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Two triterpenoids from the leaves of *Plumeria obtusa*

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Abstract

Two new and four known pentacyclic triterpenoids were isolated from the fresh leaves of *Plumeria obtusa*. The structures of the new constituents, named obtusol and zamanic acid, have been elucidated as 3β,27-dihydroxy-urs-12-ene and 3β-hydroxy-urs-30-*p*-*E*-hydroxycinnamoyl-12-en-28-oic-acid through spectroscopic studies.

Keywords: *Plumeria obtusa*; Apocynaceae; Triterpenoids; 3β,27-Dihydroxy-urs-12-ene,3β-Hydroxy-urs-30-*p*-*E*-hydroxycinnamoyl-12-en-28-oic acid

1. Introduction

Plumeria obtusa L. (champa) belongs to the Apocynaceae. It is a native of the Bahama islands, Cuba, Jamaica, Hispaniola and Pureto Rieo (Krishnamurthi, 1969). Various species of this genus are reported for their medicinal uses in the indigenous system of medicine (Nasir & Ali, 1983). The main iridoid glucoside plumieride was isolated from the stem bark of *P. rubra* and *P. lancifolia* in 1894 (Albers-Schoenberg & Schmid, 1961; Little & Johnstone, 1951). After that Coppen et al. examined various species of plumeria and identified the presence of iridoids, plumericin, isoplumericin, plumieride coumarate and plumieride coumarate glucoside in the stem and roots of *P. obtusa* (Maharan, Abdel-Wahab & Ahmed, 1973; Perude & Blomster, 1978; Rangaswami & Rao, 1960). Since the work done on *P. obtusa* was limited, we undertook studies on the chemical constituents of this species and isolated minor iridoids and triterpenoids from the leaves (Siddiqui, Siddiqui, Begum & Naeed, 1990a, 1990b). A continuation of these studies has led to the isolation of six pentacyclic triterpenoids (**1**–**6**). Compounds **1** and **2**, named as obtusol and zamanic acid, respectively, are new constituents and their structures have been elucidated as 3β-27-dihydroxy-urs-12-ene and 3β-hydroxy-urs-30-*p*-*E*-hydroxycinnamoyl-12-en-28-oic acid through spectral studies. 27-*E*-4-hydroxycinnamoyloxybetulinic acid (**3**) has been reported only from *Melilotus messanensis* (Francisco, Simonet & Galindo, 1997). Compounds **4**, **5** and **6** have been identified as ursolic acid, betulinic acid and β-amyrin, respectively. The known compounds have been indentified through comparison of their physical and spectral data with those reported in the literatures (Francisco et al., 1997; Kang, 1987; Sholiche, Ymasaki, Kasai & Tanaka, 1980).

2. Results and discussions

The HREIMS of compound **1** showed a molecular ion peak (M^+) at m/z 442.3783 corresponding to the molecular formula $C_{30}H_{50}O_2$. The ^{1}H-NMR spectrum (Table 1) displayed resonances for an olefinic proton (δ 5.12 t, J = 3.5 Hz, H-12), two secondary methyl groups (δ 0.80 d, J = 6.0 Hz and 0.92 d, J = 6.0 Hz, H-29 and H-30, respectively), five methyl groups located on quaternary carbons (δ 1.09, 0.98, 0.97, 0.93 and 0.77) apart from two oxy-methylene protons (δ 3.51 and 3.18, each 1H d, J_{gem} = 11.0 Hz, H-27a and H-27b) and a methine proton linked to oxygen (δ 3.20 dd, $J_{3\alpha,2\beta}$ = 10.5 Hz and $J_{3\alpha,2\alpha}$ = 5.0 Hz, H-3). The sig-

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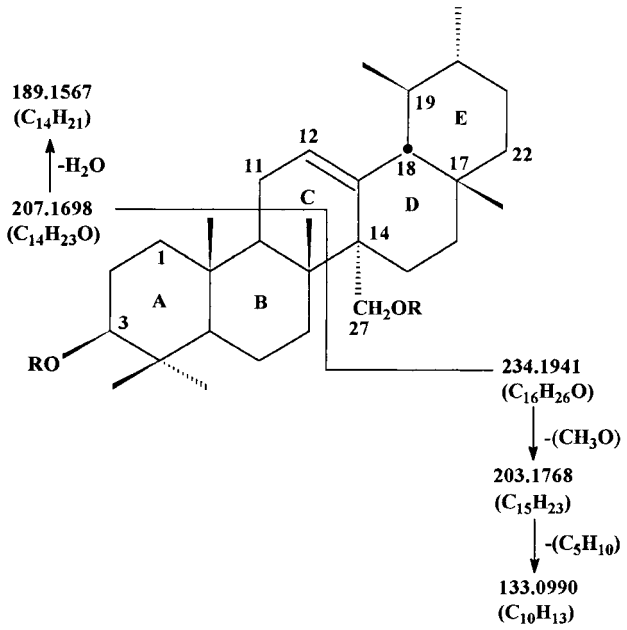
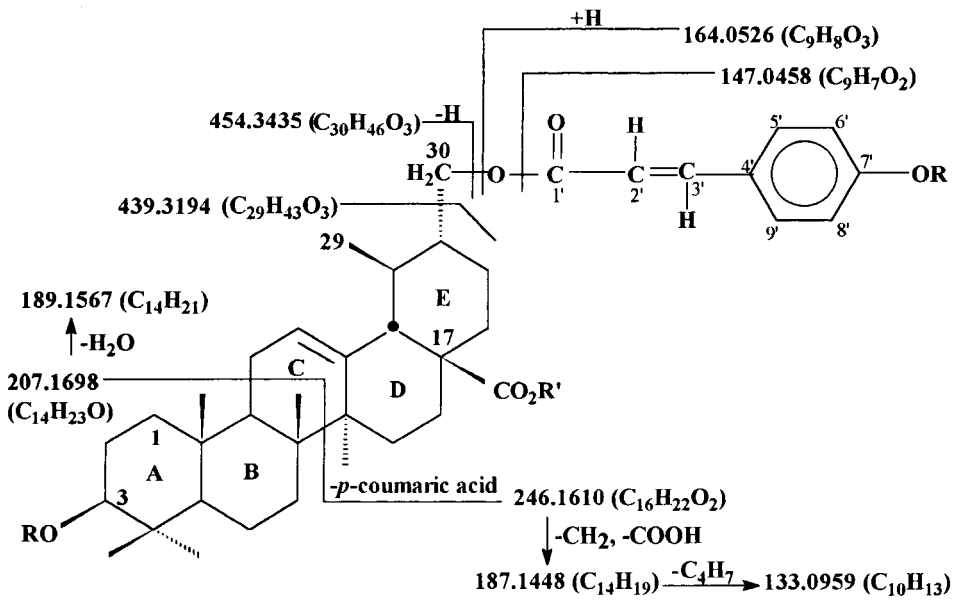


Significant mass fragments of **1** (R=H)
1a (R=Ac)

nals of two oxymethylene protons shifted to δ 4.40 (d, J = 11.0 Hz) and 3.60 (d, J = 11.0 Hz) and that of the oxymethine proton to δ 4.48 (dd, J = 11.0 and 6.5 Hz) on acetylation. One of the two hydroxyl groups in **1** was placed at C-3 on biogenetic grounds and its β-configuration was evident from the chemical shift values and coupling constants (Pyrek, 1979). The above data along with the significant mass fragments at m/z 207.1710 ($C_{14}H_{23}O$) and 234.1941 ($C_{16}H_{26}O$) derived from characteristic retro-Diels–Alder cleavage around ring C of the molecule, the fragments at m/z 203.1768 ($C_{15}H_{23}$) and 133.0990 ($C_{10}H_{13}$) resulting from m/z 234.1941 and one-proton doublet at δ 2.22 (J = 13.0 Hz, H-18) demonstrated that the compound is of urs-12-ene type (Budzikiewicz, Wilson & Djerassi, 1963). That the second hydroxyl is at either C-27 or C-28 was also discernible from the above features. Further, the base peak at m/z 203 $[234\text{-}CH_2OH]^+$ instead of at m/z 234 allowed the location of the second hydroxyl group at the labile position, i.e. C-27 (Budzikiewicz & Thomas, 1980), which secured further evidence from the 2D-NMR studies (Table 1). Thus, in the COSY spectrum correlation peaks were observed for H-9 with H-11a, H11b; H-11a with H-11b, H-12; H-27a with H-27b, H-18 and H-19; H-19 with H-29; and H-20 with H-30. The NOESY plot showed spatial relations of H-11b with H-18, H-18 with H-20 and H-27a with H-19. In the HMBC spectrum, an important relationship was observed between H-27a and C-13 along with other expected interactions. The assignments of carbons and protons could be made through BB (broad-band decoupled), DEPT, HMQC, HMBC, COSY-45 and NOESY spectra (Table 1) and by com-



Significant mass fragments of **2** (R=R'=H)
2a (R=Ac, R'=H)
2b (R=Ac, R'=CH_3)

Table 1
^{1}H and ^{13}C-NMR data of **1** and ^{1}H-NMR data of **1a**[a,b]

C/H No.	**1**		COSY correlations	NOESY correlations	HMBC correlations	**1a**, δ_H
	δ_C	δ_H				
1	38.89	1.64			H-25	
2	29.72	1.23				
3	79.08	3.20 dd (11.0, 5.0)			H-23	4.48 dd (11.0, 5.1)
4	39.44	–			H-3	
5	54.15	0.72 dd (11.5, 1.5)			H-3, H-24, H-25	
6	18.40	1.38				
7	32.92	1.40				
8	40.14	–				
9	47.79	1.24 dd (5.0, 4.0)	H-11a, H-11b	H-27a		
10	36.98	–				
11	26.09	1.90 (a) dt (12.5, 5.0)	H-9, H-11b, H-12	H-23		
		1.73 (b) td (12.5, 4.0)	H-11a, H-9	H-18		
12	125.13	5.12 t (3.5)		H-29	H-11a	5.12 t (3.4)
13	138.84	–			H-11a, H-27	
14	38.0	–			H-12	
15	30.69	1.23				
16	23.43	1.89				
17	42.17					
18	55.32	2.22 m	H-19	H-11b, H-20, H-28		
19	39.49	1.60 m	H-18, H-29	H-27a, H-30		
20	38.83	0.99	H-30	H-18		
21	27.34	1.60		–		
22	35.23	1.51		–		
23	28.18	0.97 s		H-11a		0.97
24	16.84	0.98 s		H-25		0.95
25	15.72	0.77 s		H-24, H-26		0.84
26	15.64	0.93 s		H-25	H-27a	0.84
27	69.95	3.51 (a) d (11.0)	H-27b	H-9, H-19		4.04 d (11.0)
		3.18 (b) d (11.0)	H-27a			3.60 d (11.0)
28	17.37	1.09 s	–	H-18, H-29		1.07
29	21.33	0.80 d (6.0)	H-19	H-12, H-28		0.91d (6.5)
30	23.37	0.92 d (6.0)	H-20	H-19		0.79d (6.5)
OAc	–	–	–	–		2.03, 2.04

[a] Assignments of carbon are based on BB and DEPT; correlated protons assigned on the basis of HMQC.
[b] Chemical shifts are expressed in δ-values and followed by multiplicities and coupling constant (Hz).

parison with similar compounds (Siddiqui, Siddiqui, Begum & Naeed, 1990a). Based on these data, the structure of obtusol (**1**) has been assigned as 3β,27-dihydroxy-urs-12-ene.

The EIMS of compound **2** did not show a molecular ion peak but a highest ion peak at m/z 454. The molecular formula ($C_{39}H_{54}O_6$) was deduced from exact measurement of significant mass fragments at m/z 454.3435 ($C_{30}H_{46}O_3$) and m/z 164.0526 ($C_9H_8O_3$) and confirmed by M^+-1 peak at m/z 617 in FABMS (negative mode). Compound **2** showed IR absorptions at 3500–2600 (br. OH and CO_2H), 1735–1690 (acid and ester carbonyls) and 1400–1630 cm^{-1} (four peaks, aromatic ring) while its UV spectrum showed maxima at 311.4 and 201.8 nm. The ^{1}H-NMR spectrum (Table 2) exhibited two sets of doublets, one at δ 7.64 (1H, $J_{3',2'} = 16.0$ Hz, H-3′) and 6.21 (1H, $J_{3',2'} = 16.0$ Hz, H-2′) and the other set at δ 7.40 (2H, $J_{5'/9',6'/8'} = 8.8$ Hz, H-5′ and H-9′) and 6.86 (2H, $J_{6'/8',5'/9'} = 8.8$ Hz, H-6′ and H-8′). The chemical shifts and coupling constants of these signals and significant mass fragments at m/z 147.0458 ($C_9H_7O_2$) and 164.0526 ($C_9H_8O_3$) were indicative of *p-E*-hydroxycinnamoyl (*p-E*-coumaroyloxy) moeity. Besides the signals for the *p*-coumaroyloxy substituent, the ^{1}H-NMR spectrum displayed resonances for an olefinic proton (δ 5.56 t, J = 3.6 Hz, H-12), two methylene protons (δ 4.31 dd, $J_{gem} = 12.5$ Hz, and $J_{30a,\,20} = 5.4$ Hz, H-30a, and δ 4.15 dd, $J_{gem} = 12.5$ Hz and $J_{30b,\,20} = 3.1$ Hz, H-30b), two characteristic methine protons (δ 3.16 dd, J = 11.0, 5.0 Hz, H-3 and δ 2.28 d, J = 10.9 Hz, H-18), five tertiary methyl singlets (δ 0.69, 0.83, 0.84, 0.86, 0.92) and one secondary methyl doublet (δ 0.97, J = 8.7 Hz, H-29). The signal of oxymethine proton shifted downfield (δ 4.21) on acetylation and the signals of two acetoxy methyls appeared at δ 2.08 and 2.19 as singlets. On treatment with diazomethane, diacetate (**2a**) furnished the methyl ester (**2b**), the ^{1}H-NMR spectrum of which

Table 2
^{1}H-NMR spectral data of compound **2**, **2a** and **2b**[a]

H-NO	**2**, δ_H	COSY correlations	**2a**, δ_H	**2b**, δ_H
3α	3.16 dd (11.0, 5.0)		4.21 dd (11.0, 5.5)	4.20 dd (11.0, 5.5)
12	5.56 t (3.6)		5.6 t (3.6)	5.55 t (3.6)
18	2.28 d (10.9)	H-19	2.29 d (10.9)	2.2 d (10.9)
2′	6.2 d (16.0)	H-3′	6.30 d (16.0)	6.2 d (16.0)
3′	7.64 d (16.0)	H-2′	7.68 d (16.0)	7.69 d (16.0)
5′ and 9′	7.40 d (8.8)	H-6′, H-8′	7.51 d (8.8)	7.51 d (8.8)
6′ and 8′	6.86 d (8.8)	H-5′, H-9′	7.09 d (8.8)	7.10 d (8.8)
C-methyls	0.69 s		0.74 s	0.76 s
	0.83 s		0.83 s	0.83 s
	0.84 s		0.84 s	0.84 s
	0.86 s		0.86 s	0.86 s
	0.92 s		0.92 s	0.92 s
29	0.97 d (8.7)	H-19	0.94 d (8.7)	0.97 d (8.7)
30a	4.31 dd (12.5, 5.4)	H-20, H-30b	4.29 dd (12.5, 5.4)	4.22 dd (12.5, 5.4)
30b	4.15 dd (12.5, 3.1)	H-20 H-30a	4.18 dd (12.5, 3.1)	4.02 dd (12.5, 3.1)
–OAc	–	–	2.08, 2.19 s	2.07, 2.09 s
$-OCH_3$	–	–	–	3.50 s

[a] Chemical shifts are expressed in δ-values and followed by multiplicities and coupling constant (Hz).

had a carbomethoxy singlet at δ 3.50. Thus, **2** is an ursane type triterpenoid, possessing two hydroxyl and one carboxyl groups. One of the hydroxyl was placed at C-3 on biogenetic grounds and its β-configuration was evident from the values of chemical shifts and coupling constants (Pyrek, 1979). The above data together with characteristic retro-Diels–Alder fragments (Budzikiewicz et al., 1963) at *m/z* 246.1610 ($C_{16}H_{22}O_2$) and *m/z* 207.1689 ($C_{14}H_{23}O$) suggested that **2** has a carboxylic group at C-17 (*vide* structure). These fragments and the double doublets of oxymethylene protons in the ^{1}H-NMR suggested that *p-E*-coumaroyloxy moiety in **2** is located either at C-29 or C-30. It was finally placed at C-30 on the basis of information obtained from COSY-45 results which showed the connectivities of H-18 with H-19, H-19 with H-29 and H-20, and H-20 with H-30b along with other expected correlations. On the basis of these observations and by comparison of the published spectral data of similar compounds (Siddiqui et al., 1990a; Siddiqui, Siddiqui, Naeed & Begum, 1990b), the structure of zamanic acid (**2**) has been deduced as 3β-hydroxy-urs-30-*p-E*-hydroxycinnamoyloxy-12-en-28-oic acid.

3. Experimental

3.1. General

NMR spectra (in $CDCl_3$) of **1**, **1a** and **2**: 500 MHz for ^{1}H and 75 MHz for ^{13}C (of **1**); ^{1}H-NMR spectra (in $CDCl_3$) of **3** and **4**–**6**: 300 and 400 MHz, respectively.

3.2. Plant Material

The plant was identified by Professor S.I. Ali (Department of Botany, University of Karachi) and a voucher specimen (No. 9317 KUH) has been deposited in the Herbarium of the same department.

3.3. Extraction and isolation

The fresh, undried and uncrushed leaves (12 kg) collected from Karachi region in April were repeatedly extracted with methanol (five times). The solvent from the combined extract was evaporated in vacuo and the concentrate was shaken out with EtOAc and H_2O. The EtOAc phase was treated with 4% aq. Na_2CO_3 to separate the acidic compounds from the neutral fraction. The EtOAc phase containing the neutral fraction was washed, dried and charcoaled. The charcoal bed was successively washed with EtOAc and MeOH–benzene (1:1). The EtOAc filtrate was treated as usual to give a neutral fraction (N). It was divided into petrol soluble (N-1) and petrol insoluble (N-2) fractions. The petrol insoluble (N-2) fraction was successively treated with petrol–EtOAc (1:1), petrol–EtOAc (7:3) and EtOAc to give fractions N-3, N-4 and N-5.

The fraction (N-3; 29 g) was subjected to vacuum liquid chromatography (silica gel, $CHCl_3$, $CHCl_3$–MeOH in order of inceasing polarity). The $CHCl_3$ soluble fraction was further purified over thick layer alumina plates to afford four main bands which were characterized as **1**, **4**, **5** and **6**. The fraction which eluted with $CHCl_3$–MeOH (9.7:0.3) afforded two major bands on thick layer plates (silica gel, $CHCl_3$–MeOH (9:1)) ultimately characterized as **2** and **3**.

3.4. Obtusol (*1*)

$[\alpha]_D^{27}$ +4° ($CHCl_3$, *c* 0.2); IR ν_{max} $CHCl_3$ cm^{-1}: 3250 (OH), 3050 (C–H), 1130 (C–O) and 960–940 (C=C–H,); UV λ_{max} MeOH nm: 192.4; HRMS *m/z* (rel. int.): 442.3783 [M^+; $C_{30}H_{50}O_2$] (24.1), 424.3654 [$C_{30}H_{48}O$] (5.4), 411.3576 [$C_{29}H_{47}O$] (17.7), 234.1941 [$C_{16}H_{26}O$] (68.4), 207.1689 [$C_{14}H_{23}O$] (44.27), 203.1768 [$C_{15}H_{23}$] (100), 189.1612 [$C_{14}H_{21}$] (14.8), 133.0990 [$C_{10}H_{13}$] (15.1); ^{1}H-NMR; Table 1; ^{13}C-NMR Table 1.

3.5. Acetylation of *1*

To a solution of **1** (10 mg) in pyridine (1 ml), Ac_2O (1 ml) was added and the reaction mixture kept for two days at room temp. On usual work-up, monoacetate (**1a**) was obtained as a single product. UV λ_{max} MeOH nm 202.4, IR ν_{max} $CHCl_3$ cm^{-1}; 3100 (C–H), 1730–1720 (carbonyls); EIMS *m/z:* 526 (M^+); HRMS *m/z* (rel.int): 526.3983 [M^+, $C_{34}H_{54}O_4$] (13.2), 482.3694 [$C_{32}H_{50}O_3$] (9.8), 466.3810 [$C_{30}H_{50}O_2$] (72.6), 276.2064 [$C_{18}H_{28}O_2$] (35.5), 216.1881 [$C_{16}H_{24}$] (100), 203.1811 [$C_{15}H_{23}$] (97.1), 201.1628 [$C_{15}H_{21}$] (38.3), 189.1623 [$C_{14}H_{21}$] (79.4), 133.1006 [$C_{10}H_{13}$] (78); ^{1}H-NMR Table 1.

3.6. Zamanic acid (*2*)

$[\alpha]_D^{27}$ +26° ($CHCl_3$, *c* 0.15); IR ν_{max} $CHCl_3$ cm^{-1}: 3500–2600 (OH, COOH), 2900 (C–H), 1735–1690 (C=O), 1625 (C=C), 1600–1350 (aromatic ring), 1375 (geminal dimethyl) and 1140 (C–O str.) UV λ_{max} MeOH nm: 311.4 and 201.8; HRMS *m/z* (rel.int.): 454.3435 [M^+-*p*-hydroxycinnamic acid, $C_{30}H_{46}O_3$] (99.3), 439.3194 [$C_{29}H_{43}O_3$] (5.7), 436.3284 [$C_{30}H_{44}O_2$] (5.6), 421.3079 [$C_{29}H_{41}O_2$] (4.2), 285.1870 [$C_{19}H_{25}O_2$] (25.5), 246.1610 [$C_{16}H_{22}O_2$] (6.0), 207.1689 [$C_{14}H_{23}O$] (34.6), 187.1448 [$C_{14}H_{19}$] (45.81), 164.0526 [$C_9H_8O_3$] (72.5), 147.0458 [$C_9H_7O_2$] (79.6), 133.0959 [$C_{10}H_{13}$] (47.02). ^{1}H-NMR: Table 2.

3.7. Acetylation of *2*

To a solution of **2** (7.4 mg) in pyridine (1 ml), Ac_2O (1 ml) was added and the reaction mixture kept overnight at room temperature. On usual work up, diacetate (**2a**) was obtained. EIMS *m/z*, 496 (M-*p*-acetoxycinnamic acid); ^{1}H-NMR:Table 2.

3.8. Methylation of *2a*

To a solution of **2a** (7.4 mg) in ether (1 ml), etheral CH_2N_2 was added. Removal of the solvent from reaction mixture furnished methyl ester diacetate (**2b**). EIMS *m/z*: 510 (M^+-*p*-acetoxycinnamic acid); ^{1}H-NMR: Table 2.

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