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KANERIN AND 12,13-DIHYDROURSOLIC ACID, TWO NEW PENTACYCLIC TRITERPENES FROM THE LEAVES OF *NERIUM OLEANDER*

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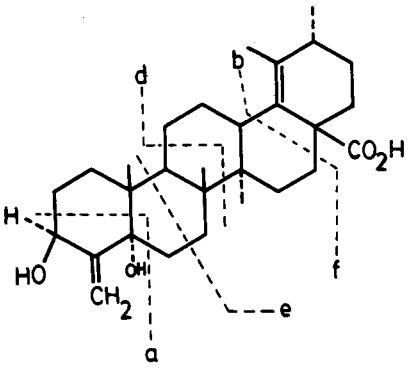
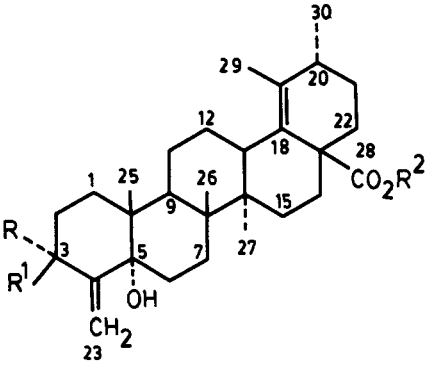
ABSTRACT.—Two new pentacyclic triterpenes, kanerin [**1**] and 12,13-dihydroursolic acid [**5**] have been isolated from the fresh, uncrushed, winter leaves of *Nerium oleander*, and their structures have been established as 24-nor-3β,5-dihydroxyursa-4(23),18-dien-28-oic acid and 3β-hydroxyursa-28-oic acid, respectively, through chemical and spectral studies.

Nerium oleander L. (syn. *Nerium odorum*, Apocynaceae), commonly known as "kaner," is distributed in the Mediterranean region and subtropical Asia and is indigenous to the Indo-Pakistan subcontinent. Its various parts are reputed to be therapeutic in the treatment of swellings, leprosy, and eye and skin diseases. The leaves also possess cardiotonic and antibacterial properties (1,2). Studies on the chemical constituents of different parts of the plant have led to the isolation of several cardiac glycosides and triterpenoids (3,4). In the course of studies on the constituents of the fresh leaves of *N. oleander*, two new cardiac glycosides and several triterpenes (5,6) have recently been reported. The present paper deals with the isolation and structure elucidation of two new,

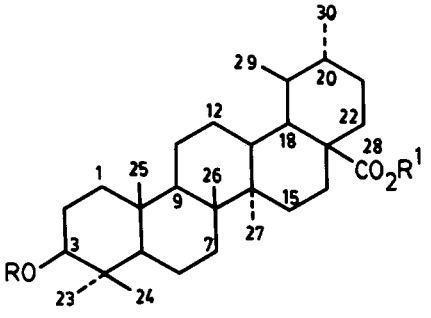


1

fragment c = fragment d − CO_2H



2 R=R^2=H, R^1=OAc
3 R=H, R^1=OAc, R^2=Me
4 R,R^1=O; R^2=H



5 R=R^1=H
6 R=Ac, R^1=H
7 R=Ac, R^1=Me

weakly acidic pentacyclic triterpenoids provisionally named as kanerin and 12,13-dihydroursolic acid. Their structures are determined as 24-nor-3β,5-dihydroxyursa-4(23),18-dien-28-oic acid [**1**] and 3β-hydroxyursa-28-oic acid [**5**], respectively, on the basis of chemical and spectral studies.

The hrms of **1** gave its molecular formula as $C_{29}H_{44}O_4$, which was confirmed by ^{13}C-nmr (broad band and gated spin echo) spectral data (Table 1) (5-Me, 11-CH_2, 4-CH, and 9C). It formed monoacetyl **2** and monomethyl **3** derivatives on reaction with Ac_2O/pyridine and CH_2N_2, respectively, showing the presence of an acetylable hydroxy function (3400 cm^{-1}) and a carboxyl group (3600–2500, 1700 cm^{-1}). The ir spectrum of **2** still showed absorption at 3550 cm^{-1}, indicating a hydroxyl function at a quaternary carbon. Appearance of five methyl groups in the ^{1}H-nmr spectrum (Table 2), four as singlets (δ 0.75, 0.82, 0.97, 1.69) and one as a doublet (δ 0.95, J = 8.0 Hz), suggested its triterpenoidal nature (7). The last signal and a fragment at *m/z* 410.2810 ($C_{27}H_{38}O_3$) resulting from the retro-Diels-Alder cleavages around ring E and subsequent loss of H_2O showed a double bond at C-18.

These features led to the conclusion that **1** is a member of the ursane series. The presence of a carboxyl group at C-17 was supported by significant fragments at *m/z* 248.1770 (fragment d), 203.1712 (fragment c), 304.2400 (fragment f), and 152.0830 (fragment b) in the mass spectrum corresponding to the formulae $C_{16}H_{24}O_2$, $C_{15}H_{23}$, $C_{20}H_{32}O_2$, and $C_9H_{12}O_2$, respectively (8). The ^{1}H-nmr spectrum further showed a double doublet at δ 4.48 for the carbinylic proton with one axial-axial (J = 10.8 Hz) and one axial-equatorial (J = 5.3 Hz) coupling. The chemical shift and the coupling constants of this proton suggested its location at C-3 with α orientation in analogy with the value of this proton in other 3β-hydroxy triterpenes of this series (9). A significant fragment at *m/z* 302.2242 (fragment e) in the mass spectrum showed that both the hydroxyl functions are in ring A and/or B. As one of the hydroxyl functions was to be located on a quaternary carbon, it was placed at C-5, and this was supported by an important fragment at *m/z* 85.0288 ($C_4H_5O_2$, fragment a) in the mass spectrum. In the ^{1}H nmr spectrum two doublets at δ 4.61 and 4.73 for the olefinic methylene protons, both with a geminal coupling constant of 1.5 Hz, suggested an exocyclic double bond, which was also indicated by the ir spectrum (880 cm^{-1}). The molecular formula and

TABLE 1. ^{13}C-nmr Spectral Data of Triterpenoid **3**, $CDCl_3$.

Carbon	Compound **3**	Carbon	Compound **3**
C-1	38.5	C-18	135.3[a]
C-2	27.9	C-19	147.4[a]
C-3	80.9	C-20	30.7
C-4	152.3	C-21	36.9
C-5	93.2	C-22	34.3
C-6	18.2	C-23	109.5
C-7	32.2	C-24	—
C-8	40.9	C-25	16.1[b]
C-9	52.5	C-26	16.4[b]
C-10	37.8	C-27	14.7
C-11	23.7	C-28	177.0
C-12	26.4	C-29	29.4
C-13	47.0	C-30	21.2
C-14	38.3	OCOMe	170.0
C-15	29.3	OCO*Me*	21.2
C-16	25.5	COO*Me*	51.1
C-17	49.6		

[a,b]Values with the same superscript may be interchanged.

TABLE 2. ^{1}H-nmr Spectral Data of Triterpenoids **1–7**.

Proton	Compound						
	1	**2**	**3**	**4**	**5**	**6**	**7**
H-3α	4.48 (dd) J_{aa} = 10.8 J_{ae} = 5.3	5.10 (dd) J_{aa} = 10.8 J_{ae} = 5.3	5.12 (dd) J_{aa} = 10.8 J_{ae} = 5.3	—	3.16 (dd) J_{aa} = 10.8 J_{ae} = 5.6	4.22 (dd) J_{aa} = 10.8 J_{ae} = 5.6	4.24 (dd) J_{aa} = 10.8 J_{ae} = 5.6
H-20β	2.98 (m)	2.97 (m)	2.99 (m)	2.99 (m)	—	—	—
H-23a	4.61 (d) J_{gem} = 1.5	4.60 (d) J_{gem} = 1.5	4.59 (d) J_{gem} = 1.5	5.83 (d) J_{gem} = 1.5	—	—	—
H-23b	4.73 (d) J_{gem} = 1.5	4.74 (d) J_{gem} = 1.5	4.72 (d) J_{gem} = 1.5	5.88 (d) J_{gem} = 1.5	—	—	—
H-23	—	—	—	—	0.97 (s)[a]	0.95 (s)[a]	0.96 (s)[a]
H-24	—	—	—	—	0.78 (s)[a]	0.83 (s)[a]	0.81 (s)[a]
H-25	0.82 (s)[a]	0.84 (s)[a]	0.83 (s)[a]	1.09 (s)[a]	0.93 (s)[a]	0.91 (s)[a]	0.91 (s)[a]
H-26	0.75 (s)[a]	0.83 (s)[a]	0.82 (s)[a]	0.84 (s)[a]	0.76 (s)[a]	0.75 (s)[a]	0.76 (s)[a]
H-27	0.97 (s)[a]	0.87 (s)[a]	0.87 (s)[a]	1.01 (s)[a]	0.98 (s)[a]	0.97 (s)[a]	0.96 (s)[a]
H-29	1.69 (s)	1.69 (s)	1.67 (s)	1.68 (s)	0.83 (d)[b] J = 7.5	0.84 (d)[b] J = 7.5	0.84 (d)[b] J = 7.5
H-30	0.95 (d) J = 8.0	0.94 (d) J = 8.0	0.96 (d) J = 8.0	0.96 (d) J = 8.0	0.89 (d)[b] J = 6.2	0.93 (d)[b] J = 6.5	0.92 (d)[b] J = 6.5
Ac	—	2.03 (s)	2.03 (s)	—	—	2.03 (s)	2.03 (s)
COOMe	—	—	3.75 (s)	—	—	—	3.73 (s)

[a,b]Values with the same superscript in a vertical column may be interchanged.

the ^{1}H-nmr spectrum indicated that one of the methyl groups of the ursane skeleton is missing, and **1** is a nortriterpenoid. This fact suggested that the exocyclic methylene group can be placed either at C-4 or C-20. However, the uv absorption (λ max 208 nm) showed that the double bonds are not conjugated. Further, a methyl group at C-4 would have resulted in a more complex pattern of H-3 rather than the double doublet observed in this case. These observations led to the placement of this double bond at C-4 (C-23), which was supported by oxidation of **1** to **4**. The uv spectrum of **4** gave absorption at 232 nm, while in the ^{1}H-nmr spectrum the signals for olefinic methylene protons shifted to δ 5.83 and 5.88. Supporting evidence was obtained from the ^{13}C-nmr spectrum in which C-3 (δ 80.9) and C-5 (δ 93.2) appeared relatively downfield, being allylic to the double bond at C-4 (C-23) (10). In the light of these observations kanerin has been assigned structure **1**.

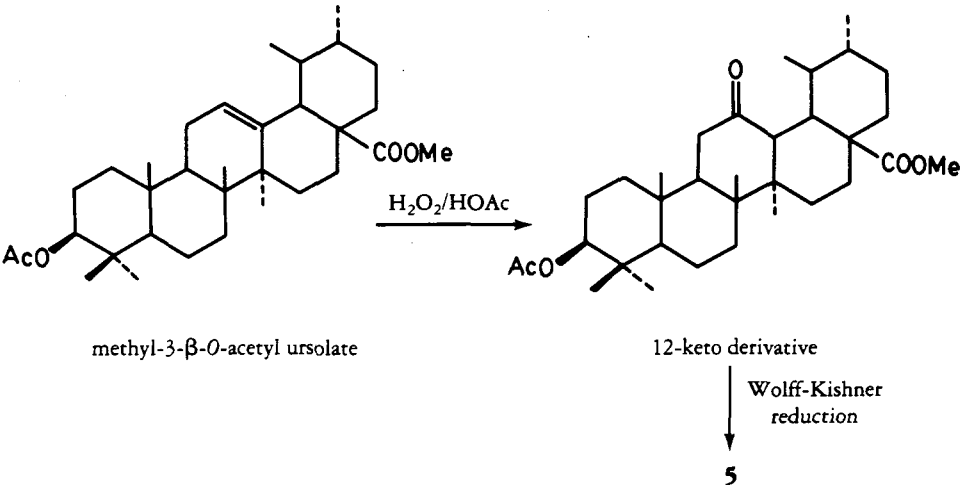
It is interesting to note that **1** is the first naturally occurring 24-nor triterpenoid of the ursane series with a double bond at C-4 (C-23). Prior to this report, only one 24-nor triterpenoid of this series, namely methyl-3-oxo-24-norurs-12-en-28-oate, has been isolated from a natural source (11). This compound was obtained after saponification of the triterpenoid fraction with methanolic KOH followed by methylation, and it was later suggested to be an artifact derived from either the corresponding 23-hydroxy-3-oxo or 24-hydroxy-3-oxo compound (12), both of which undergo formaldehyde elimination reaction under the conditions used. It has also been noted that the nor compounds isolated after the acidic hydrolysis of saponins are artifacts, formed by a reverse Prins reaction during hydrolysis (13). However, as **1** has been isolated through solvent separation and preparative tlc under mild conditions without involving hydrolysis (acidic or basic) and was also detected in the extract of fresh leaves, it is regarded as a naturally occurring compound. Moreover, **1** is also the first constituent of this series with a hydroxyl function at C-5 as well as the first naturally occurring ursolic acid derivative having a double bond at C-18.

The biosynthesis of **1** has not yet been investigated but might involve a biogenetic reverse Prins reaction as above, followed by reduction or, alternatively, the decarboxy-

lation/dehydration of a 4-carboxy-4-hydroxymethyl grouping. The latter has been encountered, e.g., in platycogenic acid (14).

Compound **5** has the molecular formula $C_{30}H_{50}O_3$ (hrms) showing six double bond equivalents in the molecule. The ^{1}H-nmr spectrum showed seven methyl signals, five as singlets at δ 0.76, 0.78, 0.93, 0.97, and 0.98 and two as doublets at δ 0.83 (J = 7.5 Hz) and 0.89 (J = 6.2 Hz), indicating the pentacyclic triterpene skeleton of the ursane series (7). The only double bond equivalent left was taken for the carbonyl function of the carboxyl group (1710 cm^{-1}), the presence of which was confirmed through the methylation (CH_2N_2) of the acetyl derivative **6** (δ Ac 2.03, δ H-3 4.22) to **7** (δ OMe 3.73). The ir spectrum further showed a hydroxyl function (3450 cm^{-1}), which was placed at C-3 on biogenetic grounds. In the ^{1}H-nmr spectrum, H-3 appeared as a double doublet at δ 3.16 (J_{aa} = 10.8 and J_{ae} = 5.6 Hz), thus corroborating its location at C-3 with β disposition (9). Further, the absence of an olefinic function in the ^{1}H-nmr and ir spectra and the lack of the characteristic fragmentation of the Δ^{12}-amyrin skeleton in the mass spectrum (8) led to the assignment of structure of **5** as 12,13-dihydroursolic acid, which was conclusively established through its partial synthesis. Thus, oxidation (H_2O_2/HOAc) of methyl-3β-*O*-acetyl ursolate, obtained from the same source as described earlier (15), furnished the corresponding 12-keto derivative which on Wolff-Kishner reduction yielded **5**, the ester groups being also hydrolyzed under the basic condition of the reaction (Scheme 1).

It may be noted that there is no earlier reference to the isolation or synthesis of dihydroursolic acid.



SCHEME 1

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were recorded in glass capillary tubes and are uncorrected. Ir (in $CHCl_3$) and uv (in MeOH) spectra were measured on JASCO IRA-I and Pye-Unicam SP-800 spectrometers, respectively; mass spectra were recorded on Finnigan MAT 112 and 312 double focusing mass spectrometers connected to a PDP 11/34 computer system. Exact masses of various fragments were obtained through their peak matchings. Nmr spectra were recorded in $CDCl_3$ on a Bruker AM 300 spectrometer, operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C nuclei. 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 (10, 16–18) and partly through the appearance of signals in gaspe spectrum. 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* (7 kg) were collected in October 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* was divided into acidic and neutral fractions. The neutral fraction (98 g, 2.81% on dry wt basis) was taken up in 90% MeOH and successively extracted with hexane and hexane-C_6H_6 (1:1). The residue obtained from the aqueous MeOH phase after usual workup was taken up in C_6H_6 and the solution treated with a little hexane. A small darkish precipitate was filtered off and the filtrate freed of the solvent under reduced pressure. The light yellow powdery residue on preparative tlc with C_6H_6-EtOAc (8:2) gave kaneroside and neriumoside as communicated earlier (5), along with a band which on further separation through preparative tlc with C_6H_6-EtOAc (9.5:0.5), furnished **1** and **5** as homogeneous constituents.

PHYSICAL CONSTANTS OF KANERIN [**1**].—Compound **1** crystallized from MeOH as irregular plates (500 mg, 0.51% of the wt of total neutral fraction), mp 280–281°; $[\alpha]_D$ 14.28° ($CHCl_3$, $c = 0.14$); uv λ max 208 nm; ir ν max 3400 (-OH), 3600–2500 (COOH), 2900–2840 (C-H), 1700 (>C=O of -COOH), 1640 (C=C), 1150–1000 (C-O), 880 cm^{-1} (>C=CH_2); eims *m/z* $[M]^+$ 456.3230 (12%), ($C_{29}H_{44}O_4$ requires 456.3239), $[M-H_2O]^+$ 438.3218 (8), $[M-33]^+$ 423 (7), 410.2810 (5) ($C_{27}H_{38}O_3$), $[M-33-14]^+$ 409 (5), $[M-33-28]^+$ 395 (6), 304.2400 (4) ($C_{20}H_{32}O_2$, fragment f), 302.2242 (5) ($C_{20}H_{30}O_2$, fragment e), 248.1770 (42) ($C_{16}H_{24}O_2$, fragment d), 208 (44), 203.1712 (50) ($C_{15}H_{23}$, fragment c), 189.1635 (100) ($C_{14}H_{21}$), 152.0830 (12) ($C_9H_{12}O_2$, fragment b), 133.0981 (50) ($C_{10}H_{13}$), 85.0288 (2) ($C_4H_5O_2$, fragment a).

ACETYLATION OF **1**.—To a solution of **1** (20 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 workup, chromatographically pure **2** was obtained: colorless, fine needles from EtOAc, mp 258–259°, ir ν max 3550, 2900–2840, 1720 (br), 1645, 885 cm^{-1}; eims *m/z* $[M]^+$ 498.3343 (4%) ($C_{31}H_{46}O_5$ requires 498.3345).

METHYLATION OF **2**.—To an Et_2O solution of **2** (20 mg), freshly prepared CH_2N_2 in Et_2O was added in excess, and the mixture was kept at room temperature overnight. Usual workup of the reaction mixture afforded **3**, which crystallized in slender rods on keeping its concentrated MeOH solution in the cold: mp 250–252°; ir ν max 3450, 2950–2830, 1720 (br), 1650, 885 cm^{-1}; eims *m/z* $[M]^+$ 512.3500 (3%) ($C_{32}H_{48}O_5$ requires 512.3501).

SARETT OXIDATION OF **1**.—A solution of **1** (10 mg) in pyridine was added to a slurry of CrO_3 (10 mg) and pyridine (1 ml) and stirred for 4 h at room temperature. The ketone **4**, obtained on workup (19), formed fine needles from EtOAc, mp 268–269°, $[\alpha]_D$ 24.3° ($CHCl_3$, $c = 0.04$); uv λ max 232 nm; ir ν max 3480, 3600–2500, 2900–2850, 1710, 1680, 1645, 1625, 1150–1050, 880 cm^{-1}; eims *m/z* $[M]^+$ 454.3082 (4%) ($C_{29}H_{42}O_4$ requires 454.3082), $[M-45]^+$ 409 (12), 248 (20), 218 (26), 203 (28), 189 (36), 69 (100).

PHYSICAL CONSTANTS OF 12,13-DIHYDROURSOLIC ACID [**5**].—Compound **5** crystallized from $CHCl_3$ as rods (450 mg, 0.46% of the wt of total neutral fraction), mp 150–152°, $[\alpha]_D$ 6.0° ($CHCl_3$, $c = 1.0$); uv λ max 208 nm; ir ν max 3450 (-OH), 3420–2500 (COOH), 2920–2860 (C-H), 1710 (>C=O of -COOH), 1150–1050 cm^{-1} (C-O); eims *m/z* $[M]^+$ 458.3747 (15%) ($C_{30}H_{50}O_3$ requires 458.3759), 205 (5), 189 (15), 135 (20), 97 (40), 57 (100).

ACETYLATION OF **5**.—Compound **5** (20 mg) was acetylated in the manner described in the case of **1** to yield **6**, which crystallized from EtOAc as colorless plates, mp 221–222°, ir ν max 3460–2500, 2900–2850, 1720 (br), 1150–1000 cm^{-1}; eims *m/z* $[M]^+$ 500.3860 (1%) ($C_{32}H_{52}O_4$ requires 500.3865).

METHYLATION OF **6**.—Compound **6** (20 mg) was methylated following the procedure described in the case of **2** to yield **7**, which crystallized from $CHCl_3$ as fine needles, mp 218–220°; ir ν max 2900–2840, 1720 (br), 1150–1020 cm^{-1}; eims *m/z* $[M]^+$ 514.4020 (2%) ($C_{33}H_{54}O_4$ requires 514.4021).

REACTION OF METHYL-3-β-*O*-ACETYL URSOLATE WITH H_2O_2/HOAc (20).—Methyl-3-β-*O*-acetyl ursolate (100 mg) in 40 ml of glacial HOAc was treated dropwise at 100° with a mixture of 4 ml of HOAc and 2 ml of H_2O_2 and was heated for 6 h. On dilution with H_2O, an insoluble mass separated out which was filtered and purified through flash chromatography ($CHCl_3$). The pure keto derivative (60 mg) was obtained as fine needles, mp 252–253°; ms *m/z* $[M]^+$ 528.

WOLFF-KISHNER REDUCTION OF KETONE DERIVATIVE.—The ketone (20 mg) obtained above was reduced with 50 mg of Na (dissolved in 2 ml of EtOH and 1 ml of anhydrous hydrazine) for 15 h at 180°. The reaction product (12 mg) obtained on usual workup was found to be identical with **5** on comparison by mp, mmp, and spectral data.

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