A number of secondary metabolites have been reported from the genus *Nerium*. Various triterpenoids, cardiac glycosides and bioactive pregnanes are isolated and characterized from the title plant. 4-Oxooctyl-2-hydroxyundecanoate \(1\), heptacosane-3-enyl-5-hydroxyhexanoate \(2\), and three known compounds, betulin \(3\), betulinic acid \(4\), and stigmasterol \(5\), and have been isolated from the stem of *Nerium oleander*. The structures of \(1\) and \(2\) are elucidated on the basis of spectral data.

**Keywords**: Apocynaceae, 4-oxooctyl-2-hydroxyundecanoate, heptacosane-3-enyl-5-hydroxyhexanoate

*Nerium oleander* is an important medicinal plant of family Apocynaceae. Several biological effects such as cardiotonic, diuretic, cytotoxic, antibacterial, antiplatelet aggregation, anti-inflammatory, hepatoprotective and antitumor activities have been reported. Due to these reported activities chemical investigation of *N. oleander* is carried out which afforded two new compounds. This is evergreen shrub and is commonly known as "Oleander" or "Kaner". It is native to North Africa, eastern Mediterranean basin and south-east Asia, and is mainly found around dry stream beds. Oleander an ornamental plant grows well in warm subtropical regions and is planted in parks due to its beautiful and fragrant flowers with different colours.

They are often, but not always, sweet scented. The fruit is a long narrow capsule which splits open on maturity to release numerous downy seeds. Over four hundred varieties have been named, with several additional flower colours including red, purple, pink, orange; white and variety of pink are the most common. Young plants grow best in spaces where they do not have to compete with other plants for nutrients.

Isolation of a number of secondary metabolites has been reported from the genus *Nerium*. Triterpenes, pregnanes, cardenolides, cardiac glycosides etc. were isolated and characterized. The genus is reported to exhibit cardiotonic, diuretic, cytotoxic, antibacterial, antiplatelet aggregation, anti-inflammatory, hepatoprotective, antitumor activity, antihyperlipidemic, antiulcer activities and depressant action on the central nervous system.

Oleander is one of the most poisonous plants and contains numerous toxic compounds. The toxicity of Oleander is extremely high due to the presence of some toxins such as oleandrin and nerine, which are cardiac glycosides.

This paper deals with the isolation and structure elucidation of two new compounds viz. 4-oxooctyl-2-hydroxyundecanoate \(1\), heptacosane-3-enyl-5-hydroxyhexanoate \(2\) (Scheme I), and three known compounds, betulin \(3\), betulinic acid \(4\) and stigmasterol \(5\).

**Result and Discussion**

Compound \(1\) was obtained as white powder with m.p. 48°C and gave a negative tetranitromethane (TNM) test for unsaturation. Its mass spectrum showed [M] + at 328 corresponding to molecular formula \(C_{19}H_{36}O_{4}\). The IR spectrum (KBr) of \(1\) confirmed the presence of hydroxyl group by showing a broad absorption at 3360 cm\(^{-1}\). It displayed characteristic absorptions at 2907, 2845 for C-H stretching, 1710 cm\(^{-1}\) for C=O stretching and 1250 cm\(^{-1}\) for C-O stretching of the ester group. The \(^1\)H NMR spectrum showed the signals at \(\delta\) 4.03 (-CH(OH)-COO-) as multiplet for the proton present at C-2 position and a triplet at \(\delta\) 4.08 (COO-CH\(_2\)-) for a methylene group attached to the ester linkage. Deformed triplets at \(\delta\) 2.41 were observed for four protons of two methylene groups adjacent to carbonyl group. The triplet at \(\delta\) 0.96 was assigned to the six protons of terminal methyl groups. Remaining methylene protons were observed at \(\delta\) 1.25 as a broad singlet. On the basis of the above evidences, compound \(1\) was identified as 4-oxooctyl-2-hydroxyundecanoate.

In the mass spectrum of compound \(2\) molecular ion peak was observed at \(m/z\) 522 [M] +. The \(^1\)H NMR spectrum showed the presence of sixty six protons. On the basis of above observations, the molecular formula of \(2\) was established as \(C_{34}H_{66}O_{3}\) with m.p.
NOTES

59°C. The important absorptions observed in the IR spectrum were 3350 (O-H stretching), 2900, 2860 (C-H stretching), 1730 (C=O stretching) and characteristic absorptions at 725 and 720 cm⁻¹ due to the (-CH₂)n bending vibrations.

In the ¹H NMR spectrum two triplets were observed at δ 4.06 (-CH₂-O-CO-) and 2.23 (-CH₂-COO-), which corresponded to the methylene groups attached at ester oxygen (C-1') and ester carbonyl (C-2) respectively. A triplet was observed at δ 5.34 for one proton at C-3 position. Other important peaks were observed at δ 2.24 (-CH₂=CH=C<), 1.68 (-CH=C (CH₃)-), and 1.87 (>CH=C (CH₂)-). Remaining methylene protons were observed at δ 1.25 as a broad singlet.

Compound 3 was obtained as colourless needles, m.p. 253-55°C and showed single spot on TLC. It developed pale-yellow colouration with TNM in chloroform indicating unsaturation. It responded positively to Liebermann-Burchard and Noller tests characteristic of triterpenoids. Its infrared spectrum showed characteristic absorption bands at 3460-3400 (broad, OH stretching), 2970-2880 (C-H stretching), 1650 cm⁻¹ (C=C stretching). ¹H NMR spectrum showed a marked resemblance with lupeol. By analogy, a pair of broad singlets at δ 4.53 and 4.67 in conjunction with a singlet at 1.67 suggested the presence of isopropenyl side chain. Appearance of a multiplet at δ 2.44 was explicable on account of C-19 proton of cyclopentane ring. The hydroxy methine proton at C-3 gave a doublet at δ 3.18 (J=12, 5 Hz). Singlets appeared at δ 0.97, 0.98 and 0.99 were on account of five tertiary methyl groups.

The mass spectrum showed an intense molecular ion peak at m/z 442 [M]⁺ corresponding to its molecular formula C₃₀H₅₀O₂ together with prominent peaks at m/z 424 [M-H₂O]⁺, 220, 207, 189, etc. which are characteristic for the triterpenoid of lupine series. Presence of an ion peak at m/z 411 [M-CH₂OH]⁺ served as further evidenced for the presence of CH₂OH group.

The above spectral data were in good agreement with the structure of betulin 3 which was confirmed.
Compound 4 was obtained as colourless crystals, m.p. 166-67°C. It responded to Liebermann-Burchard and Noller’s test for sterols and also gave positive TNM test for unsaturation. IR spectra were recorded on JEOL SX 102/BA-6000 mass spectrometer. 1H NMR and 13C NMR were recorded on a Bruker spectrometer (300 MHz) in DMSO-d6/CDCl3 as solvent and TMS as internal standard. FAB mass spectra were recorded on JEOL SX 102/BA-6000 mass spectrometer.

Plant material: The stem of the plant was collected from University Campus, University of Rajasthan, Jaipur, India. The plant was identified by Prof. N.J.Sarna, Department of Botany, University of Rajasthan, Jaipur, India.

Extraction and Isolation

The shade-dried and powdered stem (2 kg) was extracted with methanol. After evaporation of solvent the brownish mass was fractionated with petroleum ether, chloroform and ethyl acetate. The chloroform-soluble fraction (6.5 g) was chromatographed over silica gel (60-120 Mesh) with mixtures of solvents of increasing polarity to obtain 6 fractions. Fraction 1 (petroleum ether: benzene, 3:2) contained 4-oxooctyl-2-hydroxyundecanoate as white powder, 10.8 mg, m.p. 48°C. Fraction 2 (petroleum ether: benzene, 1:1) yielded heptacosane-3-enyl-5-hydroxyhexanoate as white solid, 5.7 mg, m.p. 59°C. Fraction 3 (petroleum ether: benzene, 1:3) yielded betulin as colourless needles, 4.1 mg, m.p. 254°C. Fraction 4 (petroleum ether: benzene, 5:95v/v) contained betulinic acid as colourless crystals, 2.8 mg, m.p. 317°C. Fraction 5 (benzene) afforded stigmasterol as shining needles, 2.5 mg, m.p. 167°C.

4-Oxooctyl-2-hydroxyundecanoate 1
m.p. 48°C; ms: m/z 328[M]+, 271, 243, 201, 185, 171, 157 etc; IR (KBr, cm⁻¹): 3360 (O-H stretching), 2907, 2845 (C-H stretching), 1710 (C=O stretching), 171, 157 etc; IR (KBr, cm⁻¹): 3350 (O-H stretching), 2907, 2845 (C-H stretching), 1710 (C=O stretching), 1250 cm⁻¹ (C-O stretching of ester group); 1H NMR (δ, CDCl3): δ 4.03 (m, 1H, C-2), 4.08 (t, 2H, C-1), 2.41 (t, 4H), 1.25 (brs for remaining methylene protons), 0.96 (t, 6H for terminal methyl groups).

Heptacosane-3-enyl-5-hydroxyhexanoate 2
m.p. 59°C; ms: m/z 522[M]+, 477, 463, 435, 407, 391 etc; IR (KBr, cm⁻¹): 3350 (O-H stretching), 2900, 2860 (C-H stretching), 1730 (C=O stretching), 725, 720 cm⁻¹ (-CH₂ bending vibrations); 1H NMR (δ, CDCl3): δ 4.06 (t, 2H, C-1'), 2.23 (t, 2H, C-2'), 5.34 (t,
Betulin 3

m.p. 254°C; ms: m/z 442[M⁺]⁺, 424[M-H₂O]⁺, 411[M-C₂H₅OH]⁻, 410[M-C₂H₅OH]⁻, 372, 234, 206, 203, 189, 173, 144, 133,109, 91,55; IR (KBr, cm⁻¹): 3385 (OH), 2930 (C-H stretch), 1650 (C=C stretch), 1450, 1370 cm⁻¹ (=CMe₂), °H NMR (δ, CDCl₃): 4.53 and 4.67 (≈CH₂), 3.33 and 3.85 (d, J = 11 Hz each – CH₂OH), 3.18 (dd, J = 12, 5Hz H-3a), 2.44 (m, H-19), 1.67 (s, =C-CH₃), 0.75 (s, 3H), 0.85 (s, 3H), 0.96 (s, 3H), 0.98(s, 3H), 1.02 (s, 3H) for five tertiary methyl groups; °C NMR (δ, CDCl₃): 38.8 (C-1), 27.4 (C-2), 79.0 (C-3), 38.3 (C-4), 55.4 (C-5), 18.3 (C-6), 34.3 (C-7), 41.0 (C-8), 50.6 (C-9), 37.4 (C-10), 20.9 (C-11), 25.6 (C-12), 37.0 (C-13), 42.8 (C-14), 27.1 (C-15), 29.3 (C-16), 46.4 (C-17), 47.8 (C-18), 48.8 (C-19), 150.3 (C-20), 29.8 (C-21), 34.0 (C-22), 28.0 (C-23), 15.3 (C-24), 6.1 (C-25), 6.1 (C-26), 14.7 (C-27), 60.8 (C-28), 109.6 (C-29), 19.4 (C-30).

Betulinic acid 4

m.p. 317°C; ms: m/z 456[M⁺], 423, 411, 410, 342, 248, 220, 207, 203, 189, 143, 69 IR (KBr, cm⁻¹): 3385 (OH), 3350 (COOH), 1715 cm⁻¹ (C=O); °H NMR (δ, CDCl₃): 4.56 and 4.68 (≈CH₂), 1.68 (s, =C-CH₃), 2.30 (m, H-19) 3.27 (dd, H-3a), 0.76 (s, 3H), 0.78 (s, 3H), 0.82 (s, 3H), 0.96 (s, 3H), 1.03 (s, 3H) for five tertiary methyl groups; °C NMR (δ, CDCl₃): 38.7 (C-1), 27.4 (C-2), 78.9 (C-3), 38.8 (C-4), 55.3 (C-5), 18.3 (C-6), 34.3 (C-7), 40.7 (C-8), 50.5 (C-9), 37.2 (C-10), 20.8 (C-11), 25.5 (C-12), 38.4 (C-13), 42.4 (C-14), 30.5 (C-15), 32.1 (C-16), 56.3 (C-17), 46.8 (C-18), 49.2 (C-19), 150.3 (C-20), 29.7 (C-21), 37.0 (C-22, 27.9 (C-23), 15.3 (C-24), 16.0 (C-25), 16.1 (C-26), 14.7 (C-27), 180.5 (C-28), 09.6 (C-29), 19.4 (C-30).

Stigmasterol 5

m.p. 167°C; ms: m/z 412[M⁺], 399[M-Me⁺], 384, 369, 314, 302, 273, 255; IR (KBr, cm⁻¹): 3350 (OH stretching), 1460 cm⁻¹ (≈CH=CH- bending); °H NMR (δ, CDCl₃): 5.35 (t, C-6), 5.03 (dd, J = 16, 10 Hz, C-22), 5.17 (dd, J = 16, 10 Hz, C-23) 3.50 (m, H-3a), 0.86 (t, J = 7 Hz, C-29), 1.00 (d, J = 7 Hz, C-21), 0.82 (d, J = 6.0 Hz, C-27), 0.84 (d, J = 6.0 Hz, C-26), 1.01 (s, 3H, C-19), 0.71 (s, 3H, C-18).

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