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TRITERPENES AND TRITERPENE-GLYCOSIDE FROM THE LEAVES OF *LAWSONIA INERMIS*

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ABSTRACT

*From the leaves of Lawsonia inermis (syn. L. alba), two triterpenes augustic acid (**1**) and 1β,2α,3α,19α-tetrahydroxy-12-ursen-28-oic acid (**2**), and a triterpene-glycoside suavissimoside R1 (**3**) were isolated by using various chromatoghraphies. Their structures were characterized on the basis of the spectroscopic data (1D-NMR, HSQC, HMBC, ESI-MS) in comparison with the literature. This is the first report of **1 - 3** from Lawsonia species.*

Keywords: *Lawsonia inermis, Lythraceae, Triterpene.*

I - INTRODUCTION

Lawsonia inermis L. (syn. *L. alba*) is a flowering plant, the sole species in the genus *Lawsonia* in the family Lythraceae. It is native to tropical and subtropical regions of Africa, southern Asia, and northern Australia in semi-arid zones. This is a tall shrub or small tree, 2 - 6 m high. It is glabrous, multibranched with spine tipped branchlets. Leaves are opposite, entire, glabrous, sub-sessile, elliptical, and broadly lanceolate (1.5 - 5.0 cm x 0.5 - 2 cm), acuminate, having depressed veins on the dorsal surface. During the onset of precipitation intervals, the plant grows rapidly; putting out new shoots, then growth slows. The leaves gradually yellow and fall during prolonged dry or cool intervals. Henna flowers have four sepals and a 2 mm calyx tube with 3 mm spread lobes. Petals are obvate, white or red stamens inserted in pairs on the rim of the calyx tube. Ovary is four celled, style up to 5 mm long and erect. Fruits are small, brownish capsules, 4 - 8 mm in diameter, with 32—49 seeds per fruit, and open irregularly into four splits. Lawsone content in leaves is negatively associated with the number of seeds in the fruits. *L. inermis* has many traditional and commercial uses, the most common being as a dye for hair, skin and fingernails, as a dye and preservative for leather and cloth, and as an anti-fungal [1 - 3]. Phytochemically, many components as lacoumarin [4], lawnermis acid, methyl ester of lawnermis acid [5], isoplumbagin [6], lawsonadeem, lawsonicin [7], wallichianol [8], 3-methyl-1-nonacosanol [9], 1,2,4-naphthalenetriol 4-O-β-D-glucopyranoside, lalioside, lawsoniaside [10], lawsaritol A [11], β-rosasterol [12], laxanthone II, laxanthone III, laxanthone I [13, 14] have been previously reported. This paper deals with the isolation and structural identification of two triterpenes augustic acid (**1**) and 1β,2α,3α,19α-tetrahydroxy-12-ursen-28-oic acid (**2**), and a triterpene-glycoside suavissimoside R1 (**3**) from the methanolic extract of the leaves of *L. inermis*.

II - EXPERIMENTAL

1. General experimental procedures

Optical rotations were determined on a Jasco DIP-1000 KUY polar meter. The Electrospray Ionization (ESI) mass spectrum was obtained using an AGILENT 1200 LC-MSD Trap spectrometer. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded on Bruker AM500 FT-NMR spectrometer. Chemical shifts (δ) are reported in ppm using tetramethylsilane (TMS) as an internal standard. Column chromatography (CC) was performed on silica gel 230 - 400 mesh (0,040 - 0,063 mm, Merck) or YMC RP-18 resins (30-50 μm, Fujisilisa Chemical Ltd.). Thin layer chromatography (TLC) was performed on DC-Alufolien 60 F_{254} (Merck 1.05715) or RP_{18} F_{254s} (Merck) plates. Spots were visualized by spraying 10% H_2SO_4 aqueous and heating for 5 minutes.

2. Plant material

The leaves of *Lawsonia inermis* L. (Lythraceae) were collected in Ngoc Truc, Ha Dong, Ha Tay in March 2008 and identified by Dr Nguyen Van Duc, Hanoi University of Natural Science. A voucher specimen (No. LB1) was deposited at National Institute of Medicinal Materials and at Hanoi University of Pharmacy.

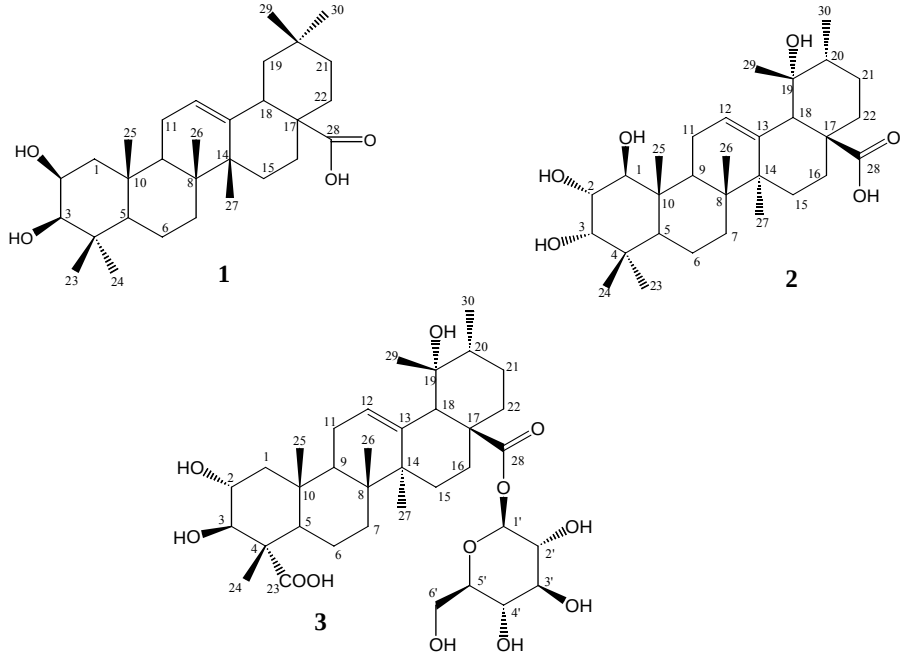

Fig. 1: The structures of **1** - **3**

3. Extraction and isolation

Dried leaves (5.0 kg) of *Lawsonia inermis* were powdered and then extracted three times with MeOH. After removal the solvent, the extract (31 g) was suspended in water and partitioned in turn with *n*-hexane, chloroform, and ethyl acetate to obtained *n*-hexane (14 g), chloroform (7 g), ethyl acetate (5.4 g) fractions, and the water layer. The chloroform fraction (7 g) was crudely separated on normal silica gel column gradient concentration of MeOH in chloroform from 50/1 to 50/50 (v/v) to give four sub-fractions (LIC1 to LIC5). The LIC1 sub-fraction was further separated on normal silica gel column eluted with chloroform/methanol 10/1 to give **1** (10 mg) as amorphous powder. The LIC2 sub-fraction was chromatoghraphed on a YMC RP-18 column using methanol:water:acetone (1/1/0.5; v/v/v) as eluent to give compound **2** (11 mg) as amorphous powder. The LIC3 sub-fraction was

chromatoghraphed on a YMC RP-18 column using methanol:water (1/1; v/v) as eluent to give compound **3** (13 mg) as needles.

2β,3β-Dihydroxy-12-oleanen-28-oic acid (augustic acid) (1): Amorphous powder; mp. 259-260ºC; positive ESI-MS: *m/z* 473 $[M+H]^+$, 454 $[M-H_2O+H]^+$, negative ESI-MS: *m/z* 471 $[M-H]^-$ ($C_{30}H_{48}O_4$, M = 472); ^{13}C-NMR (125MHz, Pyridin-d_5) see table 1, and ^{1}H-NMR (500 MHz, Pyridin-d_5) see table 2.

Table 1: The ^{13}C-NMR data of **1** - **3** in comparison with the literature

C	**1**		**2**		**3**	
	δ_C [#]	δ_C [a. b]	δ_C [##]	δ_C [b,c]	δ_C [###]	δ_C [a. b]
1	46.52	46.65	71.2	78.98	48.3	47.05
2	67.15	68.03	78.9	69.83	68.7	68.73
3	82.23	82.91	80.7	78.26	81.0	80.93
4	41.44	41.30	40.6	37.39	54.8	54.89
5	54.84	55.07	53.1	47.16	52.3	51.30
6	18.11	18.07	18.3	17.77	21.5	20.69
7	32.41	32.46	32.6	32.77	33.3	32.36
8	47.14	38.99	42.9	42.51	40.7	39.88
9	47.14	47.35	47.8	47.36	48.2	46.94
10	37.72	37.67	37.7	37.39	38.6	37.75
11	22.66	22.91	25.5	26.64	24.2	23.49
12	121.54	121.79	129.8	128.14	128.2	127.81
13	143.95	144.09	137.3	137.21	139.2	138.53
14	41.44	39.10	41.1	40.95	42.1	41.35
15	27.44	27.52	28.2	27.97	29.1	28.28
16	23.08	22.91	26.0	25.16	26.1	25.36
17	45.76	46.32	47.9	46.82	48.6	48.09
18	40.88	41.47	53.1	53.04	54.4	53.57
19	45.52	45.95	73.2	71.57	72.7	72.78
20	30.44	30.20	41.1	41.21	42.1	41.35
21	33.40	33.44	27.3	25.84	26.7	25.90
22	32.17	32.36	37.4	37.03	37.7	37.06
23	28.85	28.50	28.2	28.50	180.1	183.47
24	16.94	16.74	16.1	21.68	13.4	13.80
25	16.34	15.94	12.0	12.03	17.4	16.56
26	17.14	16.82	16.6	16.79	17.5	16.67
27	25.69	25.48	24.6	23.93	24.5	23.95
28	178.51	178.20	178.4	178.61	176.9	177.47
29	32.89	32.67	27.4	26.25	27.0	26.35
30	23.42	23.15	17.1	16.02	16.7	16.03
1’					95.8	94.97
2’					74.0	72.31
3’					78.9	77.25
4’					71.3	70.17
5’					79.2	77.94
6’					62.4	61.39

[#]δ_C of augustic acid [15], [##]δ_C of 1α,2α,3β,19α-tetrahydroxyurs-12-ene-28-oic acid [16], [###]δ_C of suavissimoside R1 [19], [a]Measured in Pyridine-d_5, [b]125 MHz, [c]Measured in DMSO, δ: ppm.

1β,2α,3α,19α-Tetrahydroxy-12-ursen-28-oic acid (2): Amorphous powder; mp. 234 - 235ºC; positive ESI-MS: *m/z* 505 $[M+H]^+$, 487.2 $[M+H-H_2O]^+$, 469.2 $[M+H-2H_2O]^+$, 451.2 $[M+H-3H_2O]^+$ ($C_{30}H_{48}O_6$, M = 504). ^{13}C-NMR (125 MHz, DMSO-d_6) see table 1, and ^{1}H-NMR (500 MHz, DMSO-d_6), see table 2.

Suavissimoside R1 (3): Needles, mp 254 - 255ºC, positive ESI-MS: *m/z* 703 $[M+Na]^+$, negative ESI-MS: *m/z* 679 $[M-H]^-$ ($C_{36}H_{56}O_{12}$, M = 680); ^{13}C-NMR (125MHz, Pyridin-d_5) see table 1, and ^{1}H-NMR (500 MHz, Pyridin-d_5) see table 2.

III - RESULTS AND DISCUSSION

Compound **1** was obtained as an amorphous powder. The molecular formula of **1** was

Table 2: The ^{1}H-NMR data and HMBC results of **1** - **3**

C	**1**		**2**		**3**	
	δ_H [a, c] (*J* in Hz)	HMBC H to C	δ_H [a, c] (*J* in Hz)	HMBC H to C	δ_H [a, c] (*J* in Hz)	HMBC H to C
1	1.08/2.05 m	2, 3, 5	3.30 d (9.5)	2, 3	1.85/2.05 m	
2	3.93 m		3.48 dd (9.5, 3.0)		4.04 m	
3	3.22 d (4.5)	2, 4, 5, 23	3.22*	1, 4	4.45 d, 9.5	2, 4, 24
5	0.75 m		1.22 m		2.01 m	
6	1.31/1.14 m		1.36 m		1.42/1.62 m	
7	1.11/0.82 m		1.22/1.44 m		1.20/1.68 m	
9	1.53 m	5	1.82		1.32 m	
11	1.92/1.78 m		2.00/2.52		1.85 m	
12	5.26 m	9, 11, 14	5.16 br t		5.30 br s	14, 18
15	1.14/1.06 m		0.90/1.66		0.99/2.04 m	
16	1.91/1.77 m		1.38/2.47		1.75/2.76 m	
18	3.04 m	13	2.37 br s		2.66 m	13, 14, 28
19	1.60/1.07 m		-			
20			1.27		1.17 m	
21	1.63/1.25 m		1.14/1.62		1.08/1.74 m	
22	1.60/1.77 m		1.51/1.58		1.66/1.84 m	
23	1.04 s	3, 4	0.88 s	2, 4, 5		
24	0.77 s	4, 23	0.80 s	3, 4, 5	1.43 s	3, 4, 5, 23
25	0.78 s	1, 5, 9, 10	0.91 s	1, 5, 9	0.82 s	1, 5, 9, 10
26	0.86 s	7, 8, 9, 14	0.71 s	8, 9, 14	0.87 s	7, 8, 9, 14
27	1.03 s	8, 13, 14, 15	1.30 s	8, 13, 15	1.41 s	8, 13, 14, 15
29	0.84 s	19, 20, 30	1.07 s	18, 19, 20	1.24 s	18, 19, 20
30	0.87 s	20, 21	0.85 d (6.5)	19, 20, 21	0.95 d (6.5)	19, 20, 21
1’					5.91 d (8.0)	28
2’					4.00 m	1’, 3’
3’					4.13 t (9.0)	2’, 4’
4’					4.01 m	6’
5’					3.88 m	
6’					4.11 dd (5.5, 12.0)/ 4.28 dd (2.0, 12.0)	5’

Table 3: The C-1, C-2 and C-3 chemical shifts of **2** and of the corresponding compounds

Compounds	δ_{C-1}	δ_{C-2}	δ_{C-3}	ref.
1β,2α,3β,19α-tetrahydroxyurs-12-ene-28-oic acid methyl ester	74.9	74.6	79.9	[16]
1β,2β,3β,19α-tetrahydroxyurs-12-ene-28-oic acid methyl ester	77.0	74.7	79.9	[17]
1α,2α,3β,19α-tetrahydroxyurs-12-ene-28-oic acid	71.2	78.9	80.0	[18]
1β,2α,3α,19α-tetrahydroxyurs-12-ene-28-oic acid (**2**)	78,98	69.83	78.26	

suggested to be $C_{30}H_{48}O_4$ from the exhibition of the quasi ion peaks at *m/z* 473 $[M+H]^+$, 454 $[M-H_2O+H]^+$ (positive ion mode) and *m/z* 471 $[M-H]^-$ (negative ion mode) in the ESI-MS. The ^{13}C-NMR spectrum of **1** showed signals of 30 carbon atoms including 7 methyl, 9 methylene, 6 methine, and 8 quaternary carbons, determining from the DEPT 95° and DEPT 135° experiments. A carbonyl group was assigned at δ 178.20, two oxymethine carbons were confirmed at δ 68.03 and 82.91, a trisubstituted double bond was determined at δ 121.79 (CH) and 144.09 (C). The above data led to suggest that compound **1** should be a pentacyclic-triterpenoid with C-28 carboxylic acid, the double bond at C-12/C-13 and two hydroxyl groups at C-2 and C-3, which has a molecular formula as $C_{30}H_{48}O_4$. The ^{1}H-NMR spectrum of **1** displayed a broad singlet of the trisubstituted double bond at δ 5.26, two oxymethine groups at δ 3.93 (multiplet) and 3.22 (doublet, J = 4.5 Hz), and seven singlets at δ 0.77, 0.84, 0.86, 0.87, 1.03, 1.04 and 1.08 were assigned to seven quaternary methyl groups. All the NMR data of **1** were compared to those of 2β,3β-dihydroxy-12-oleanen-28-oic acid (augustic acid) [15] and found to match well (Table 1). Furthermore, the HSQC and HMBC experiments were taken to determine all the chemical shifts of **1**. All the carbons were assigned to relevant protons through an HSQC experiment. The key correlations observed in the HMBC spectrum were shown in Table 2. The two hydroxyl groups at C-2 and C-3 were determined as *β*-orientation from their chemical shifts and proton-proton coupling constants, which were resemble to those of augustic acid [15] and of the other corresponding compounds [20]. Thus, compound **1** was identified as 2β,3β-dihydroxy-12-oleanen-28-oic acid. To the best of our knowledge, this is the first report of *Lawsonia* species.

The ^{1}H-NMR spectrum of **2** indicated the presence of signals at δ 3.30 (d, J = 9.5 Hz), 3.48 (dd, J = 9.5, 3.0 Hz) and 3.22 (overlapped), corresponding to three protons of the oxymethine groups. Seven methyl groups were identified at δ 0.71 (s), 0.80 (s), 0.88 (s), 0.91 (s), 1.07 (s), 1.30 (s), and 0.85 (d, J = 6.5 Hz). A broad triplet at δ 5.16 was typical for the trisubstituted double bond. The doublet methyl signal at δ 0.85 suggested the ursane skeleton of **2**. The ^{13}C-NMR spectrum exhibited 30 carbon signals, including 8 quaternary carbons, 8 methine, 7 methylene and 7 methyl groups. In which, a carboxylic carbon signals resonated at δ 178.61, a double bond at δ 128.14 and 137.21, three oxymethine at δ 78.98, 78.26, and 69.83, and one tertiary carbon bearing oxygen atom at δ 71.57. All the carbon and proton chemical shifts were assigned from DEPT 90°, DEPT 135°, HSQC and HMBC experiments. These data suggested that **2** have molecular formula of $C_{30}H_{48}O_6$, which was further confirmed from the exhibition of the quasi molecular ion peaks at *m/z* 505 $[M+H]^+$, 487.2 $[M+H-H_2O]^+$, 469.2 $[M+H-2H_2O]^+$, and 451.2 $[M+H-3H_2O]^+$ in the positive ESI-MS. Comparison the NMR data of **2** with those of the corresponding structures [16-20] led to suggest that the structure of **2** was 1β,2α,3α,19α-tetrahydroxy-12-ursen-28-oic acid. The location of three hydroxyl group at C-1, C-2 and C-3 were confirmed by HSQC and HMBC experiments. The correlation were observed between H-25 (δ 0.71) and C-1 (δ

78.98), and between H-23 (δ 0.88)/H-24 (δ 0.80) and C-3 (δ 78.26) in the HMBC spectrum of **2** confirming that two hydroxyl groups were at C-1 and C-3. Furthermore, the HMBC cross peaks was observed from H-1 (δ 3.30) and C-2 (δ 69.83)/C-3 (δ 78.26) confirming the hydroxyl group at C-2. The forth hydroxyl group was determined at C-19 as α-orientation from its carbon chemical shift and the HMBC cross peaks was observed between H-30/H-29 and C-19. More detail comparison results of the carbon chemical shifts at C-1, C-2, and C-3 of the corresponding structural parts were shown in Table 3. In which, the carbon chemical shifts of C-1, C-2, and C-3 in **2** were differed from that of the others (table 3) suggesting that the stereochemistry of three hydroxyl groups of **2** were 1β,2α,3α, which were further confirmed by the proton coupling constants of H-1/H-2 and H-2/H-3. The $J_{H-1}/_{H-2}$ = 9.5 Hz, and $J_{H-2}/_{H-3}$ = 3.0 Hz suggested that H-1 and H-2 orientation were *axial*, and H-3 was *equatorial*. From the above data, compound **2** was identified as 1β,2α,3α,19α-tetrahydroxy-12-ursen-28-oic acid, which was first isolated from *Lawsonia* species.

Compound **3** was obtained as needles. The ^{1}H-NMR spectrum of **3** displayed 5 quaternary methyl groups at δ 1.43, 1.41, 1.24, 0.87, and 0.82 as singlets, and a tertiary methyl group at δ 0.95 as a doublet (J = 6.5 Hz), suggesting the presence of a ursane skeleton like **2**. The olephilic proton at δ 5.30 as a broad singlet was assigned for a trisubstituted double bond, and the signals at δ 4.04 (m) and 4.45 (d, J = 9.5 Hz) displayed the presence of two oxymethine groups. In addition, a sugar moiety was confirmed from the signals at δ 5.91 (d, J = 8.0 Hz), 4.00 (overlapped), 4.13 (t, J = 9.0 Hz), 4.01 (m), 3.88 (m), and from the oxymethylene group signals at δ 4.11 (dd, J = 5.5, 12.0 Hz) and 4.28 (dd, J = 2.0, 12.0 Hz). The lager proton coupling constant ($J_{H-1'/H-2'}$ = 8.0 Hz) and the above data suggested the presence of a β-D-glucopyranose. The ^{13}C-NMR spectrum of **3** showed signals of 36 carbon atoms. Of which, 30 carbons belong to a pentacyclic triterpene, and six carbons belong to a sugar moiety. A carboxylic group was identified at δ 183.47 and a carboxylate group was identified at δ 177.47; the C-12/C-13 double bond was identified at δ 127.01 and 138.53 [20]; two ox methyl groups were at δ 68.73 and 80.93 confirming two hydroxyl group as α-OH and β-OH at C-2 and C-3, respectively [19, 20]. The 2-OH and 3-OH were further confirmed by the HMBC cross peaks from H-24 (δ 1.43) and C-3 (δ 80.93), and from H-2 (δ 4.04 and C-4 (δ 54.89). The other signals of the aglycone were assigned by comparing the NMR data of **3** with those of **2** and of suavissimoside R1 [19], The carbon chemical shifts at δ 94.94, 72.31, 77.25, 70.17, 77.94 and 61.39 were typical for a β-D-glucopyranose (from C-1' to C-6'). The carboxylate group was confirmed at C-28, and the sugar was attached to C-28 because the HMBC cross peak from H-19 to C-28 and H-1' to C-28 were observed. The other carboxyl group was assigned at C-23 by the H-C long rang correlation between H-24 and C-23. All the NMR data of **3** were assigned from the HSQC, HMBC spectra in comparison with those of suavissimoside R1 and shown in Table 1 and 2. Moreover, the ESI-MS exhibited the quasi molecular ion peaks at *m/z* 703 $[M+Na]^+$ (positive) and *m/z* 679 $[M-H]^-$ (negative), corresponding to the molecular formula of suavissimoside R1 ($C_{36}H_{56}O_{12}$, M= 680). Obviously, compound **3** was identified as suavissimoside R1**,** which was first isolated from *Lawsonia* species.

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