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THREE PENTACYCLIC TRITERPENOIDS FROM THE LEAVES OF *PLUMERIA OBTUSA*

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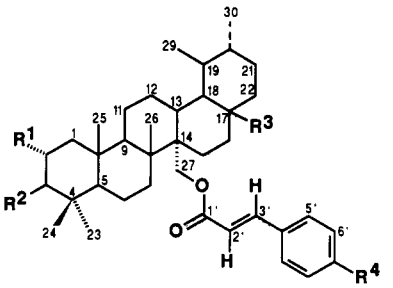
ABSTRACT.—Two new pentacyclic triterpenoids, coumarobtusanoic acid [**2**] and coumarobtusane [**3**], have been isolated from the leaves of *Plumeria obtusa* along with a known triterpene, 27-*p*-*E*-coumaroyloxyursolic acid. The new structures have been established as 27-*p*-*E*-coumaroyloxy-2α,3β-dihydroxyurs-28-oic acid [**2**] and 27-*p*-*E*-coumaroyloxy-2α,3β-dihydroxyursane [**3**] through chemical and spectroscopic studies.

The genus *Plumeria* (Apocynaceae) is a native of tropical America, and various ornamental species are grown in the warmer regions of the world (1,2). *Plumeria obtusa* L. has not been studied earlier for its chemical or pharmacological aspects, although other species of *Plumeria* have been investigated for their chemical constituents, and plumeiride (3), plumericins (4,5), and fulvoplumierin (6,7) have been reported along with α- and β-amyrin (8), lupeol, steroids (9,10), and cardiac glycosides (11). *P. obtusa* was subjected to chemical investigation, and the isolation and structure of three *p*-coumaroyloxy pentacyclic triterpenoids **1**, **2**, and **3** form the subject of the present paper. Compounds **2** and **3** are hitherto unreported, while **1** has been reported earlier (12).



1 R^1=H, R^2=R^4=OH, R^3=COOH, Δ^{12}
1a R^1=H, R^2=R^4=OAc, R^3=COOH, Δ^{12}
1b R^1=H, R^2=R^4=OAc, R^3=COOMe, Δ^{12}
2 R^1=R^2=R^4=OH, R^3=COOH
2a R^1=R^2=R^4=OAc, R^3=COOH
2b R^1=R^2=R^4=OAc, R^3=COOMe
3 R^1=R^2=R^4=OH, R^3=Me
3a R^1=R^2=R^4=OAc, R^3=Me

RESULTS AND DISCUSSION

The spectral data of **1** (12 mg) showed its identity with 27-*p*-*E*-coumaroyloxy ursolic acid [**1**] (12). The ^{1}H-nmr spectrum (Table 1) of **1**, which was recorded in pyridine-d_5, showed slight downfield shifts of all the protons as compared to those reported by Budzikiewicz and Thomas (12). The chemical shifts (Table 2) of C-17–C-22, C-29, and C-30, which are comparable with those of ursolic acid (13), provided additional evidence of the respective positions of COOH and ester functions at C-17 and C-27 (14).

The spectral data of **2** and formation of the triacetyl **2a** and triacetyl monomethyl **2b** derivatives on reaction with Ac_2O/pyridine and CH_2N_2, respectively, showed its close similarity with **1**. The olefinic signal of H-12 was, however, missing in the ^{1}H-nmr of **2** which instead showed two carbinylic protons, one as a doublet at δ 3.38 (J = 10.2 Hz) and the other as a doublet of double doublet at δ 4.06 (J = 10.2, 10.2, and 4.6 Hz). One of the hydroxyl groups was placed at C-3 on biogenetic grounds, and, as the multiplicities of these protons indicated their vicinal nature, the second hydroxyl group was placed at C-2. The coupling constants of the protons further exhibited their axial nature. These data led to the definition of the structure of coumarobtusanoic acid as 27-*p*-*E*-coumaroyloxy-2α,3β-dihydroxyurs-28-oic acid [**2**], which was further substantiated by the ^{13}C-nmr

TABLE 1. ^{1}H-nmr Chemical Shifts of Triterpenoids **1**, **2**, and **3**, Their Corresponding Acetates **1a**, **2a**, and **3a**, and Acetyl Methyl Derivatives **1b** and **2b**.[a, b]

Proton	Compound							
	1	**1a**	**1b**	**2**	**2a**	**2b**	**3**	**3a**
H-2	—	—	—	4.06 ddd (10.2) (10.2) (4.6)	5.18 ddd (10.2) (10.2) (4.6)	5.08 ddd (10.2) (10.2) (4.6)	4.05 ddd (10.2) (10.2) (4.6)	5.09 ddd (10.2) (10.2) (4.5)
H-3	3.38 dd (7.8) (8.0)	4.43 dd (7.8) (8.0)	4.43 dd (7.8) (8.0)	3.38 d (10.2)	4.74 d (10.2)	4.73 d (10.2)	3.39 d (10.2)	4.75 d (10.2)
H-12	5.46 br s	5.57 t (3.4)	5.56 t (3.4)	—	—	—	—	—
H-18	2.60 d (11.6)	2.30 d (11.6)	2.29 d (11.6)	—	—	—	—	—
H-23	1.00 s	0.85 s	0.85 s	1.00 s	0.92 s	0.92 s	1.03 s	0.96 s
H-24	0.98 s	0.83 s	0.83 s	0.99 s	0.77 s	0.74 s	0.97 s	0.84 s
H-25	0.99 s	0.84 s	0.84 s	0.98 s	0.79 s	0.83 s	0.98 s	0.85 s
H-26	1.05 s	0.88 s	0.87 s	1.05 s	0.95 s	0.93 s	1.08 s	0.87 s
H-27a	4.73 d (13.0)	4.32 d (13.1)	4.30 d (13.0)	4.71 d (12.5)	4.33 d (13.1)	4.31 d (13.1)	4.72 d (12.5)	4.33 d (13.1)
H-27b	4.54 d (13.0)	4.17 d (13.1)	4.17 d (13.0)	4.55 d (12.5)	4.15 d (13.1)	4.11 d (13.1)	4.54 d (12.5)	4.18 d (13.1)
H-28	—	—	—	—	—	—	1.00 s	0.97 s
H-29	1.19 d (7.2)	0.95 d (7.2)	0.96 d (7.2)	1.10 d (6.3)	0.86 d (6.9)	0.87 d (7.0)	1.18 d (6.6)	0.95 d (6.0)
H-30	0.93 d (7.1)	0.91 d (7.1)	0.91 d (7.2)	0.97 d (5.8)	0.85 d (6.3)	0.86 d (6.5)	1.05 d (6.4)	0.91 d (6.2)
H-2′	6.67 d (15.9)	6.30 d (16.4)	6.30 d (16.2)	6.67 d (15.8)	6.33 d (16.1)	6.30 d (16.0)	6.55 d (15.8)	6.33 d (15.8)
H-3′	7.96 d (15.9)	7.61 d (16.4)	7.60 d (16.2)	7.97 d (15.8)	7.62 d (16.1)	7.62 d (16.0)	7.95 d (15.8)	7.60 d (15.8)
H-5′/H-9′	7.60 d (8.5)	7.53 d (8.7)	7.53 d (8.6)	7.59 d (8.6)	7.53 d (8.8)	7.52 d (8.7)	7.58 d (8.6)	7.53 d (8.7)
H-6′/H-8′	7.13 d (8.5)	7.11 d (8.7)	7.12 d (8.6)	7.12 d (8.6)	7.13 d (8.8)	7.12 d (8.7)	7.10 d (8.6)	7.12 d (8.7)
OAc	—	2.01 s	2.02 s	—	1.96, 1.97 s	1.97 (6H) s	—	2.00 (6H) s
7′-OAc	—	2.29 s	2.29 s	—	2.28 s	2.08 s	—	2.30 s
OMe	—	—	3.57 s	—	—	3.59 s	—	—

[a]Coupling constants are in parentheses.

[b]The chemical shifts of the methyl protons in a vertical column may be interchanged.

(BB, DEPT, and hetero COSY) spectral data (Table 2), and mass spectral fragments (see Experimental).

The ^{1}H- and ^{13}C-nmr spectral data of **3** showed that it differs from **2** only in having a methyl group at C-17 instead of the carboxyl function. Thus, the ^{1}H-nmr spectrum showed seven methyl signals, five as singlets and two as doublets (Table 1), and the same number of methyl carbons were observed in the DEPT spectrum (Table 2). Further, it formed the triacetate derivative **3a**, indicating the presence of three hydroxyl groups. A substantial amount of evidence was obtained from the significant fragments at *m/z* 424, 218, 206, and 205 in the ei mass spectrum, which led us to characterize coumarobtusane as 27-*p*-*E*-coumaroyloxy-2α,3β-dihydroxyursane [**3**].

In compounds **1**, **2**, and **3**, the molecular ion peak was not observed in the ei or fab spectra. The molecular formulae of **2** and **3** ($C_{39}H_{56}O_7$ and $C_{39}H_{58}O_5$, respectively) were arrived at through the exact mass measurement of various fragments and ^{13}C-nmr spectral data.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir (KBr) and uv (MeOH) spectra were measured on JASCO IRA-I and Pye-Unicam SP-800 spectrometers, respectively; mass spectra were recorded on Finnigan MAT 112 and MAT 312 double focussing mass spectrometers connected to a PDP 11/34 computer system. ^{1}H-nmr spectra (**1**, **2**, and **3** in pyridine-d_5 and **1a**, **2a**, and **3a** in $CDCl_3$) were recorded on a Bruker AM 400 spectrometer, operating at 400 MHz. The chemical shifts are reported in δ (ppm), and the coupling constants are in Hz. The ^{13}C-nmr spectral assignments (Table 2) have been made partly through a comparison of the chemical shifts with published data for similar compounds (13, 15, 16) and partly through DEPT and hetero COSY spectra. The ^{13}C-nmr measurements were made at 75 MHz on a Bruker 300 MHz nmr spectrometer over 77000.00 Hz, using 32K data points for BB decoupled SFORD and DEPT nmr experiments. The 2D ^{1}H-^{13}C chemical shift correlation spectra were obtained using the DEPT 2D pulse sequence with ^{1}H broad band decoupling throughout the acquisition period.

PLANT MATERIAL.—The plant material collected in the month of April (1987) from the Karachi region, was identified by Prof. S.I. Ali,

TABLE 2. ^{13}C-nmr Chemical Shifts of **1**, **2**, and **3**.

Carbon	Compound			Carbon	Compound		
	1	**2**	**3**		**1**	**2**	**3**
C-1	37.4	46.5	45.6	C-21	30.7	31.1	31.1
C-2	28.1	68.6	68.6	C-22	37.4	37.4	37.4
C-3	78.1	83.9	83.9	C-23	29.3	28.3	28.3
C-4	38.5	39.1	39.1	C-24	16.0	17.0	17.6
C-5	56.0	55.9	55.9	C-25	16.6	16.9	16.9
C-6	18.9	18.9	18.9	C-26	16.9	17.5	17.5
C-7	33.2	33.6	33.2	C-27	66.2	66.2	66.2
C-8	39.8	39.6	39.8	C-28	179.9	179.9	18.9
C-9	48.2	48.2	48.0	C-29	17.0	16.6	15.8
C-10	38.5	38.5	38.5	C-30	21.4	21.4	21.4
C-11	23.8	23.8	23.9	C-1′	167.2	167.2	167.2
C-12	125.6	28.1	29.3	C-2′	115.6	116.0	115.7
C-13	134.6	39.1	39.4	C-3′	145.2	145.2	145.2
C-14	47.2	42.0	42.1	C-4′	134.6	133.6	134.6
C-15	28.3	28.7	30.0	C-5′	130.6	130.6	130.4
C-16	24.1	24.2	24.1	C-6′	116.9	116.8	116.9
C-17	48.2	48.2	34.2	C-7′	161.5	161.5	161.5
C-18	53.3	48.3	49.2	C-8′	116.9	116.8	116.9
C-19	39.4	39.6	39.6	C-9′	130.6	130.6	130.4
C-20	39.1	39.5	39.5				

Department of Botany, University of Karachi, and a voucher specimen (No. 9317 KUH) has been deposited in the Herbarium.

EXTRACTION AND ISOLATION.—The fresh, undried, and uncrushed leaves (12 kg) were five times percolated with MeOH at room temperature, and the combined extracts after concentration under reduced pressure were partitioned between EtOAc and H_2O. The former layer was treated with a 4% aqueous solution of Na_2CO_3 to separate the acidic from the neutral fractions. The EtOAc layer containing the neutral fraction was washed with H_2O, dried (anhydrous Na_2SO_4), and freed of the solvent. The residue was divided into petroleum-ether-soluble and petroleum-ether-insoluble portions, and the petroleum-ether-insoluble fraction was successively treated with petroleum ether-EtOAc (7:3) and petroleum ether-EtOAc (1:1). The residue (3.50 g) obtained on removal of the solvent from the petroleum ether-EtOAc (1:1) soluble portion was subjected to flash cc (Si gel E. Merck 9385; $CHCl_3$, $CHCl_3$/MeOH in order of increasing polarity). The fractions eluted with $CHCl_3$-MeOH (9.6:0.4–9.5:0.5) furnished a fraction containing a mixture of the weakly acidic constituents **1** and **2**, along with **3**. Through subsequent purification of the crude fraction on precoated thin layer cards of Si gel SIF-254 [$CHCl_3$-MeOH (9.5:0.5)] pure **1**, **3**, and **2**, in order of polarity, were ultimately obtained.

COUMAROBTUSANOIC ACID [**2**].—Amorphous (15 mg): ν max 3400–2650 br (OH, COOH), 1725–1690 br (ester and acid), 1600–1335 cm^{-1} (aromatic C=C); λ max 282.5 nm; eims *m/z* (%) $[M-164-18]^+$ 454.3448 ($C_{30}H_{46}O_3$, calcd 454.3446) (9), 248 (100), 205 (14), 204 (4), 187 (24), 164.0467 ($C_9H_8O_3$, calcd 164.0473) (14), 147.0446 ($C_9H_7O_2$, calcd 147.0445) (38); ^1H nmr see Table 1; ^{13}C nmr see Table 2.

COUMAROBTUSANE [**3**].—Amorphous (7 mg): ν max 3450–3200 br (OH), 1725 (ester), 1600–1350 cm^{-1} (aromatic C=C); λ max 282 nm; eims *m/z* (%) $[M-164-18]^+$ 424.3715 ($C_{30}H_{48}O$, calcd 424.3705) (10), 218 (12), 206 (18), 205 (22), 187 (32), 164.0467 ($C_9H_8O_3$, calcd 164.0473) (38), 147.0446 ($C_9H_7O_2$, calcd 147.0445) (100), 119 (44), 81 (44); ^1H nmr see Table 1; ^{13}C nmr see Table 2.

ACETYLATION OF **1**, **2**, AND **3**.—These triterpenes (5 mg each) were taken up in pyridine (1 ml) and treated with Ac_2O (1 ml) at room temperature overnight. Usual workup gave the corresponding acetates (**1a**, **2a**, and **3a**) in almost theoretical yield. Compound **1a**: eims *m/z* (%) $[M-206]^+$ 496.3573 ($C_{32}H_{48}O_4$, calcd 496.3552) (20), 451.3548 ($C_{31}H_{47}O_2$, calcd 451.3575) (46), 421.3117 ($C_{29}H_{41}O_2$, calcd 421.3106) (12), 391.3332 ($C_{29}H_{43}$, calcd 391.3364) (38), 246 (20), 206 (59), 164 (42), 147 (100), 119 (64), 95 (72), 87 (78); ^1H nmr see Table 1. Compound **2a**: eims *m/z* (%) $[M-206]^+$ 556.3772 ($C_{34}H_{52}O_6$, calcd 556.3763) (4), 510.3708 ($C_{33}H_{50}O_4$, calcd 510.3709) (1.7), 436.3333 ($C_{30}H_{44}O_2$, calcd 436.3341) (8), 421.3109 ($C_{29}H_{41}O_2$, calcd 421.3106) (2), 307 (2), 248 (100), 206 (63), 249 (18), 164 (12), 147 (10), 119 (96); ^1H nmr see Table 1. Compound **3a**: eims *m/z* (%) $[M-206-60]^+$ 466.3812 ($C_{32}H_{50}O_2$, calcd 466.3810) (4), 309 (12), 249 (10), 218 (14), 206 (79), 164 (32), 147 (78), 83 (100), 69 (85); ^1H nmr see Table 1.

METHYLATION OF **1a** AND **2a**.—Compounds **1a** (3 mg) and **2a** (3 mg) were dissolved in Et_2O, treated with an excess of freshly prepared CH_2N_2, and kept at room temperature overnight. Removal of the solvent from the reaction mixtures afforded **1b** and **2b**, respectively. Compound **1b**: eims *m/z* (%) 510 (22), 497 (16), 451 (4), 437 (14), 377 (8), 261 (10), 201 (50), 167 (30), 83 (100); ^1H nmr see Table 1. Compound **2b**: eims *m/z* (%) 570 (1), 510 (6), 450 (7), 435 (3), 391 (5), 308 (1), 262 (80), 203 (100), 189 (35); ^1H nmr see Table 1.

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