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TRITERPENOIDS FROM THE LEAVES OF *NERIUM OLEANDER*

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Key Word Index—*Nerium oleander*; Apocynaceae; pentacyclic triterpenes.

Abstract—Two new triterpenoids have been isolated from the fresh, uncrushed leaves of *Nerium oleander* and their structures elucidated as 3β,27-dihydroxy-urs-18-en-13,28-olide and 3β,22α,28-trihydroxy-25-nor-lup-1 (10),20(29)-dien-2-one. Elucidation of the structures was based on spectroscopic methods including one-dimensional and two-dimensional NMR (COSY-45, NOESY and *J*-resolved). Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Nerium oleander L. (Syn. *N. odorum* Soland; *N. indicum* Mill) commonly known as 'kaner', is a native of the Indo-Pakistan subcontinent, widely distributed in the Mediterranean region and sub-tropical Asia, and is also cultivated elsewhere [1–5]. It is a poisonous plant, used with caution medicinally from South China to South-East India. Recent investigations on this plant have revealed the presence of several glycosides, triterpenes and straight chain compounds [6–12]. Several triterpenoids have previously been reported by this group, namely kaneric acid [13], nericoumaric acid, isoneriucoumaric acid [14], oleanderol [15], oleanderen [16], kanerodione, oleanderolic acid [17], kanerin, 12,13-dihydroursolic acid [18] and kanerocin [19]. We now report on the isolation of two new pentacyclic triterpenes, neriumin and neriuminin, characterized respectively as 3β,27-dihydroxy-urs-18-en-13, 28-olide (**1**) and 3β,22α,28-trihydroxy-lup-1 (10), 20 (29)-dien-2-one (**2**).

RESULTS AND DISCUSSION

The HREI-mass spectrum of neriumin (**1**) showed the $[M]^+$ peak at *m/z* 470.3374 corresponding to the formula $C_{30}H_{46}O_4$. Its triterpenoidal nature was indicated by the molecular formula, the presence of six methyl signals in the ^1H NMR spectrum and the other spectral data discussed below. The IR spectrum showed absorptions suggesting the presence of a five-membered lactone ring (1760 cm^{-1}) and double bonds (1570 cm^{-1}). The molecular formula showed eight double bond equivalents, five of which were accounted for by the pentacyclic ring nucleus, two by the lactone ring and one by a C=C. These features suggested that the remaining oxygen atoms are present as hydroxyl groups. The ^1H NMR spectrum (Table 1) showed a double doublet at δ 3.18 (J = 11.4 and 4.8 Hz) attributable to the proton geminal to the hydroxyl group placed at C-3 on biogenetic consideration [20]. The coupling constants and the chemical shifts [21] of H-3 favoured the β-orientation (equatorial) of the hydroxyl group at C-3. The ^1H NMR spectrum further showed two AB doublets at δ 3.73 and 3.55 each with a geminal coupling of 11.0 Hz, indicating a methylene carbon linked to an oxygen function. Six methyl signals were also observed in the ^1H NMR spectrum, five as singlets at δ 1.88, 0.99, 0.97, 0.94 and 0.78 and one as a doublet at δ 1.05 (J = 7.1 Hz). These data indicated that compound **1** belonged to the ursane series of triterpenes, with two methyls functionalized, one involved in the lactone ring and the other forming a hydroxymethyl function. The hydroxyl group placed at C-3 was further supported by mass spectral fragment a which showed that one hydroxyl group is located in ring A/B. Conversely, this fragment and fragments b and c (see **1**) exemplified the lactone ring and the remaining hydroxyl groups in rings C/D. The lactone ring between C-13 and C-28, and the hydroxyl group at

Table 1. ^1H NMR chemical shifts and coupling constants [δ (ppm) in $CDCl_3$ (500 MHz)] of compound **1**

H-3	3.18	*dd* (J = 11.4, 4.8 Hz)
H_3-23	0.94*	*s*
H_3-24	0.97*	*s*
H_3-25	0.99*	*s*
H_3-26	0.78*	*s*
H-27a	3.55	*d* (J = 11.0 Hz)
H-27b	3.73	*d* (J = 11.0 Hz)
H_2-29	1.88	*s*
H_3-30	1.05	*d* (J = 7.1 Hz)

*Assignments may be interchanged.

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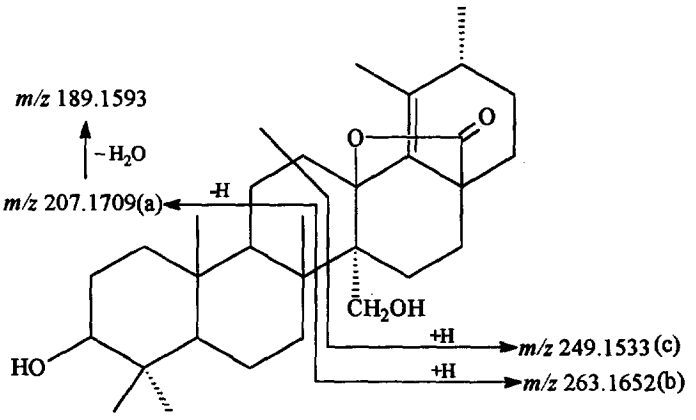


1

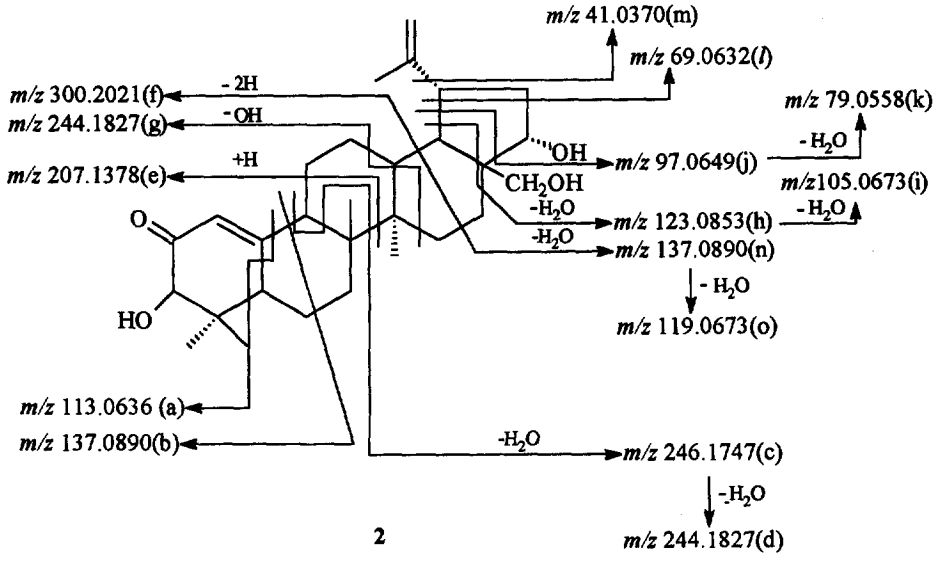
C-27 were supported by the NOESY interaction between H-27a and H-27b with H-30. Finally, the tetra-substituted double bond was placed at C-18 since the ^{1}H NMR spectrum did not show any olefinic proton and instead showed a downfield methyl singlet (δ 1.88). In the light of these observations, the structure of compound **1** has been established as 3β,27-dihydroxy-urs-18-en-13,28-olide. The ^{13}C NMR data (Table 2) are consistent with the structure. The assigments of these shifts are based on comparison with those of similar compounds [22–26].

Neriuminin (**2**) was assigned the molecular formula $C_{29}H_{44}O_4$ (HR-mass spectrometry, $[M]^+ = m/z$ 456.3239). The UV spectrum exhibited absorptions at 237.2, 213.4 and 202.2 nm while the IR spectrum contained bands at 3300 (OH), 2900 (C-H), 1660 (conjugated C=O), 1600 (C=C) and 1140 cm^{-1} (C–O). The ^{1}H NMR spectrum (Table 3) showed five methyl

Table 2. ^{13}C NMR spectral data of compounds **1** and **2** (in $CDCl_3$, TMS as int. standard)

C	1	2	C	1	2
1	37.7	125.0	16	25.7	25.6
2	27.3	203.0	17	48.2	50.8
3	78.9	82.0	18	136.7	47.8
4	38.8	48.0	19	N.O.	49.1
5	55.2	42.5	20	37.1	150.2
6	18.2	18.5	21	30.7	40.8
7	33.1	32.5	22	31.4	75.5
8	38.1	41.3	23	28.1	29.2
9	47.6	43.0	24	15.5	15.8
10	36.4	165.0	25	16.1	–
11	23.8	20.5	26	17.1	16.2
12	28.1	25.5	27	62.5	14.9
13	88.1	26.8	28	178.9	65.5
14	46.8	42.0	29	19.5	109.5
15	26.7	26.8	30	20.7	19.5

N.O.: not observed.



2

Table 3. ^{1}H NMR chemical shifts and coupling constants of compound **2**

H-1	5.73	*s*
H-3	4.02	*s*
H-5	1.55	*dd* (J = 10.0, 3.0 Hz)
H-19	2.96	*m*
H-22	3.11	*dd* (J = 11.5, 4.9 Hz)
H_3-23	0.93*	*s*
H_3-24	0.82*	*s*
H_3-26	0.98*	*s*
H_3-27	1.15*	*s*
H-28a	3.55	*d* (J = 9.8 Hz)
H-28b	3.50	*d* (J = 9.5 Hz)
H-29a	4.68	*m*
H-29b	4.54	*m*
H_3-30	1.67	*s*

Coupling constants were calculated from ^{1}H NMR and two-dimensional J-resolved spectra.

*Assignments may be interchanged within a vertical column.

singlets at δ 0.93, 0.82, 0.98, 1.15 (H-23, H-24, H-26, H-27) and 1.67 (H-30). The signal at δ 1.67 appeared as a broad singlet which along with the two one-proton multiplets at δ 4.68 (H-29a) and 4.54 (H-29b) indicated an isopropylene group in a lupane type skeleton [27]. The molecular formula of compound **2** showed that it is a nor-triterpenoid. Two one-proton mutually coupled (COSY-45) doublets at δ 3.55 (J_{gem} = 9.8 Hz, H-28 a) and δ 3.50 (J_{gem} = 9.8 Hz, H-28b) in the ^{1}H NMR spectrum and clearly identified in the two-dimensional J-resolved spectrum established the presence of an hydroxymethyl group. The fragments e–j (see **2**) showed that the methylene carbon bearing the hydroxyl group is C-28 while another hydroxyl group is at C-22 (δ_H 3.11, *dd*, J = 11.5 and 4.9 Hz), and that the remaining two oxygens are in ring A/B. An interaction between the protons at δ 3.11 (H-22) and δ 2.96 (H-19) in the NOESY plot demonstrated that these lie in one plane, i.e. β, and hence the hydroxyl group at C-22 is α. One oxygen was placed at C-3 as a hydroxyl group on biogenetic grounds [20] and the chemical shift and singlet of H-3 demonstrated that the carbonyl group is at C-2 conjugated with a C=C at C-1 (C-10) since H-1 appeared as a singlet at δ 5.73. This was substantiated by the fragment a at m/z 113.0636 ($C_6H_9O_2$). In the NOESY plot an interaction was observed between H-3 and H-5 α at δ 1.55 (*dd*, J = 10.0, 3.0 Hz) displaying the β-orientation of the hydroxyl group at C-3. In the light of these data, the structure of **2** has been determined as 3β,22α-28-trihydroxy-25-nor-lup-1(10),20(29)-dien-2-one which was substantiated by various mass fragments (see **2**) and ^{13}C NMR assignments (Table 2) which were made through comparison with those of compounds having similar structures [28, 29].

EXPERIMENTAL

General. UV: MeOH; IR: $CHCl_3$; EIMS: 70 eV; HRMS: 70 eV. ^{1}H NMR. 500 MHz with $CDCl_3$ as solvent and spectra referenced to residual solvent signals. ^{13}C NMR: 125 MHz for compound **1** and 100 MHz for compound **2** with $CDCl_3$ as solvent. Unambiguous assignment of proton chemical shifts of structure **2** were made through COSY-45 and NOESY expts. TLC: silica gel PF_{254}. Flash CC: silica gel 9385. The leaves of *N. oleander* were collected from the Karachi region and were identified by Dr S. I. Ali, Department of Botany, University of Karachi. A voucher specimen (No. 1-1) has been deposited in the Herbarium of the Botany Department, University of Karachi.

Extraction and isolation. Fresh and uncrushed leaves (40 kg) were extracted with MeOH at room temp. The concentrated syrupy residue obtained on removal of the solvent from the combined extracts under red. pres. was shaken out with EtOAc and H_2O. The EtOAc layer was extracted with 4% aq. Na_2CO_3 soln to separate the acidic from the neutral fr. The EtOAc layer containing the neutral fr. was washed, dried over anhydrous Na_2SO_4 and passed through charcoal. The charcoal bed was washed successively with EtOAc and MeOH–benzene (1:1). The solvent from the combined EtOAc filtrate and washings was evapd and the fr. was designated N-1 (390 g). The residue from the MeOH–benzene eluate was designated N-2 (22 g). N-1 was divided into petrol-soluble (NPE) (142 g) and insoluble (NIPE) (248 g) frs. The petrol-insoluble fr. was dissolved in a minimum quantity of MeOH and kept cold overnight. A white crystalline residue settled out which was filtered and the filtrate was again kept for crystallization. Several crystalline crops thus obtained were combined and recrystallized from the same solvent. The flowers of needles ultimately obtained were identified as ursolic acid (114 g) [30, 31]. The residue (134 g) from the filtrate was again treated with petrol to give petrol-soluble (B-3) and insoluble (B-2) frs. B-3 (30 g) was subjected to further sepn using VLC (petrol-EtOAc, followed by $CHCl_3$–MeOH, in order of increasing polarity). On combining the eluates on the basis of TLC, four frs (Fr-1 to Fr-4) were ultimately obtained. The main fr., Fr-1 (14 g), was subjected to further purification through VLC (petrol–EtOAc, in increasing order of polarity) which yielded nine frs (Fr-1-I to Fr-1-IX) on pooling together the eluates on the basis of TLC. Fr-1-VI (4.2 g) was further purified through FCC ($CHCl_3$–MeOH, increasing order of polarity). On usual follow up 11 fractions (Fr-1-VI-1 to Fr-1-VI-11) were ultimately obtained. Fr-1-VI-1 (300 mg) showed five spots on TLC ($CHCl_3$–MeOH, 39:1) which were sepd into five bands (Fr-1-VI-1-1 to Fr-1-VI-1-5) by means of thick-layer chromatography. Fr. Fr-1-VI-1-2 (35.9 mg) was the major fraction and was resolved on silica cards precoated on aluminium sheets with $CHCl_3$–MeOH (49:1) into three components. The middle band (Fr-1-VI-1-2-2; 4.3 mg) consisted of a single compound charcaterized as 3β,27-dihydroxy-urs-18-en-13,28-olide (**1**).

Fractions Fr-1-VI-2 and Fr-1-VI-3 from FCC showed almost similar TLC spectra. These were combined and

taken up in a small quantity of $CHCl_3$–MeOH (1:1) and kept at room temp. overnight. A crystallizate separated out which was filtered and recrystallized from the solvent mixt. to give pure oleanolic acid. The mother liquor showed six spots (Fr-1-VI-2-1 to Fr-1-VI-2-6) on TLC ($CHCl_3$-MeOH, 49:1), the main band (Fr-1-VI-2-3; 89.8 mg) was further purified by TLC ($CHCl_3$–MeOH, 49:1) ultimately yielding compound **2** (6.5 mg) which was characterized as 3β,22α-28-trihydroxy-lup-1(10),20(29)-dien-2-one.

Neriumin (**1**). Rectangular plates (4.3 mg), mp 165–166°. HR-MS *m/z*: 470.3374 ($C_{30}H_{46}O_4$; calcd 470.3396) $[M]^+$, 452.3283 ($C_{30}H_{44}O_3$) $[M-H_2O]^+$, 263.1652 ($C_{16}H_{23}O_3$, fragment b), 249.1533 ($C_{15}H_{21}O_3$, fragment c), 235.1749 ($C_{16}H_{23}O_2$) [fragment b $-CO_2$], 220.1805 ($C_{15}H_{24}O$) [fragment b $-CO_2+H$], 207.1709 ($C_{14}H_{23}O$, fragment a), 205.1593 ($C_{14}H_{21}O$) [fragment c $-CO_2]^+$, 189.1593 ($C_{14}H_{21}$) [fragment a $-H_2O]^+$, 187.1457 ($C_{14}H_{19}$) [fragment d $-H_2O]^+$, 69.0694 (C_5H_9); UV λ_{max}^{MeOH} nm: 204.6; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 2980, 1760 and 1570 cm^{-1}; ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Neriuminin (**2**). Small rods (6.5 mg), mp 169-170°. HR-MS *m/z*: 456.3239 ($C_{29}H_{44}O_4$; calculated 456.3239) $[M]^+$, 300.2029 ($C_{20}H_{28}O_2$), 246.1747 ($C_{17}H_{26}O$), 244.1827 ($C_{17}H_{24}O$), 207.1378 ($C_{13}H_{19}O_2$), 137.0890 ($C_9H_{13}O$), 123.0853 ($C_8H_{11}O$), 119.0855 (C_9H_{11}), 113.0636 ($C_6H_9O_2$), 105.0673 (C_8H_9), 97.0649 (C_6H_9O), 79.0558 (C_6H_7), 69.0632 (C_5H_9), 41.0370 (C_3H_5); UV λ_{max}^{MeOH} nm: 237.2, 213.4; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300, 2900, 1660, and 1440 and 1140; ^1H NMR: Table 3; ^{13}C NMR: Table 2.

REFERENCES

1. Nasir, E. and Ali, S. I. (1982) *Flora of West Pakistan*, Vol. 148, p. 19. Pakistan Agriculture Research Council, Islamabad.
2. Dymock, W., Warden, C. J. H. and Hooper, D. (1891) *Pharmacographia Indica*, Vol. II, p. 398. The Institute of Health and Tibbi Research (republished under the auspices of Hamdard National Foundation of Pakistan) Karachi, Pakistan.
3. Chopra, R. N., Nayar, S. L. and Chopra, I. C. (1956) *Glossary of Indian Medicinal Plants*, p. 175. Council of Scientific and Industrial Research, New Delhi.
4. Manjunath, B. L. (1966) *The Wealth of India*, Vol. VII, p. 15. Council of Scientific Industrial Research, New Delhi.
5. Nadkarni, K. M. (1976) *The Indian Materia Medica*, Vol. I, p. 847 (revised by A. K. Nadkarni). Popular Prakashan Ltd, Bombay.
6. Yamauchi, T., Takata, N. and Mimusa, T. (1975) *Phytochemistry* **14**, 1379.
7. Yamauchi, T., Abe, F., Ogata, Y. and Takahashi, M. (1974) *Chem. Pharm. Bull.* **22**, 1680.
8. Rittel, W. and Reichstein, T. (1954) *Helv. Chim. Acta* **37**, 1361.
9. Chen, K. K., Henderson, F. G. and Anderson, R. C. (1951) *J. Pharmacol. Exptl Therap.* **103**, 420.
10. Rittel, W. and Reichtein, T. (1953) *Helv. Chim. Acta* **36**, 787.
11. Fauconnet, L. and Pouly, P. L. (1962) *Pharm. Acta Helv.* **37**, 301.
12. Siddiqui, S., Siddiqui, B. S., Begum, S. and Hafeez, F. (1990) *Pak. J. Sci. Ind. Res.* **33**, 127.
13. Siddiqui, S., Hafeez, F., Begum, S. and Siddiqui, B. S. (1986) *J. Nat. Prod.* **49**, 1086.
14. Siddiqui, S., Siddiqui, B. S., Hafeez, F. and Begum, S. (1987) *Planta Med.* **53**, 424.
15. Siddiqui, S., Hafeez, F., Begum, S. and Siddiqui, B. S. (1988) *J. Nat. Prod.* **51**, 229.
16. Siddiqui, S., Siddiqui, B. S., Hafeez, F. and Begum, S. (1988) *Planta Med.* **54**, 232.
17. Siddiqui, S., Begum, S., Hafeez, F. and Siddiqui, B. S. (1989) *Phytochemistry* **28**, 1187.
18. Siddiqui, S., Begum, S., Siddiqui, B. S. and Hafeez, F. (1989) *J. Nat. Prod.* **52**, 57.
19. Siddiqui, S., Siddiqui, B. S., Begum, S. and Hafeez, F. (1989) *Planta Med.* **55**, 292.
20. Kojima, H. and Ogura, H. (1989) *Phytochemistry* **28**, 1703.
21. Siddiqui, S., Siddiqui, B. S, Naeed, A., Begum, S. (1989) *Phytochemistry* **28**, 3143.
22. Kang, S. S. (1987) *Kor. J. Pharmacogn.* **18**, 151.
23. Siddiqui, S., Siddiqui, B. S., Naeed, A. and Begum, S. (1990) *J. Nat. Prod.* **53**, 1332.
24. Numata, A., Takahashi, C., Miyamoto, T., Yoneda, M. and Yang, P. (1990) *Chem. Pharm. Bull.* **38**, 942.
25. Siddiqui, S., Siddiqui, B. S., Naeed, A. and Begum, S. (1992) *Phytochemistry* **31**, 4279.
26. Begum, S., Adil, Q., Siddiqui, B. S. and Siddiqui, S. (1993) *J. Nat. Prod.* **56**, 613.
27. Cheung, H. T. and Williamson, G. D. (1969) *Tetrahedron* **25**, 119.
28. Sholichin, M., Yamasaki, K., Kasai, R. and Tanak, O. (1980) *Chem. Pharm. Bull.* **28**, 1006.
29. Wehrli, F. W. and Nishida, T. (1979) in *Progress in the Chemistry of Organic Natural Products*, Vol. 36. *The Use of Carbon-13 Nuclear Magnetic Reasonance Spectroscopy in Natural Products Chemistry* (Herz, W., Grisebach, H. and Kirby, G. W., eds), pp. 80, 100–101. Springer, Wien.
30. Yamaguchi, K. (1970) *Spectral Data of Natural Products*, Vol. 1, p. 158. Elsevier, Amsterdam.
31. Seo, S., Tomita, Y. and Tori, K. (1975) *Tetrahedron Letters* 7.