

Isolation and Structure Elucidation of Obtusilin, a New Triterpenoid and 27-*p*-Z-Coumaroyloxursolic Acid from the Leaves of *Plumeria obtusa*

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Summary: From the fresh spring leaves of *Plumeria obtusa* a new triterpenoid, obtusilin, has been isolated along with 27-*p*-Z-coumaroyloxursolic acid reported earlier. Their structures have been established through chemical and spectroscopic methods as 3 β -hydroxy-27-*p*-Z-coumaroyloxylea-12-en-28-oic acid and 3 β -hydroxy-27-*p*-Z-coumaroyloxurs-12-en-28-oic acid.

Introduction

As a result of investigation on the chemical constituents of the methanolic extract of the fresh leaves of *Plumeria obtusa* L. (Apocynaceae), isolation of four triterpenes has been communicated earlier [1]. In the present paper the isolation of a new pentacyclic triterpenoid (1) is described along with (2) [2] which is hitherto unreported from this plant. The structures of these have been elucidated as 3 β -hydroxy-27-*p*-Z-coumaroyloxylea-12-en-28-

oic acid (1) and 3 β -hydroxy-27-*p*-Z-coumaroyloxurs-12-en-28-oic acid (2) through chemical and spectral studies.

Experimental

IR (CHCl₃) and UV (MeOH) spectra were measured on JASCO IRA-1 and Pye- Unicam SP-800 spectrophotometers, respectively. Mass spectra

were recorded on Finnigan MAT 112 and MAT 312 double focussing spectrometers connected to a PDP 11/34 computer system. NMR spectra were recorded in CD₃OD on Bruker AM 300 MHz spectrometer operating at 300.13 MHz for ¹H and 75.43 MHz for ¹³C nuclei. The ¹³C-NMR spectral assignments (Table-II) have been made partly through a comparison of the chemical shifts with the published data for similar compounds [3,4] and partly through the appearance of signals in DEPT and hetero-COSY spectra. The purity of samples was checked on TLC (silica gel SF-I-254 precoated aluminium cards). 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.

Extraction and isolation

The fresh uncrushed spring leaves of *Plumeria obtusa* (12 kg), collected in the month of April (1987), were repeatedly percolated (5 times) with methanol at room temperature. The methanolic extract was concentrated under reduced pressure and shaken out with ethyl acetate and water. The ethyl acetate soluble phase was treated with 4% aqueous solution of Na₂CO₃ to separate the acidic from the neutral fraction. The ethyl acetate layer containing the neutral fraction was washed with water, dried (anhydrous Na₂SO₄) and freed of the solvent. The gummy residue was divided into petrol soluble and petrol insoluble portions and the petrol insoluble fraction was succes-

sively treated with petrol-ethyl acetate (7:3) and petrol-ethyl acetate (1:1). The petrol-ethyl acetate (1:1) soluble residue (3.50 g) was subjected to flash column chromatography (silica gel E. Merck 9385; CHCl₃, CHCl₃-MeOH in order of increasing polarity). The fraction eluted with CHCl₃-MeOH (9.5:0.5) furnished pure (1) and (2) through subsequent flash column chromatography (silica gel E. Merck 9385; petrol-ethyl acetate 8:2), (2) being more polar constituent.

Physical constants of (1)

Amorphous; EIMS *m/z* (rel.int.) : 454.3441 (Calc. for C₃₀H₄₆O₃:454.3446, *M-p*-coumaric acid, 28), 246.2076 (C₁₆H₂₂O₂, 58), 207.1771 (C₁₄H₂₃O, 22), 201.1551 (C₁₅H₂₁, 60), 189.1656 (C₁₄H₂₁, 47), 164.0453 (C₉H₈O₃, 26), 147.0450 (C₉H₇O₂, 8) and 57.0343 (C₃H₅O, 100); ¹H-NMR : Table I; ¹³C-NMR: Table II.

Acetylation of (1)

To a solution of (1) (40mg) in pyridine (1 ml) Ac₂O (1 ml) was added and the reaction mixture was allowed to stand at room temperature overnight. It was worked-up in the usual manner affording the diacetyl derivative (1a). Amorphous powder; EIMS *m/z* (rel.int.) : 496.3549 (Calc. for C₃₂H₄₈O₄: 496.3552, *M-p-O*-acetyl cinnamic acid, 26), 483 (30), 451 (15), 436 (20), 249 (32), 246 (20), 206 (10), 201 (20), 189 (60), 164 (60) and 147 (100); ¹H-NMR: Table I.

Table-1: ¹H-NMR spectral data of triterpenes (1) and (2) (δ ppm/Hz)

Proton	(1)	(1a)	(1b)	(2)	(2a)	(2b)
3α	3.02t (7.5)	4.18t (7.7)	4.14t (7.5)	3.07t (7.7)	4.16t (7.9)	4.15dd (12.7,5.6)
12	5.52t (3.4)	5.53t (3.6)	5.56t (3.1)	5.55t (3.7)	5.55t (3.5)	5.54t (3.5)
18	2.87dd (13.4,4.8)	2.85dd (12.8,4.6)	2.89dd (12.9, 4.5)	2.27d (10.6)	2.30d (10.2)	2.28d (11.0)
Methyls	0.73s 0.87s 0.88s 0.89s 0.92s 0.94s	0.74s 0.76s 0.77s 0.84s 0.91s 0.95s	0.73s 0.77s 0.77s 0.84s 0.91s 0.94s	0.72d(6.5) 0.73s 0.84s 0.88d(6.5) 0.92s 0.93s	0.73d(5.9) 0.74s 0.84s 0.87d (6.3) 0.93s 0.95s	0.72d (6.0) 0.73s 0.84s 0.87d (6.4) 0.94s 0.97s
27a	4.26d (12.5)	4.44d(10.6)	4.40d (11.9)	4.34d (12.9)	4.47d (11.9)	4.44d (12.8)
27s	4.12d (12.5)	4.12d(10.6)	4.12d (11.9)	4.11d (12.9)	4.14d (11.9)	4.14d (12.8)
2'	5.75d (12.6)	5.86d (12.7)	5.85d (12.7)	5.75d (12.7)	5.86d (12.8)	5.84 (12.7)
3'	6.77d (12.6)	6.89d (12.7)	6.89d (12.7)	6.76d (12.7)	6.89d (12.6)	6.90d (12.7)
5'	7.62d (8.6)	7.58d (8.6)	7.61d (8.4)	7.65d (8.6)	7.71d (8.5)	7.73d (8.6)
6'	6.80d (8.6)	7.11d (8.6)	7.07d (8.4)	6.81d (8.6)	7.09d (8.5)	7.08d (8.6)
8'	6.80d (8.6)	7.11d(8.6)	7.07 d (8.4)	6.81d (8.6)	7.09d (8.5)	7.08d (8.6)
9'	7.62d (8.6)	7.58d (8.6)	7.61d (8.4)	7.65d (8.6)	7.71d (8.5)	7.73d (8.6)
COOCH ₃	-	-	3.60s	-	-	3.61s
OCOCH ₃	-	2.01s	2.02s	-	2.02s	2.02s
Ar-OCOCH ₃	-	2.28s	2.31s	-	2.29s	2.30s

Methylation of (1a)

Compound (1b) was obtained on reaction of (1a) (30 mg) with CH_2N_2 (in excess) in ether at room temperature overnight. Usual work-up afforded (1b). EIMS m/z (rel.int.) : 510.3704 (Calc. for $\text{C}_{33}\text{H}_{50}\text{O}_4$: 510.3708, M-*p*-O-acetyl cinnamic acid, 12), 497(25), 464 (15), 450 (20), 260 (32), 201 (42) and 189 (100); $^1\text{H-NMR}$: Table I.

Physical constants of (2)

Amorphous; EIMS m/z (rel.int.) : 454.3448 (Calc. for $\text{C}_{30}\text{H}_{46}\text{O}_3$: 454.3446, M-*p*-coumaric acid, 42), 246.2076 ($\text{C}_{16}\text{H}_{22}\text{O}_2$, 20), 207.1809 ($\text{C}_{14}\text{H}_{23}\text{O}$, 38), 201.1552 ($\text{C}_{15}\text{H}_{21}$, 45), 189.1556 ($\text{C}_{14}\text{H}_{21}$, 100), 164.0452 ($\text{C}_9\text{H}_8\text{O}_3$, 10) and 147.0501 ($\text{C}_9\text{H}_7\text{O}_2$, 30); IR $\nu_{\text{max}}\text{-CHCl}_3 \text{ cm}^{-1}$: 3600- 2600 (br., OH and COOH), 1730 (br., acid and ester carbonyls), 1645 (C=C) and 1612-1370 cm^{-1} (four peaks, aromatic ring); UV $\lambda_{\text{max}}\text{MeOH nm}$: 206 and 311 nm; $^1\text{H-NMR}$: Table I; $^{13}\text{C-NMR}$: Table II.

Tabl-2 $^{13}\text{C-NMR}$ spectral data (δ/ppm) of (1) and(2).

Carbon	(1)	(2)	Carbon	(1)	(2)
1	39.0	38.1	26	18.2	18.2
2	27.2	27.7	27	65.8	66.7
3	78.9	79.5	28	177.6	182.5
4	38.6	38.4	29	28.1	18.9
5	55.2	56.6	30	21.0	21.6
6	18.3	19.4	1'	166.0	168.3
7	32.5	34.7	2'	115.9	116.9
8	40.3	39.8	3'	143.0	144.5
9	48.4	49.6	4'	134.2	134.6
10	38.3	38.2	5'	132.5	133.6
11	24.2	24.5	6'	115.2	116.0
12	129.9	131.5	7'	160.8	160.0
13	133.0	134.6	8'	115.2	116.0
14	47.6	46.8	9'	132.5	133.6
15	24.2	25.4			
16	23.6	24.7			
17	45.4	48.8			
18	39.0	53.9			
19	44.9	40.5			
20	32.5	39.9			
21	33.7	27.7			
22	33.4	38.1			
23	28.0	28.6			
24	15.5	16.3			
25	15.6	16.6			

Acetylation of (2)

Compound (2) on acetylation according to the procedure described for (1a) yielded (2a). EIMS m/z (rel.int.): 496.3557 (Calc. for $\text{C}_{32}\text{H}_{48}\text{O}_4$:

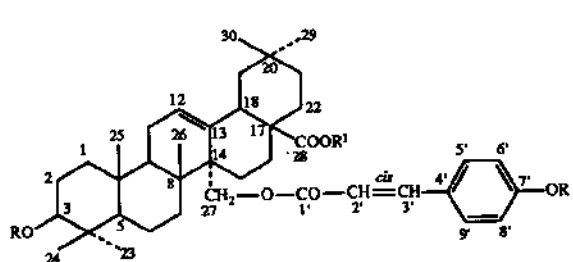
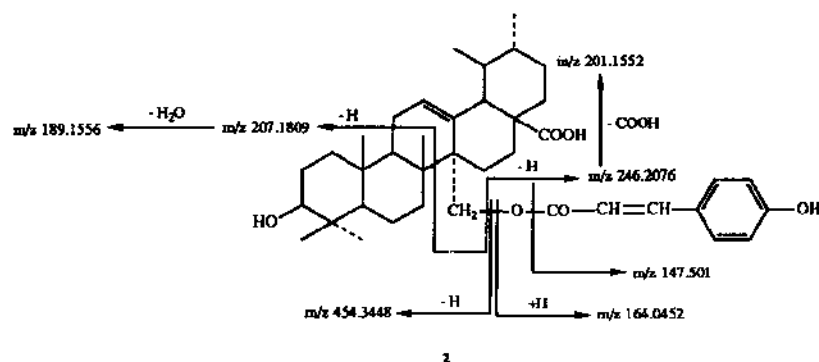
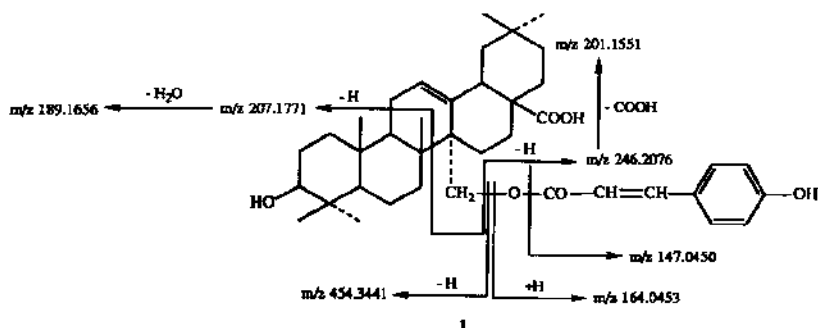
496.3552, M-*p*-O-acetyl cinnamic acid, 23), 483 (17), 436 (9), 421(8), 285 (17), 249 (4), 246 (10), 206 (12), 201 (48) and 189 (100); $^1\text{H-NMR}$: Table I-

Methylation of (2a)

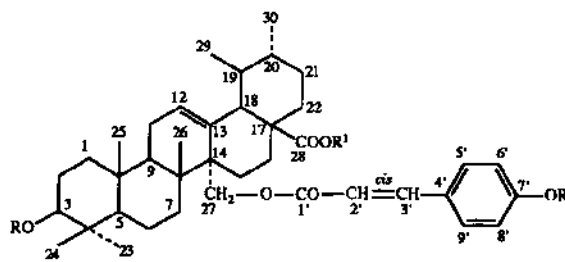
A solution of (2a) in ether was mixed with ethereal CH_2N_2 and kept overnight at room temperature. Usual work-up furnished (2b) as amorphous powder. EIMS m/z (rel.int.) : 510.3711 (Calc. for $\text{C}_{33}\text{H}_{50}\text{O}_4$: 510.3708, M-*p*-O-acetyl cinnamic acid, 50), 497 (77), 464 (65), 450 (20), 260 (40), 206 (20), 201 (75), and 189 (100); $^1\text{H-NMR}$: Table I.

Results and Discussion

(1) and (2) were obtained from the petrol-ethyl acetate (1:1) soluble neutral fraction of the methanolic extract of the fresh leaves through flash column chromatography as described in the experimental. Compound (1) showed IR absorptions at 3650-2600 (br., OH, COOH), 1730 (br., ester and acid carbonyls), 1640 (C=C) and 1620-1370 cm^{-1} (four peaks, aromatic ring) and UV absorptions at 205.7 and 311.5 nm. It yielded a diacetyl derivative (1a) on reaction with Ac_2O in pyridine which afforded the diacetyl monomethyl derivative (1b) on treatment with CH_2N_2 showing the presence of two hydroxyl groups - one aromatic (δ OCOCH_3 , 2.28, s) and the other aliphatic (δ OCOCH_3 , 2.01, s) -, and a carboxyl function in the molecule. The $^1\text{H-NMR}$ spectrum of (1) displayed resonances for a *p*-Z-coumaroyloxy substituent (Table I) which was supported by the $^{13}\text{C-NMR}$ chemical shifts (Table II) and the HRMS fragments at m/z 164.0453 ($\text{C}_9\text{H}_8\text{O}_3$) and 147.0450 ($\text{C}_9\text{H}_7\text{O}_2$). Furthermore, the $^1\text{H-NMR}$ spectrum of 1 showed signals of six methyls as singlets (δ 0.73, 0.87, 0.88, 0.89, 0.92 and 0.94), an olefinic proton (δ 5.52 t, $J = 3.4$ Hz, H-12), apart from the signals of H-2 and H-3 (Table I), two methylene protons (δ 4.26 d, $J = 12.5$ Hz, H-27a and 4.12 d, $J = 12.5$ Hz, H-27b) and two methine protons (δ 3.02 t, $J = 7.5$ Hz, H-3 α and 2.87 dd, $J = 13.4$ and 4.8 Hz, H-18). The aliphatic hydroxyl group was placed at C-3 on biogenetic grounds and its β -disposition was decided on the basis of δ value and coupling constants of H-3 [5]. The characteristic retro-Diels-Alder fragmentation of (1) at m/z 246, 207, 201 and 189 (vide structure) showed that the *p*-coumaroyloxy group and the car-



1a R = Ac, R¹ = H
1b R = Ac, R¹ = CH₃



1a R = Ac, R¹ = H
1b R = Ac, R¹ = CH₃

boxyl function are located at C-27 and C-17 with the double bond at C-12 in the oleanane skeleton. The C-27 substituent was supported by the chemical shifts of H-12 (δ 5.52), C-12 (δ 129.9) and C-13 (δ 133.0) since substitution at C-27 causes a downfield shift of H-12, C-12 and an upfield shift of C-13 from those of their values in oleanolic acid, in analogy with other 27-substituted oleanolic acid derivatives [3,6,7]. The comparable chemical shift of C-17 (δ 45.4) of (1) with that of oleanolic acid and other C-17 carboxyl derivatives [3] and the down-field resonance of C-14 (δ 47.6) as against ca. δ 42.2 demonstrated that the carboxyl group is located at C-17 and the *p*-coumaroyloxy group is at C-27. In the alternate arrangement C-14 would appear at more downfield (ca. δ 56-59) [3,8] while C-

17 at more upfield (ca. δ 36) [3]. Compound (1) did not give the molecular ion peak in the mass spectrum (EI, FD and FAB) but showed significant fragments (EI, HRMS) at m/z 454.3491 (C₃₀H₄₆O₃) and 164.0453 (C₉H₈O₃) corresponding to the molecular formula C₃₉H₅₄O₆ which was confirmed by ¹³C-NMR spectral data (BB, DEPT). On the basis of above observations, the structure of (1) has been assigned as β -hydroxy-27-*p*-Z-coumaroyloxyolean-12-en-28-oic acid.

The spectral data of (2) showed its close resemblance with compound (1) and exhibited that it differs in the basic skeleton only. Thus the ¹H-NMR spectrum of (2) demonstrated an olefinic proton (δ 5.55 t, J = 3.7 Hz, H-12), two methylene

protons (δ 4.34 d, $J = 12.9$ Hz, H-27a and δ 4.11 d, $J = 12.9$ Hz, H-27b), two methine protons (δ 3.07, t, $J = 7.7$ Hz, H-3; δ 2.27 d, $J = 10.6$ Hz, H-18) and a *p*-Z-coumaroyloxy group (Table I). It formed a diacetyl derivative (2a, δ OCOCH₃ 2.02, 2.29) on treatment with Ac₂O/pyridine which could be converted to the diacetyl monomethyl derivative (2b) (δ OCOCH₃ 2.30, 2.03, δ OCH₃ 3.61) on reaction with diazomethane. These transformations justified the coumaroyloxy hydroxyl group, the secondary hydroxyl group and the carboxyl function. The secondary hydroxyl group was placed at C-3 on biogenetic grounds and its β -orientation was decided through the δ value and coupling constants of H-3 (loc.cit). In contrast to (1) the ¹H-NMR of 2 showed four methyls as singlets (δ 0.73, 0.84, 0.92, 0.93) and two methyls as doublets (δ 0.72, 0.88). These features together with the H-18 doublet and the fragments at m/z 246, 207, 201 and 189 resulting through retro-Diels-Alder cleavages around ring C from molecular ion minus *p*-coumaric acid fragment (vide structure) indicated that (2) has a Δ^{12} - α -amyrin skeleton. The location of the carboxyl group at C-17 and the *p*-Z-coumaroyloxy group at C-27 could be done on the basis of discussion made earlier in the case of (1). The molecular formula

C₃₉H₅₄O₆ of compound (2) was also arrived at through HRMS fragments at m/z 454.3448 (C₃₀H₄₆O₃) and 164.0452 (C₉H₈O₃) and ¹³C-NMR (BB, DEPT) spectro-metry. These observations led to define the structure of (2) as 3 β -hydroxy-27-*p*-Z-coumaroyloxyurs-12-en-28-oic acid.

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