CONSTITUENTS OF *AZADIRACHTA INDICA*: ISOLATION AND STRUCTURE ELUCIDATION OF A NEW ANTIBACTERIAL TETRANORTRITERPENOID, MAHMOODIN, AND A NEW PROTOLIMONOID, NAHEEDIN

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ABSTRACT.—Mahmoodin [1], a new limonoid, has been isolated from *Azadirachta indica* (neem) oil, along with seven known tetranortriterpenoids, azadirone, epoxyazadiradione, nimbin, gedunin, azadiradione, deacetylnimbin, and 17-hydroxyazadiradione. A new protolimonoid, naheedin [3], has been obtained from the neem fruits along with azadirachtol. Their structures have been elucidated through chemical and spectral analyses including 2D nmr studies. The absolute configuration of 1 was established by comparison of its cd spectrum with those of the known tetranortriterpenoids. Mahmoodin showed significant antibacterial activity against various Gram-positive and Gram-negative organisms. Four hydrocarbons, icosane, docosane, 2-methyltricosane, and docosene, have also been identified by gc-ms of the EtOH extract of the fruit coats. Only docosane has earlier been reported from neem, while the remaining three are unreported from this plant.

Azadirachta indica A. Juss. (Meliaceae) grows in tropical Asia, America, Australia, and Africa. Due to its therapeutic (1–4), and pesticidal (5–7) properties, the extractives of its various parts have been the subject of extensive investigations leading to the isolation of a plethora of interesting constituents (8–12). The present paper reports the isolation of mahmoodin [1] along with azadirone (13), epoxyazadiradione (13), nimbin (14–16), gedunin (13), azadiradione (13,17), deacetylnimbin (16,18), and 17-hydroxyazadiradione (17) from neem oil as well as naheedin [3] and azadirachtol from the fresh whole fruit. Mahmoodin belongs to the gedunin class of limonoids (8,13), and only five such meliacins have been reported from neem (13, 19–21). Naheedin is a protolimonoid (8) (apoeuphol or apotirucallol) with an intact C-8 side chain, a rarely occurring type of triterpenoid (8). Four hydrocarbons, icosane, docosane, 2-methyltricosane, and docosene, have been identified in the EtOH extract of fresh fruit coats through gc-ms.

RESULTS AND DISCUSSION

Mahmoodin [1] was isolated from neem oil employing the classical method of isolation (see Experimental). The eims and fdms spectra of 1 showed a molecular ion peak at *m/z* 526, the exact mass measurement of which gave the formula $C_{30}H_{38}O_8$. The ir spectrum exhibited peaks at 1670 (α,β-unsaturated carbonyl), 1715 (carbonyl of α,β-unsaturated δ-lactone), 1740 (ester carbonyl), 3350 (OH), and 870, 1500, 3155 cm^{-1} (β-substituted furan ring). The uv spectrum displayed maxima at 210 and 237 nm. The functionalities indicated by the ir and uv spectra were also confirmed by the ^{1}H-nmr spectral data (Table 1), which are similar to those of nimolicinol isolated earlier from neem fruits (20). Thus, it showed resonances attributable to a β-substituted furan, a ring A 1-en-3-one, an α,β-unsaturated δ-lactone, an acetate function at C-7, and five tertiary methyl singlets. ^{1}H-nmr assignments were confirmed through ^{1}H-^{1}H homonuclear decoupling experiments.

The molecular formula showed 12 double bond equivalents, all of which were accounted for by the deoxygedunin skeleton (20,22) of the molecule. A $C_2H_5O_2$ unit was left to be incorporated; it could be identified as an -O-CH$_2$-CH$_2$-OH moiety in the light of the following observations. The ^{1}H-nmr spectrum exhibited two triplets at δ 3.68 ($J = 4.7$ Hz, H-2') and 3.53 ($J = 4.7$ Hz, H-1') each integrating for two pro-
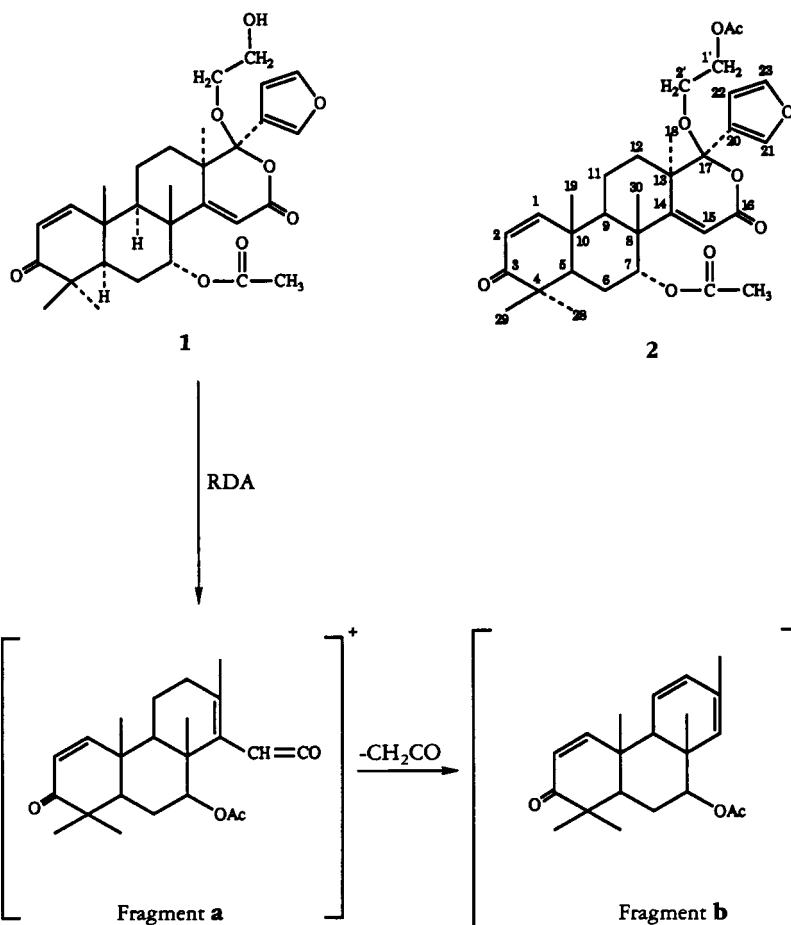
SCHEME 1

tons. That these signals were due to two mutually coupled methylene protons was evident from the homonuclear decoupling experiments. Thus irradiation of each of these converted the triplet of the other into a singlet.

The glycolyl side chain could be placed at C-17, since the characteristic H-17 signal of the gedunin type of meliacins (13,19,22) was lacking in **1**. This substitution was
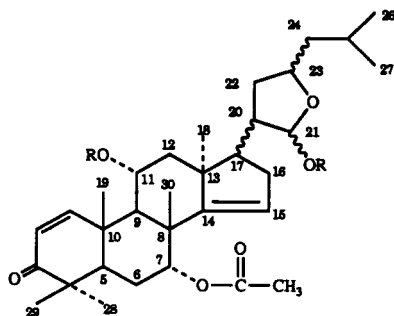
3   R=H
4   R=Ac

TABLE 1. ^{1}H-nmr Spectral Data of Triterpenoids **1–4**.[a]

Proton	Compound			
	1	**2**	**3**	**4**
H-1	7.10 (d)	7.03 (d)	7.12 (d)	7.10 (d)
	$J_{1,2} = 10.2$	$J_{1,2} = 10.2$	$J_{1,2} = 10.1$	$J_{1,2} = 10.1$
H-2	5.85 (d)	5.86 (d)	5.80 (d)	5.85 (d)
	$J_{2,1} = 10.2$	$J_{2,1} = 10.2$	$J_{2,1} = 10.1$	$J_{2,1} = 10.1$
H-5	2.17 (dd)	2.06 (m)	2.13 (m)	2.22 (m)
	$J_{5,6\beta} = 11.3$			
	$J_{5,6\alpha} = 3.3$			
H-6α	2.07 (ddd)	2.06 (m)	1.89 (m)	1.91 (m)
	$J_{gem} = 18.0$			
	$J_{6\alpha,5} = 3.3$			
	$J_{6\alpha,7} = 2.8$			
H-6β	1.83 (ddd)	1.83 (m)	1.80 (m)	1.85 (m)
	$J_{gem} = 18.0$			
	$J_{6\beta,5} = 11.3$			
	$J_{6\beta,7} = 2.8$			
H-7	5.26 (t)	5.26 (m)	5.20 (m)	5.32 (m)
	$J_{7,6\alpha} = 2.8$	Wh/2 = 7.5 Hz	Wh/2 = 7.0 Hz	Wh/2 = 7.0 Hz
	$J_{7,6\beta} = 2.8$			
H-9	2.23 (dd)	2.08 (m)	2.35 (d)	2.36 (m)
	$J_{9,11\beta} = 12.4$		$J_{9,11\beta} = 12.5$	
	$J_{9,11\alpha} = 3.2$			
H-11α	2.04 (m)	2.00 (m)	—	—
H-11β	1.86 (m)	1.86 (m)	3.51 (ddd)	4.95 (m)
			$J_{11,12\alpha} = 12.5$	
			$J_{11,9} = 12.5$	
			$J_{11,12\beta} = 5.5$	
H-12α	1.53 (ddd)	1.56 (m)	1.50 (dd)	1.55 (m)
	$J_{gem} = 15.1$		$J_{gem} = 14.0$	
	$J_{12\alpha,11\beta} = 9.4$		$J_{12\alpha,11} = 12.5$	
	$J_{12\alpha,11\alpha} = 4.0$			
H-12β	1.68 (ddd)	1.87 (m)	2.22 (dd)	2.31 (m)
	$J_{gem} = 15.1$		$J_{gem} = 14.0$	
	$J_{12\beta,11\alpha} = 5.5$		$J_{12\beta,11} = 5.5$	
	$J_{12\beta,11\beta} = 1.8$			
H-15	5.66 (s)	5.64 (s)	5.12 (m)	5.13 (m)
H-16α	—	—	2.35 (m)	2.40 (m)
H-16β	—	—	2.30 (m)	2.32 (m)
H-17	—	—	2.25 (m)	2.26 (m)
H-21	7.75 (dd)	7.55 (dd)	5.50 (m)	6.20 (m)
	$J_{21,22} = 0.7$	$J_{21,22} = 0.9$		
	$J_{21,23} = 1.7$	$J_{21,23} = 1.8$		
H-22	6.59 (dd)	6.32 (dd)	u.i.	u.i.
	$J_{22,21} = 0.7$	$J_{22,21} = 0.9$		
	$J_{22,23} = 1.7$	$J_{22,23} = 1.8$		
H-23	7.34 (t)	7.41 (dd)	3.65 (m)	3.66 (m)
	$J_{23,22} = 1.7$	$J_{23,22} = 1.8$		
	$J_{23,21} = 1.7$	$J_{23,21} = 1.8$		
OAc	1.95 (s)	2.05 (s)	1.95 (s)	2.05 (s)
		2.06 (s)		2.10 (s)
				1.93 (s)
OH	n.o.	—	3.90 (m)	—
			2.81 (m)	
13-Me	1.24 (s)	1.13 (s)	1.17 (s)	1.03 (s)
10-Me	1.22 ()s	1.22 (s)	1.25 (s)	1.10 (s)

TABLE 1.   Continued.

Proton	Compound			
	1	**2**	**3**	**4**
25-Me	—	—	0.92 (d) (6H) $J_{25Me,25}$ = 7.0	0.90 (d) (6H) $J_{25Me,25}$ = 7.0
4α-Me	1.08 (s)	1.09 (s)	1.07 (s)	1.07 (s)
4β-Me	1.06 (s)	1.07 (s)	1.07 (s)	1.07 (s)
8-Me	1.38 (s)	1.38 (s)	1.30 (s)	1.20 (s)
H-1'	3.53 (t) $J_{1',2'}$ = 4.7	4.36 (m)	—	—
H-2'	3.68 (t) $J_{2',1'}$ = 4.7	3.71 (m)	—	—

[a]δ_H, J in Hz. n.o. = not observed; u.i. = unidentified.

corroborated by (i) the ^{13}C-nmr spectra (BB and DEPT, Table 2), which showed a quaternary carbon at δ 100.00 attributable to C-17 and two secondary carbinylic carbons at δ 71.96 and 61.12 for the two methylenes (C-2' and C-1', respectively), (ii) downfield chemical shifts of furan ring protons (H-21 and H-22), and (iii) appearance of H-21 as a double doublet instead of doublet of double doublets (16). These observations were supported by significant fragments in the ms at m/z 370.2127 ($C_{23}H_{30}O_4$, fragment **a**), arising from the retro-Diels-Alder cleavage around ring C, and the base peak at m/z 328.2029 ($C_{21}H_{28}O_3$, fragment **b**) resulting from the loss of a ketene molecule from the fragment **a** (Scheme 1). Other important fragments in the mass spectrum were at m/z 496, 495, 137, and 95.

On the basis of these spectral data the structure of mahmoodin was defined as 17-glycolyldeoxygedunin {**1**}. In conformity with this structure, mahmoodin gave a monoacetyl derivative **2** ([M]$^+$ 568.2662, H-1' δ 4.36 m, OAc δ 2.05 s, and 2.06 s) on acetylation with Ac$_2$O and pyridine at room temperature.

TABLE 2. ^{13}C-nmr Chemical Shifts of Triterpenoids **1** and **3**.

Carbon	Compound		Carbon	Compound	
	1	**3**		**1**	**3**
C-1	157.13	158.20	C-18	23.48	21.16
C-2	125.98	126.00	C-19	19.22	19.35
C-3	204.09	204.50	C-20	125.68	45.00[d]
C-4	45.02[a]	44.21[c]	C-21	144.00	97.61
C-5	45.72	45.21[d]	C-22	110.75[b]	31.91
C-6	23.54	23.23	C-23	142.55	70.90
C-7	74.14	73.93	C-24	—	41.21
C-8	44.02[a]	45.21[c]	C-25	—	29.90
C-9	38.11	38.71	C-26	—	31.20
C-10	40.76	38.81	C-27	—	26.71
C-11	15.90	72.80	C-28	20.97	22.76
C-12	29.72	42.32	C-29	27.08	27.31
C-13	45.02[a]	46.32[c]	C-30	24.83	24.35
C-14	175.17	159.21	OCOCH$_3$	21.39	21.60
C-15	110.05[b]	119.33	OCOMe	170.19	170.10
C-16	166.01	34.92	C-1'	61.12	—
C-17	100.00	57.30	C-2'	71.96	—

[a-d]Assignments may be reversed.

The stereochemistry of various centers of mahmoodin [1] has been established through NOESY spectral analysis, which showed the spatial proximity of H-21 with H-1', H-2', and 13-Me; H-22 with H-23 and H-2'; H-7 with 8-Me and 10-Me; the methylene protons H-1' and H-2' with 10-Me; H-5 with H-9 and 4α-Me; OAc with 13-Me; 8-Me and H-15 with H-1' and H-2'; H-11β and H-6β with 10-Me; and H-1 with H-2. Connectivities indicated a typical trans A/B ring junction with 13-Me on the α side of the molecule. The spatial proximity of H-21 with 13-Me and that of both 8-Me and H-15 with H-1' and H-2' demonstrated that its furan ring is α-oriented and that the glycolyl chain is β-oriented.

The cd spectral data (λ max 354.0 nm, Δε −1.242; λ max 343.2 nm, Δε −1.301; and λ max 265.4, Δε 1.869) of mahmoodin, which are comparable with those reported for similar limonoids (13), confirmed the absolute stereochemistry as drawn in structure **1**.

It is noteworthy that there has been no report of a tetranortriterpenoid with a C-17 glycolyl side chain, although a few C-17 oxygenated limonoids including isonimolicinolide (23) bearing a 17-*O*-acetyl group have been reported from neem fruits (8,9,17,20). Biogenetically, formation of **1** may be considered from isonimolicinolide through oxidation of ring D to δ-lactone, as observed in the case of epoxyazadiradione-gedunin conversion (13), and transformation of the -OAc group to -O-CH$_2$-CH$_2$-OH.

Mahmoodin and its mother fraction (SF), 10 mg each, were tested for antimicrobial activity against eight Gram-negative organisms, *Escherichia coli* (a), *Enterobacter aerogenes* (b), *Shigella sonnei* (c), *Salmonella schotmuelleri* (d), *Klebsiella pneumoniae* (e), *Klebsiella ozaenae* (f), *Serratia marcescens* (g), *Proteus vulgaris* (h), and nine Gram-positive bacteria, *Bacillus cereus* (i), *Bacillus subtilis* (j), *Corynebacterium diphtheriae* (k), *Corynebacterium pseudodiphtheriticum* (l), *Corynebacterium xerosis* (m), *Staphylococcus aureus* (n), *Staphylococcus epidermidis* (o), *Streptococcus pyogenes* (p), and *Streptococcus faecalis* (q). Mahmoodin [1] showed significant inhibitory effects (zone of inhibition in mm is indicated in parentheses) against c (16), d (20), e (15), i (18), j (20), m (17), n (17), p (28), and q (22) while it is moderately active against a,f,g,k,l, and o. On the other hand SF is only weakly effective against d,f,h,i,j,k,m,o,p, and q. All organisms used in this study are clinical isolates. Data were not compared with control antibiotics; details of these studies will be published elsewhere.

Naheedin [3] has the molecular formula C$_{32}$H$_{48}$O$_6$ (hrms). Its uv spectrum showed maxima at 202 and 226 nm, and the ir spectrum displayed peaks at 3440 (OH), 1740, 1260 (ester), 1665 (α,β-unsaturated carbonyl), 1645, and 825 cm^{-1} (trisubstituted double bond). The ^{1}H-nmr spectrum of **3** (Table 1) showed a pair of doublets related to H-1 and H-2, a six-line pattern ascribed to a proton geminal to the α-oriented hydroxyl group (δ$_{OH}$ 3.90 exchangeable with D$_2$O) at C-11, a multiplet (Wh/2 = 7.0 Hz) at δ 5.20 for H-7 geminal to the acetoxy function (δ 1.95, s), a multiplet at δ 5.12 for the olefinic proton of the C-14 double bond, five tertiary methyl singlets, and one six-proton doublet at δ 0.92 (6H, *J* = 7.0 Hz) for two secondary methyls. These values are in agreement with those reported for the same protons in azadirachtol (24). However, the signal for the C-20 (22) double bond was missing in the ^{1}H- and ^{13}C-nmr spectra, and H-17, H-21, and H-23 were observed at comparatively high field. These facts indicated that in **3** the composition of the C-17 side chain is C$_8$H$_{15}$O$_2$, i.e., a saturated cyclic hemiacetal. This was further corroborated by the mass spectrum, which showed [M]$^+$ at *m/z* 528.3430, i.e., two mass units higher than that of azadirachtol (24), and fragments at *m/z* 143.1071 and 125.0955 corresponding to C$_8$H$_{15}$O$_2$ and C$_8$H$_{13}$O, respectively. Thus all the 9 double bond equivalents of the molecule were accounted for. The ms further showed important fragments at *m/z* 510, 492, 468.2491 [M − 4 × Me]$^+$, 450, and 408.2292 (468-HOAc). Another ion at *m/z* 137.0967 arises from the cleav-

age of ring A. Thus the structure of naheedin was established as 20,22-dihydroazadirachtol [3], which was confirmed through formation of the diacetyl derivative 4, the ^{1}H-nmr spectrum of which showed a shifting of resonance of H-11 from δ 3.51 to δ 4.95 and that of H-21 from δ 5.50 to δ 6.20, while the signals for the two hydroxyl groups at δ 3.90 and 2.81 were replaced by two sharp singlets of the acetoxy methyls at δ 2.05 and 2.10 in addition to a singlet at δ 1.93 present in 3. ^{13}C-nmr chemical shifts (Table 2) further confirmed the structure as 3, the assignments of which have been made through comparison with those of azadirachtol (24) and other model compounds (17,25,26).

HYDROCARBONS.—The petroleum ether-EtOAc (99:1) eluate, obtained by flash cc of the mother liquor of epoxyazadiradione, furnished a fraction M-3, showing a single spot on tlc, while the ^{1}H-nmr and mass spectra indicated that it is a mixture of hydrocarbons. From gc-ms, four hydrocarbons were identified in M-3: icosane, docosane, 2-methyltricosane, and an unsaturated docosene. Identification of docosane has earlier been reported in the leaves of *A. indica* (27).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined in glass capillary tubes and are uncorrected. Mass spectra were recorded on double focussing Finnigan MAT-112 spectrometer connected to PDP 11/34 computer system. Exact mass measurements were carried out through peak matching. Gc-ms were recorded on Hx-110 Jeol mass spectrometer connected to HP 3890 J gas chromatograph fitted with a packed column of OV 101. Temperature programming was from 100 to 250° at 5°/min. The ei energy and ion source temperature of the mass spectrometer were 70 eV and 250° respectively. Ir (CHCl$_3$) and uv (MeOH) spectra were measured on JASCO IRA-1 and uv 240 spectrophotometers, respectively. For mahmoodin, nmr spectra (CDCl$_3$) were recorded on a Bruker Aspect AM 400 spectrometer, operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C nuclei. The NOESY spectrum was recorded on a Bruker Aspect AM-300 using the standard Bruker microprograms NOESY AUR at delay time 2 msec. Assignments of various protons, particularly in the upfield region, were made through double resonance and 2D nmr experiments (COSY-45, NOESY, J-resolved and hetero-COSY). The ^{13}C-nmr spectral assignments have been made partly through a comparison of the chemical shifts with the published data for similar compounds (9, 17, 19, 20, 24–26), and partly through the appearance of signals in DEPT and hetero-COSY spectra. For naheedin, ^{1}H and ^{13}C-nmr (BB and off-resonance) spectra were run in CDCl$_3$ on a Bruker WP-100-SY Ft-nmr spectrometer. Chemical shifts are recorded in ppm (δ) and coupling constants (J) are in Hz. Purity of samples was checked by tlc (Si gel 60 GF$_{254}$ and Al$_2$O$_3$ PF$_{254}$ Type E).

ISOLATION OF MAHMOODIN [1].—Neem oil (1 liter), supplied by the courtesy of Hamdard Foundation India, was partitioned between petroleum ether and 80% EtOH, and the latter was repeatedly extracted with petroleum ether. The fat-free lower phase thus obtained was concentrated, saturated with saline, and extracted with EtOAc. The EtOAc layer was dried (anhydrous Na$_2$SO$_4$) and evaporated under reduced pressure to yield a residue which was divided into C$_6$H$_6$-soluble and C$_6$H$_6$-insoluble (CL) fractions. The residue obtained on removal of the solvent from the former fraction was divided into 60% aqueous EtOH-soluble and EtOH-insoluble portions. The 60% EtOH-soluble fraction was treated with charcoal and filtered, and the charcoal bed was eluted with MeOH and MeOH-C$_6$H$_6$ (1:1). The last eluate (SF) was subjected to thick layer chromatography [Al$_2$O$_3$, CHCl$_3$-MeOH (95:5)] affording tlc-pure amorphous mahmoodin (50 mg). On flash cc (28) (Si gel, petroleum ether/EtOAc in the order of increasing polarity) of fraction CL, pure azadirone, epoxyazadiradione, nimbin, gedunin, azadiradione, deacetylnimbin, and 17-hydroxyazadiradione were obtained.

Mahmoodin [1].—Fdms *m/z* (%) [M]$^+$ 526 (82), 483 (24), 370 (100), 328 (12); eims *m/z* (%), [M]$^+$ 526.2554 (C$_{30}$H$_{38}$O$_8$ requires [M]$^+$ 526.2566) (6), 509 (2), 496 (4), 495 (2), [M − C$_3$H$_2$O$_2$]$^+$ 456.2514 (7), 412 (2), [fragment **a**]$^+$ 370.2127 (C$_{23}$H$_{30}$O$_4$) (8), [fragment **b**]$^+$ 328.2029 (C$_{21}$H$_{28}$O$_3$) (100), 310 (8), 284 (4), 268 (2), 191 (5), 156 (10), 150 (25), 137 (36), 109 (24), 95 (73), 69 (30).

ACETYLATION OF MAHMOODIN [1].—To a solution of 1 (20 mg) in pyridine (2 ml), Ac$_2$O (8 ml) was added, and the reaction mixture was kept overnight at room temperature. The usual workup furnished the diacetyl derivative 2, (15 mg) as an amorphous powder: λ max 236, 211 nm; ν max 1668, 1720, 1740 (br), 1520, 875 cm^{-1}; eims *m/z* (%) [M]$^+$ 568.2662 (C$_{34}$H$_{40}$O$_{10}$ requires [M]$^+$ 568.2672) (8), 524 (12), 508 (4), 482 (5), 466 (4), 465 (5), 422 (7), 328 (40), 151 (30), 137 (34), 95 (96), 69 (100).

ISOLATION OF NAHEEDIN [3].—Fresh, unruptured, ripe fruits of neem (10 kg), collected in the Karachi region, (The tree was identified by Prof. S.I. Ali, and a voucher specimen [no. NM-1] has been deposited in the Herbarium of the Botany Department of Karachi University.) were repeatedly percolated with EtOH at room temperature. Removal of solvent from the combined extracts under reduced pressure gave a dark green residue, which was partitioned between EtOAc and H_2O. The EtOAc layer was repeatedly extracted with 1% NaOH to separate the acidic from the neutral constituents. The residue obtained, on removal of solvent from the EtOAc layer, was partitioned between 50% EtOH and Et_2O-petroleum ether (1:1). The upper layer was treated with charcoal and freed of solvent in vacuo. The residue thus obtained was divided into petroleum-ether-soluble and petroleum-ether-insoluble fractions, of which the latter showed four uv active spots on tlc. On subjecting this fraction to preparative layer chromatography [Si gel, C_6H_6-EtOAc (80:20)], naheedin [3] (needles, mp 170–171°, 30 mg) was obtained, along with azadirachtol (250 mg) (24).

Naheedin [3].—Eims m/z (%) $[M]^+$ 528.3430 ($C_{32}H_{48}O_6$ requires $[M]^+$ 528.3451) (4), 510 (4), 492 (6), 483 (5), 480 (2), $[M - 4 \times Me]^+$ 468.2491 (6), 450 (5), 438 (3), 432 (2), $[468 - HOAc]^+$ 408.2292 (8), 395 (4), 370 (2), 170 (11), 152 (25), 150 (70), 143.1071 ($C_8H_{15}O_2$) (12), 137.0967 ($C_9H_{13}O$) (29), 125.0955 ($C_8H_{13}O$), (8), 69 (100), 57 (11).

ACETYLATION OF NAHEEDIN [3].—On acetylation with Ac_2O (6 ml) and pyridine (1 ml) at room temperature for 6 h, naheedin (10 mg) furnished the diacetyl derivative 4 (9 mg) which formed fine needles on crystallization from MeOH: mp 145–146°; λ max 204, 220 nm; ν max 1735 (broad), 1660, 1642, 1380, 1260, 1020, 825 cm^{-1}; eims m/z (%) $[M]^+$ 612.3640 ($C_{36}H_{52}O_8$ requires $[M]^+$ 612.3662) (4), 569 (2), $[M - HOAc]^+$ 552.3439 (4), 509 (3), 368 (6), 309 (4), 137 (100), 69 (80), 55 (55), 43 (60).

ISOLATION OF HYDROCARBONS.—Extraction of the coats of fresh neem fruits and fractionation of the EtOH extract followed by flash cc have been described earlier in the context of isolation of azadirol, epoxyazadiradione, and kulactone (29). The petroleum ether -EtOAc (99:1) eluate, obtained on flash cc in the above procedure, constituted fraction M-3, showing a single spot on Si gel GF_{254} glass coated plates. Four major peaks were observed in the gc-ms spectrum of M-3. The ms of each of these led to the identification of three saturated hydrocarbons, icosane, docosane, and 2-methyltricosane, and one unsaturated hydrocarbon, docosene.

ACKNOWLEDGMENTS

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