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PENTACYCLIC TRITERPENOIDS FROM THE LEAVES OF *PLUMERIA OBTUSA*

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Key Word Index—*Plumeria obtusa*; Apocynaceae; triterpenoids; 3β,27-dihydroxylup-12-ene (obtusalin); betulinic, oleanolic and ursolic acids.

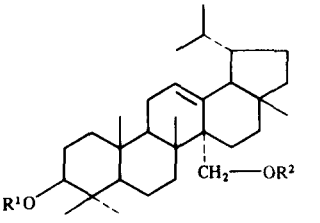
Abstract—Four pentacyclic triterpenoids have been isolated from the fresh, undried and uncrushed spring leaves of *Plumeria obtusa*. The new triterpene, obtusalin, has been characterized as 3β,27-dihydroxylup-12-ene through chemical and spectral studies while the other three have been identified as known betulinic acid, oleanolic acid and ursolic acid. This is the first report of any chemical investigation on this species.

INTRODUCTION

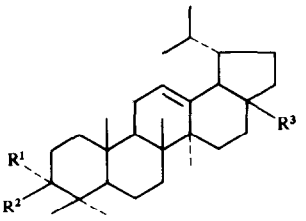
Plumeria obtusa is a native of tropical America and grows in the warmer regions of the world. The genus has a medicinal value in the indigenous system of medicine and its various species have shown antibiotic effects [1, 2]. The extracts of different parts of the plants have been used as purgative, emmenagogue, and febrifuge. This species has not been investigated for chemical constituents or pharmacological action, although other members of the genus have been studied extensively and plumierides [3], plumericins [4, 5] and fulvoplumierin [6, 7] have been reported along with the detection of α-and β-amyrins [8], lupeol, steroids [9, 10] and cardiac glycosides [11].

RESULTS AND DISCUSSION

The petrol–ethyl acetate (7:3) soluble neutral fraction of the methanolic extract of the fresh leaves furnished triterpenoids **1–4** on flash column chromatography. Physical and spectral data of the latter three compounds agreed well with those published for betulinic [12–14], oleanolic, and ursolic acids [15, 16] respectively. Compound **1**, which we have named obtusalin, had the molecular formula $C_{30}H_{50}O_2$ (HRMS) and showed UV absorption at 210 nm. The ^{1}H NMR spectrum (Table 1) showed resonances for an olefinic proton (δ5.13*t*, J = 3.6 Hz), two secondary methyl groups (δ0.93 and 0.80 *d*, J = 5.8 and 5.9 Hz), and five tertiary methyl groups apart from methylene and methine protons geminal to one primary (δ3.52, 3.18 each 1H *d*, J_{gem} = 10.9 Hz) and one secondary (δ3.21 *dd*, $J_{3\alpha,2\beta}$ = 10.8, $J_{3\alpha,2\alpha}$ = 4.9 Hz) hydroxyl group indicated by the IR absorption at 3300–3250 cm^{-1} (*br s*) and supported by the formation of the diacetate derivative **1a** (δ4.05, 3.62, each 1H *d*, J = 11.0 Hz, H-27a and H-27b and δ4.46 *dd*, $J_{3\alpha,2\beta}$ = 10.0, $J_{3\alpha,2\alpha}$ = 5.5 Hz). The coupling constants of the methine proton and the chemical shift [17] were in favour of the secondary hydroxyl group at C-3 with β-orientation (equatorial). The ^{1}H NMR spectrum further showed a one-proton doublet at δ1.36 (*d*, J = 12.0 Hz) for H-18. Significant absorption at 1043 and 1000 cm^{-1} were due to the −COH stretching vibrations of an A/B *trans*-triterpene [18, 19]. In addition, absorption at 1370, 1343 and 1330 cm^{-1} were suggestive of bending vibration due to *gem*-dimethyl and/or an isopropyl group in the molecule. These data along with the significant fragments at *m/z* 234 and 207 derived from the characteristic retro-Diels–Alder cleavages of the molecule, and the ion at *m/z* 191 [234 − isopropyl group]$^+$, strongly indicated that the compound is of a lup-12-ene type. Further, the base peak at *m/z* 203 [234 − CH_2OH]$^+$ instead of *m/z* 234 allowed the location of the second hydroxyl group at C-27 [20, 21]. The mass fragmentation pattern of the diacetate **1a** also confirmed one of the hydroxyl groups at C-27. On Sarett oxidation, **1** gave a mixture of four products, which after separation on precoated thin layer cards, were



1 R¹ = R² = H
2 R¹ = R² = —COMe



9 R¹ = H, R² = OH, R³ = —CH_2OH
10 R¹, R² = O, R³ = —CHO

Table 1. ^{1}H NMR data of triterpenes **1**, **1a**, **5**, **9–11** (δ/Hz)

H	1	1a	5	9	10	11
2α	—	—	2.37 *ddd* (16.2) (7.0) (3.8)	—	—	—
2β	—	—	2.53 *ddd* (16.2) (11.0) (7.4)	—	—	—
3α	3.21 *dd* (10.8) (4.9)	4.46 *dd* (10.0) (5.5)	—	—	—	3.20 *dd* (10.0) (4.7)
5α	0.72 *dd* (11.6) (1.5)	—	—	—	—	—
9	1.54 *dd* (10.0) (3.4)	—	—	—	—	—
11α	1.84 *ddd* (13.2) (3.6) (3.4)	—	—	—	—	—
11β	1.61 *ddd* (13.2) (10.0) (3.6)	—	—	—	—	—
12	5.13 *t* (3.6)	5.13 *t* (3.6)	5.33 *t* (3.4)	5.14 *br t*	5.34 *br s*	—
23	1.01 *s*	1.08 *s*	1.03 *s*	1.00 *s**	1.09 *s**	0.92 *s*
24	0.98 *s*	0.96 *s*	0.96 *s*	1.00 *s**	1.06 *s**	0.75 *s*
25	0.78 *s*	0.85 *s*	0.81 *s*	0.79 *s**	0.84 *s**	0.81 *s*
26	0.94 *s*	0.86 *s*	0.95 *s*	0.96 *s**	1.05 *s**	0.96 *s*
27a	3.52 *d* (10.9)	4.05 *d* (11.0)	—	—	—	—
27b	3.18 *d* (10.9)	3.62 *d* (11.0)	—	—	—	—
27	—	—	9.32 *s*	0.96 *s**	0.93 *s*	1.01 *s*
28a	—	—	—	—	—	3.32 *d* (11.0)
28b	—	—	—	—	—	3.78 *d* (11.0)
28	1.10 *s*	0.98 *s*	1.04 *s*	3.5 *m*	9.30 *s*	—
29a	—	—	—	—	—	4.60 *d* (1.5)
29b	—	—	—	—	—	4.72 *d* (1.5)
29/30	0.93 *d* (5.8)	0.93 *d* (7.8)	1.08 *d* (5.2)	0.92 *d*	1.08 *d*	—
30/29	0.80 *d* (5.9)	0.83 *d* (7.8)	0.87 *d* (6.5)	0.92 *d*	1.08 *d*	1.68 *s*

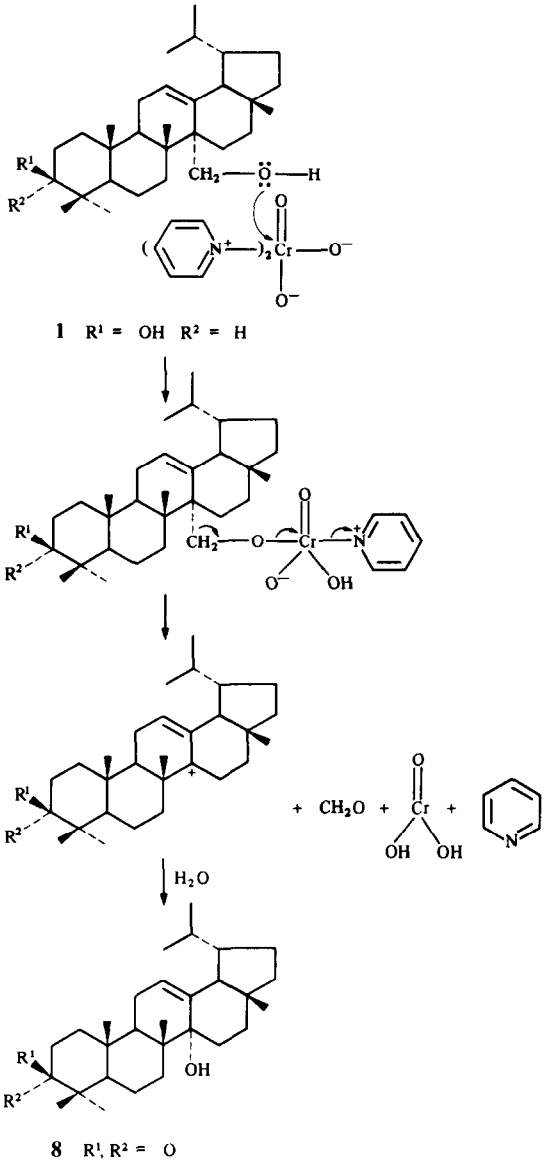
*Values in a verticle column may be interchanged.

characterized as 3,27-dioxolup-12-ene (**5**), 3,11,27-trioxolup-12-ene (**6**), 27-hydroxy-3-oxolup-12-ene (**7**) and 3-oxo-14-hydroxy-27-norlup-12-ene (**8**) through mass and ^{1}H NMR spectral data. These oxidation products were also in support of the presence of two hydroxyl groups at C-3, and C-27, and a double bond at C-12 in obtusalin. The formation of **8** as one of the oxidation products deserves special comment as there is no earlier record of formation of an alcohol through C–C bond cleavage by chromium trioxide/pyridine, although similar reactions have been noted to occur during oxidation of secondary alcohols with chromium trioxide in acidic conditions [22, 23]. Following the mechanism of these reactions, the mechanism in Scheme 1 is suggested for the formation of (**8**).

Further support of the above was obtained from the NOESY spectrum (Table 2). The connectivities of H-5 with H-9, H-23 and H-6α; H-9 with H-23, H-3, H-27a and H-27b; H-27b with H-20; H-18 with H-19; and H-18 with H-28 showed that the primary hydroxyl and isopropyl groups both have α-dispositions, whereas the hydroxyl function at C-3 is β-oriented. In the light of these findings obtusalin has been assigned the structure as 3β,27-dihydroxylup-12-ene (**1**). Evidence in favour of **1** has also been obtained through a comparison of the ^{1}H NMR spectral data of **1** with those of the 28-hydroxy isomer **9** [24] and betulin (**11**) [25]. Further, a comparison of the ^{1}H NMR spectral data of the oxidation product (**5**) of **1** with those of its 28-isomer (**10**) reported in the literature

Table 2. Interactions observed in the NOESY plot of **1**

		Connected with the proton	
H	δ	H	δ
5	0.72	9	1.54
5	0.72	23	1.01
5	0.72	6α	1.53
9	1.54	6α	1.53
9	1.54	23	1.01
9	1.54	27a	3.18
9	1.54	27b	3.52
18	1.36	28	1.10
18	1.36	19	1.38
27b	3.52	20	0.88



Scheme 1.

(Table 1) also coroborated the hydroxyl group at C-27 in **1**. A survey of the literature shows that this compound is an addition to the relatively few naturally occurring pentacyclic triterpenoids possessing a 27-hydroxyl group along with a double bond at C-12 in the lupane series.

EXPERIMENTAL

Mps: uncorr. MS: Finnigan MAT 112 and 312 double focussing mass spectrometers connected to a PDP 11/34 computer system; NMR spectra ($CDCl_3$): 400 MHz for ^1H and 100 MHz for ^{13}C. The chemical shifts are reported in δ(ppm) and the coupling constants are in Hz. The ^{13}C NMR spectral assignments (Table 3) have been made partly through a comparison of the chemical shifts with the published data for similar compounds [25, 26] and partly through the appearance of signals in DEPT and heteroCOSY spectra (Table 3). Optical rotation: 24° in $CHCl_3$. Precoated thin layer cards (DC-karten SIF) were used for TLC. The plant was identified by Prof. S. I. Ali (Department of Botany, University of Karachi) and a voucher specimen (No. 9317 KUH) has been deposited in the Herbarium.

Fresh undried and uncrushed leaves (12 kg) of *P. obtusa* collected from the Karachi region in April 1987 were repeatedly extracted with MeOH at room temp. The concd syrupy residue obtained on removal of the solvent from the combined extracts under red. pres. was shaken out with EtOAc and water. The former layer was then extracted with 4% aq. soln of Na_2CO_3 to separate the acidic from the neutral fraction. The EtOAc layer was washed with H_2O, dried (Na_2SO_4) and the residue left on removal of the solvent was divided into petrol-soluble and petrol-insoluble portions. The petrol–EtOAc (7:3) soluble part (6 g) of the petrol-insoluble fraction was subjected to flash CC [27] (silica gel, E. Merck 9385, petrol, petrol–EtOAc in order of increasing polarity). Fractions eluted with petrol–EtOAc (7:3) successively furnished pure obtusalin (**1**, 70 mg), a fraction containing betulinic acid (**2**) with some allied impurities along with pure oleanolic acid (**3**, 290 mg) and ursolic acid (**4**, 285 mg). The fraction containing betulinic acid (**2**) was resubjected to flash CC (silica gel, E. Merck, 9385, petrol–EtOAc 9:1) giving betulinic acid (**2**; 41.3 mg) in a pure state.

Obtusalin (**1**). Needles (MeOH), mp 194–196°, $[\alpha]_D^{24}+67.9°$ ($CHCl_3$; *c* 0.412); EIMS *m/z* (rel. int.): 442.3807 $[M]^+$ ($C_{30}H_{50}O_2$ requires: 442.3810, 4), 412.3668 $[C_{29}H_{48}O]^+$ (4), 234 (20), 207 (18), 203 (100), 189 (10), 175 (6), 133 (44), 95 (20) and 69 (18); UV λ_{max}^{MeOH} nm: 210; IR ν_{max}^{KBr} cm^{-1}: 3300–3250, 1600, 1370, 1343, 1330, 1125 and 1000.

Acetylation of obtusalin. Compound **1** (20 mg) was dissolved in pyridine (1 ml) and Ac_2O (1 ml) added. The reaction mixture was left at room temp overnight and worked-up in the usual manner affording the diacetate derivative **1a**; amorphous powder, EIMS *m/z* (rel. int.): 526.4024 $[M]^+$ ($C_{34}H_{54}O_4$ requires: 526.4021, 6), 465 (50), 450 (7), 422 (5), 276 (12), 255 (8), 249 (10), 216 (100), 203 (98), 190 (60), 189 (54), 133 (50), 119 (44), 95 (46), 81 (43) and 69 (64); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720 (br), 1640 (br), 1600, 1380, 1010 and 990.

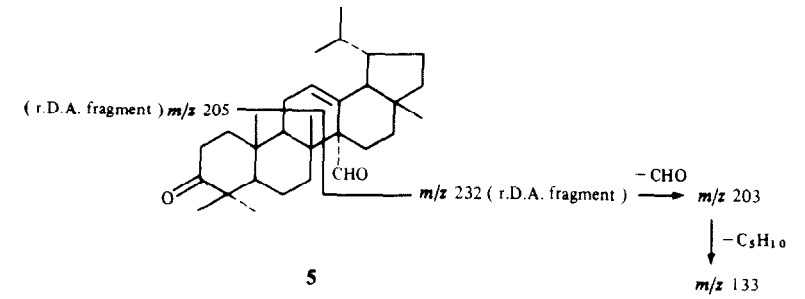
Sarett oxidation of obtusalin. A soln of obtusalin (20 mg) in pyridine was added to a slurry of CrO_3 (20 mg) in pyridine (4 ml) and the reaction mixture kept stirring at room temp. for 6 hr. On usual work-up, a mixture of four products was obtained which, after separation on precoated thin layer cards ($CHCl_3$–MeOH, 9:1), were characterized as **5–8**.

Physical constants of **5**. Needles ($CHCl_3$), mp 159–160°, 5.4 mg; EIMS *m/z* (rel. int.): 438.3508 $[M]^+$ ($C_{30}H_{46}O_2$ requires: 438.3497, 4), 299 (2), 273 (6), 232 (4), 218 (12), 205 (4), 203 (10), 189 (8), 149 (42), 135 (22), 133 (10), 97 (18), 95 (24), 85 (72), 83 (100), 71 (50), and 57 (64).

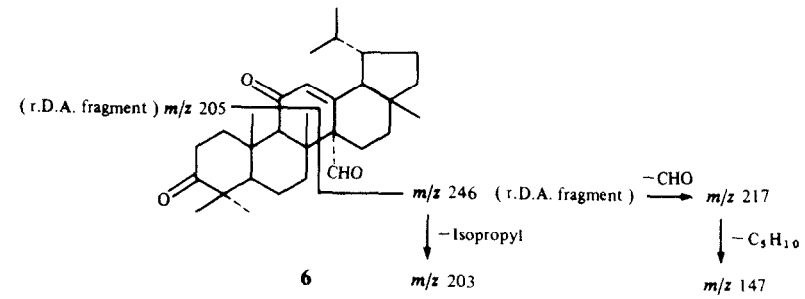
Physical constants of **6**. Amorphous, 4.6 mg; EIMS *m/z* (rel. int.): 452.3301 $[M]^+$ ($C_{30}H_{44}O_3$ requires: 452.3290, 12), 437 (10), 425 (14), 424 (12), 408 (24), 238 (4), 287 (38), 269 (20), 257 (18), 246 (60), 217 (38), 205 (40), 203 (38), 191 (40), 175 (44), 161 (70), 135 (80), and 119 (100); ^1H NMR ($CDCl_3$) δ:9.32 (1H, *s*, CHO), 5.60 (1H, *s*, H-12), 2.52 (1H, *m*, H-2β) and 2.37 (1H, *m*, H-2α).

Physical constants of **7**. Rods ($CHCl_3$–MeOH, 1:1), mp 122–124°, 4.4 mg; EIMS *m/z* (rel. int.): 440.3599 $[M]^+$ ($C_{30}H_{48}O_2$ requires: 440.3654, 5), 422 (10), 407 (7), 275 (8), 257 (8), 234 (18), 216 (18), 205 (10), 203 (19), 149 (38), 133 (22), 121 (30), 109 (28), 97 (32), 95 (54), 83 (64), 69 (70), 57 (88) and 55 (100); ^1H NMR ($CDCl_3$) δ: 5.13 (1H, *t*, $J_{12,11\alpha}=J_{12,11\beta}=3.5$ Hz, H-12), 3.17 (1H, *d*, $J=11.0$ Hz, H-27a), 3.51 (1H, *d*, $J=11.0$ Hz, H-27b), 2.53 (1H, *m*, H-2β) and 2.38 (1H, *m*, H-2α).

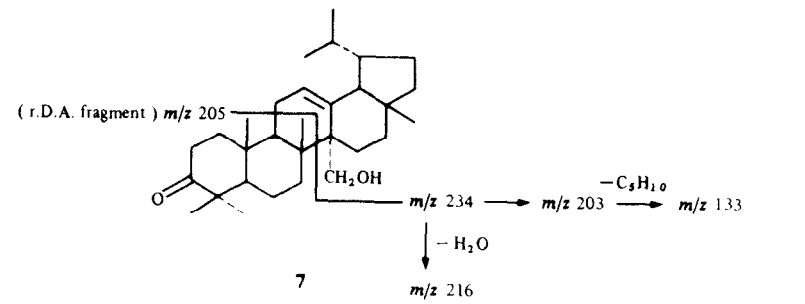
Physical constants of **8**. Amorphous, 4.2 mg. EIMS *m/z* (rel. int.): 426.3522 $[M]^+$ ($C_{29}H_{46}O_2$ requires 426.3497, 10), 408 (30), 393 (5), 338 (4), 220 (14), 203 (100), 189 (14), 133 (30), 132 (98), 107


(r.D.A. fragment) m/z 205
CHO
m/z 232 (r.D.A. fragment)
−CHO
m/z 203
−C5H10
m/z 133

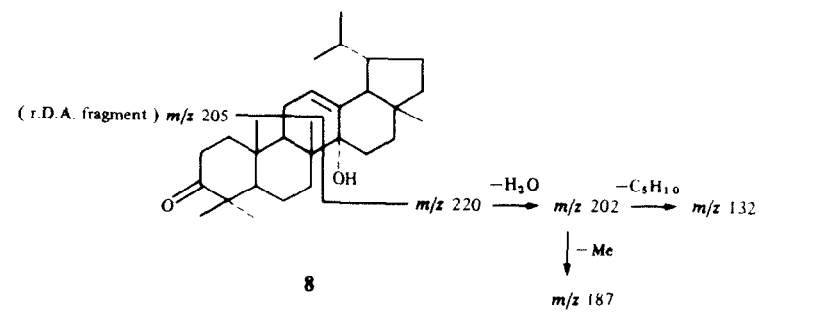
5


(r.D.A. fragment) m/z 205
CHO
m/z 246
(r.D.A. fragment)
−CHO
m/z 217
−Isopropyl
m/z 203
−C5H10
m/z 147

6


(r.D.A. fragment) m/z 205
CH2OH
m/z 234
−C5H10
m/z 203
m/z 133
−H2O
m/z 216

7


(r.D.A. fragment) m/z 205
OH
m/z 220
−H2O
m/z 202
−C5H10
m/z 132
−Me
m/z 187

8

Table 3. ^1H–^{13}C-Heterocosy of **1**

C	$\delta^{13}C$	δ ^1H	Multiplicity* ($J_{H,H}$ Hz)	C	δ ^{13}C	δ ^1H	Multiplicity ($J_{H,H}$ Hz)
1	38.8	1.66	*m*	16	26.0	1.20	*m*
2	27.3	1.62	*m*	17	38.8†	—	—
3	79.1	3.21	*dd* (10.8, 4.9)	18	54.0	1.36	*d* (12.0)
4	38.0†	—	—	19	39.5	1.38	*m*
5	55.2	0.72	*dd* (11.6, 1.5)	20	39.4	0.88	*m*
6	18.4	1.44	*m*	21	30.7	1.46	*m*
		1.53	*m*	22	35.2	1.60	*m*
7	32.9	1.35	*m*	23	28.2	1.01	*s*
		1.55					
8	40.1†	—	—	24	16.8	0.98	s
9	47.7	1.54	*dd* (10.0, 3.4)	25	15.6	0.78	*s*
10	36.9†	—	—	26	15.7	0.94	*s*
11	23.4	1.61	*ddd* (13.2, 10.0, 3.6)	27	69.9	3.18	*d* (10.9)
		1.84	*ddd* (13.2, 3.6, 3.4)			3.52	*d* (10.9)
12	125.1	5.13	*t* (3.6)	28	23.3	1.1	*s*
13	138.8†	—	—	29	21.3	0.93	*d* (5.8)
14	42.1†	—	—	30	17.3	0.80	*d* (5.9)
15	23.4	1.09	*m*				
		1.18	*m*				

*Observed in normal ^1H NMR spectrum.
†Observed in the broad band spectrum only.

(32), 95 (42), 83 (82), 69 (24) and 55 (65); ^1H NMR ($CDCl_3$) δ: 5.22 (1H, *t*, $J_{12,11\alpha}=J_{12,11\beta}=3.6$ Hz, H-12), 2.52 (1H, *m*, H-2β), and 2.38 (1H, *m*, H-2α).

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