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TWO CYTOTOXIC PENTACYCLIC TRITERPENOIDS FROM *NERIUM OLEANDER*

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Key Word Index—*Nerium oleander*; Apocynaceae; pentacyclic triterpenoids; 3β-hydroxy-28-*Z*-*p*-coumaroyloxy-urs-12-en-27-oic acid (*cis*-karenin); 3β-hydroxy-28-*E*-*p*-coumaroyloxy-urs-12-en-27-oic acid (*trans*-karenin); cytotoxicity.

Abstract—The isolation and structure elucidation of two novel cytotoxic pentacyclic triterpenoids *cis*-karenin (3β-hydroxy-28-*Z*-*p*-coumaroyloxy-urs-12-en-27-oic acid) and *trans*-karenin (3-β-hydroxy-28-*E*-*p*-coumaroyloxy-urs-12-en-27-oic acid) from the leaves of *Nerium oleander* is described.

INTRODUCTION

Nerium oleander distributed in the Mediterranean region and subtropical Asia, is indigenous to the Indo-Pakistan subcontinent. Its various parts are reputed to have cardiotonic and antibacterial properties [1, 2]. We herein report the isolation and structure elucidation of two new cytotoxic pentacyclic triterpenoids, *cis*-karenin (**1**) and *trans*-karenin (**2**), with ED_{50} 15.0 and 7.5 $\mu g\,ml^{-1}$, respectively, on KB cell line. Their structures have been elucidated on the basis of detailed ^1H and ^{13}C NMR studies including 2D experiments (COSY-45, NOESY, *J*-resolved and heteroCOSY) which allowed a complete assignment of all of the protons and carbons.

RESULTS AND DISCUSSION

cis-Karenin (**1**) ($C_{39}H_{54}O_6$, confirmed by ^{13}C NMR; broad band and DEPT) produces an HR-mass spectral fragment at *m/z* 454.3443 ($C_{30}H_{46}O_3$) corresponding to the molecular ion minus coumaric acid ($C_9H_8O_3$; HR-MS *m/z* 164.0469). The structures of the moieties $C_{30}H_{46}O_3$ and $C_9H_8O_3$ as an ursane derivative [3, 4] and *cis*-coumaric acid, respectively, were deduced from the spectral data (see Experimental, and Tables 1 and 2). The resonance of the H-12 triplet at δ5.5 and the shifts of C-12 (δ130.7), C-13 (δ134.8) and C-14 (δ57.8) were not comparable with those of ursolic acid [5]. These features were consistent with a carboxyl group (ν_{max} 3400–2670, 1710 cm^{-1}) at C-14 [6]. An interaction of H-28a with H-18 in the NOESY spectrum established the position of the *cis*-*p*-hydroxy cinnamoyloxy moiety at C-28 (Table 2). The retro-Diels–Alder cleavage and other significant fragments drawn on the structure fully support the structure of **1** as 3β-hydroxy-28-*cis*-*p*-coumaroyloxy-urs-12-en-27-oic acid.

The main features differentiating the spectral data (Tables 3 and 4) of **2** from those of **1** were the coupling constant ($J = 15.99$ Hz) and chemical shifts of H-2′ (δ6.29) and H-3′ (δ7.57) and the absence of an interaction of these protons in the NOESY spectrum. Thus a *trans*-*p*-coumaroyloxy moiety was considered to be present in **2**, cf. *cis*-*p*-coumaroyloxy group in **1** in which H-2′ and H-3′ resonated at δ5.74 and 6.84, respectively, with a coupling constant of 12.84 Hz and showed an interaction in the NOESY spectrum (Table 2).

These and other spectral observations (see Experimental) led to the structure of **2** as 3β-hydroxy-28-*trans*-*p*-coumaroyloxy-urs-12-en-27-oic acid, the geometric isomer of **1**.

All the protons and ^{13}C signals of **1** and **2** have been assigned on the basis of heteroCOSY (Tables 1 and 3) and COSY-45° (Tables 2 and 4) experiments. The stereochemical assignments are based on coupling constants and NOESY experiments (Tables 2 and 4).

EXPERIMENTAL

Mps: uncorr.; MS: Finnigan MAT 112 and 312 double focussing mass spectrometers connected to a PDP 11/34 computer system; NMR (Me_2CO-d_6): 300 MHz for ^1H and 75 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 have been made partly through a comparison of the chemical shifts with the published data for similar compounds [6, 7] and partly through heteronuclear correlation and the appearance of signals in the DEPT spectrum (Tables 1 and 3). VLC/TLC and flash CC: silica gel PF_{254} and silica gel E.

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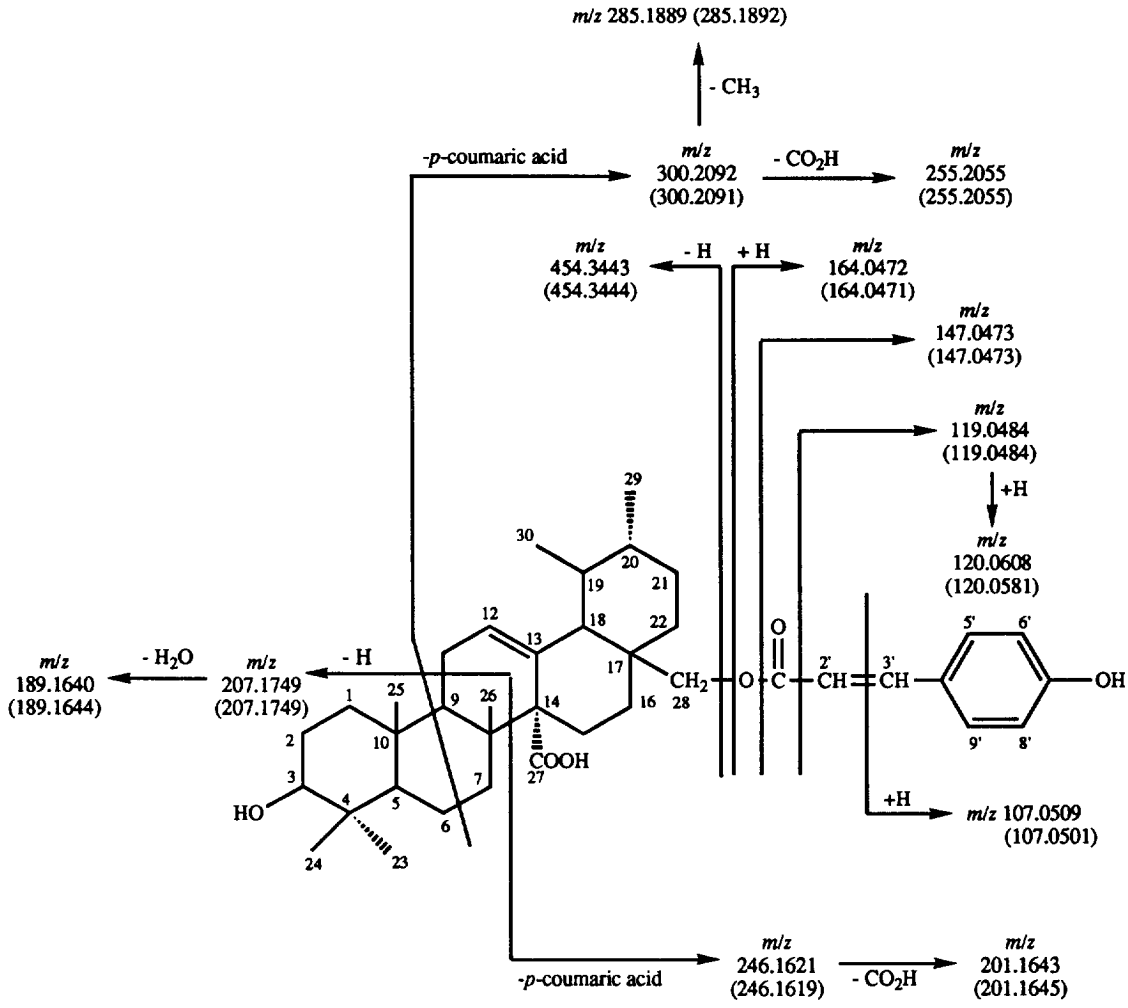

Scheme 1. Mass spectral fragmentation of **1** and **2** (*m/z* values in parentheses).

Merck 9385, respectively. The plant was identified by Prof. S. I. Ali (Department of Botany, University of Karachi) and a voucher specimen (N.OL-1) is deposited in the Herbarium.

The residue left on removal of the solvent from the combined methanolic percolates of the fresh, undried and uncrushed leaves (20 kg) of *N. oleander* collected in July from the Karachi region, was divided into acidic, basic and neutral fractions. From the neutral petrol-insoluble portion a mixture of ursolic and oleanolic acid was separated according to the procedure described earlier [8]. The mother liquor thus obtained was again divided into petrol-soluble and -insoluble fractions. The latter fraction was subjected to VLC [9] ($CHCl_3$, $CHCl_3$–MeOH in order of increasing polarity). The eluate from $CHCl_3$–MeOH (19:1) showed two major spots on TLC. This fraction was then subjected to thick layer chromatography ($CHCl_3$–MeOH, 19:1) which furnished **1** and **2** as homogeneous constituents in the order of increasing polarity in 0.00134 and 0.000485% yield, respectively, per 20 kg of fresh leaves.

3β-*Hydroxy*-28-Z-p-*coumaroyloxy-urs*-12-*en*-27-*oic acid* (**1**). Needles; mp 205–206°; UV λ_{max}^{MeOH} nm: 205, 312; IR ν_{max}^{KBr} cm^{-1}: 3600–2670, 3450, 1710, 1630, 1600–1420 (4 peaks); ^1H and ^{13}CNMR: Table 1; COSY-45 and NOESY: Table 2; HR-MS *m/z*: 454.3443 ($C_{30}H_{46}O_3$) [M − *p*-coumaric acid]$^+$, 439.3229 ($C_{29}H_{43}O_3$), 421.3082 ($C_{29}H_{41}O_2$), 410.3428 ($C_{25}H_{46}O_4$), 393.3148 ($C_{28}H_{41}O$), 300.2093 ($C_{20}H_{28}O_2$), 285.1889 ($C_{19}H_{25}O_2$), 255.2055 ($C_{19}H_{27}$), 246.1621 ($C_{16}H_{22}O_2$), 207.1749 ($C_{14}H_{23}O$), 201.1643 ($C_{15}H_{21}$), 189.1640 ($C_{14}H_{21}$), 164.0472 (C_9H_8O), 147.0473 ($C_9H_7O_2$), 120.0608 (C_8H_8O), 119.0484 (C_8H_7O) and 107.0509 (C_7H_6O).

3β-*Hydroxy*-28-E-p-coumaroyloxy-urs-12-en-27-oic acid (**2**). Rods; mp 230–231°; UV λ_{max}^{MeOH} nm: 205, 280; IR ν_{max}^{KBr} cm^{-1}: 3600–2650, 3445, 1715, 1625, 1600–1435 (4

Table 1. ^{13}C and ^{1}H NMR spectral data of compound **1** (heteroCOSY)

C	δ	δ	Correlated ^{1}H		
1	39.5	1.25	2H	*m*	
2	28.3	1.50, 1.55	2H	*m*	
3	78.4	3.10	1H	*dd*	(10.5, 5.3)
4	40.1	—			
5	56.3	0.80	1H	*dd*	(11.5, 1.5)
6	19.2	1.75	2H	*m*	—
7	37.8	1.7, 1.72	2H	*m*	—
8	41.2	—	—		
9	49.3	1.7	1H	*dd*	(10.2, 3.6)
10	38.0	—			
11	24.6	1.95, 1.97	2H	*m*	—
12	130.8	5.51	1H	*t*	—
13	134.8	—			
14	57.8	—			
15	25.3	0.95	2H	*m*	—
16	28.1	0.99	2H	*m*	—
17	38.2	—			
18	53.6	2.30	1H	*d*	(11.0)
19	39.5	1.25	1H	*m*	—
20	40.1	1.15	1H	*m*	—
21	31.2	2.1	2H	*m*	—
22	34.6	0.85	2H	*m*	—
23	28.6	0.99	3H	*s*	—
24	18.9	0.84	3H	*s*	—
25	16.3	0.76	3H	*s*	—
26	18.1	0.93	3H	*s*	—
27	180.6	—			
28	66.3	4.40	1H	*d*	(12.72)
		4.13	1H	*d*	(12.72)
29	16.4	0.75	3H	*d*	(7.26)
30	21.5	0.92	3H	*d*	(6.24)
1′	166.8	—			
2′	117.0	5.74	1H	*d*	(12.84)
3′	143.9	6.84	1H	*d*	(12.84)
4′	127.4	—			
5′/9′	133.6	7.75	2H	*d*	(8.55)
6′/8′	115.8	6.83	2H	*d*	(8.55)
7′	159.7	—			

Multiplicities and coupling constants were measured from normal ^{1}H NMR and 2D-*J* resolved spectra.

Table 2. Selected correlations of *cis*-karenin (**1**) from NOESY and COSY-45 experiments

		Spatial connectivities with protons (NOESY spectrum)		Correlated protons (COSY-45 spectrum)	
H	δ	H	δ	H	δ
3	3.10	—	—	2	1.50, 1.55
9	1.7	—	—	11	1.97
12	5.51	—	—	11	1.97
18	2.3	28a	4.40	19	1.25
		3′	6.84	—	—
		5′, 9′	7.75	—	—
19	1.25	—	—	20	1.15
26	0.93	5′, 9′	7.75	—	—
28a	4.40	—	—	28b	4.13
2′	5.74	3′	6.84	3′	6.84
6′, 8′	6.83	5′, 9′	7.75	5′, 9′	7.75

Table 3. ^{13}C and ^{1}H NMR spectral data of compound **2** (heteroCOSY)

C	δ	δ	Correlated ^{1}H		
1	39.6	1.25	2H	*m*	
2	28.6	1.50, 1.55	2H	*m*	
3	78.5	3.08	1H	*dd*	(11.28, 4.83)
4	40.1	—			
5	56.2	0.79	1H	*dd*	(11.4, 1.6)
6	19.1	1.75	2H	*m*	—
7	37.7	1.72	2H	*m*	—
8	41.0	—			—
9	49.3	1.69	1H	*dd*	(10.4, 3.4)
10	38.0	—			—
11	24.4	2.10	2H	*m*	—
12	130.6	5.54	1H	*t*	(3.39)
13	134.6	—			—
14	59.0	—			—
15	25.2	0.98	2H	*m*	—
16	28.1	1.01	2H	*m*	—
17	38.1	—			—
18	53.6	2.36	1H	*d*	(11.40)
19	39.4	1.26	1H	*m*	—
20	40.1	1.17	1H	*m*	—
21	31.2	2.06	2H	*m*	—
22	34.5	0.87	2H	*m*	—
23	28.6	0.97	3H	*s*	—
24	19.1	0.85	3H	*s*	—
25	16.3	0.75	3H	*s*	—
26	18.2	0.96	3H	*s*	—
27	179.5	—			—
28	66.2	4.39	1H	*d*	(12.80)
		4.18	1H	*d*	(12.80)
29	18.2	0.86	3H	*d*	(7.02)
30	21.5	0.90	3H	*d*	(6.37)
1′	167.1	—			—
2′	115.9	6.29	1H	*d*	(15.99)
3′	145.2	7.57	1H	*d*	(15.99)
4′	127.0	—			—
5′/9′	130.8	7.48	2H	*d*	(8.67)
6′/8′	116.8	6.90	2H	*d*	(8.67)
7′	161.0	—		—	

Multiplicities and coupling constants were measured from normal ^{1}H NMR and 2D-*J* resolved spectra.

Table 4. Selected correlations of *trans*-karenin (**2**) from NOESY and COSY-45 experiments

		Spatial connectivities with protons (NOESY spectrum)		Correlated protons (COSY-45 spectrum)	
H	δ	H	δ	H	δ
3	3.08	—	—	2	1.55
12	5.54	—	—	11	2.10
18	2.36	28a	4.39	—	—
28a	4.39	—	—	28b	4.18
2′	6.29	—	—	3′	7.57
6′, 8′	6.90	5′, 9′	7.48	5′, 9′	7.48

peaks); ^{1}H and ^{13}C NMR: Table 3; COSY-45 and NOESY: Table 4; HR-MS *m/z*: 454.3444 ($C_{30}H_{46}O_3$) [M − *p*-coumaric acid]$^+$, 439.3224 ($C_{29}H_{43}O_3$), 421.3087 ($C_{29}H_{41}O_2$), 410.3426 ($C_{25}H_{46}O_4$), 393.3143 ($C_{28}H_{41}O$), 300.2091 ($C_{20}H_{28}O_2$), 285.1892 ($C_{19}H_{25}O_2$), 255.2055 ($C_{19}H_{27}$), 246.1619 ($C_{16}H_{22}O_2$), 207.1749 ($C_{14}H_{23}O$), 201.1645 ($C_{15}H_{21}$), 189.1644 ($C_{14}H_{21}$), 164.0471 ($C_9H_8O_3$), 147.0473 ($C_9H_7O_2$), 120.0581 (C_8H_7O), 119.0484 (C_8H_7O) and 107.0501 (C_7H_6O).

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