Two New Insect Growth Regulator Meliacins From *Azadirachta indica* A. Juss (Meliaceae)

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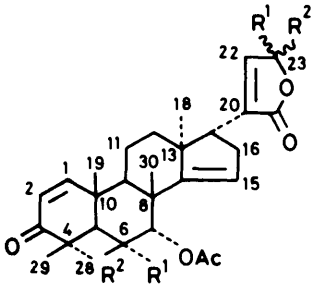
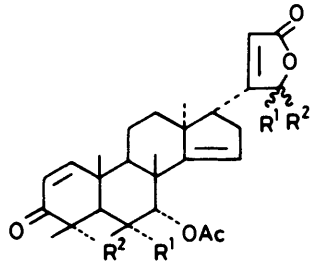
Two new bitter meliacins, nimocinolide (**1**) and isonimocinolide (**2**), have been isolated from the fresh leaves of *Azadirachta indica* (neem). A new meliacin, nimocin (**6**) has further been isolated from its fresh fruits along with the known azadirone, gedunin, epoxyazadiradione, 7-deacetyl-7-benzoylazadiradione, azadiradione, 17-hydroxyazadiradione, and β-sitosterol. The structures of these tetranortriterpenoids were elucidated through chemical and spectral studies. Compounds (**1**) and (**2**) acted as insect growth regulators against houseflies (*Musca domestica*) and mosquitoes (*Aedes aegypti*).

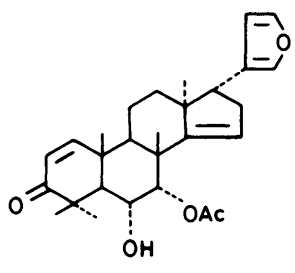
Azadirachta indica (neem) is widely distributed in Asia and Africa and almost every part of the tree has been used for the treatment of a variety of human ailments, particularly diseases of bacterial and fungal origin.[1,2] It has recently been shown that nimbidin, an amorphous factor isolated by Siddiqui,[3] has anti-arthritic and anti-inflammatory properties and possesses significant anti-ulcer potential.[4] Various other fractions of the tree have been found to have anti-tumour,[5] antipyretic, and anti-inflammatory properties.[6] More recent studies have further shown that some of the constituents of neem have pronounced pesticidal activity.[7]

We have already reported the isolation and structure elucidation of the triterpenoids nimolicinol,[8] azadirachtol,[9] nimocinol,[10] and nimbocinone;[11] the present paper deals with the isolation and structure elucidation of two new bitter tetranortriterpenoids, nimocinolide (**1**) and isonimocinolide (**2**) (from the fresh winter leaves), and a new tetranortriterpenoid nimocin (**6**), along with azadirone,[12] gedunin,[12] epoxyazadiradione,[12] 7-deacetyl-7-benzoylazadiradione,[13] azadiradione,[12,14] 17-hydroxyazadiradione,[14] and β-sitosterol (from the fresh fruits). The structures of these compounds have been established through detailed ^1H and ^{13}C n.m.r. spectroscopy and chemical transformations.

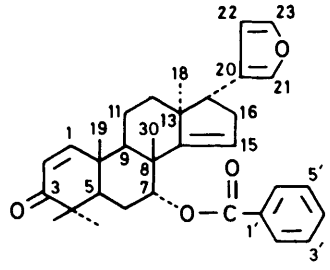




(**1**) R^1 = OH, R^2 = H (**2**) R^1 = OH, R^2 = H
(**3**) R^1 = OAc, R^2 = H (**4**) R^1 = OAc, R^2 = H
(**5**) R^1 = R^2 = O



Nimocinol



(**6**)

Results and Discussion

Fresh winter leaves of neem were extracted with ethanol and the dark greenish extract was separated into acidic and neutral fractions. The residue obtained on work-up of the neutral fraction was subjected to solvent fractionation followed by purification by successive preparative t.l.c. on silica gel and aluminium oxide to give two uniform meliacin butenolides, nimocinolide (**1**) and isonimocinolide (**2**).

Nimocinolide (**1**) has the molecular formula $C_{28}H_{36}O_7$ (high resolution mass spectroscopy and elemental analysis). Its u.v. spectrum showed maxima at 230 nm, while the i.r. spectrum showed peaks at 3 450 (OH), 1 765 (α,β-unsaturated-γ-lactone), 1 730 (ester carbonyl), 1 660 (cyclohexenone), 1 650, and 820 cm^{-1} (trisubstituted double bonds).

The triterpenoidal nature of (**1**) is indicated by the presence in the ^1H n.m.r. spectrum of five three-proton singlets at δ 0.92, 1.13, 1.26, 1.30, and 1.41 (Table 1). The spectrum also showed a pair of doublets at δ 7.10 and 5.89 (*J* 10 Hz), a characteristic feature of ring A 1-en-3-ones; this was further supported by a fragment at *m/z* 137.0969 ($C_9H_{13}O$) in the mass spectrum. The ^1H n.m.r. spectrum was consistent with the presence of a hydroxy and an acetoxy substituent at C-6 and C-7, respectively, both possessing α-orientation. Thus, two one-proton doublets [δ 2.20 (*J* 11.6 Hz) and 5.34 (*J* 2.4 Hz)] and a one-proton doublet of doublets [δ 4.35 (*J* 11.6, 2.4 Hz)] have been assigned to 5-H, 7-H, and 6-H, respectively. A three-proton singlet at δ 2.04 has been attributed to the acetoxy protons, while a two-proton broad multiplet at δ 2.50—2.85 (exch. with D_2O) is due to the two hydroxy protons.

The data so far recorded show that the rings A—D of nimocinolide (**1**) are identical with those of nimocinol.[10] However, the signals of the furan ring are missing, a γ-hydroxy-α,β-unsaturated-γ-lactone being indicated instead by the ^1H and ^{13}C n.m.r. spectral data[15,16] (Tables 1 and 2). Thus two one-proton signals at δ 6.95 and 6.14 have been assigned to 22-H and 23-H respectively, the latter being shifted to δ 6.90 upon acetylation. This is corroborated by the ^{13}C n.m.r. spectrum (Table 2) which shows a hemiacetal carbon at δ 96.80 (C-23) and an α,β-unsaturated-γ-lactone [δ 137.50 (C-20), 169.50 (C-21), and 145.80 (C-22)]. The presence of double signals for C-20, C-21, C-22, and C-23 (Table 2) indicates that it is epimeric at C-23; this has also been observed for other γ-hydroxy butenolides.[15] Moreover, the chemical shifts of the ring A—D carbons are comparable with those reported for nimocinol (Table 2).

Table 1. ^{1}H N.m.r. spectral data (δ_H p.p.m. and J/Hz) of tetranortriterpenoids

Assignment	Nimocinol[a]	(1)	(2)	(3)	(4)	(5)[a]
1-H	7.06 (d) $J_{1,2}$ 10.0	7.10 (d) $J_{1,2}$ 10.0	7.09 (d) $J_{1,2}$ 10.0	7.12 (d) $J_{1,2}$ 10.0	7.09 (d) $J_{1,2}$ 10.0	7.03 (d) $J_{1,2}$ 10.0
2-H	5.82 (d) $J_{2,1}$ 10.0	5.89 (d) $J_{2,1}$ 10.0	5.91 (d) $J_{2,1}$ 10.0	5.92 (d) $J_{2,1}$ 10.0	5.93 (d) $J_{2,1}$ 10.0	5.85 (d) $J_{2,1}$ 10.0
5-H	2.17 (d) $J_{5,6}$ 11.2	2.20 (d) $J_{5,6}$ 11.6	2.18 (d) $J_{5,6}$ 11.6	2.50 (d) $J_{5,6}$ 11.6	2.49 (d) $J_{5,6}$ 11.6	3.45 (s)
6-H	4.30 (dd) $J_{6,5}$ 11.2 $J_{6,7}$ 2.5	4.35 (dd) $J_{6,5}$ 11.6 $J_{6,7}$ 2.4	4.36 (dd) $J_{6,5}$ 11.6 $J_{6,7}$ 2.4	5.40 (dd) $J_{6,5}$ 11.6 $J_{6,7}$ 2.2	5.34 (dd) $J_{6,5}$ 11.6 $J_{6,7}$ 2.2	
7-H	5.30 (d) $J_{7,6}$ 2.5	5.34 (d) $J_{7,6}$ 2.4	5.35 (d) $J_{7,6}$ 2.4	5.44 (d) $J_{7,6}$ 2.2	5.45 (d) $J_{7,6}$ 2.2	4.92 (s)
9-H	2.00—2.35 (m)	2.17 (dd) $J_{9,11\alpha}$ 2.9 $J_{9,11\beta}$ 11.3	2.17 (dd) $J_{9,11\alpha}$ 3.5 $J_{9,11\beta}$ 11.5	2.35 (dd) $J_{9,11\alpha}$ 4.0 $J_{9,11\beta}$ 10.8	2.46 (dd) $J_{9,11\alpha}$ 3.2 $J_{9,11\beta}$ 10.8	2.28—2.33 (m)
11-H$_\alpha$	2.55—2.90 (m)	2.75 (m)	2.85 (m)	2.85 (m)	2.78 (m)	2.28—2.33 (m)
11-H$_\beta$	2.00—2.35 (m)	2.21 (dddd) J_{gem} 14.0 $J_{11\beta,9}$ 11.3 $J_{11\beta,12\alpha}$ 11.3 $J_{11\beta,12\beta}$ 3.3	1.95 (dddd) J_{gem} 16.0 $J_{11\beta,9}$ 11.5 $J_{11\beta,12\alpha}$ 11.5 $J_{11\beta,12\beta}$ 4.3	1.78 (dddd) J_{gem} 14.6 $J_{11\beta,9}$ 10.8 $J_{11\beta,12\alpha}$ 10.8 $J_{11\beta,12\beta}$ 4.4	1.85 (dddd) J_{gem} 15.5 $J_{11\beta,9}$ 10.8 $J_{11\beta,12\alpha}$ 10.8 $J_{11\beta,12\beta}$ 4.0	1.61 (m)
12-Hα	2.55—2.90 (m)	2.52 (ddd) J_{gem} 14.6 $J_{12\alpha,11\alpha}$ 2.0 $J_{12\alpha,11\beta}$ 11.3	2.50 (m)	2.52 (ddd) J_{gem} 13.5 $J_{12\alpha,11\alpha}$ 1.7 $J_{12\alpha,11\beta}$ 10.8	2.65 (ddd) J_{gem} 14.0 $J_{12\alpha,11\alpha}$ 2.2 $J_{12\alpha,11\beta}$ 10.8	2.50—2.56 (m)
12-Hβ	2.55—2.90 (m)	2.83 (m)	2.72 (ddd) J_{gem} 14.5 $J_{12\beta,11\alpha}$ 4.3 $J_{12\beta,11\beta}$ 4.3	2.77 (m)	2.67 (ddd) J_{gem} 13.3 $J_{12\beta,11\alpha}$ 4.0 $J_{12\beta,11\beta}$ 4.0	2.50—2.56 (m)
15-H	5.37 (m)	5.38 (m)	5.42 (m)	5.38 (m)	5.39 (m)	5.34 (m)
16-Hα	2.00—2.35 (m)	2.02 (ddd) J_{gem} 16.0 $J_{16\alpha,15}$ 4.0 $J_{16\alpha,17\beta}$ 4.0	1.99 (m)	2.02 (m)	2.02 (ddd) J_{gem} 13.2 $J_{16\alpha,15}$ 3.4 $J_{16\alpha,17\beta}$ 3.4	1.74 (m)
16-Hβ	2.00—2.35 (m)	2.18 (m)	2.16 (m)	2.20 (m)	2.18 (m)	2.20 (m)
17-H	2.00—2.35 (m)	2.11 (m)	2.07 (dd) $J_{17,16\alpha}$ 4.0 $J_{17,16\beta}$ 8.0	2.28 (dd) $J_{17,16\alpha}$ 4.0 $J_{17,16\beta}$ 7.8	2.10 (m)	2.07 (m)
21-H	7.30 (m)		5.93 (m)		6.87 (m)	
22-H	6.22 (m)	6.95 (m)	6.02 (m)	6.95 (m)	6.01 (m)	6.65 (s)
23-H	7.18 (m)	6.14 (m)		6.90 (m)		
OAc	1.97 (s)	2.04 (s)	2.05 (s)	2.00, 2.04, 2.16 (3s)	2.01, 2.04, 2.18 (3s)	2.03 (s)
OH	2.50 (br m)	2.50—2.85 (br m)	2.61—2.90 (br m)			
13-Me	1.07 (s)	0.92 (s)	0.96 (s)	0.90 (s)	0.95 (s)	0.85 (s)
10-Me	1.21 (s)	1.30 (s)	1.32 (s)	1.25 (s)	1.18 (s)	1.15 (s)
4-Meα	1.18 (s)	1.13 (s)	1.13 (s)	1.18 (s)	1.17 (s)	0.87 (s)
4-Meβ	1.25 (s)	1.26 (s)	1.28 (s)	1.25 (s)	1.26 (s)	1.25 (s)
8-Me	1.35 (s)	1.41 (s)	1.42 (s)	1.32 (s)	1.32 (s)	1.25 (s)

[a] Spectra recorded on a 100 MHz instrument.

The i.r., u.v., and mass spectra of isonimocinolide (**2**) showed its similarity to nimocinolide (**1**). The presence of a 21-hydroxybut-20(22)-ene-21,23-γ-lactone side chain in (**2**) was indicated by the presence of two one-proton signals at δ 5.93 (21-H) and 6.02 (22-H), the former being shifted to δ 6.87 upon acetylation in the ^{1}H n.m.r. spectrum (Table 1), and by the presence of signals at δ 157.80 (C-20), 98.60 (C-21), 119.06 (C-22), and 169.60 (C-23) in the ^{13}C n.m.r. spectrum of (**2**) (Table 2). The C-21 epimeric nature of isonimocinolide was also indicated by the appearance of double signals for the side chain carbons.

The spectral data of the two compounds inevitably showed that they have identical carbocyclic nuclei and differ only in the nature of the side chain. This fact was finally confirmed through oxidation experiments when a mixture (A) of compounds (**1**) and (**2**) afforded the common oxidation product (**5**).

The stereochemistry of various centres of nimocinolide (**1**) was established through n.O.e. difference and 2-D n.O.e. (NOESY) spectral analysis. Thus, irradiation at δ 1.41 (8-Me) influenced the signals of 4-Me β (δ 1.26), 10-Me (δ 1.30), and 6-H (δ 4.35), and irradiation at δ 1.30 (10-Me) affected the signals of 4-Me β (δ 1.26) and 8-Me (δ 1.41), showing the spatial proximity of 10-Me to both 4β-Me and 8-Me. Similarly, irradiation at δ 1.26 (4β-Me) affected the signals of 6-H (δ 4.35) and 7-H (δ 5.34). These observations showed that 4β-Me, 8-Me, and 10-Me are oriented in the same plane as 6-H and 7-H, *i.e.* β, since the β-orientation of 6-H and 7-H has already been shown by the coupling constants observed in the ^{1}H n.m.r. spectrum of

Table 2. ^{13}C N.m.r. shifts (δ_C/p.p.m.) of tetranortriterpenoids

Carbon	Nimocinol [10]	(1)	(2)	Carbon	Nimocinol [10]	(1)	(2)
1	157.30	157.40	157.10	17	51.64	50.03[c]	52.90
2	126.10	126.30	126.40			51.00[c]	53.80
3	205.90	206.00	206.00	20	124.36	137.50	157.80[f]
4	40.50	40.50	40.60			137.60	157.60[f]
5	49.80	50.70[c]	51.60	21	142.55	169.50	98.60
6	68.00	68.20	68.20			169.10	98.80
7	79.00	78.90	79.10	22	110.93	145.60	119.04[g]
8	45.43	45.50	45.60			145.80	119.06[g]
9	37.15	37.10	37.00	23	139.63	96.80	169.60
10	43.11	43.40	43.50			96.90	169.20
11	16.30	16.40	16.50	O ‖ O–C–Me	171.97	171.90	172.00
12	33.60[a]	32.70	33.30[e]	O ‖ O–C–*Me*	21.20[b]	21.20[d]	21.60[h]
13	47.08	47.50	47.50	CMe	27.07	27.20	27.30
14	158.00	157.20	157.50[f]		20.79[b]	20.90[d]	21.10[h]
15	119.55	119.50	119.60[g]		20.22[b]	20.30[d]	20.40[h]
16	34.32[a]	34.01	33.40[e]		19.64	19.30	19.50
		34.06	33.80[e]		14.04	16.60	16.70

[a–h] Assignments may be reversed.

(**1**) (Table 1). However, irradiation of these methyl signals did not influence the signals of 5-H and 9-H, showing that they are on the other side of the plane, *i.e.* α. Furthermore, irradiation at δ 1.13 (4-Me α) affected the signal of 5-H (δ 2.20) and irradiation at δ 0.92 (13-Me) influenced the 22-H signal (δ 6.95), while irradiation at δ 6.95 (22-H) affected 13-Me (δ 0.92) and 9-H (δ 2.17), showing the α-orientation of 13-Me, 4α-Me, 5-H, 9-H, and the side chain at C-17. These results were corroborated by a NOESY experiment which showed the connectivities of 13-Me with OAc, 9-H, 16-Hα, and 22-H; 4β-Me with 6-H; 6-H with 7-H; 9-H with 12α-H; and also of 23-H with 22-H, 2-H with 1-H, and 1-H with 22-H.

The n.O.e. difference and 2-D n.O.e. (NOESY) experiments showed that the stereochemistry of various centres in isonimocinolide (**2**) is the same as that observed in nimocinolide (**1**). Irradiation at δ 1.42 (8-Me) influenced the signals of 10-Me (δ 1.32), 4β-Me (δ 1.28), and 6-H (δ 4.36); while irradiation at δ 1.32 (10-Me) affected the signals of 8-Me (δ 1.42), 6-H (δ 4.36), 7-H (δ 5.35), and 1-H (δ 7.09); and irradiation at δ 1.28 (4β-Me) influenced the signals of 8-Me (δ 1.42), 10-Me (δ 1.32), 6-H (δ 4.36), and 7-H (δ 5.35). Irradiation at δ 1.13 (4α-Me) affected the signals of 5-H (δ 2.18) and irradiation at δ 0.96 (13-Me) influenced the signals of 9-H (δ 2.17), OAc (δ 2.05), and 22-H (δ 6.02). When the signal of 22-H (δ 6.02) was irradiated, the 13-Me (δ 0.96) and OAc (δ 2.05) signals were affected while irradiation of the OAc signal (δ 2.05) showed an effect at δ 0.96 (13-Me). Apart from these spatial relationships, the NOESY spectrum also showed the connectivities of 10-Me with 11-Hβ, 2-H with 1-H, and OAc with 22-H.

It has to be noted in this context that butenolides (**1**) and (**2**) were initially obtained as a mixture (A) showing a single spot on t.l.c. (silica gel, benzene–acetone 8:2). However, the ^1H n.m.r. spectrum recorded on a 300 MHz instrument and double signals observed in the ^{13}C n.m.r. spectrum indicated that it is a mixture of two isomers. This was unequivocally shown by its acetylation which yielded the acetyl derivatives showing two distinct spots on t.l.c. with very close R_F values. These, after separation on preparative t.l.c. (silica gel, benzene–ethyl acetate 75:25), were characterised as (**3**) and (**4**). After many attempts to separate the isomeric mixture (A), it was ultimately separated by preparative t.l.c. (aluminium oxide, chloroform–methanol 95:5) to give (**1**) and (**2**), in a ratio of 1:2.

In order to confirm that the two butenolides (**1**) and (**2**) are genuine natural products and were not derived from light-induced oxygen attack on the furan ring, a solution of nimocinol [10] in benzene was irradiated with a Pyrex-filtered u.v. light in a stream of oxygen.[17] The reaction product upon purification through preparative t.l.c. (aluminium oxide, chloroform–methanol 95:5) yielded nimocinolide (**1**), and no isonimocinolide (**2**) was detected in the reaction mixture. This observation, together with the fact that the two butenolides were detected in the fresh neem leaves extract, provided conclusive evidence that they are naturally occurring compounds and not artefacts. Compounds (**1**) and (**2**) affect fecundity in houseflies (*Musca domestica*) at doses of 100—500 p.p.m. and show mutagenic properties in mosquitoes (*Aedes aegypti*) producing intermediates.

The powdery, hexane-insoluble portion of the neutral fraction obtained on work-up of the ethanolic extract of fresh, uncrushed, undried, ripe fruits was chromatographed on a column of silica gel and eluted with benzene and benzene–ethyl acetate. Eight uniform components were ultimately obtained, seven of which have been identified as azadirone, β-sitosterol, epoxyazadiradione, gedunin, 7-deacetyl-7-benzoylazadiradione, azadiradione, and 17-hydroxyazadiradione, both by m.p. studies and by comparison of their spectral data (u.v., i.r., high resolution mass, and ^1H n.m.r.) with those recorded in the literature (*loc. cit.*).

The hitherto unrecorded new constituent, which has molecular formula $C_{33}H_{38}O_4$ (high resolution mass) has been named as nimocin (**6**). Its u.v. spectrum showed maxima at 207, 220, 273, and 300 nm, while the i.r. spectrum showed peaks at 3 200br (furan and aromatic C–H), 2 860 and 2 830 (aliphatic C–H), 1 720 (ester carbonyl), 1 665 (cyclohexenone), 1 600—1 400 (4 peaks, benzene ring), 1 270 and 1 100 (C–O), and 870 cm^{-1} (furan). The ^1H n.m.r. spectral data of (**6**) and azadirone (*loc. cit.*) were very similar, although the signal of the acetoxy group was missing in (**6**) and instead signals corresponding to a benzoyloxy group were observed. These data led to the characterisation of (**6**) as 7-deacetyl-7-benzoylazadirone, which

was further supported by significant fragments observed in the mass spectrum (see Experimental section).

Experimental

M.p.s were recorded in glass capillary tubes and are uncorrected. I.r. (in $CHCl_3$) and u.v. (in MeOH) spectra were measured on JASCO IRA-I and Pye-Unicam SP-800 spectrometers respectively; mass spectra were recorded on Finnigan MAT 112 and 312 double focussing mass spectrometers. N.m.r. spectra were recorded in $CDCl_3$ on a Bruker Aspect 3000 spectrometer operating at 300 MHz for ^1H and 75 MHz for ^{13}C nuclei. Chemical shifts are reported in p.p.m. relative to tetramethylsilane. Optical rotations were measured at 24 °C in $CHCl_3$, on a Polartronic-D polarimeter. Merck Kieselgel 60 PF_{254} and Aluminium oxide 60 PF_{254} coated on glass plates were used for analytical (thin layer) and preparative (thick layer) chromatography.

Isolation of Nimocinolide (**1**) *and Isonimocinolide* (**2**).—An ethanolic extract of fresh, undried, unruptured neem leaves (40 kg), collected in winter from the Karachi region, was separated into acidic and neutral fractions (1 kg, 4.5% on dry wt. basis). The residue obtained on work-up of the neutral fraction was separated into 50% methanol soluble and insoluble portions, and the former was shaken out with ether. The ethereal phase was dried (Na_2SO_4) and evaporated and the residue was taken up in moist methanol and the solution kept cold for 24 h when a precipitate was formed. The supernatant liquid was decanted off and slowly evaporated at room temperature to afford a crystalline material which was subjected to preparative t.l.c. (silica gel, benzene–acetone 8:2) to yield a crystalline product (A). This was separated into (**1**) and (**2**) by preparative thick layer chromatography on plates coated with aluminium oxide (chloroform–methanol 19:1).

Nimocinolide (**1**). Needles (0.68 g, 0.068% on the wt. of total neutral fraction), m.p. 160 °C (from chloroform), $[\alpha]_D$ 86.66° (*c* 0.6 in $CHCl_3$) (Found: C, 69.4; H, 7.35%; M^+, 484.2451. $C_{28}H_{36}O_7$ requires C, 69.42; H, 7.43%; *M*, 484.2461); $\lambda_{max.}$ 230 nm (ε 3 562); $\nu_{max.}$ 3 450, 1 765, 1 730, 1 660, 1 650, and 820 cm^{-1}; *M* − $MeCO_2H$), 409 (34, *M* − $MeCO_2H$-Me), 384 (5, *M* − side chain), 371 (4, *M* − $C_5H_5O_3$), 357 (5, *M* − $C_6H_7O_3$), 245.1185 (26, *M* − $C_{13}H_{19}O_4$), 137.0969 (100, ring A + H), 127 (25, $C_6H_7O_3$), 113 (42, side chain + CH_2), and 100 (70, side chain + H).

Isonimocinolide (**2**). Rods (1.28 g, 0.128% on the wt. of total neutral fraction), m.p. 165 °C (from $CHCl_3$), $[\alpha]_D$ 85° (*c* 0.8 in $CHCl_3$) (Found: C, 69.4; H, 7.45%; M^+, 484.2469. $C_{28}H_{36}O_7$ requires C, 69.42; H, 7.43%; *M*, 484.2461); $\lambda_{max.}$ 232 nm (ε 3 064); $\nu_{max.}$ 3 455 (OH), 1 765 (α,β-unsaturated-γ-lactone), 1 735 (ester carbonyl), 1 660 (cyclohexenone), 1 650, and 820 cm^{-1} (trisubstituted double bonds); *m/z* 484.2469 (M^+, 5%), 466.2365 (10, *M* − H_2O), 424.2268 (15, *M* − $MeCO_2H$), 409 (30, *M* − $MeCO_2H$-Me), 384.2277 (10, *M* − side chain), 371 (6, *M* − $C_5H_5O_3$), 357 (4, *M* − $C_6H_7O_3$), 245.1180 (30, *M* − $C_{13}H_{19}O_4$), 137.0970 (100, ring A + H), 127 (20, $C_6H_7O_3$), 113 (48, side chain + CH_2), and 100.0224 (85, side chain + H).

Acetylation of Nimocinolide (**1**).—Acetic anhydride (2 ml) was added to a solution of (**1**) (30 mg) in pyridine (1 ml) and the reaction mixture was kept overnight at room temperature. Work-up gave the acetylated product (**3**) which crystallized from methanol as white plates (24 mg, 80%), m.p. 72—75 °C (from MeOH), $[\alpha]_D$ 25° (*c* 0.04 in $CHCl_3$); $\lambda_{max.}$ 225 nm (ε 4 132); $\nu_{max.}$ 1 770 (α,β-unsaturated-γ-lactone), 1 738 br (ester carbonyls), 1 665 (cyclohexenone), 1 660, and 820 cm^{-1} (trisubstituted double bonds); *m/z* 568.2660 (M^+, 3%), 508.2444 (12, *M* − $MeCO_2H$), 448.2234 (14, *M* − 2 × $MeCO_2H$), 406.2144 (8, $C_{26}H_{30}O_4$), 388.2043 (6, $C_{26}H_{28}O_3$), 373 (10), 142 (6, side chain), and 137 (26).

Acetylation of Isonimocinolide (**2**).—Compound (**2**) (30 mg) was acetylated in the same manner as above to yield (**4**), which crystallized from chloroform as fine needles (21 mg, 70%), m.p. 88—90 °C (from $CHCl_3$), $[\alpha]_D$ 28.57° (*c* 0.07 in $CHCl_3$); $\lambda_{max.}$ 233 nm (ε 3 692); $\nu_{max.}$ 1 780 (α,β-unsaturated-γ-lactone), 1 735 br (ester carbonyls), 1 665 (cyclohexenone), 1 660, and 820 cm^{-1} (trisubstituted double bonds); *m/z* 568.2666 (M^+, 5%), 508.2444 (10, *M* − $MeCO_2H$), 448.2234 (12, *M* − 2 × $MeCO_2H$), 406.2146 (10, $C_{26}H_{30}O_4$), 388.2043 (5, $C_{26}H_{28}O_3$), 373 (8), 142 (10, side chain), and 137 (20).

Sarett Oxidation of Nimocinolide (**1**) *and Isonimocinolide* (**2**).—A solution of (A) (40 mg) in pyridine (1 ml) was oxidized with Sarett's reagent (40 mg CrO_3 in 1 ml pyridine) with stirring at room temperature for 8 h. Work-up gave (**5**) as the major oxidation product, which was purified by preparative t.l.c. (silica gel, benzene–acetone 75:25), needles (16 mg, 40%), m.p. 98—100 °C (from MeOH), $[\alpha]_D$ 10° (*c* 0.2 in $CHCl_3$); $\lambda_{max.}$ 220 nm (ε 2 468); $\nu_{max.}$ 1 780—1 740 (anhydride carbonyls), 1 725 (ester carbonyl), 1 715 (cyclohexanone), 1 660 (cyclohexenone), 1 650, and 820 cm^{-1} (trisubstituted double bonds); *m/z* 480.2176 (M^+, 2%).

Photo-oxidation of Nimocinol.—A solution of nimocinol (120 mg) in benzene (20 ml) was irradiated with a u.v. lamp (Hanovia 1L Photochemical Reacter) in a Pyrex flask under a stream of oxygen. After disappearance of nimocinol (5 h) as shown by t.l.c., the solvent was evaporated and the residue subjected to preparative t.l.c. (aluminium oxide, chloroform–methanol 95:5) which afforded nimocinolide (**1**) (36 mg, 30%), m.p. 160 °C (from chloroform).

Isolation of Nimocin (**6**).—The ethanolic extract of fresh, uncrushed, undried, ripe fruits (nimoli, 20 kg) of neem was divided into acidic and neutral fractions. The powdery hexane insoluble portion of the latter was chromatographed on column (silica gel 40, 70—230 mesh). On successive elution with benzene and benzene–ethyl acetate (9:1 v/v), eight uniform components were obtained in the following order of elution: azadirone,[12] nimocin, β-sitosterol, gedunin,[12] epoxyazadiradione,[12] 7-deacetyl-7-benzoylazadiradione,[13] azadiradione,[12,14] and 17-hydroxyazadiradione.[14] The structure of nimocin has been established as 7-deacetyl-7-benzoylazadirone (**6**). Needles (10 mg, 0.005% on the wt. of total neutral fraction); m.p. 190—195 °C (from $CHCl_3$); $\lambda_{max.}$ 207, 220, 230 sh, 273, and 300 nm; $\nu_{max.}$ 3 200, 2 860, 2 830, 1 720, 1 665, 1 600—1 400 (4 peaks), 1 270, 1 100, and 870 cm^{-1}; δ 7.80—7.50 (m, 2′- and 6′-H), 7.53—7.33 (m, 3′-, 4′-, 5′-, 21-, and 23-H), 7.12 (d, *J* 10 Hz, 1-H), 5.95 (d, *J* 10 Hz, 2-H), 5.85 (m, 22-H), 5.45 (m, 7- and 15-H), 2.50—2.20 (m, 5-and 9-H), 2.21—1.80 (m, 6-, 11-, and 12-H), 1.80—1.50 (m, 17-H), 1.30, 1.20, 0.98, 0.93, and 0.88 (each s, 5 Me); *m/z* 498.2800 (M^+, 25%), 499 (*M* + 1, 10%), 500 (*M* + 2, 3%), 430 (3, *M*-furan + H), 417 (4, *M* − furan + CH_2), 393 (3, *M* − PhCO), 376 (12, *M* − $PhCO_2H$), 150 (8, ring A + CH_2), 137.0958 (30, ring A + H), 108 (50, C_7H_8O), 105.0338 (100, C_6H_5CO), 95 (54, C_6H_7O), and 81 (60, furan + CH_2).

Acknowledgements

For the biological results our thanks are due to Dr. S. N. H. Naqvi and co-workers from the Toxicology Laboratory of Zoology Department, University of Karachi. One of us (T. M.) expresses grateful thanks to the Hamdard Foundation of Pakistan for the provision of a research fellowship in the course of this work.

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Received 16*th August*. 1985; *Paper* 5/1423