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KANERIC ACID, A NEW TRITERPENE FROM THE LEAVES OF *NERIUM OLEANDER*

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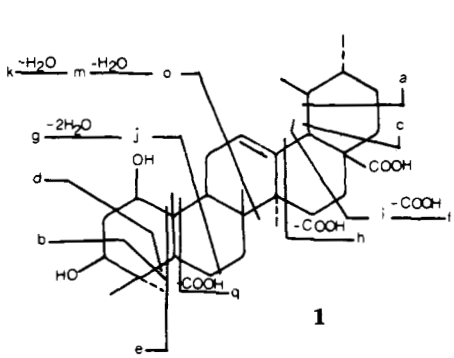
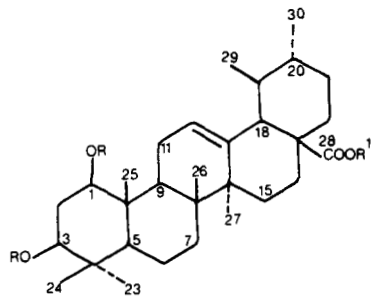
ABSTRACT.—A new triterpene, kaneric acid, along with uvaol, has been isolated from the leaves of *Nerium oleander*. The structure of kaneric acid has been established as 1β,3β-dihydroxy-urs-12-ene-28-oic acid (**1**) through chemical and spectral studies, while uvaol, which is hitherto unreported from this source, has been identified as 3β,28-dihydroxy-12-ursene (**4**).

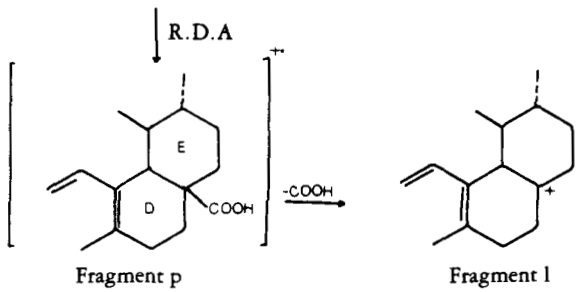
Leaves of *Nerium oleander* L. (syn. *Nerium odorum*, Apocynaceae) possess cardiotonic and antibacterial properties and are used in the treatment of swellings, leprosy, and eye and skin diseases in the indigenous system of medicine (1,2). Different parts of the plant have yielded several new cardiac glycosides and triterpenes (3,4). Studies on the constituents of fresh leaves revealed various triterpenoidal (5,6), glycosidal (7), and non-terpenoidal (8) constituents. The present paper deals with the isolation and structure elucidation of the novel compound kaneric acid (**1**) and uvaol (**4**) from the MeOH



1

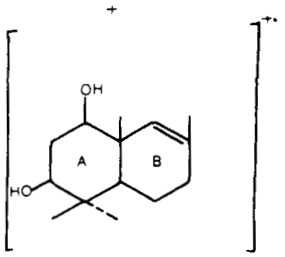


2 R=Ac, R^1=H
3 R=Ac, R^1=Me



Fragment p

Fragment l



Fragment n

extract of the fresh leaves. Uvaol (3β,28-dihydroxy-12-ursene) has previously been isolated from *Arctostaphylos uva-ursi* Spreng. (9) and characterized through ir, uv, and ms. In the present paper high resolution mass (Table 1), ^{1}H- and ^{13}C-nmr (Table 2) spectral data of **4** are also described.

TABLE 1. High Resolution Mass Spectral Data of **1** and **4**

Fragments	High resolution mass spectral data of **1**	Corresponding formulae	High resolution mass spectral data of **4**	Corresponding formulae
a	70.0830	C_5H_{10}	56.0699	C_4H_8
b	72.0559	C_4H_8O	70.0805	C_5H_{10}
c	84.0968	C_6H_{12}	72.0671	C_4H_8O
d	85.0658	$C_5H_9O(-H)$	84.0899	C_6H_{12}
e	98.0693	$C_6H_{10}O(C_6H_{12}O_2-H_2O)$	99.0833	$C_6H_{11}O(-H)$
f	123.1133	$C_9H_{15}(C_{10}H_{16}O_2-CO_2H)$	108.0913	C_8H_{12}
g	133.0981	$C_{10}H_{13}(C_{10}H_{17}O_2\text{-}2\times H_2O)$	139.1104	$C_9H_{15}O(-H)$
h	137.1288	$C_{10}H_{17}(C_{11}H_{18}O_2-CO_2H)$	153.1233	$C_{10}H_{17}O(-H)$
i	167.1067	$C_{10}H_{15}O_2(-H)$	166.1380	$C_{11}H_{18}O(-2H)$
j	169.1250	$C_{10}H_{17}O_2(-H)$	203.1770	$C_{15}H_{23}$
k	201.1548	$C_{15}H_{21}(-H)(C_{15}H_{26}O_2\text{-}2\times H_2O)$	206.1696	$C_{14}H_{22}O$
l	203.1712	$C_{15}H_{23}$	222.1954	$C_{15}H_{26}O$
m	210.1767	$C_{15}H_{23}O(-H)(C_{15}H_{26}O_2-H_2O)$	234.1959	$C_{16}H_{26}O$
n	223.1778	$C_{14}H_{24}O_2$	273.2239	$C_{19}H_{29}O(-H)$
o	239.2054	$C_{15}H_{27}O_2(+H)$	288.2239	$C_{20}H_{32}O$
p	248.1730	$C_{16}H_{24}O_2$	—	—
q	271.2291	$C_{20}H_{31}(C_{20}H_{32}O_2\text{-}CO_2H)$	—	—

Compound **1** has the molcular formula $C_{30}H_{48}O_4$ (hrms) showing seven double bond equivalents. The ^{1}H-nmr spectrum showed seven methyl signals, five as singlets at δ 0.82, 0.95, 0.96, 0.99, and 1.00, two as doublets at δ 0.84 (J=6.5 Hz) and 0.86 (J=7.0 Hz), and a one-proton multiplet at δ 5.22 for an olefinic proton. The ir spectrum showed bands at 3400 (-OH) and 1700 cm^{-1} (carbonyl of the carboxyl group), and the uv spectrum showed a band at 208 nm. In the mass spectrum, fragments were

TABLE 2. ^{13}C-nmr Chemical Shifts of **1** and **4** (75 MHz), $CDCl_3$[a]

Carbon	Compounds		Carbon	Compounds	
	1	**4**		**1**	**4**
C-1	79.0[b]	38.8	C-16	24.5	22.6
C-2	29.3	27.3	C-17	48.1	36.8
C-3	78.3[b]	79.0	C-18	52.8	54.1
C-4	38.5	38.8	C-19	39.1	38.9
C-5	55.4	55.4	C-20	39.1	39.4
C-6	18.7	18.4	C-21	30.1	30.7
C-7	33.3	32.9	C-22	36.3	30.6
C-8	39.4	39.4	C-23	28.2	28.1
C-9	47.5	47.8	C-24	15.6	15.4
C-10	37.4	37.2	C-25	17.6	15.6
C-11	24.5	23.4	C-26	16.5	16.9
C-12	128.0	125.0	C-27	23.6	23.4
C-13	138.0	138.0	C-28	177.7	69.7
C-14	42.2	42.8	C-29	17.6	16.2
C-15	28.0	29.2	C-30	21.6	21.3

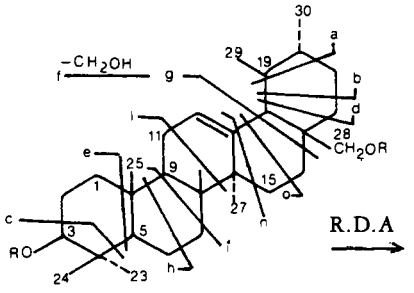
[a]All values are in (ppm).
[b]Values may be interchanged.

observed at *m/z* 248.1730 ($C_{16}H_{24}O_2$), 203.1712 ($C_{15}H_{23}$), and 133.0981 ($C_{10}H_{13}$) which are derived from the retro-Diels-Alder cleavages of Δ^{12}-amyrin skeleton (10). All these data showed that kaneric acid belongs to the ursane series of triterpenoids.

The molecular formula of **1** showed four oxygen atoms in the molecule, two of which were taken for the carboxyl group and confirmed through methylation (CH_2N_2) to **3** (δ OCH_3=3.69). The ms fragments mentioned above suggested the location of the acid group at C-17 which was supported by other important ions a-q in the hrms (Table 1). Acetylation (Ac_2O/pyridine) of **1** to the diacetyl derivative (**2**) showed that the remaining oxygen atoms are accounted for by two hydroxyl groups. The fragments at *m/z* 248.1730 and 223.1778, corresponding to $C_{16}H_{24}O_2$ and $C_{14}H_{24}O_2$ in the mass spectrum of **1**, indicated that both the hydroxyl groups are present in rings A and/or B. Their positions at C-1 and C-3 were conclusively established through ions b,d, and e observed in the high resolution mass spectrum (Table 1) and the multiplicities of the carbinylic protons. Thus, the ^{1}H-nmr spectrum showed two double doublets at δ 4.29 (J_{aa}=11.5 and J_{ae}=6.5 Hz) and δ 4.14 (J_{aa}=11.5 and J_{ae}=6.5 Hz) for H-1 and H-3, and their coupling constants corresponded to one axial-axial and one axial-equatorial coupling showing that both the hydroxyl groups are equatorial and have β-disposition.

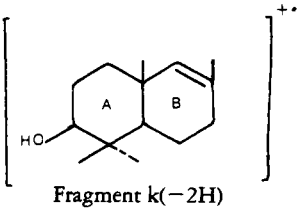
The ^{1}H-nmr spectrum of **2** showed two singlets at δ 2.05 and 2.14 for the methyl groups of the acetyl functions, while H-1 and H-3 appeared as double doublets at δ 4.59 and 4.20 (J_{aa}=11.5 and J_{ae}=6.5 Hz). In light of these observations structure **1** has been assigned to kaneric acid.

Compound **4** $C_{30}H_{50}O_2$ (hrms) showed five singlets and two doublets in the ^{1}H-nmr spectrum for seven methyls indicating its ursane-type triterpenoidal skeleton. A double bond at C-12 and a hydroxyl group at C-28 were indicated by fragments m and j (Table 1) in the mass spectrum and were supported by a signal at δ 5.12 (t, J=3.42 Hz)

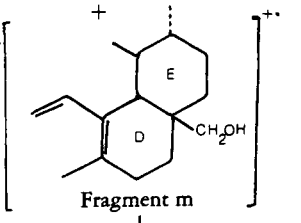


4 R=H
5 R=Ac

R.D.A

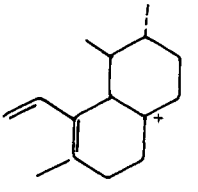


Fragment k(−2H)



Fragment m

−CH_2OH



Fragment j

and two AB doublets at δ 3.77 (H-28a) and 3.31 (H-28b) (J_{gem} = 10.7 Hz) in the ^{1}H-nmr spectrum. The second hydroxyl group, indicated by the formation of the diacetate, was placed at C-3 on biogenetic grounds and corroborated by the mass (peak at *m/z* 206.1696) and ^{1}H-nmr spectra (δ 3.18, d, J_{aa} = 10.6 and J_{ae} = 5.5 Hz). In light of these data, **4** has been identified as 3β-28-dihydroxy-12-ursene (uvaol).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were recorded in glass capillary tubes and are uncorrected. Ir and uv spectra were measured on JASCO, IRA-1, and Pye-Unicam SP-800 spectrometers. Mass spectra were recorded on Finnigan MAT 112 and MAT 312 double focusing mass spectrometers connected to a PDP 11/34 computer system. ^{1}H- and ^{13}C-nmr (broad band and spin echo) spectra were recorded in $CDCl_3$ on Bruker WP-100 SY FT-nmr and AM-300 MHz spectrometers. ^{13}C-nmr spectral assignments have been made partly through a comparison of the chemical shifts with the published data for similar compounds (11-13) and partly through the appearance of signals in the spin echo spectra. The purity of samples was checked on tlc (Silica gel, E. Merck, SIF-254 precoated aluminum cards).

PLANT MATERIAL.—Leaves of *N. oleander* were collected in July 1985 from the Karachi region, and identified by Dr. Saeeda Qureshi, 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.—Fresh, uncrushed, and undried *Nerium* leaves (7 kg) were repeatedly extracted with MeOH at room temperature, and the residue obtained from the combined methanolic extracts was divided into neutral and acidic fractions. The residue from the neutral fraction was partitioned between 80-90% MeOH and hexane/hexane-C_6H_6 (1:1) phase. The C_6H_6-soluble fraction of the residue obtained from the 90% MeOH phase was subjected to preparative thick layer chromatography, furnishing kaneroside and neriumoside (7). The combined hexane and hexane-C_6H_6 phase yielded neriumol and nerifol (8) through fractional crystallization and preparative thick layer chromatography. In another working, kanerocin (5), neriucoumaric acid (6), and isoneriucoumaric acid (6) were obtained from the hexane-insoluble neutral fraction through fractional crystallization and preparative thick layer chromatography. The residue obtained on removal of the solvent from the combined mother liquors of these constituents was divided into hexane-soluble and insoluble fractions. The hexane-soluble portion was taken in MeOH and kept at room temperature overnight, when colorless shining plates of uvaol (**4**) separated out, were filtered, and recrystallized from MeOH.

The hexane insoluble fraction was subjected to column chromatography (Si gel; Merck 70-230 mesh; 3×112 cm), and the column was eluted with hexane and hexane/EtOAc in order of increasing polarity. The fraction eluted with hexane-EtOAc (8:2) showed a major spot on tlc which was purified by preparative thick layer chromatography [Si gel; $CHCl_3$-MeOH (97:3)] yielding **1** as a colorless crystallizate.

PHYSICAL CONSTANTS OF KANERIC ACID (**1**).—Colorless, irregular plates (1.5 g) (MeOH), mp 122°, $[\alpha]^{24}D$ = 16.66° ($CHCl_3$, *c*=0.6); eims (*m/z* 472.3482 (M^+, $C_{30}H_{48}O_4$ requires 472.3552); uv λ max (MeOH) 208 nm; ir ν max ($CHCl_3$) 3400 (-OH), 3420-2500 (COOH), 2900-2840 (C-H), 1700 (carbonyl of the carboxyl group), 1640 (C=C), 1000-1150 (C-O) cm^{-1}; ^{1}H nmr (300 MHz $CDCl_3$) δ 0.82 (s), 0.84 (d, *J*=6.5 Hz), 0.86 (d, *J*=7.0 Hz), 0.95 (s), 0.96 (s), 0.99 (s), 1.00 (s), 2.12 (1H, dt, $J_{2\alpha,2\beta}$ = 16.0, $J_{2\alpha,1\alpha}$, = $J_{2\alpha,3\alpha}$ = 6.5 Hz, H-2α), 2.29 (1H, dt, $J_{2\beta,2\alpha}$ = 16.0, $J_{2\beta,1\alpha}$ = $J_{2\beta,3\alpha}$ = 11.5 Hz, H-2β), 4.14 (1H, dd, J_{aa} = 11.5 and J_{ae} = 6.5 Hz, H-3), 4.29 (1H, dd, J_{aa} = 11.5 and J_{ae} = 6.5 Hz, H-1), 5.22 (1H, m, H-12).

ACETYLATION OF **1**.—To a solution of pyridine (1 ml) and Ac_2O (1 ml), 30 mg of **1** was added and the reaction mixture kept for 24 h at room temperature. On usual work-up, chromatographically pure **2** was obtained as colorless irregular plates (EtOAc), mp 118°; eims (*m/z* 496.3550 [M^+-CH_3COOH, $C_{32}H_{48}O_4$ requires 496.3552]; ir ν max ($CHCl_3$) 2900, 2840, 1720 (br), 1645 cm^{-1}; ^{1}H nmr (300 MHz $CDCl_3$); δ 0.82 (s), 0.84 (d, *J*=6.5 Hz), 0.86 (d, *J*=7.0 Hz), 0.95 (s), 0.96 (s), 0.99 (s), 1.00 (s), 2.05 (3H, s, $COCH_3$), 2.14 (3H, s, $COCH_3$), 2.29 (1H, dt, $J_{2\alpha,2\beta}$ = 16.0 and $J_{2\alpha,1\alpha}$ = $J_{2\alpha,3\alpha}$ = 6.5 Hz, H-2α), 2.88 (1H, dt, $J_{2\beta,2\alpha}$ = 16.0 and $J_{2\alpha,1\alpha}$ = $J_{2\beta,3\alpha}$ = 11.5 Hz, H-2β), 4.20 (1H, dd, J_{aa} = 11.5 and J_{ae} = 6.5 Hz, H-3), 4.59 (1H, dd, J_{aa} = 11.5 and J_{ae} = 6.5 Hz, H-1), 5.22 (1H, m, H-12).

METHYLATION OF **2**.—To an ethereal solution of **2** (30 mg) freshly prepared CH_2N_2 was added in excess and kept at room temperature overnight. Usual work-up of the reaction mixture afforded **3**, which crystallized in colorless plates on keeping its concentrated methanolic solution in the cold; mp 115°; eims (*m/z*) 510.3700 [M^+-CH_3COOH, $C_{33}H_{50}O_4$ requires 510.3708]; ir ν max ($CHCl_3$) 2900, 2840, 1720 (br), 1645 cm^{-1}; ^{1}H nmr (300 MHz $CDCl_3$) δ 0.82 (s), 0.84 (d, *J*=6.5 Hz), 0.86 (d, *J*=7.0 Hz), 0.95

(s), 0.96 (s), 0.99 (s), 1.00 (s), 2.05 (3H, s, $COCH_3$), 2.14 (3H, s, $COCH_3$), 3.69 (3H, s, OCH_3), 2.29 (1H, dt, $J_{2\alpha,2\beta}$=16.0 and $J_{2\alpha,1\alpha}$=$J_{2\alpha,3\alpha}$=6.5, H-2α), 2.85 (1H, dt, $J_{2\beta,2\alpha}$=16.0 and $J_{2\beta,1\alpha}$=$J_{2\beta,3\alpha}$=11.5 Hz, H-2β), 4.20 (1H, dd, J_{aa}=11.5 and J_{ae}=6.5 Hz, H-3), 4.59 (1H, dd, J_{aa}=11.5 and J_{ae}=6.5 Hz, H-1), 5.22 (1H, m, H-12).

PHYSICAL CONSTANTS OF UVAOL (**4**).—Colorless plates (MeOH) (100 mg) mp 222-224°; $[\alpha]^{24}D$=+70° ($CHCl_3$, *c*=0.9); eims (*m/z*) 442.2807 (M^+, $C_{30}H_{50}O_2$ requires 442.3810); uv λ max (MeOH) 205 nm; ir ν max ($CHCl_3$) 3400 (-OH), 2900-2840 (C-H), 1640 (C=C), 1000-1150 (C-O cm^{-1}; ^1H nmr (300 MHz $CDCl_3$) δ 0.81 (s), 0.82 (d, *J*=6.5 Hz), 0.94 (d, *J*=5.1 Hz), 0.95 (s), 0.96 (s), 0.98 (s), 1.01 (s), 3.18 (1H, dd, J_{aa}=10.6 and J_{ae}=5.5 Hz, H-3), 3.31 (1H, d, J_{gem}=10.7 Hz, H-28b), 3.77 (1H, d, J_{gem}=10.7 Hz, H-28a), 5.12 (1H, t, *J*=3.42 Hz, H-12).

ACETYLATION OF **4**.—To a solution of pyridine (1 ml) and Ac_2O (1 ml) 30 mg of **4** was added, and the reaction mixture was kept for 24 h at room temperature. On usual work-up, chromatographically pure **5** was obtained as colorless plates (EtOAc) , mp 150°; eims (*m/z* 526.4020 [M^+, $C_{34}H_{54}O_4$ requires 526.4021]; ir ν max ($CHCl_3$) 2900-2840 (C-H), 1720 (ester carbonyl), 1640 (C=C) cm^{-1}; ^1H nmr (300 MHz $CDCl_3$) δ 0.84 (s), 0.97 (d, *J*=5.1 Hz), 0.92 (d, *J*=6.5 Hz), 0.96 (6H, s), 0.98 (s), 1.01 (s), 2.03 (6H, s, 2×$COCH_3$), 4.00 (1H, d, J_{gem}=10.7 Hz, H-28b), 4.28 (1H, d, J_{gem}=10.7 Hz, H-28a), 4.44 (1H, dd, J_{aa}=10.6 and J_{ae}=5.5 Hz, H-3), 5.13 (1H, t, *J*=3.42 Hz, H-12).

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