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OLEANDEROL, A NEW PENTACYCLIC TRITERPENE FROM THE LEAVES OF *NERIUM OLEANDER*

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ABSTRACT.—A new pentacyclic triterpene, oleanderol, and the known betulin, betulinic acid, ursolic acid, and oleanolic acid have been isolated from the fresh leaves of *Nerium oleander*. The structure elucidation of oleanderol and identification of betulin, betulinic acid, ursolic acid, and oleanolic acid have been carried out through chemical and spectral studies.

Nerium oleander L. (syn. *Nerium odorum*, Apocynaceae), distributed in the Mediterranean region and subtropical Asia, is indigenous to the Indo-Pakistan subcontinent. The plant is commonly known as "kaner", and its various parts are reputed as therapeutic agents in the treatment of swellings, leprosy, and eye and skin diseases. The leaves possess cardiotonic and antibacterial properties (1,2). Studies carried out by Siddiqui *et al.* on the constituents of fresh, undried, and uncrushed leaves of *N. oleander* have resulted in the isolation and structure elucidation of various triterpenoidal (3,4), glycosidal (5), and non-triterpenoidal (6) constituents. The present paper deals with the isolation and structure elucidation of a new triterpene oleanderol [**1**], along with betulin [**3**] and weakly acidic pentacyclic triterpenes, betulinic acid [**5**], ursolic acid, and oleanolic acid from the neutral fraction of the MeOH extract of the fresh leaves of *N. oleander*. The isolation of betulin and betulinic acid is hitherto unreported from this source. For the characterization of **1**, hrms, ir, uv, ^{1}H-nmr, 2D-nmr, nOe difference, and ^{13}C-nmr spectral data have been used, whereas the identification of betulin (7–9) and betulinic acid (8–10) has been made through comparison of their spectral data with those reported in the literature. In addition, ursolic and oleanolic acids have been characterized through comparison of spectral data of acetyl methyl derivatives with those reported in the literature (11,12). These triterpenes are of potential pharmacological significance because the fraction containing these constituents showed CNS depressant activity in mice. After treatment with the fraction, the animals were observed for a period of 2 h, and the behavior was scored according to a modified version of the Irwin procedure (13).

The molecular formula, $C_{30}H_{48}O_3$, of **1** was determined through peak matching of the molecular ion at *m/z* 456, observed through ei and fd sources, and hrms. Formation of the triacetate **2** showed the presence of three hydroxyl groups, whereas five methyl singlets in the ^{1}H-nmr spectrum at δ 0.74, 0.81, 0.92, 0.96, and 1.68 (H-30) and two doublets at δ 4.60 and 4.73 (J_{gem} = 1.5 Hz, H-29a and H-29b), and the bands in the ir spectrum at 1640 and 880 cm^{-1} clearly showed that it is a lupane type of triterpenoid (14). Further, a triplet at δ 5.26 (J = 5.2 Hz, H-12) and the characteristic fragments at 208.1769 (fragment **i**), 248.1776 (fragment **k**), and 217.1590 (fragment **j**) resulting from the retro-Diels-Alder cleavages around ring C exhibited the double bond at C-12 (15). In view of biogenetic considerations, one of the hydroxyl functions was placed at C-3, the β orientation of which was supported by a double doublet at δ 3.19 (J_{aa} = 10.0 and J_{ae} = 4.7 Hz, H-3). That the other two hydroxyl groups are present as -CH_2OH was obvious from two sets of doublets at δ 3.32 and 3.79 (J_{gem} = 11.0 Hz) and 3.54 and 3.65 (J_{gem} = 9.4 Hz). The relationship between each pair was confirmed through COSY-45 and ^{1}H-^{1}H homonuclear decoupling experiments. Their positions at C-27 and C-28 were exhibited by fragments **a** and **c–k**, observed in hrms (Table 1). The exact assignments of H-27 (δ 3.54 and 3.65) and H-28 (δ 3.32 and 3.79) were possible through nOe difference measurements (Table 2). Thus, irradiation at δ 3.32 (H-28a) enhanced the methyl signals at δ 0.81 (H-25), 0.96 (H-26), and δ 3.79 (H-28b),

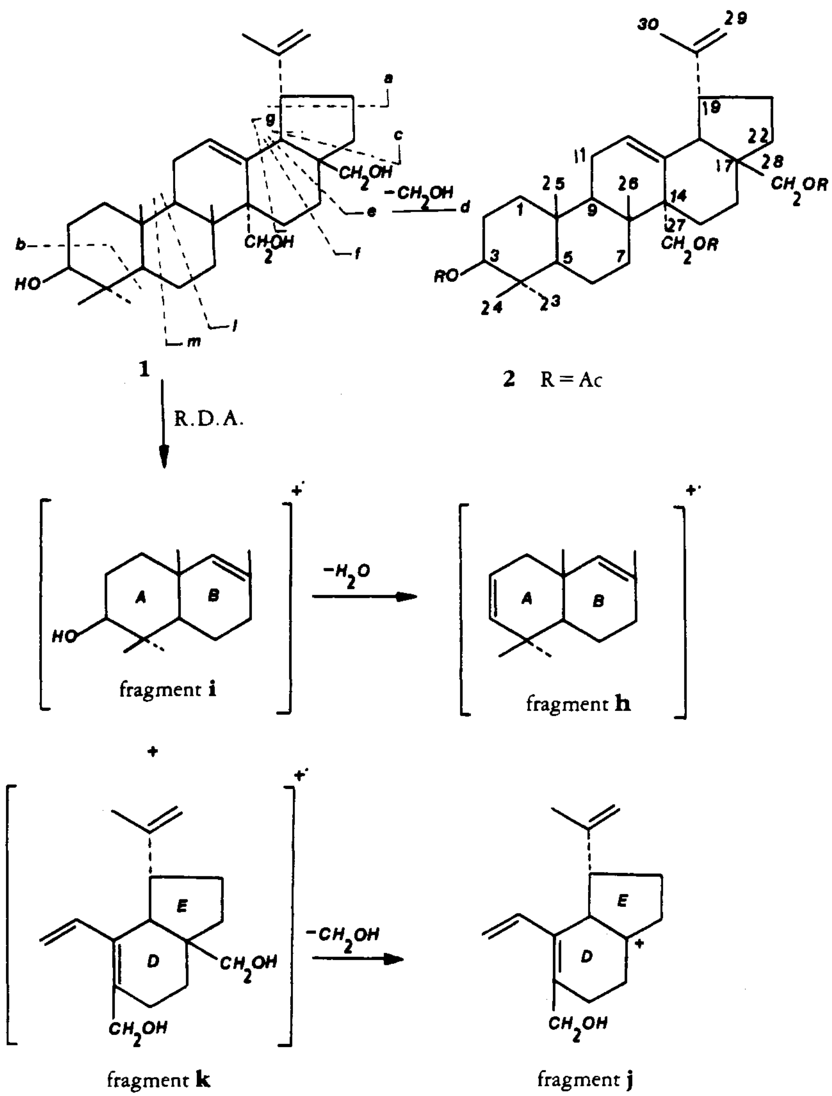


TABLE 1. Hrms Data for Oleanderol [**1**].

Fragment	Exact mass	Corresponding formula
a	68.0612	C_5H_8
b	72.0544	C_4H_8O
c	82.0763	C_6H_{10}
d	107.0879	C_8H_{11} ($C_9H_{14}O-CH_2OH$)
e	138.1044	$C_9H_{14}O$
f	152.1193	$C_{10}H_{16}O$
g	166.1366	$C_{11}H_{18}O$
h	190.1720	$C_{14}H_{22}$ ($C_{14}H_{24}O-H_2O$)
i	208.1769	$C_{14}H_{24}O$
j	217.1590	$C_{15}H_{21}O$ ($C_{16}H_{24}O_2-CH_2OH$)
k	248.1776	$C_{16}H_{24}O_2$
l	302.2287	$C_{20}H_{30}O_2$
m	316.2408	$C_{21}H_{32}O_2$
$[M]^+$	456.3587	$C_{30}H_{48}O_3$

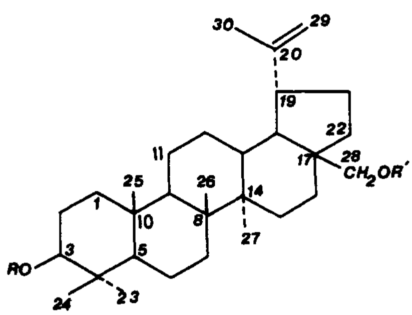
TABLE 2. The nOe Difference of Triterpenoids **1** and **3**.

Proton irradiated	nOe observed in **1**		nOe observed in **3**	
	Proton affected (δ)	nOe[a] (%)	Proton affected (δ)	nOe[a] (%)
H-3α	H-23 (0.92)	1.75	H-23 (0.92)	s
	H-27a (3.54)	1.42	H-27 (1.01)	s
	H-27b (3.65)	1.78	—	—
H-23	H-3α (3.19)	3.21	H-3α (3.20)	4.20
H-24	H-23 (0.92)	s	H-23 (0.92)	s
	H-25 (0.81)	s	H-25 (0.81)	s
	H-26 (0.96)	s	H-26 (0.96)	s
H-25	H-24 (0.74)	1.50	H-24 (0.75)	1.75
H-27	—	—	H-3α (3.20)	<1
	—	—	H-23 (0.92)	2.9
H-27a	H-3α (3.19)	2.21	—	—
	H-23 (0.92)	<1	—	—
H-28a	H-25 (0.81)	1.20	H-28b (3.78)	7.14
	H-26 (0.96)	1.65	—	—
	H-28b (3.79)	2.14	—	—
H-28b	H-28a (3.32)	2.00	H-28a (3.32)	6.50
H-29a	H-29b (4.73)	10.00	H-29b (4.72)	14.28
	H-30 (1.68)	3.21	H-30 (1.68)	4.76
H-29b	H-29a (4.60)	11.25	H-29a (4.60)	12.50
H-30	H-29a (4.60)	4.28	H-29a (4.60)	3.25

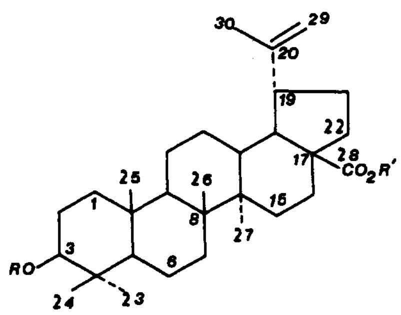
[a]s: Significant nOe; percentage could not be calculated due to the combined integration of these protons with very close chemical shifts.

whereas on irradiation at δ 3.54 (H-27a) these signals remained unaffected. In the ^{1}H-nmr spectrum of the acetyl derivative **2**, three singlets for the acetoxy methyl protons appeared at δ 2.03, 2.04, and 2.06. The carbinylic protons at δ 3.19, 3.32, 3.79, and 3.54, 3.65 shifted to δ 4.45 (dd, J_{aa} = 10.0 and J_{ae} = 4.7 Hz), 3.85, 4.20 (J_{gem} = 11.0 Hz), and δ 4.04, 4.29 (J_{gem} = 9.4 Hz), respectively. The structural assignments made above were confirmed by ^{13}C-nmr spectral data (Table 3).

These data led to the assignment of structure **1** for oleanderol [lupa-12,20(29)-dien-3β,27,28-triol]. It is interesting to note that the isolation of **1** is the first report of a lupane triterpene from the leaves of *N. oleander*. Further, the present paper describes the ^{1}H-nmr spectral data of betulin and its acetyl derivative **4**, betulinic acid and its methyl **6** and acetyl methyl **7** derivatives recorded on 300 MHz and nOe difference measurements of **3**.



3 R = R′ = H
4 R = R′ = Ac



5 R = R′ = H
6 R = H, R′ = Me
7 R = Ac, R′ = Me

TABLE 3. ^{13}C-nmr Chemical Shifts of **1**, **3**, and **5** (75 MHz), $CDCl_3$.[a]

Carbon	Compound			Carbon	Compound		
	1	**3** (9)	**5** (9)		**1**	**3**	**5**
C-1	38.7	38.8	38.7	C-16	29.7	29.3[d]	32.1
C-2	27.3	27.4[b]	27.4	C-17	48.9	47.8	56.3
C-3	79.0	79.0	78.9	C-18	49.3	47.8	46.8
C-4	38.4	38.3	38.8	C-19	46.9	48.8	49.2
C-5	55.4	55.4	55.3	C-20	151.0	150.3	150.3
C-6	18.3	18.3	18.3	C-21	30.6	29.8[d]	29.7
C-7	34.4	34.3[c]	34.3	C-22	32.2	34.0[c]	37.0
C-8	41.0	41.0	40.7	C-23	28.0	28.0	27.9
C-9	50.6	50.6	50.5	C-24	15.3	15.3	15.3
C-10	37.0	37.4	37.2	C-25	16.1[b]	16.1[e]	16.0[b]
C-11	23.4	20.9	20.8	C-26	16.1[b]	16.1[e]	16.1[b]
C-12	125.0	25.6	25.5	C-27	69.9	14.7	14.7
C-13	140.0	37.0	38.4	C-28	60.6	60.8	180.5
C-14	50.5	42.8	42.4	C-29	109.6	109.6	109.6
C-15	27.4	27.1[b]	30.5	C-30	19.4	19.4	19.4

[a]All values are in δ (ppm).
[b–e]Values in a vertical column may be interchanged.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were recorded in glass capillary tubes and are uncorrected. Ir and uv 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 focusing mass spectrometers connected to PDP 11/34 computer system. Exact masses of various fragments were obtained through their peak matchings and high resolution mass spectra. ^{1}H-nmr spectra were recorded in $CDCl_3$ on a Bruker AM 300 spectrometer operating at 300 MHz. ^{13}C-nmr (broad band and spin echo) spectra were recorded in $CDCl_3$ on a Bruker AM 300 spectrometer operating at 75 MHz, and the chemical shifts are reported in δ (ppm). The ^{13}C-nmr spectral assignments have been made partly through a comparison of the chemical shifts with the published data for similar compounds (9,12) and partly through chemical shift rules (16). Optical rotations were measured at 24° in $CHCl_3$ on a polartronic-D polarimeter. Merck Si gel 60 PF_{254} coated on glass plates was used for tlc.

PLANT MATERIAL.—Leaves of *N. oleander* were collected in July 1986, from the Karachi region and identified by Dr. S.I. Ali, Department of Botany, University of Karachi. A voucher specimen (N.OL-1) has been deposited in the Herbarium of the Botany Department, University of Karachi.

EXTRACTION AND ISOLATION.—The residue left on removal of the solvent from the combined MeOH percolates of the fresh and uncrushed leaves of *N. oleander* (10 kg) was divided into acidic and neutral fractions. The neutral, hexane-insoluble fraction was taken up in MeOH and kept cold overnight, yielding a colorless crystallizate that was filtered and ultimately identified as a mixture of ursolic and oleanolic acid. The mother liquor was subjected to preparative thick layer chromatography (Si gel, $CHCl_3$-MeOH; 9.5:0.5), and the major band (R_f 0.59) was rechromatographed on thick layer plated (Si gel; hexane-EtOAc, 9:1) to give **1** (R_f 0.44) as a pure constituent. The other diffuse band on purification through preparative thick layer chromatography (Si gel, hexane-EtOAc, 8:2) afforded **3** (40 mg) and **5** (35 mg) as uniform constituents.

PHYSICAL CONSTANTS OF OLEANDEROL [**1**].—Colorless, irregular plates (32.5 mg) ($CHCl_3$), mp 206–208°, $[\alpha]^{24}D=6.15°$ ($CHCl_3$, $c=0.3$); uv λ max (MeOH) 208 nm; ir ν max ($CHCl_3$) 3400 (-OH), 2900–2840 (C-H), 1640 (>C=C), 1150–1020 (C-O) and 880 (>C=CH_2) cm^{-1}; hrms see Table 1; ^{1}H nmr see Table 4; ^{13}C nmr see Table 3.

ACETYLATION OF **1**.—To a solution of **1** (10 mg) in pyridine (1 ml), Ac_2O (1 ml) was added, and the reaction mixture was kept for 24 h at room temperature. On usual work-up, chromatographically pure **2** was obtained as colorless, irregular plates (EtOAc), mp 260–262°; eims *m/z* $[M-60]^+$ 520 (2%), 498 (5), 466 (18), 438 (18), 423 (8), 395 (6), 216 (40), 203 (56), 189 (80), 133 (52), 119 (54), 95 (78), 81 (80), 69 (100); ir ν max ($CHCl_3$) 2900–2850 (C-H), 1720 (br), 1640 (>C=C), 1150–1000 (C-O), 880 (>C=CH_2) cm^{-1}; ^{1}H nmr see Table 4.

TABLE 4. [1]H-nmr Spectral Data of Compounds **1–7**.[a,b]

Proton	Compound						
	1	**2**	**3** (7)	**4** (8)	**5** (10)	**6**	**7** (8)
H-3α	3.19,dd	4.45,dd	3.20,dd	4.29,dd	3.19,dd	3.19,dd	4.20,dd
H-12	5.26,t	5.25,t	—	—	—	—	—
H-19	2.99,ddd	2.98,ddd	2.99,ddd	2.97,ddd	2.99,ddd	2.98,ddd	2.99,ddd
H-23	0.92,s	0.96,s	0.92,s	0.92,s	0.93,s	0.93,s	0.94,s
H-24	0.74,s	0.82,s	0.75,s	0.76,s	0.75,s	0.72,s	0.82,s
H-25	0.81,s	0.84,s	0.81,s	0.81,s	0.82,s	0.80,s	0.83,s
H-26	0.96,s	0.93,s	0.96,s	0.97,s	0.96,s	0.95,s	0.93,s
H-27	—	—	1.01,s	1.01,s	0.97,s	0.96,s	0.96,s
H-27a	3.54,d	4.04,d	—	—	—	—	—
H-27b	3.65,d	4.29,d	—	—	—	—	—
H-28a	3.32,d	3.85,d	3.32,d	4.00,d	—	—	—
H-28b	3.79,d	4.20,d	3.78,d	4.28,d	—	—	—
H-29a	4.60,d	4.60,d	4.60,d	4.67,d	4.60,d	4.59,d	4.59,d
H-29b	4.73,d	4.72,d	4.72,d	4.72,d	4.73,d	4.73,d	4.72,d
H-30	1.68,s	1.67,s	1.68,s	1.67,s	1.68,s	1.68,s	1.67,s
$OCOCH_3$	—	2.03,s 2.04,s 2.06,s	—	2.03,s (2×3H)	—	—	2.03,s
$COOCH_3$	—	—	—	—	—	3.73,s	3.73,s

[a]Multiplicities: $J_{3\alpha,2\beta}=10.0$ Hz, $J_{3\alpha,2\alpha}=4.7$ Hz, $J_{12,11\alpha}=J_{12,11\beta}=5.2$ Hz, $J_{19,18}=J_{19,21\beta}=11.0$ Hz, $J_{19,21\alpha}=5.5$ Hz, $J_{27a,27b}=9.4$ Hz, $J_{28a,28b}=11.0$ Hz, $J_{29a,29b}=1.5$ Hz.

[b]The assignments of methyls and other protons are based on COSY-45, NOESY, nOe difference (Table 2), 2D*J*-resolved spectra, and the multiplicities observed in the [1]H-nmr spectra.

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