Three New Tetranortriterpenoids from Neem Seed Oil

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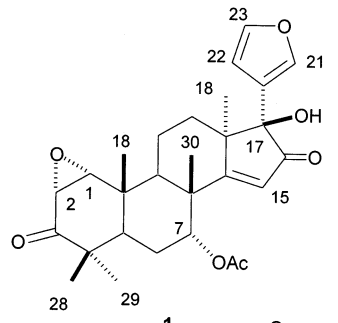
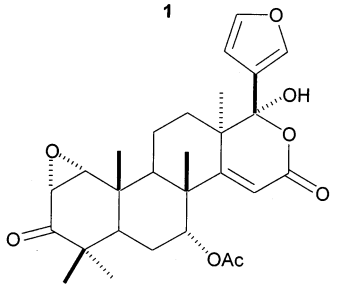
Three new tetranortriterpenoids, 1α,2α-epoxy-17β-hydroxyazadiradione (**1**), 1α,2α-epoxynimolicinol (**2**), and 7-deacetylnimolicinol (**3**), have been isolated from a methanol extract of neem oil (*Azadirachta indica*, seed oil) along with the known compounds epoxyazadiradione, 17β-hydroxyazadiradione, gedunin, nimbin, and nimolicinol (**4**). Spectral studies and chemical transformations were used to establish the structure of compounds **1**−**3**. The characterization of the epoxides **1** and **2** in neem oil is of biogenetic significance, as they may be considered as intermediates between A-ring enones and 1,3-diols among the *A. indica* tetranortriterpenoids.

The Indian neem tree, *Azadirachta indica* A. Juss. (synonyms *Melia azadirachta* L.; *Melia indica* Margosa) (Meliaceae), has been used for the treatment of a variety of human ailments and is also widely used as an insecticide. A large number of triterpenoids and tetranortriterpenoids have been isolated and identified from neem oil.[1−3] The present study deals with the isolation and characterization of three new tetranortriterpenoids, 1α,2α-epoxy-17β-hydroxyazadiradione (**1**), 1α,2α-epoxynimolicinol (**2**), and 7-deacetylnimolicinol (**3**), along with the known compounds epoxyazadiradione, gedunin, 17β-hydroxyazadiradione, nimbin, and nimolicinol (**4**), from a methanol extract of neem oil.

The FABMS of 1α,2α-epoxy-17β-hydroxyazadiradione (**1**) showed a protonated molecular ion peak at *m/z* 483 $[M + H]^+$, which was consistent with the molecular formula $C_{28}H_{34}O_7$ as obtained by elemental analysis. Its IR spectrum showed peaks at 3460 (OH), 1740 (ester carbonyl), 1704 (six-membered epoxy ketone), 1680 (cyclopentenone), and 876 cm^{-1} (furan ring). The UV spectrum exhibited a λ_{max} at 246 nm (log ∈ 4.07). The ^1H NMR spectrum (Table 1) showed singlets at δ 1.01, 1.05, 1.06, 1.25, and 1.32, indicative of five tertiary methyl groups. Three one-proton multiplets at δ 7.59, 6.40, and 7.43 corresponding to H-21, H-22, and H-23, respectively, were characteristic of a β-substituted furan ring in **1**. The presence of an epoxy group in ring A was indicated by two doublets at δ 3.43 and 3.62 (J = 4.5 Hz), corresponding to the H-2 and H-1 protons, respectively. There was no further coupling observed for the H-1 and H-2 protons; therefore the oxirane ring was connected to quaternary carbon atoms at C-3 and C-10. A one-proton multiplet at δ 5.28 ($W_{1/2}$ = 7.5 Hz) was attributed to H-7, and a one-proton signal at δ 4.46, which disappeared with D_2O exchange, indicated the presence of a hydroxyl group. The absence of a characteristic H-17 signal suggested that the hydroxyl group could be placed at C-17. These data and the ^{13}C NMR data (Table 1) showed a close structural similarity of **1** to 17β-hydroxyazadiradione.[4] To confirm the orientation of the 1,2-epoxy and 17-hydroxy groups in **1**, the epoxidation of 17β-hydroxyazadiradione was conducted using H_2O_2 (30%) and NaOH (5%). This reaction gave a 1α,2α-epoxy derivative, which was identical in all respects (mp, mmp, TLC, IR, NMR, and FABMS) to **1**. This conversion supported the assignment of the β-orientation of the 17-hydroxy group and the α-orientation of the 1,2-epoxy group in **1**.



1



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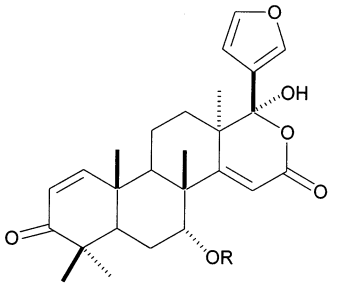

3: R = H
4: R = Ac

The FABMS of 1α,2α-epoxynimoilicinol (**2**) showed a protonated molecular ion peak at *m/z* 499 $[M + H]^+$, which was consistent with the molecular formula $C_{28}H_{34}O_8$ as obtained by elemental analysis. The IR spectrum showed peaks at 3440 (OH), 1742 (ester carbonyl), 1720 (α,β-unsaturated six-membered lactone), 1709 (six-membered epoxy ketone), and 880 cm^{-1} (furan ring). In the UV spectrum, a λ_{max} appeared at 239 nm (log ∈ 4.27). The ^1H NMR spectrum (Table 1) showed three one-proton multiplets at δ 7.57, 7.43, and 6.47, corresponding to H-21, H-23, and H-22, respectively. A one-proton multiplet at δ 5.20 ($W_{1/2}$ = 7.5 Hz) was assigned to H-7. Five tertiary methyl

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Table 1. ^{13}C and ^{1}H NMR Spectral Data of Compounds **1**−**3**

	1		**2**		**3**	
position	δ_C	δ_H m (J in Hz)	δ_C	δ_H m (J in Hz)	δ_C	δ_H m (J in Hz)
1	62.9	3.62 d (4.5)	62.6	3.53 d (4.5)	157.1	7.05 d (10.2)
2	56.5	3.42 d (4.5)	56.4	3.40 d (4.5)	125.2	5.85 d (10.2)
3	211.3		211.4		204.7	
4	44.1		44.1		44.1	
5	39.9	2.45 m	39.3	2.4 m	40.8	2.4 dd (12.3,5)
6	25.1	2.0−2.2 m	24.1	2.1−2.3 m	25.3	2.2 m
7	73.8	5.28 m	73.2	5.2 m	70.6	4.16 m
8	47.0		44		46.5	
9	44.4	2.6 m	44.2	2.8 dd (12.6,6.5)	46.5	2.05 dd (12.6,6.5)
10	41.4		42.1		42.3	
11	16.2	1.6 m	15.3	1.6 m	18.2	1.5−1.65 m
12	38.8	1.8 m	38.8	1.8 m	35.6	1.8 m
13	44.42		44.6		44.1	
14	193.0		170.7		173.2	
15	120.0	5.8 s	110.9	5.6 s	111.2	5.9 s
16	206.2		164.9		164.4	
17	80.5		104.4		104.7	
18	23.4	1.01 s	23.8	1.22 s	23.5	1.12 s
19	21.3	1.06 s	20.3	1.17 s	19.1	1.17 s
20	125.7		125.0		126.0	
21	142.7	7.59 m	142.7	7.57 m	143.1	7.57 m
22	109.5	6.40 m	110.1	6.47 m	110.0	6.47 m
23	141.3	7.43 m	141.7	7.43 m	141.8	7.43 m
28	21.2	0.99 s	20.8	1.03 s	21.5	1.11 s
29	27.3	1.05 s	26.9	1.15 s	27.2	1.15 s
30	24.9	1.32 s	23.2	1.3 s	23.2	1.30 s
$-OCOCH_3$	169.8		170.1			
$-OCOCH_3$	21.0	1.99 s	20.8	2.02 s		

group signals appeared at δ 1.03, 1.15, 1.17, 1.22, and 1.30. A one-proton broad singlet at δ 3.58, which disappeared with D_2O exchange, was ascribed to a hydroxyl proton. The absence of two one-proton AB doublets of a 1-en-3-one system of ring A and the presence of two one-proton doublets at δ 3.40 and 3.53 (J = 4.5 Hz), attributed to H-2 and H-1, supported the presence of an epoxy group in ring A. The absence of the characteristic H-17 signal in the ^{1}H NMR spectrum suggested that the hydroxyl group was at C-17. Thus, the data obtained for **2** and its ^{13}C NMR data (Table 1) showed a close structural resemblance to those of nimolicinol (**4**).[5] Moreover epoxidation of nimolicinol with H_2O_2 (30%) and NaOH (5%) gave a 1α,2α-epoxy derivative, which was identical in all respects (mp, mmp, TLC, UV, IR, NMR, FABMS) to **2**. This conversion supported the α-orientation of both the epoxide unit and the 17-hydroxy group in **2**.

7-Deacetylnimolicinol (**3**) showed peaks at 3450 (OH), 1722 (α,β-unsaturated six-membered lactone), 1675 (α,β-unsaturated six-membered ketone), and 878 cm^{-1} (furan ring) in the IR spectrum and a UV maximum at 239 nm (log ∈ 4.05). The FABMS of **3** showed a protonated molecular ion peak at m/z 441 $[M + H]^+$, which is consistent with the molecular formula of $C_{26}H_{32}O_6$ as obtained by elemental analysis. Its ^{1}H NMR spectrum (Table 1) showed two one-proton AB doublets at δ 7.05 and 5.85 (J = 10.2 Hz, H-1 and H-2, respectively), characteristic of a ring-A 1-ene-3-one system, and a one-proton singlet at δ 5.90 for H-15. Three one-proton multiplets at δ 7.57, 7.43, and 6.47, corresponding to H-21, H-23, and H-22, respectively, were observed. A singlet at δ 4.06 (1H) and a doublet at δ 0.92 (1H), which disappeared on shaking with D_2O, indicated the presence of two hydroxyl groups. The absence of both a characteristic H-17 signal and an acetyl group at C-7 in the ^{1}H NMR spectrum inferred that the hydroxyl groups were at C-17 and C-7, which was supported from the ^{13}C NMR spectral data (Table 1). Thus, the data obtained for **3** showed a close structural resemblance with nimolicinol (**4**).[5] Acetylation of **3** with pyridine and acetic anhydride afforded an acetate, which was identical in all respects (mp, UV, IR, NMR, FABMS, and TLC) with nimolicinol (**4**).[5] Thus, **3** was assingned as 7-deacetylnimolicinol.

The remaining compounds were identified as epoxyazadiradione,[6] nimbin,[7,8] gedunin,[9] 17β-hydroxyazadiradione,[10] and nimolicinol (**4**)[5] (mp, UV, IR, ^{1}H NMR, ^{13}C NMR, MS, and TLC).

The isolation and identification of epoxides **1** and **2** in neem oil is of biogenetic significance, as these components may be considered as the intermediates between A-ring enones and 1,3-diols among the *Azadirachta indica* tetranortriterpenoids.

Experimental Section

General Experimental Procedures. Melting points were determined on a Kofler hot-stage instrument and are uncorrected. Optical rotations were determined on a JASCO DIP-370 digital polarimeter. UV spectra were recorded on a Shimadzu 160A spectrophotometer. IR spectra were recorded on a Nicolet 400 FT IR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ with TMS as an internal standard. Chemical shifts are given in parts per million (ppm) downfield from TMS. FABMS were recorded on a JEOL SX 102/DA-6000 mass spectrometer (positive FABMS ion mode) using xenon (6 kV, 10 mA) as the FAB gas. Microanalysis was carried out on a Carlo Erba Flash EA 1112 instrument.

Plant Material. The commercial sample of neem (*Azadirachta indica*) seed oil investigated in this study was extracted from seeds collected in May 2000 from Western Maharashtra, India. This oil was obtained from Godrej Agrovet Co. (Mumbai, India), where it was authenticated.

Extraction and Isolation. The neem oil (500 g) was extracted initially with MeOH (2 L) at room temperature. The concentrated MeOH extract was shaken with petroleum ether (2 L) in portions to remove the fatty material. The insoluble residue obtained after the petroleum ether wash was subjected to column chromatography over silica gel (100−200 mesh). The fractions collected during the elution with *n*-hexane−EtOAc (10:3, 5:2, and 1:1) were concentrated to give mixture A, mixture B, and mixture C, respectively. Mixture A was

subjected to flash column chromatography over silica gel (230−400 mesh). The column was successively eluted with *n*-hexane−EtOAc mixtures of increasing polarity to obtain epoxyazadiradione (4.5 g), gedunin (4.8 g), 17*β*-hydroxyazadiradione (0.642 g), and 1α,2α-epoxy-17*β*-hydroxyazadiradione (**1**) (0.126 g). Similarly, flash column chromatography of mixture B using elution with *n*-hexane and EtOAc yielded nimolicinol (**4**) (0.185 g) and 1α,2α-epoxynimolicinol (**2**) (0.087 g), whereas mixture C yielded nimbin (0.872 g) and 7-deacetylnimolicinol (**3**) (0.048 g).

1α,2α-Epoxy-17*β*-hydroxyazadiradione (1): colorless needles (MeOH); mp 120−122 °C; $[\alpha]_D^{30}$ +54° (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} 246 nm (log ∈ 4.07); IR (KBr) ν_{max} 3466, 1740, 1704, 1680, 1526, 1426, 1383, 928, 878 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) (Table 1); ^{13}C NMR (125 MHz, $CDCl_3$) (Table 1); FABMS *m*/*z* 483 $[M + H]^+$; *anal*. C 69.87%, H 7.42%, calcd for $C_{28}H_{34}O_7$, C 69.69%, H 7.10%.

1α,2α-Epoxynimolicinol (2): colorless needles (MeOH); mp 230−234 °C; $[\alpha]_D^{30}$ +73° (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} 239 nm (log ∈ 4.27); IR (KBr) ν_{max} 3440, 1742, 1720, 1709, 1637, 1597, 1759, 1387, 985, 878 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) (Table 1); ^{13}C NMR ($CDCl_3$, 125 MHz) (Table 1); FABMS *m*/*z* 499 $[M + H]^+$; *anal*. C 67.18%, H 6.52%, calcd for $C_{28}H_{34}O_8$, C 67.45%, H 6.87%.

7-Deacetylnimolicinol (3): colorless needles (MeOH); mp 246−248 °C; $[\alpha]_D^{30}$ +101° (*c* 1.0, $CHCl_3$); UV (MeOH) λ_{max} 242 nm (log ∈ 4.05); IR (KBr) ν_{max} 3450, 1722, 1675, 1532, 1462, 1378, 932, 878 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) (Table 1); ^{13}C NMR ($CDCl_3$, 125 MHz) (Table 1); FABMS *m*/*z* 441 $[M + H]^+$; *anal*. C 70.57%, H 7.63%, calcd for $C_{26}H_{32}O_6$, C 70.88%, H 7.32%.

Epoxidation of 17*β*-Hydroxyazadiradione and Nimolicinol (4). To a mixture of 17*β*-hydroxyazadiradione or nimolicinol (**4**) (20 mg) in MeOH (5 mL) and 5% aqueous NaOH (1 drop using a syringe needle) was added 30% H_2O_2 solution (1 mL). The mixture was stirred at room temperature for 3 h. After the reaction, MeOH was removed under vacuum and the residue was extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with brine, dried over Na_2SO_4, and then concentrated to give the corresponding epoxide, 1α,2α-epoxy-17*β*-hydroxyazadiradione (**1**, 78% yield) or 1α,2α-epoxynimolicinol (**2**, 82% yield), respectively.

Acetylation of 7-Deacetylnimolicinol (3). A mixture of **3** (20 mg), Ac_2O (1 mL), and pyridine (1 mL) was stirred at room temperature for 1 h. After the usual workup, the acetate derivative was obtained. It was identical with the sample nimolicinol (**4**).

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Supporting Information Available: HPLC profile of the hexane-insoluble residue from a methanol extract of neem seed oil (Figure 1). This information is available free of charge via the Internet at http://pubs.acs.org.

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