of compound **3**

Position	δ_H (J in Hz)	δ_C , mult.	Position	δ_H (J in Hz)	δ_C , mult.
1	2.10,m;0.88,m	46.8,CH ₂	16	1.20-1.64,o	33.4,CH ₂
2	3.71,m	69.3,CH	17	-	47.9,qC
3	2.99 (d,8.94)	83.9,CH	18	-	136.7,qC
4	-	39.2,qC	19	5.20,s	133.5,CH
5	1.39,o	55.6,CH	20	-	32.1,qC
6	1.20-1.64,o	18.2,CH ₂	21	1.20-1.64,o	33.5,CH ₂
7	1.20-1.64,o	34.4,CH ₂	22	1.20-1.64,o	33.4,CH ₂
8	-	40.7,qC	23	1.01,s	28.4,CH ₃
9	1.20-1.64,o	51.1,CH	24	0.80,s	16.5,CH ₃
10	-	38.5,qC	25	0.93,s	19.7,CH ₃
11	1.20-1.64,o	21.0,CH ₂	26	0.98,s	16.0,CH ₃
12	1.20-1.64,o	25.9,CH ₂	27	0.78,s	14.9,CH ₃
13	1.20-1.64,o	41.3,CH	28	-	179.8,qC
14	-	42.6,qC	29	0.98,s	29.1,CH ₃
15	1.20-1.64,o	29.3,CH ₂	30	1.00,s	30.4,CH ₃

Note: "o" means "overlapped".

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