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TWO NEW TERPENOIDS FROM ROOT BARK OF *AZADIRACHTA INDICA*

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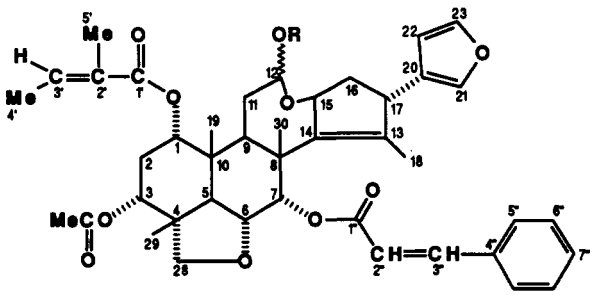
ABSTRACT.—Studies in the chemical constituents of root bark of *Azadirachta indica* have resulted in the isolation and structure elucidation of a new tetranortriterpenoid, nimbilin [**1**], along with a new aromatic diterpene, nimolinin [**3**]. The structures of these compounds have been established through chemical transformations and spectral studies.

In view of the enormous therapeutic and economic importance (1–3) attributed to *Azadirachta indica* A. Juss. (Meliaceae), also known as "neem", studies carried out by several groups of workers on its various parts have led to the isolation of a series of a new tri- (4–8) and di- (9) terpenoidal constituents. Extension of our investigation on the terpenoidal constituents of the root bark has led to the isolation of a new tetranortriterpene named nimbilin [**1**] and a new tricyclic diterpene named nimolinin [**3**].

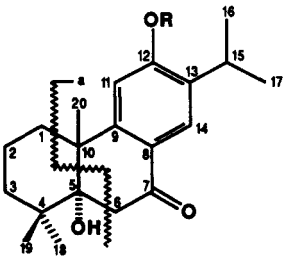
RESULTS AND DISCUSSION

The CH_2Cl_2 extract of neem root bark was divided into acidic and neutral fractions following the procedures described in the Experimental section. The neutral fraction yielded nimbilin [**1**] and the acidic fraction yielded nimolinin [**3**]. The structures of these constituents have been deduced as **1** and **3** respectively through spectral studies and chemical reactions.

Nimbilin [**1**] has the molecular formula $C_{42}H_{50}O_{10}$ (through ms peak matching of the molecular ion) and showed uv absorptions at 215, 220, and 278 nm, while its ir



1 R=H
2 R=Ac



3 R=H
4 R=Me

TABLE 1. ^{1}H-nmr Spectral Data (δ_H and J in Hz) of Triterpenes **1** and **2**.

Proton	Compound	
	1	**2**
H-1	4.88 (t) $J_{1,2\alpha} = J_{1,2\beta} = 2.96$	4.93 (m)
H-2α	2.08 (m)	2.01 (m)
H-2β	2.19 (m)	2.10 (m)
H-3	4.81 (t) $J_{3,2\alpha} = J_{3,2\beta} = 2.96$	4.83 (m)
H-5	2.82 (d) $J_{5,6} = 12.50$	2.80 (m)
H-6	4.14 (dd) $J_{6,5} = 12.50$ $J_{6,7} = 2.92$	4.10 (m)
H-7	5.85 (d) $J_{7,6} = 2.92$	5.84 (m)
H-9	3.47 (dd) $J_{9,11\beta} = 11.50$ $J_{9,11\alpha} = 2.60$	3.48 (m)
H-11α	1.71 (m)	1.72 (m)
H-11β	2.27 (m)	2.26 (m)
H-12	5.29 (m)	5.62 (m)
H-15	5.26 (dd) $J_{15,16\alpha} = 8.00$ $J_{15,16\beta} = 2.82$	5.11 (m)
H-16α	2.20 (ddd) $J_{gem} = 13.32$ $J_{16\alpha,17} = 9.92$ $J_{16\alpha,15} = 8.00$	2.20 (m)
H-16β	1.49 (dd) $J_{gem} = 13.32$ $J_{16\beta,15} = 2.82$	1.46 (m)
H-17	3.27 (d) $J_{17,16\alpha} = 9.92$	3.25 (m)
H-18	1.78 (s)	1.69 (s)
H-19	1.01 (s)	1.02 (s)
H-21	7.20 (m)	7.17 (m)
H-22	6.32 (m)	6.31 (m)
H-23	7.27 (m)	7.28 (m)
H-28a	3.48 (d) $J_{gem} = 8.5$	3.46 (d) $J_{gem} = 7.02$
H-28b	3.53 (d) $J_{gem} = 8.5$	3.52 (d) $J_{gem} = 7.02$
H-29	1.18 (s)	1.18 (s)
H-30	1.47 (s)	1.46 (s)
H-3′	6.89 (qq) $J_{3',4'} = 7.30$ $J_{3',5'} = 1.51$	6.89 (m)
H-4′	1.89 (d) $J_{4',3'} = 7.30$	1.86 (m)
H-5′	1.93 (d) $J_{5',3'} = 1.51$	1.93 (br s)
H-2″	6.38 (d) $J_{2'',3''} = 16.00$	6.38 (d) $J_{2'',3''} = 16.00$
H-3″	7.73 (d) $J_{3'',2''} = 16.00$	7.70 (d) $J_{3'',2''} = 16.44$
H-9″,5″	8.08 (d) $J_{5'',6''} = J_{9'',8''} = 8.00$	7.90 (m)
H-8″,6″	7.46 (dd) $J_{8'',9''} = J_{6'',5''} = 8.00$ $J_{8'',7''} = J_{6'',7''} = 8.00$	7.46 (m)
H-7″	7.38 (m)	7.41 (m)
OAc	2.04 (s)	2.06, 2.11 (2 × s)

spectrum displayed peaks at 3400 (OH), 2850 (C-H), 3120, 1720 (ester carbonyls), 1505, 1100 cm^{-1} (ether linkage), and 860 (furan ring). The presence of a β-substituted furan ring at C-17, a common feature of meliacins, was evident in nimbilin from its nmr spectral signals at δ H 7.20, 6.32, and 7.27 (Table 1) and δ C 139.0, 110.5, and 142.9 (Table 2), assigned to H-21, H-22, H-23 and C-21, C-22, C-23, respectively. Four tertiary methyls at δ 1.78 (H-18), 1.01 (H-19), 1.18 (H-29), and 1.47 (H-30); two signals at δ 5.29 (H-12) and 5.26 (H-15); and two AB doublets ($J = 8.5$ Hz) at δ 3.48 and 3.53 (H-28a, H-28b), showed that **1** contained the carbocyclic skeleton of nimbolin B (9) with one of the geminal methyls oxidized to an oxymethylene being involved in an ether linkage with C-6. This latter feature was also observed in salannin, a tetranortriterpenoid possessing a seco ring C but with a different arrangement of ring D (10). The hydroxyl group was also supported by acetylation of **1** to **2** on reaction with Ac_2O/pyridine.

TABLE 2. ^{13}C-nmr Chemical Shifts (δ_C/ppm) of Compound **1**.

Carbon	1	Carbon	1
C-1	71.2[a]	C-21	139.0
C-2	29.7	C-22	110.5
C-3	72.1[a]	C-23	142.9
C-4	42.5	C-28	78.0
C-5	40.3	C-29	19.2
C-6	72.7[a]	C-30	20.8[e]
C-7	75.0[b]	1′	169.5[f]
C-8	45.5	2′	129.5[d]
C-9	36.5	3′	137.0
C-10	40.3	4′	14.5
C-11	32.9	5′	12.2
C-12	91.7	1″	169.8[f]
C-13	142.8	2″	119.3
C-14	144.0	3″	146.2
C-15	75.8[b]	4″	134.0
C-16	37.3	9″,5″	130.4
C-17	46.5	8″,6″	128.3
C-18	16.2[c]	7″	144.0
C-19	16.5[c]	COMe	21.1[e]
C-20	129.1[d]	*C*OMe	170.0[f]

[a–f]Values with the same superscript may be interchanged.

The ^1H-nmr spectrum further showed a tigloyloxy (δ 6.89, qq, $J_{3',4'} = 7.30$, $J_{3',5'} = 1.51$ Hz, H-3′; 1.89, d, $J_{4',3'} = 7.30$ Hz, H-4′; 1.93, d, $J_{5',3'} = 1.51$ Hz, H-5′) and an acetoxy function (δ 2.04, s), which were not vicinal, because the protons geminal to these did not show mutual coupling. They were, however, individually coupled with the same methylene protons (H-2) which established their location at C-1 and C-3. Placement of the tigloyloxy function at C-1 and acetoxy at C-3 could be established through comparison of the chemical shifts of H-1 (δ 4.88) and H-3 (δ 4.81) triplets with those of salannin (10). Three one-proton signals at δ 5.85 (d, $J = 2.92$ Hz, H-7), 4.14 (dd, $J_{6,5} = 12.50$ Hz, $J_{6,7} = 2.92$ Hz, H-6), and 2.82 (d, $J_{5,6} = 12.50$ Hz, H-5) are reminiscent of H-7, H-6, and H-5, respectively, of nimbolin B (9), demonstrating that H-6 is vicinal to the ether oxygen while H-7 is at the base of the cinnamoyloxy group [δ 6.38 and 7.73 (each 1H, d, $J = 16.00$ Hz, H-2″, H-3″, respectively), 7.90 (2H, d, $J = 8.00$ Hz, H-9″, -5″), 7.46 (2H, dd, $J = 8.00$ Hz, H-8″, -6″), 7.38 (1H, m, H-7″)].

These assignments were corroborated by a COSY-45 spectrum which showed throughbond connectivities of H-6 with H-5 and H-7, both H-1 and H-3 with H-2α and H-2β, H-15 with H-16α and H-16β, H-12 with H-11α and H-11β, H-2″ with H-3″, and H-21 with H-23. In the light of these data, nimbilin has been assigned structure **1**.

The stereochemistry of various centers of nimbilin has been established through NOESY spectral analysis, which showed the spatial connectivities of H-28 with OAc, H-6 with H-29, H-7 with H-18, H-22 with H-16α, H-2″ with H-3″, and H-1 with H-19.

Nimolinin [**3**] has the molecular formula $C_{20}H_{28}O_3$ (through peak matching of the molecular ion). Its uv spectrum showed absorptions at 205, 225, and 270 nm and its ir spectrum displayed peaks at 3600–3100 (OH), 2900 (C-H), 1710 (α,β-unsaturated ketone), 1600 (aromatic double bond), 1380 (gem dimethyl), and 1100 cm^{-1} (C-O).

The molecular formula of **3** showed 7 double bond equivalents, four being justified by the aromatic ring, one by the carbonyl function and two by the remaining two rings of the skeleton. The ^{1}H-nmr spectrum showed three singlets of three protons each at δ 1.14, 1.26, and 1.27 for the geminal and angular methyl groups. Two one-proton singlets were observed at δ 6.67 and 7.90 attributable to the olefinic protons at C-11 and C-14, respectively. A one-proton seven-line pattern at δ 3.12 (J = 6.88 Hz) and a six-proton doublet at δ 1.28 (J = 6.88 Hz) showed the presence of an isopropyl group, which was further confirmed by the homonuclear decoupling experiment and COSY-45 plot. Another substituent of the aromatic ring of **3** was taken to be a hydroxyl group, which was indicated by the ir spectrum and molecular formula and confirmed by methylation of nimolinin [**3**] with freshly prepared CH_2N_2 to **4** (δ H 3.95, OMe).

The carbonyl function was placed at C-7 in the light of the chemical shifts of H-11 and H-14, which are comparable with those reported for sugiol (11). The remaining oxygen function indicated by the molecular formula could be identified as a hydroxyl group because no further unsaturation was indicated by the molecular formula. This could be placed at C-5 because a carbinylic proton was not observed in the ^{1}H-nmr spectrum and the signal of H-5 was also missing. Appearance of H-6α and H-6β as two doublets at δ 2.84 (J_{gem} = 15.68 Hz) and δ 2.63 (J_{gem} = 15.68 Hz), respectively, instead of typical double doublets, and failure to acetylate the methylated product **4** further confirmed the location of the hydroxyl function at C-5.

Keeping in view the above observations, the structure of nimolinin has been defined as **3**. There is only one report (12) of the isolation of an abietane diterpenoid with a hydroxyl function at C-5 prior to this communication.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were recorded on an air-bath-type melting point apparatus and are uncorrected. Ir (in $CHCl_3$) and uv (in MeOH) spectra were measured on a JASCO-IRA-I and Pye-Unicam SP-800 spectrometer, respectively; ms was recorded on a Finnigan MAT 311A double focussing mass spectrometer; ^{1}H-nmr spectra were recorded in $CDCl_3$ on a Bruker Aspect AM 400 spectrometer operating at 400 MHz. ^{13}C-nmr (Broad Band and DEPT) spectra were recorded in $CDCl_3$ on a Bruker Aspect-300 spectrometer operating at 75 MHz. ^{13}C chemical shift assignments have been made partly through DEPT spectra and partly through comparison with similar compounds (13,14). Chemical shifts are reported in ppm (δ). Merck Kieselgel 60 PF_{254} coated on glass plate was used for analytical (thin layer) and preparative (thick layer) chromatography.

PLANT MATERIAL.—Neem root bark (28 kg) was collected from the Karachi region in June of 1987 and identified by Professor S.I. Ali, Department of Botany, University of Karachi. A voucher specimen (No. NM-1) has been deposited in the Herbarium of the Botany Department, University of Karachi.

EXTRACTION AND ISOLATION.—Defatted neem root bark (28 kg) was repeatedly percolated with CH_2Cl_2 at room temperature. The CH_2Cl_2 extract, obtained on removal of the solvent under reduced pressure, was reextracted with EtOAc and H_2O. The EtOAc layer was repeatedly extracted with 4% Na_2CO_3

to separate the acidic and neutral fractions. The neutral fraction (0.5% of the weight of root bark) obtained on removal of the solvent after usual workup of the EtOAc layer was treated with hexane to give hexane-soluble (A) and hexane-insoluble fractions. The latter was successively extracted with Et_2O and EtOAc, furnishing Et_2O- and EtOAc-soluble fractions. Each of these was concentrated and treated with an excess of hexane, affording Et_2O-hexane-soluble (B) and Et_2O-hexane-insoluble fractions, and EtOAc-hexane-soluble (C) and EtOAc-hexane-insoluble fractions, respectively. Fractions A, B, and C were combined on the basis of tlc, freed of the solvent, and partitioned between 90% MeOH and hexane. The 90% MeOH phase, after addition of NaCl, was reextracted with EtOAc, concentrated, and again treated with an excess of hexane, giving an amorphous powder (3.6 g). This powder (650 mg) was subjected to flash cc (Eyela, Si gel, E. Merck 9385) with hexane, then mixtures of hexane/EtOAc of increasing polarity. Fractions eluted with 70% hexane in EtOAc furnished nimbilin [**1**] and some allied impurities. Compound **1** was finally purified by repeated Si gel flash cc with 87.5% hexane in EtOAc.

The Na_2CO_3 phase referred to above was acidified with dilute HCl, extracted with EtOAc, washed, dried (anhydrous Na_2SO_4), and charcoaled. The residue (1.5% of the dry wt of root bark) obtained on removal of the solvent was treated successively with hexane, Et_2O, and EtOAc. The concentrated EtOAc-soluble-portion (2 g) was taken in a flask containing Si gel (E. Merck 9385) and successively eluted with C_6H_6 and mixtures of C_6H_6/EtOAc with increasing polarity. Fractions obtained with C_6H_6-EtOAc (9:1–6:4) were combined and again subjected to flask chromatography (Si gel, C_6H_6/EtOAc mixtures with increasing polarity). The C_6H_6-EtOAc (8:2–7:3) eluates were combined and subjected to flash cc (Si gel, E. Merck 9385; hexane, hexane/EtOAc), yielding nimolinin as a pure product with 90% hexane in EtOAc.

NIMBILIN [**1**].—Plates (11.9 mg; 0.047% of the dry wt of neutral fraction) from hexane: mp 149–150°; eims *m/z* (%) $[M]^+$ 714.3932 (1) (calcd for $C_{42}H_{50}O_{10}$, 714.3925), $[M-Me-Ac]^+$ 656.2976 (0.5), $[C_{34}H_{44}O_9]^+$ 596.2927 (2.2), $[C_{33}H_{44}O_7]^+$ 552.3142 (6.5), $[C_{30}H_{36}O_7]^+$ 508.2444 (2.5), 57 (100).

ACETYLATION OF NIMBILIN [**1**].—To a solution of **1** (5.5 mg) in pyridine (1 ml), Ac_2O (2 ml) was added and the reaction mixture kept over night at room temperature. The acetylated product **2** (3.2 mg) obtained on usual workup crystallized from $CHCl_3$ as irregular plates, mp 171–172°; uv λ max 215, 278 nm; ir ν max 2850 (C-H), 1720 (br, carbonyls), 3120, 1505, and 800 (furan ring), 1100 cm^{-1} (C-O); eims *m/z* (%) $[M]^+$ 756 (1).

NIMOLININ [**3**].—Irregular plates from hexane (4 mg, 0.001% of the dry wt of total acid fraction): mp 113–114°; eims *m/z* (%) $[M]^+$ 316.2029 (2.2) (calcd for $C_{20}H_{28}O_3$, 316.2038), $[M-C_7H_{13}]^+$ 219.1019 (18), $[M-C_7H_{13}O]^+$ (fragment a) 203.1068 (100); ^1H nmr δ 7.90 (1H, s, H-14), 6.67 (1H, s, H-11), 3.12 (1H, h, $J=6.88$ Hz, H-15), 2.84 (1H, d, $J=15.68$ Hz, H-6α), 2.63 (1H, d, $J=15.68$ Hz, H-6β), 1.28 (6H, d, $J=6.88$ Hz, H-16, H-17), 1.27, 1.26, and 1.14 (each 3H, s, 3 × Me).

METHYLATION OF NIMOLININ [**3**].—An ethereal solution of nimolinin (2.9 mg) was treated with freshly prepared CH_2N_2 and the reaction mixture was kept at room temperature for 4–5 h. On evaporation of the solvent, **4** (3.1 mg) was obtained as a pure product (needles): mp 96–97°; uv λ max 204, 235 nm, ir ν max 3400 (OH), 1600 (C=C), 1380 (gem, dimethyl), 1100 (C-O) cm^{-1}, eims *m/z* (%) $[M]^+$ 330; ^1H-nmr δ 3.95 (3H, s, OMe).

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