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TETRACYCLIC TRITERPENOIDS OF THE FRUIT COATS OF *AZADIRACHTA INDICA*

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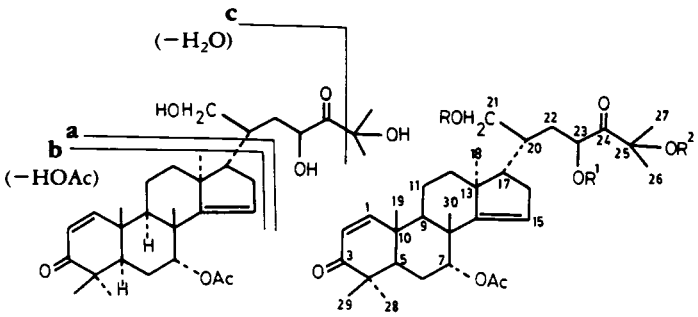
ABSTRACT.—From the EtOH extract of the coats of fresh, undried, uncrushed, ripe fruits of neem, *Azadirachta indica*, a new tetracyclic triterpenoid azadirol [**1**] and a known triterpenoid, kulactone [**5**], hitherto unreported from neem, have been isolated. Their structures have been elucidated through spectroscopic methods and chemical transformations. Azadirol [**1**] is the first naturally occurring apotirucallol (apoeuphol) derivative with the C-8 side chain uncyclized and represents an immediate intermediate in the biosynthesis from tirucallol (euphol) of apotirucallol (apoeuphol) triterpenoids with a degraded or cyclized side chain.

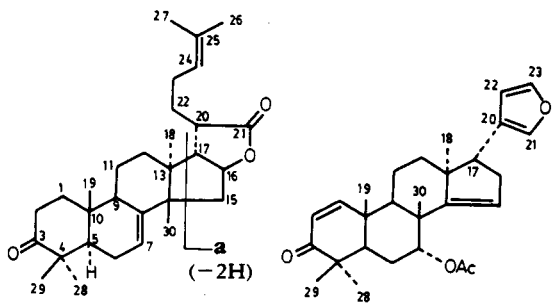
Azadirachta indica A. Juss. (Meliaceae) (neem) is indigenous to the Indo-Pakistan subcontinent, and almost every part of the tree is known in the folklore for the treatment of a variety of human ailments (1,2). Chemical studies undertaken by various groups of workers on its different parts have resulted in the isolation of a host of new constituents (3–7). Some of its factors have recently been shown to possess significant anti-inflammatory, anti-arthritic, anti-ulcer, antipyretic (8,9), antitumor (10), and pesticidal properties (11–14).

We have isolated a new tetracyclic triterpenoid, azadirol [**1**], from the neem fruit coats along with the known triterpenoid, kulactone [**5**] (15), which has previously been isolated from *Melia azedarach*.



1

2 R=R^1=Ac, R^2=H
3 R=R^1=R^2=Ac



5 **4**

RESULTS AND DISCUSSION

The eims spectrum of **1** did not show the molecular ion peak, which was, however, observed in the positive fabms spectrum at *m/z* 545 $[MH]^+$. High resolution mass spectrum showed significant ions at *m/z* 526.3294 $[M-H_2O]^+$, 509.3282 $[M-(H_2O+OH)]^+$ and 508.3184 $[M-2\times H_2O]^+$ which are in agreement with the molecular formula of **1** as $C_{32}H_{48}O_7$. The uv spectrum exhibited absorptions at 228 and 204 nm, while the ir spectrum displayed peaks at 3450 (OH), 1720 (C=O), 1660 (cyclohexenone), 1600 (C=C), and 1375 (Me) cm^{-1}. The ^{1}H-nmr spectrum showed a pair of doublets at δ 7.13 and 5.84 (J = 10.2 Hz) (Table 1), while the ^{13}C-nmr spectrum showed signals at δ 158.36, 125.53, and 204.80 (Table 2) characteristic of ring A 1-en-3-ones (3). This was supported by fragments at *m/z* 137.0912 ($C_9H_{13}O$) and 150.1047 ($C_{10}H_{14}O$) in the high resolution mass spectrum. Four one-proton double doublets, at δ 2.15 ($J_{5,6\beta}$ = 13.0, $J_{5,6\alpha}$ = 2.6 Hz), 2.18 ($J_{9,11\beta}$ = 12.2, $J_{9,11\alpha}$ = 6.3 Hz), 5.21 ($J_{7,6\beta}$ = $J_{7,6\alpha}$ = 2.9 Hz) and 5.27 ($J_{15,16\beta}$ = 3.4, $J_{15,16\alpha}$ = 1.5 Hz) have

TABLE 1. ^{1}H-nmr Spectral Data (δ_H/ppm and J in Hz) of Tetracyclic Triterpenoids.

Proton	Compound				
	1	**2**	**3**	**4**	**5**
H-1	7.13 d $J_{1,2}$ = 10.2	7.12 d $J_{1,2}$ = 10.2	7.12 d $J_{1,2}$ = 10.2	7.13 d $J_{1,2}$ = 10.2	—
H-1α	—	—	—	—	1.96 m
H-1β	—	—	—	—	1.47 m
H-2	5.84 d $J_{2,1}$ = 10.2	5.84 d $J_{2,1}$ = 10.2	5.84 d $J_{2,1}$ = 10.2	5.83 d $J_{2,1}$ = 10.2	—
H-2α	—	—	—	—	2.75 m
H-2β	—	—	—	—	2.25 m
H-5	2.15 dd $J_{5,6\beta}$ = 13.0 $J_{5,6\alpha}$ = 2.6	2.15 dd $J_{5,6\beta}$ = 13.0 $J_{5,6\alpha}$ = 2.2	2.17 dd $J_{5,6\beta}$ = 13.0 $J_{5,6\alpha}$ = 2.5	2.18 dd $J_{5,6\beta}$ = 12.2 $J_{5,6\alpha}$ = 2.7	1.74 dd $J_{5,6\beta}$ = 13.7 $J_{5,6\alpha}$ = 2.3
H-6α	1.88 m	1.92 m	1.92 m	1.90 ddd J_{gem} = 17.0 $J_{6\alpha,7}$ = 3.4 $J_{6\alpha,5}$ = 2.7	2.40 m
H-6β	1.75 m	1.77 m	1.77 m	1.78 ddd J_{gem} = 17.0 $J_{6\beta,5}$ = 12.2 $J_{6\beta,7}$ = 3.4	2.12 m
H-7	5.21 dd $J_{7,6\beta}$ = $J_{7,6\alpha}$ = 2.9	5.21 t $J_{7,6\beta}$ = $J_{7,6\alpha}$ = 3.0	5.21 dd $J_{7,6\beta}$ = 3.1 $J_{7,6\alpha}$ = 2.2	5.25 t $J_{7,6\beta}$ = $J_{7,6\alpha}$ = 3.4	5.33 ddd $J_{7,6\beta}$ = $J_{7,6\alpha}$ = $J_{7,9}$ = 3.3
H-9	2.18 dd $J_{9,11\beta}$ = 12.2 $J_{9,11\alpha}$ = 6.3	2.17 dd $J_{9,11\beta}$ = 10.9 $J_{9,11\alpha}$ = 4.2	2.18 dd $J_{9,11\beta}$ = 11.7 $J_{9,11\alpha}$ = 4.8	2.21 dd $J_{9,11\beta}$ = 11.5 $J_{9,11\alpha}$ = 2.8	2.49 m
H-11α	1.65 m	1.69 m	1.71 m	1.75 m	2.09–2.13 m
H-11β	1.94 dddd J_{gem} = 15.0 $J_{11\beta,9}$ = $J_{11\beta,12\alpha}$ = 12.2 $J_{11\beta,12\beta}$ = 2.0	1.90 m	1.89 m	1.95 m	2.09–2.13 m
H-12α	1.53 m	1.55 ddd J_{gem} = 16.7 $J_{12\alpha,11\beta}$ = 12.9 $J_{12\alpha,11\alpha}$ = 6.0	1.56 m	1.65 m	1.48 m
H-12β	1.78 m	1.85 m	1.87 m	1.85 m	1.96 m
H-15	5.27 dd $J_{15,16\beta}$ = 3.4 $J_{15,16\alpha}$ = 1.5	5.30 dd $J_{15,16\beta}$ = 3.7 $J_{15,16\alpha}$ = 2.5	5.29 dd $J_{15,16\beta}$ = 3.5 $J_{15,16\alpha}$ = 1.4	5.35 dd $J_{15,16\beta}$ = 3.4 $J_{15,16\alpha}$ = 2.1	—
H-15α	—	—	—	—	1.75 m
H-15β	—	—	—	—	2.28 m
H-16α	1.95 m	2.01 m	2.08 m	2.41 ddd J_{gem} = 16.3 $J_{16\alpha,17}$ = 10.8 $J_{16\alpha,15}$ = 2.1	4.13 ddd $J_{16\alpha,15\beta}$ = $J_{16\alpha,17}$ = 10.1 $J_{16\alpha,15\alpha}$ = 7.6

TABLE 1. Continued.

Proton	Compound				
	1	2	3	4	5
H-16β	2.25 ddd J_{gem} = 16.0 $J_{16\beta,17}$ = 8.0 $J_{16\beta,15}$ = 3.4	2.33 ddd J_{gem} = 15.2 $J_{16\beta,17}$ = 7.2 $J_{16\beta,15}$ = 3.7	2.32 ddd J_{gem} = 15.0 $J_{16\beta,17}$ = 7.5 $J_{16\beta,15}$ = 3.5	2.31 ddd J_{gem} = 16.3 $J_{16\beta,17}$ = 7.4 $J_{16\beta,15}$ = 3.4	—
H-17	1.97 m	1.80 ddd $J_{17,16\alpha}$ = 11.0 $J_{17,16\beta}$ = 7.2 $J_{17,20}$ = 5.0	1.78 m	2.78 dd $J_{17,16\alpha}$ = 10.8 $J_{17,16\beta}$ = 7.4	2.13 m
H-18	0.96 s	1.02 s	1.01 s	0.77 s	1.01 s
H-19	1.15 s	1.15 s	1.15 s	1.20 s	0.95 s
H-20	2.10 m	2.00 m	1.95 m	—	2.44 m 2.44 m
H-21	—	—	—	7.21 m	—
H-21a	3.82 m	4.26 dd J_{gem} = 11.4 $J_{21a,20}$ = 3.0	4.23 dd J_{gem} = 11.4 $J_{21a,20}$ = 3.1	—	—
H-21b	3.60 m	3.96 dd J_{gem} = 11.4 $J_{21b,20}$ = 5.4	3.93 dd J_{gem} = 11.4 $J_{21b,20}$ = 5.3	—	—
H-22	—	—	—	6.59 dd $J_{22,23}$ = 1.7 $J_{22,21}$ = 0.7	—
H-22a	1.70 m	1.75 m	1.76 m	—	1.75 m
H-22b	1.91 ddd J_{gem} = 15.0 $J_{22b,23}$ = 11.0 $J_{22b,20}$ = 2.8	1.92 m	2.03 m	—	1.75 m
H-23	3.86 m	5.61 dd $J_{23,22a}$ = 9.0 $J_{23,22b}$ = 2.8	5.56 dd $J_{23,22a}$ = 8.4 $J_{23,22b}$ = 4.4	7.34 t $J_{23,22}$ = $J_{23,21}$ = 1.7	2.03 m
H-24	—	—	—	—	5.10 qt $J_{24,23}$ = 6.8 $J_{24,27}$ = 1.4
25-OH	2.87 s	2.71 s	—	—	—
H-26	1.26 s	1.38 s	1.51 s	—	1.60 d $J_{26,24}$ = 1.1
H-27	1.40 s	1.42 s	1.59 s	—	1.68 d $J_{27,24}$ = 1.4
H-28	1.06 s	1.06 s	1.06 s	1.05 s	1.04 s
H-29	1.06 s	1.06 s	1.06 s	1.06 s	1.11 s
H-30	1.16 s	1.17 s	1.17 s	1.23 s	1.24 s
O ‖ O−C−CH_3	1.92 s	1.94 s 2.06 s 2.07 s	1.94 s 2.04 s 2.06 s 2.07 s	1.93 s	—

been related to H-5, H-9, H-7, and H-15, respectively, while a three-proton singlet at δ 1.92 has been attributed to the acetoxy protons. The ^{1}H- and ^{13}C-nmr spectral data revealed that the rings A–D of **1** are identical with those of azadirone (16), which was corroborated by the fragments at *m/z* 369.2425 (fragment **a**) and 309.2276 (fragment **b**) corresponding to formulae $C_{24}H_{33}O_3$ and $C_{22}H_{29}O$, respectively. However, the signals of the furan ring, which is a common feature of meliacins (3,7,14,16), were missing in the ^{1}H- and ^{13}C-nmr spectra. The doublet of doublets of H-17 of azadirone [**4**] at δ 2.78 was also absent in the ^{1}H-nmr spectrum of **1** (Table 1). On the other hand, the ^{1}H-nmr spectrum showed seven tertiary methyl singlets at δ 0.96, 1.06, 1.06, 1.15, 1.16, 1.26, and 1.40, compared to five in azadirone, indicating the triterpenoidal nature of **1** with the intact eight-carbon side chain at C-17. Fragment **a** $[M - C_8H_{15}O_4]^+$ at *m/z* 369.2425 indicated the composition of the side chain as $C_8H_{15}O_4$. The ^{1}H-nmr spectrum showed two multiplets at δ 3.82 (H-21a) and 3.60 (H-21b) assignable to

TABLE 2. ^{13}C-nmr Chemical Shifts (δ_C/ppm) of Tetracyclic Triterpenoids.

Carbon	Compound					Carbon	Compound				
	1	**2**	**3**	**4**	**5**		**1**	**2**	**3**	**4**	**5**
C-1	158.36	157.97	158.04	158.20	38.40	C-17	51.85	55.53	55.57	51.72	58.15
C-2	125.53	125.63	125.55	125.53	34.92	C-18	19.96	19.54	19.49	20.69	12.50
C-3	204.80	204.43	204.48	204.58	215.10	C-19	19.02	19.02	19.03	19.07	21.42
C-4	44.17	44.19	44.16	44.18	47.90	C-20	35.43	36.69	35.94	124.62	45.52
C-5	46.17	46.24	46.17	46.21	52.71	C-21	65.39	65.61	65.50	139.74	180.30
C-6	23.82	23.87	23.84	23.85	26.00	C-22	32.86	31.32	31.60	111.09	29.85
C-7	74.72	74.65	74.61	74.61	118.75	C-23	67.56	73.54	73.17	142.60	26.11
C-8	42.80	42.82	42.76	42.88	143.60	C-24	215.00	211.23	204.92	—	123.50
C-9	38.53	38.58	38.50	38.76	48.00	C-25	76.81	77.23	83.25	—	132.69
C-10	39.82	39.88	39.84	40.01	35.09	C-26	24.40	27.47	23.64	—	17.75
C-11	16.75	16.82	16.79	16.56	24.33	C-27	23.17	27.40	24.87	—	25.72
C-12	33.92	34.08	34.03	33.09	29.25	C-28	21.26	21.29	21.29	21.34	24.30
C-13	46.55	46.69	46.59	47.22	39.71	C-29	27.06	27.11	27.09	27.10	21.46
C-14	158.83	159.18	159.08	158.95	55.26	C-30	27.35	27.77	27.39	27.36	32.15
C-15	119.26	119.18	119.11	119.16	35.81	OCOMe	170.15	169.96	169.95	170.11	—
								170.07	170.01		
								170.67	170.17		
									171.09		
C-16	34.96	34.84	34.78	34.46	82.50	$OCOCH_3$	21.15	21.16	20.78	21.16	—
									20.93		
									21.19		
									21.29		

methylene protons attached to an oxygen atom and a one-proton multiplet at δ 3.86 (H-23) due to a tertiary carbinylic proton. The downfield shifts of H-26 (δ 1.26) and H-27 (δ 1.40) singlets indicated the presence of another hydroxyl group at C-25, which was supported by fragment **c** ($C_{29}H_{39}O_5$) resulting from the loss of C_3H_7O plus an H_2O molecule from the molecular ion. These observations were supported by the ^{13}C-nmr spectrum (BB and DEPT, Table 2) which showed peaks at δ 65.39 ($-CH_2O-$), 67.56 ($-\overset{|}{C}H-O-$), and 76.81 ($-\overset{|}{\underset{|}{C}}-O-$) attributable to C-21, C-23, and C-25, respectively, besides a peak at δ 74.72 for C-7. The nature of the fourth oxygen atom in the side chain was determined as oxo by the signal at δ 215.00 in the ^{13}C-nmr spectrum. Placement of various functional groups in the side chain was finally supported by the ^1H-^1H homodecoupling experiments and COSY-45, which showed through bond interaction of H-21a with H-21b and H-20, H-21b with H-20, H-23 with H-22a and H-22b, and H-20 with H-17, H-22a, and H-22b.

In light of these spectral data, the structure of azadirol has been arrived at as 21,23,25-trihydroxy-7-acetoxy-$\Delta^{1,14}$-apotirucalla-3,24-dione [**1**], which was chemically confirmed by acetylation of **1** (Ac_2O/pyridine, room temperature, 2 days), which gave two products identified as diacetyl **2** (major) and triacetyl **3** (minor) derivatives. The eims of the diacetyl derivative **2** showed important ions at *m/z* 569, 551, and 525 corresponding to the loss of $C_2H_3O_2$, C_2H_3O plus H_2O, and $C_2H_4O_2$ plus C_2H_3O, respectively, from the parent ion, while ^1H- and ^{13}C-nmr spectra showed that its tetracyclic nucleus is identical to **1**, and the changes were observed in the side chain signals only. Thus, the signals of carbinylic protons H-21a, H-21b, and H-23 shifted to δ 4.26 (dd, $J_{gem}=11.4$, $J_{21a,20}=3.0$ Hz), 3.96 (dd, $J_{gem}=11.4$, $J_{21b,20}=5.4$ Hz), and 5.61 (dd, $J_{23,22a}=9.0$, $J_{23,22b}=2.8$ Hz), respectively, with the appearance of three acetoxy methyl singlets at δ 1.94, 2.06, and 2.07 in the ^1H-nmr spectrum. Minor shifts in the H-26 and H-27 signals were also noted (Table 1). The fdms spectrum of the triacetyl derivative **3** showed a molecular ion peak at *m/z* 670 $[M]^+$, while the eims spectrum showed important peaks at *m/z* 610, 551, 491, and 310 corresponding to the loss of $C_2H_4O_2$, $C_2H_4O_2$ plus $C_2H_5O_2$, $2\times C_2H_4O_2$ plus $C_2H_3O_2$, and side chain plus $C_2H_3O_2$, respectively. In the ^1H-nmr spectrum, H-26 and H-27 resonated at further down field (δ 1.51 and 1.59) as compared to **1** and **2**, showing that in **3** the tertiary hydroxyl group at C-25 has also been acetylated. The rest of the ^1H-nmr spectral data were identical with those of **2**, and the ^{13}C-nmr spectral data of **3** were also similar to those of **2** with the addition of two carbons for one more acetyl group. In going from **1** to the diacetyl **2** and triacetyl **3** derivatives, a pronounced β effect of the acetyl function was noted on the carbonyl carbon (C-24) (17). However, this effect was not significant on C-22 (β to 23-OAc), and in the case of C-20 (β to 21-OAc) and C-26, C-27 (β to 25-OAc) a reversed order was observed. The configuration of C-23 as *R* has been determined through Horeau's method (18) and was supported by the negative cd curve (19,20).

Stereochemistry of various centers of **1** has been established through NOESY experiments, which showed through bond connectivities of H-1 with H-2, and H-19; H-2 with both H-29 and H-28; H-15 with H-30; H-7 with H-19, H-30, and OAc; H-23 with 25-Me, H-22a, and H-21b; H-21a with H-21b; H-18 with H-22b; H-17 with H-30; H-11β with H-12β, H-19, and H-30; H-19 with H-29; and H-5 with H-28, H-11α, and H-6α. The interaction of H-17 with H-30 and that of H-18 with H-22b showed that in **1**, H-17 is β- and H-20 is α-oriented; therefore, compound **1** belongs to the apotirucallanes.

It was originally suggested that the limonoids (C-26 compounds related to limonin) might arise from apoeuphol (apotirucallol) (21). As such, **1** represents the first

naturally occurring apotirucallane (apoeuphane) intermediate with an uncyclized C-8 side chain in the biosynthesis of apotirucallane apoeuphane triterpenoids from tirucallanes (euphanes), since prior to this the isolation of such an apotirucallane apoeuphane derivative has not been reported. Subsequently, cyclization of the side chain might lead to C_{30} apo derivatives like grandifoliolenone (22) and azadirachtol (23), whereas degradation of the side chain would give tetra; penta; or hexa-nor tetracyclic triterpenoids (3).

Azadirol [**1**] was subjected to antibacterial testing against seven Gram positive organisms, *Bacillus subtilis, Bacillus pumilis, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus citerus, Streptococcus pyogenes,* and *Serratia marcescens,* and nine Gram negative organisms, *Escherichia coli, Shigella dysenteriae, Shigella sonnei, Salmonella typhi, Salmonella paratyphi* A, *Salmonella schottmuelleri, Klebsiella pneumoniae, Proteus vulgaris,* and *Pseudomonas aeruginosa*. It showed no significant activity against these bacteria.

The eims spectrum of **5** showed a molecular ion peak at *m/z* 452.3294 corresponding to the formula $C_{30}H_{44}O_3$ (calcd 452.3290) $[M]^+$. Its uv spectrum showed absorption at 206 nm, while the ir spectrum displayed peaks at 2850 (CH), 1775 (γ-lactone), 1710 (C=O), 1670 (C=C), and 1375 (Me) cm^{-1}. The ^{1}H-nmr spectrum showed resonances for H-7 (δ 5.33, ddd, $J_{7,6\alpha}=J_{7,6\beta}=J_{7,9}=3.3$ Hz), H-16α (δ 4.13, ddd, $J_{16\alpha,17}=J_{16\alpha,15\beta}=10.1$, $J_{16\alpha,15\alpha}=7.6$ Hz), H-24 (δ 5.10 qt, $J_{24,23}=6.8$, $J_{24,27}=1.4$ Hz) and quaternary methyls (δ 0.95, 1.01, 1.04, 1.11, and 1.24) along with two vinyl methyls at δ 1.60 (d, $J_{26,24}=1.1$ Hz, H-26) and 1.68 (d, $J_{27,24}=1.4$ Hz, H-27). On the basis of these spectral data (Table 1) **5** was identified as kulactone, which has earlier been isolated from *M. azedarach* (15). The structure was corroborated by the mass spectrum which showed significant fragments at *m/z* 437.3021 ($C_{29}H_{41}O_3$) and 325.2531 ($C_{23}H_{33}O$) corresponding to the loss of Me and C_6H_{11} plus CO_2 along with fragment **a** at *m/z* 178.0994 ($C_{11}H_{14}O_2$). Final evidence of the structure was provided by the hitherto unreported ^{13}C-nmr chemical shifts (Table 2) observed in BB and DEPT spectra.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were determined in glass capillary tubes and are uncorrected. Uv (in MeOH) and ir (in $CHCl_3$) spectra were measured on Hitachi-U-3200 and JASCO-A-302 spectrophotometers, respectively. Mass spectra were recorded on a Finnigan MAT-112 spectrometer, exact masses have been measured through hrms, and fabms (positive) spectra were run on a JMS HX-110 double focussing mass spectrometer, operating at an accelerating voltage of 10 kV, using MeOH as a solvent and glycerol as a matrix on the target. The samples were ionized by bombardment with xenon (gas) atoms. The ^{1}H-nmr spectra were recorded in $CDCl_3$ on a Bruker Aspect AM-400 spectrometer operating at 400 MHz. ^{13}C-nmr spectra (BB and DEPT) were recorded in $CDCl_3$ on a Bruker Aspect AM-300 spectrometer operating at 75 MHz. The chemical shifts are recorded in ppm (δ) and coupling constants (*J*) are in Hz. ^{13}C-nmr spectral assignments of **1–3** and **5** have been made partly through DEPT and hetero-COSY spectra and partly through a comparison of chemical shifts with those of azadirone (Table 2). Exact assignments of the ^{1}H-nmr chemical shifts, particularly for the upfield region for **1–3** and **5**, were made through double resonance experiments and 2D studies (COSY-45, *J*-resolved, and hetero-COSY). Flash cc was performed on Eyela EF-10 (Si gel, E. Merck 9385). Si gel GF_{254} was used for vacuum liquid chromatography (24,25). The purity of compounds was checked on Si gel GF_{254} coated plates. Petroleum ether used was of the boiling range 60–80°.

PLANT MATERIAL.—The coatings (20 kg) were obtained from fresh, undried, uncrushed, ripe neem fruits collected from the Karachi region in July 1988. The tree was identified by Prof. S.I. Ali, Dean, Faculty of Science, and a voucher specimen (No. NM-1) has been deposited in the herbarium of the Botany Department of Karachi University.

EXTRACTION AND ISOLATION.—The fresh, undried, uncrushed, ripe neem fruit coatings were repeatedly extracted with EtOH at room temperature. The thickish residue obtained on removal of the solvent from the combined extracts under vacuo was partitioned between EtOAc and H_2O. The EtOAc layer was extracted with 4% Na_2CO_3 solution to separate the acidic from the neutral fractions. The EtOAc layer

was washed, dried (Na_2SO_4), and evaporated under vacuum. The residue from the neutral EtOAc fraction was successively treated with petroleum ether and Et_2O. The Et_2O-soluble portion was treated with 90% MeOH-petroleum ether (1:1). The 90% MeOH phase was extracted with EtOAc after addition of saline. The EtOAc phase was washed, dried (Na_2SO_4), and freed of the solvent, affording 200 g of a gummy residue, 180 g of which was subjected to vacuum liquid chromatography (Si gel GF_{254}, petroleum ether, petroleum ether/EtOAc in order of increasing polarity.) The petroleum ether-EtOAc (6:4) eluate (C) was concentrated and kept at room temperature. A white crystalline material separated out, which on recrystallization with $CHCl_3$-MeOH (1:1) afforded a limonoid (18 g) as white needles, mp 204–205°, which were identified as epoxyazadiradione through comparison of spectral data (uv, ir, mass, ^1H and ^{13}C nmr) with those reported in the literature (16,26). The mother liquor of epoxyazadiradione was subjected to flash (Eyela) cc (Si gel E. Merck 9385, petroleum ether/EtOAc in the order of increasing polarity). The petroleum ether-EtOAc (9.6:0.4) eluate furnished a triterpenoid (20 mg) as rods, mp 163–164°, which was identified as kulactone through comparison of its mass, ^1H-nmr, uv, and ir spectral data with those reported in the literature (15) and the ^{13}C-nmr and 2D spectral data. The petroleum ether-EtOAc (2:8 and 1:9), EtOAc, and MeOH-EtOAc (1:9, 2:8, 3:7, and 4:6) eluates were combined together on the basis of tlc. This constituted fraction E (30 g), which was again subjected to vacuum liquid chromatography (Si gel GF_{254}, $CHCl_3$/MeOH in order of increasing polarity). The $CHCl_3$-MeOH (9.9:0.1) eluate furnished fractions 7 and 8 with one major and five minor spots. These were subjected to thick layer chromatography [Si gel GF_{254}, $CHCl_3$-MeOH (9.65:0.35)] affording azadirol [**1**] (0.4 g) as a major component which on crystallization with MeOH-C_6H_6 (1:1) formed irregular buff white plates (0.4 g, mp 109–112°).

Azadirol [**1**].—Fabms (positive) *m/z* $[MH]^+$ 545, 527, 509, 485, 449; hrms *m/z* (rel. int.) $[M-H_2O]^+$ 526.3294 (calcd for $C_{32}H_{46}O_6$, 526.3294) (9.8), 509.3282 ($C_{32}H_{45}O_5$) (10.4), 508.3184 ($C_{32}H_{44}O_5$) (27.1), 469.2911 ($C_{29}H_{41}O_5$) (6.6), 468.2870 ($C_{29}H_{40}O_5$) (17.9), 467.2798 ($C_{29}H_{39}O_5$; fragment **c**) (7.9), 426.2731 ($C_{27}H_{38}O_4$) (15.0) 425.2713 ($C_{27}H_{37}O_4$) (46.2), 408.2682 ($C_{27}H_{36}O_3$) (5.8), 369.2425 ($C_{24}H_{33}O_3$; fragment **a**) (8.5), 368.2365 ($C_{24}H_{32}O_3$) (22.5), 309.2276 ($C_{22}H_{29}O$; fragment **b**) (11.2), 259.1690 ($C_{17}H_{13}O$) (6.8), 137.0912 ($C_9H_{13}O$) (9.3); ^1H nmr see Table 1; ^{13}C nmr see Table 2; cd $[\theta]^{232}+18.78°$, $[\theta]^{342}-13.76°$ ($CHCl_3$).

Acetylation of **1**.—To a solution of **1** (100 mg) in pyridine (1.5 ml), Ac_2O (4 ml) was added, and the reaction mixture was kept for 2 days at room temperature. On usual workup the reaction product showed one major and one minor spot on tlc. These were separated by thick layer chromatography [Si gel GF_{254}, $CHCl_3$-MeOH (9.7:0.3)] affording the diacetate **2** (50 mg) and the triacetate **3** (18 mg) as white amorphous powders.

Diacetyl azadirol [**2**].—Uv λ max (MeOH) nm 228, 206; ir ν max ($CHCl_3$) cm^{-1} 3450 (OH), 1700–1740 (C=O), 1660 (cyclohexenone), 1600 (C=C), 1365(Me); ^1H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* (rel. int.) $[M]^+$ 628 ($C_{36}H_{52}O_9$), 569 (2), 551 (2), 526 (2), 511 (18), 510 (4), 451 (3), 369 (2), 310 (16), 273 (9), 259 (2), 159 (28), 150 (96), 137 (50), 121 (32), 106 (28), 105 (40), 91 (38), 59 (100), 69 (51).

Triacetyl azadirol [**3**].—Uv λ max (MeOH) nm 228, 205; ir ν max ($CHCl_3$) cm^{-1} 1700–1740, 1660, 1600, 1365; ^1H nmr see Table 1; ^{13}C nmr see Table 2; fdms $[M]^+$ 670 ($C_{38}H_{54}O_{10}$); eims *m/z* (rel. int.) 610 (2), 551 (16), 536 (8), 491 (28), 431 (5), 401 (58), 402 (32), 310 (37), 281 (20), 253 (14), 150 (83), 137 (60), 101 (78), 81 (50), 69 (100).

Application of Horeau's method to azadirol [**1**].—To 1 mg azadirol [**1**], 0.5 ml of dry pyridine and 2 ml of α-phenyl butyric anhydride were added and kept at room temperature for 24 h. After adding H_2O (1 ml), the reaction mixture was left for 2 days and worked up employing the modified procedure (27): $[\alpha]^{25}$ [in 5 ml C_6H_6 (0.5 dm tube)] + 0.02°, $[\alpha]^+ = 2.85$; configuration = 23*R*.

Kulactone [**5**].—Rods (58 mg): mp 163–164°; eims *m/z* (rel. int.) $[M]^+$ 452.3294 (calcd for $C_{30}H_{44}O_3$, 452.3290) (6), 437.3021 ($C_{29}H_{41}O_3$) (48), 370 (10), 355 (8), 325 (19), 313 (31), 297 (15), 271 (19), 257 (18), 178 ($C_{11}H_{14}O_2$; fragment **a**) (19), 121 (32), 107 (36), 95 (40), 83 (44), 82 (100), 69 (82); ^1H nmr see Table 1; ^{13}C nmr see Table 2.

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