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NOTE

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

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A new oleanane-type triterpenoid, 2α, 3β-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 medicine[1]. The plant is reported to contain flavonoids, tannins and phenolic compounds, alkaloids, terpenoids, quinones, coumarins, xanthones and fatty acids[2-4]. 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 ^{1}H NMR, 150 MHz for ^{13}C 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 × 20 mm i.d., S-5 μm, 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-H_2O (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) → (2: 1, v/v) → (1: 2, v/v)] → $CHCl_3$-MeOH [(10: 1, v/v)] → $CHCl_3$-MeOH-H_2O [(65: 35: 10, v/v/v, lower layer)] → MeOH} to give 16 fractions. Fr. 11 (5.03 g) was subjected to ordinary-phase silica gel column chromatography {hexane-$CHCl_3$ [(75: 25, v/v) → (50: 50, v/v) → (25: 75, v/v)] → $CHCl_3$ → $CHCl_3$-MeOH [(90: 10, v/v) → (80: 20, v/v) → (50: 50, v/v)] → 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 × 20 mm i.d., S-5 μm, 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 × 20 mm i.d., S-5 μm, 12 nm)] to give oleanolic acid (**2**, 6.5 mg). Fr. 11-5 (154.5 mg) was purified by HPLC [MeOH: H_2O (90: 10), HPLC column: YMC-Pack ODS-A (250 × 20 mm i.d., S-5 μm, 12 nm)] to give 2α,3β-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: H_2O (90: 10), HPLC column: YMC-Pack ODS-A (250 × 20 mm i.d., S-5 μm, 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).

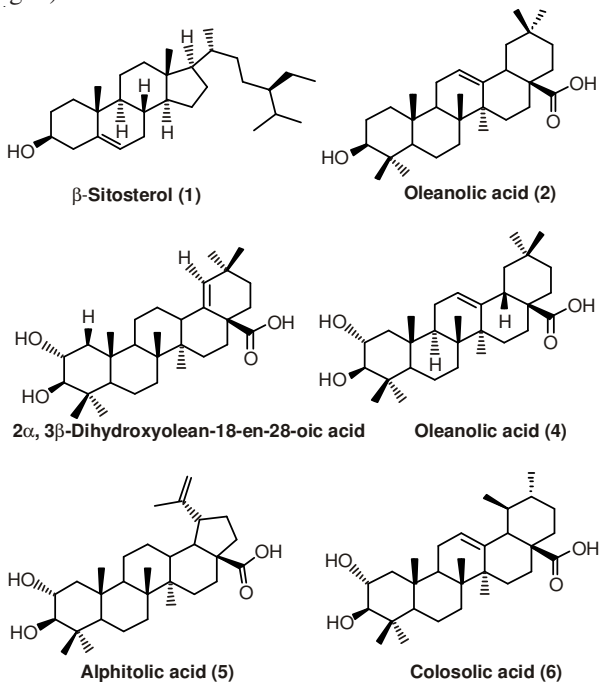

Fig. 1. structures of isolated triterpenoids **1-6**.

Compounds **3** were isolated as amorphous powders, it showed positive optical rotation ($[\alpha]_D^{30}$ = +18.82° 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 acetate[14], 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-1β; 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 δH 3.71 (1H, m) and δH 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 coss-peaks from H-3 to C-2 and H-23/24 to C-3. Additionally, comparison of spectral data of **3** with 2α, 3β-dihydroxyolean-28-oic acid, revealed that **3** differed from 2α,3β-dihydroxyolean-28-oic acid[15] only in the absence of Gao *et al.*[12] double bond, so that, the structure of **3** was formulated as 2,3-dihydroxyolean-18-en-28-oic acid. The relative configuration of **3** was determined through the NOESY correlations (Fig. 2). between H-1β(δ_H 2.10, m)/H-2(δ_H 3.71, m), H-2(δ_H 3.71, m)/H_3-24β (δ_H 0.8, s), H-3 (δ_H 2.99, d, *J* = 8.94)/H_3-23α (δ_H 1.01, s). Thus, the structure of **3** was established as 2α, 3β-dihydroxyolean-18-en-28-oic acid.

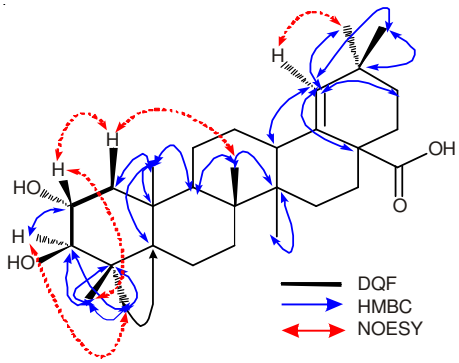

Fig. 2. Key 2D NMR correlation of compound **3**

TABLE-1
NMR DATA ($CDCl_3$) OF COMPOUND **3**

Position	δH (*J* in Hz)	δC, mult.	Position	δH (*J* in Hz)	δC, 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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