New insect-growth-regulator meliacin butenolides from the leaves of *Azadirachta indica* A. Juss

Bina Shaheen Siddiqui,*[a] Farhana Afshan,[a] Ghiasuddin,[a] Shaheen Faizi,[a] Syed Naeem-ul-Hassan Naqvi[b] and Rajput Mohammad Tariq[c]

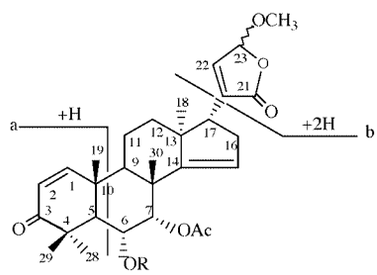
[a] *H.E.J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan*
[b] *Department of Pharmacology, Baqai Medical University, Tool Plaza, Super Highway, Karachi, Pakistan*
[c] *Department of Zoology, University of Karachi, Karachi-75270, Pakistan*

Received (in Cambridge) 23rd March 1999, Accepted 23rd June 1999

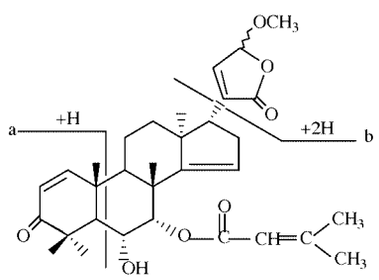
Two new triterpenoids, 23-*O*-methylnimocinolide [7α-acetoxy-6α-hydroxy-23ξ-methoxy-3-oxo-24,25,26,27-tetranorapotirucalla(apoeupha)-1,14,20(22)-trieno-21,23-lactone] **1**, 7-*O*-deacetyl-23-*O*-methyl-7α-*O*-senecioylnimocinolide [6α-hydroxy-23ξ-methoxy-3-oxo-7α-senecioyloxy-24,25,26,27-tetranorapotirucalla(apoeupha)-1,14,20(22)-trieno-21,23-lactone] **2** have been isolated from the methanolic extract of the fresh leaves of *Azadirachta indica* (neem). Their structures were elucidated through spectral studies, including 2D NMR (NOESY, COSY-45, HMQC and HMBC). Compounds **1** and **2** show insect-growth-regulating effect on mosquitoes (*Aedes aegypti*) with LC_{50} 53 ppm and 2.14 ppm, respectively. The senecioyloxy substituent at C-7 in **2** results in a significant increase of activity.

Introduction

Azadirachta indica A. Juss (syn. *Melia azadirachta* Linn; *Melia indica*, *Margosa*), known in the vernacular as Neem and Nimba, belongs to the Meliaceae family. It is widely distributed in Asia and Africa and almost every part of the plant is used in the indigenous systems of medicine.[1–4] In view of the attributed therapeutic and pesticidal[5] importance of this plant, comprehensive investigations on its different parts have been carried out by various groups of workers, leading to the isolation and structure elucidation of a series of constituents.[5–12] The present studies have been undertaken on the neutral fraction of the methanolic extract of the fresh leaves which resulted in the isolation of two new tetranortriterpenoids, namely, 7α-acetoxy-6α-hydroxy-23ξ-methoxy-3-oxo-24,25,26,27-tetranorapotirucalla(apoeupha)-1,14,20(22)-trieno-21,23-lactone (23-*O*-methylnimocinolide) **1**, and 6α-hydroxy-23ξ-methoxy-3-oxo-7α-senecioyloxy-24,25,26,27-tetranorapotirucalla(apoeupha)-1,14,20(22)-trieno-21,23-lactone (7-*O*-deacetal-23-*O*-methyl-7-*O*-senecioylnimocinolide) **2**.† Their structures have been elucidated through spectral studies including 1D and 2D NMR (COSY-45, NOESY, *J*-resolved, HMQC and HMBC) and chemical transformations.



1 R = H
1a R = Ac



2

Results and discussion

Two new tetracyclic triterpenoids, 23-*O*-methylnimocinolide **1** and 7-*O*-deacetyl-23-*O*-methyl-7-*O*-senecioylnimocinolide **2** were obtained from the neutral part of the methanolic leaves extract. Compound **1** formed colourless rods from $CHCl_3$–MeOH (1:1), mp 119–120 °C, and showed the molecular ion peak at *m/z* 498 in the EIMS and at *m/z* 498.2590 in the HRMS, corresponding to the molecular formula $C_{29}H_{38}O_7$. Its UV spectrum exhibited absorption at 230 nm, consistent with an α,β-unsaturated carbonyl and an α,β-unsaturated γ-lactone system, while the IR spectrum showed peaks at 3450 (OH), 1760 (α,β-unsaturated γ-lactone), 1680–1720 (α,β-unsaturated ketone and ester), 1600, 840 (C=C stretch and C–H bend of R_2C=CR) and 1375 (geminal methyls) cm^{-1}. The molecular formula of **1** suggested the presence of eleven double-bond equivalents, four of which were accounted for by two α,β-unsaturated carbonyl systems, one by the acetyl function, one by an isolated C=C double bond, one by the lactone ring and the remaining four by the four rings (A–D) of the azadirone nucleus.[8]

The ^{1}H NMR spectrum (Table 1) showed two one-proton AB doublets at δ 7.12 and 5.90 (J = 10.2 Hz) attributed to H-1 and H-2, respectively, of the 1-en-3-one system of ring A,[8] while the ^{13}C NMR spectrum indicated the corresponding signals at δ_C 157.1 (C-1) and 126.0 (C-2) along with a signal at δ 200.0 (C-3). This moiety was further supported by the mass fragment **1A** at *m/z* 137.0966 ($C_9H_{13}O$). The ^{1}H NMR spectrum (Table 1) indicated the triterpenoidal nature of 23-*O*-methylnimocinolide **1** by the presence of five quaternary methyl singlets at δ 0.89 (H_3-18), 1.18 (H_3-19), 1.26 (H_3-28), 1.30 (H_3-29) and 1.39 (H_3-30). The ^{1}H NMR spectrum further showed the presence of α-oriented hydroxy and acetoxy substituents at C-6 and C-7,

† Senecioyl = 3-methylbut-2-enoyl.

Table 1 ^{1}H (300 MHz) (data in parentheses are multiplicity, *J*/Hz) and ^{13}C (125 MHz) NMR spectral data of **1** with single-bond (HMQC) and multiple-bond (HMBC) correlations

Position	^{13}C	^{1}H	HMBC correlations
1	157.1	7.12 (d, 10.2)	1.18 (H_3-19), 5.90 (H-2)
2	126.0	5.90 (d, 10.2)	
3	200.0		7.12 (H-1)
4	34.0		
5	50.6	2.11 (d, 11.7)	5.29 (H-7), 1.26 (H_3-28), 1.30 (H_3-29)
6	68.3	4.25 (dd, 11.7, 2.9)	2.11 (H-5), 1.26 (H_3-28), 5.29 (H-7)
7	79.0	5.29 (d, 2.9)	1.39 (H_3-30)
8	46.5		
9	33.7	2.32 (dd, 12.4, 3.7)	
10	37.7		
11	16.3	2.82 (m), 2.20 (m)	
12	32.7	2.46 (m), 2.82 (m)	
13	47.4		
14	158.6		
15	118.0	5.36 (m)	
16	33.9	2.16 (m), 2.23 (m)	
17	50.1	2.20 (m)	0.89 (H_3-18)
18	20.7	0.89 (s)	
19	20.9	1.18 (s)	
20	137.0		
21	171.1		6.89 (H-22)
22	142.0	6.89 (m)	
23	102.2	5.75 (m)	6.89 (H-22)
28	21.2	1.26 (s)	
29	19.3	1.30 (s)	
30	27.3	1.39 (s)	
OCO*C*H_3	21.5	2.08 (s)	
O*C*OCH$_3$	171.9		
OCH_3	57.0	3.57 (s)	

Table 2 ^{1}H (500 MHz) (data in parentheses are multiplicity, *J*/Hz) and ^{13}C (125 MHz) NMR spectral data of **2** with single-bond (HMQC) and multiple-bond (HMQC) correlations

Position	^{13}C	^{1}H	HMBC correlations
1	157.1	7.10 (d, 10.1)	1.11 (H_3-19), 5.89 (H-2)
2	126.3	5.89 (d, 10.1)	
3	205.6		1.29 (H_3-29), 1.24 (H_3-28)
4	43.4		
5	36.8	2.17 (d, 11.6)	1.24 (H_3-28), 1.11 (H_3-19)
6	68.3	4.37 (dd, 2.5, 11.6)	2.17 (H-5), 5.33 (H-7)
7	79.1	5.33 (d, 2.5)	4.37 (H-6), 2.17 (H-5)
8	47.5		1.38 (H_3-30), 2.28 (H-9)
9	33.9	2.28 (dd, 7.0, 3.4)	1.11 (H_3-19)
10	40.6		1.11 (H_3-19)
11	32.6	2.80 (m), 2.34 (m)	
12	16.4	2.86 (m), 2.52 (m)	
13	45.5		2.26 (H-17)
14	158.6		5.36 (H-15)
15	119.0	5.36 (m)	0.89 (H_3-18), 2.59 (H_2-16)
16	34.0	2.32 (m), 2.59 (m)	
17	50.0	2.26 (m)	
18	21.0	0.89 (s)	
19	21.0	1.11 (s)	
20	138.3		
21	172.0		
22	144.3	6.88 (t, 1.5)	
23	102.5	5.75 (d, 1.5)	3.58 (OCH_3), 6.88 (H-22)
28	27.3	1.24 (s)	
29	31.9	1.29 (s)	
30	20.3	1.38 (s)	
OCH_3	57.5	3.58 (s)	
C-1′	171.0		
C-2′	113.0	5.67 (m)	
C-3′	158.8		
C-4′	21.1	1.94 (s)	2.16 (H_3-5′)
C-5′	27.1	2.16 (s)	1.94 (H_3-4′)

respectively. Thus, it contained two one-proton doublets at δ 2.11 (J = 11.7 Hz) and δ 5.29 (J = 2.9 Hz) and a one-proton double doublet at δ 4.25 (J = 11.7, 2.9 Hz) attributed to H-5, H-7 and H-6, respectively. The signal at δ 4.25 shifted to δ 5.42 (dd, J = 11.7, 2.9 Hz) on acetylation. A muliplet at δ 5.36 in the ^{1}H NMR spectrum, and CH and C carbon shifts at δ_C 118.0 and 158.6 in the ^{13}C NMR spectrum further showed an isolated C=C at C-14.[9] The ^{1}H and ^{13}C NMR spectral data revealed that the rings A–D of **1** are identical with those of nimocinol.[9] However, the signals of a furan ring, a usual feature of meliacins,[8] were not observed and instead a γ-methoxy-α,β-unsaturated γ-lactone was indicated by the mass fragment **1B** and ^{1}H and ^{13}C NMR data (Table 1). Thus two one-proton signals at δ 6.89 and 5.75 were attributable to H-22 and H-23, respectively, and a three-proton singlet at δ 3.57 was due to OCH_3 protons. Their corresponding carbon signals were discernible from the HMQC spectrum at δ_C 142.0, 102.2 and 57.0, along with other signals in the broad-band spectrum at δ_C 137.0 (C-20) and 171.1 (C-21). The presence of double signals for H-22, H-23, C-20, C-21, C-22, C-23 and OCH_3 indicated that **1** exists in two epimers at C-23, which has also been observed in other γ-substituted butenolides.[10] The assignments of protons and carbons were further supported by the interactions observed in the 2D-NOE (NOESY) and HMBC plots. Thus, the NOESY spectrum showed spatial proximity of H-1 to H-2; H-7 to H-6 and H_3-30; H_3-18 to H-22; and H-22 to H-23. The interaction between H_3-18 and H-22 suggested the α-orientation of the γ-butenolide ring at C-17. In the HMBC spectrum cross-peaks were noted for correlation between C-29/H-6; C-6/H-7; C-7/H_3-30; C-17/H-15 and C-17/H_3-18. In light of these spectral data the structure of **1** has been elucidated as 7α-acetoxy-6α-hydroxy-23ξ-methoxy-3-oxo-24,25,26,27-tetranorapotirucalla(apoeupha)-1,14,20(22)-trieno-21-23-lactone, which is consistent with the significant mass spectral fragments (see Experimental section). It may be noted that **1** is the 23-*O*-methyl derivative of nimocinolide reported earlier as an insect growth regulator against *Musca domestica* and *Aedes aegypti*.[10] This is, however, the first reported isolation of **1** as a natural product.

Keeping in view the possibility of methylation of nimocinolide to furnish **1** during extraction, nimocinolide was treated with methanol with a trace of hydrochloric acid, and the contents left at room temperature. No change was observed on TLC after up to six days. However, on refluxing the contents for 1.5 hours, nimocinolide was transformed into **1**, M^+ 498. The mass fragments were consistent with the methylation occurring at the 23-hydroxy group. This observation suggests that **1** is a naturally occurring constituent since the extraction was done at room temperture and nimocinolide does not undergo methylation at room temperature under normal conditions. Conversion of nimocinolide to **1** provides structural evidence of 23-*O*-methylnimocinolide being the isolated product.

The EIMS spectrum of **2** did not show the molecular ion peak, which was, however, observed in the FABMS spectrum at 539 *m*/*z* (M^+ + H). The molecular formula $C_{32}H_{42}O_7$ could be deduced by a combined application of high-resolution mass spectroscopy and ^{13}C NMR spectral data (broad band (BB), DEPT; Table 2). The UV spectrum showed an absorption maximum at 233 nm and the IR spectrum exhibited absorption bands at 3450 (OH), 1765 (α,β-unsaturated γ-lactone), 1660 (conjugated cyclohexenone), 1600 (double bonds) and 1375 (geminal methyls) cm^{-1}. The ^{1}H and ^{13}C NMR data (Table 2) showed five tertiary methyls (δ_H 0.89, 1.11, 1.24, 1.29, 1.38; δ_C 21.0 × 2, 27.3, 31.9, 20.3), a secondary hydroxy group (δ_{Hgem} 4.37; δ_C 68.3) and a senecioyloxy function [δ_H 1.94, H_3-4′; 2.16, H_3-5′; 5.67, H-2′; δ_C 171.0 (C-1′); 113.0 (C-2′); 158.8 (C-3′); 21.1 (C-4′); 27.1 (C-5′)]. This ester moiety was further supported by the significant mass fragments at *m*/*z* 421.2311 ($C_{27}H_{33}O_4$, $M^+ - C_5H_8O_2 - OH$) and at *m*/*z* 438.2414 ($C_{27}H_{34}O_5$, $M^+ - C_5H_8O_2$). The ^{1}H NMR spectrum showed two more trisubstituted (δ_H 5.36 and 6.88; H-15 and H-22 respectively)

and one disubstituted (δ_H 7.10 and 5.89; H-1 and H-2, respectively) double bonds. The signals at δ 7.10 and 5.89, appearing as AB doublets (J = 10.1 Hz), were attributable to H-1 and H-2, respectively, and had corresponding ^{13}C NMR signals at δ_C 157.1 (C-1) and 126.3 (C-2) in the HMQC. These signals and a carbon at δ_C 205.6 in the broad-band spectrum indicated the characteristic ring A, 1-en-3-one system.[8,9] It was also supported by the mass fragment at *m*/*z* 137.0931 ($C_9H_{13}O$, fragment **2A**). The signals at δ 5.36 and 6.88 had cross-peaks at δ_C 119.0 and 144.3 in the HMQC spectrum and were assigned to H-15/C-15 and H-22/C-22, respectively. The NMR data led us to place the hydroxy group at C-6 and the senecioyloxy moiety at C-7, both possessing α-dispositions. Thus, in the ^{1}H NMR spectrum two one-proton doublets were observed at δ 2.17 (J = 11.6 Hz; H-5) and 5.33 (J = 2.5 Hz; H-7) besides a one-proton double doublet at δ 4.37 (J = 11.6, 2.5 Hz; H-6). These signals had cross-peaks at δ_C 36.8, 79.1 and 68.3 in the HMQC spectrum (Table 2). The data recorded so far showed that the rings (A–D) of **2** were structurally close to those of nimocinol,[9] nimocinolide[10] and **1**, with the difference that the C-7 acetoxy group was replaced by a senecioyloxy function. Furthermore, a γ-methoxy-α,β-unsaturated γ-lactone was indicated by the IR (1765 cm^{-1}) and ^{1}H and ^{13}C NMR spectral data (Table 2). Thus there were present a one-proton triplet at δ 6.88 ($J_{22,23} = J_{22,17}$ = 1.5 Hz; H-22) and a one-proton doublet at δ 5.75 (J = 1.5 Hz; H-23) in the ^{1}H NMR spectrum which showed their connectivity with a carbon at δ_C 144.3 (C-22) and a hemiacetal carbon at δ_C 102.5 (C-23), respectively, in the HMQC. The signals for C-20 and C-21 were found at δ_C 138.3 and 172.0, respectively, in the broad-band spectrum. A diagonastic mass fragment **2B** at *m*/*z* 115.0454 ($C_5H_7O_3$) in the mass spectrum of **2** also confirmed the presence of an α,β-unsaturated γ-methoxy γ-lactone.

The stereochemistry of various centres has been confirmed through NOESY, which showed spatial connectivities of H-1 with H-2, H-2 with H_3-19, H-5 with H-6 and H_3-28, H-15 with H-17; H-17 with H_3-18 and H_3-18 with H-22.

In light of these spectral data the structure of **2** was elucidated as 6α-hydroxy-23ξ-methoxy-3-oxo-7α-senecioyloxy-24,25,26,27-tetranorapotirucalla(apoeupha)-1,14,20(22)-trieno-21,23-lactone.

All precise assignments of ^{1}H/^{13}C NMR chemical shifts of **1** and **2** have been carried out through 1D and 2D NMR spectral studies including COSY-45, NOESY, HMQC, HMBC and *J*-resolved experiments, and are consistent with the reported values of compounds with similar partial structures.[7–12]

The insecticidal activity of **1** and **2** was determined on *Aedes aegypti* 4th instar larvae. Both these compounds showed insect-growth-regulating (IGR) effects on these larvae, with LC_{50} 53 and 2.14 ppm, respectively. Compound **1** is the 23-*O*-methyl derivative of nimocinolide reported earlier[10] which also showed IGR effect on mosquitoes (*Aedes aegypti*) and house flies (*Musca domestica*), with an LC_{50} greater than 100 ppm. The 23-*O*-methyl group in **1** has increased its toxicity. Furthermore, in compound **2**, which differs from **1** at position 7 only and has a senecioyloxy substituent instead of an acetoxy group, the toxicity has significantly increased (LC_{50} 2.14 ppm).

Experimental

General

UV (in MeOH) and IR (in $CHCl_3$) spectra were measured on Hitachi 3200 and JASCO A302 spectrophotometers, respectively. Optical rotations (in $CHCl_3$) were measured on a JASCO DIP-360 polarimeter; $[\alpha]_D^{27}$-values are in units of 10^{-1} deg cm^2 g^{-1}. EI mass spectra were measured on Finnigan MAT 112 and MAT 312 double-focussing mass spectrometers connected to a DEC PDP 11/34 computer system. High-resolution mass measurements were carried out by peak matching using perfluorokerosine (LPFK) as internal standard and by accurate mass measurements on the DEC PDP 11/34 computer system linked to a Finnigan MAT 312 mass spectrometer. The FAB mass (positive) spectrum was run on a JMS HX-110 double-focussing mass spectrometer operating at an accelerating voltage of 10 kV, using MeOH as solvent and glycerol as matrix on the target. The ^{1}H NMR spectra were recorded in $CDCl_3$, on a Bruker Aspect AM 300 for compound **1** and AM-500 for compound **2** operating at 300 and 500 MHz, respectively, while ^{13}C NMR spectra (BB and DEPT) of both **1** and **2** were recorded in $CDCl_3$ on a Bruker Aspect AM-500 spectrometer operating at 125 MHz. The ^{13}C NMR spectral assignments of both compounds were made through BB, DEPT, HMQC and HMBC spectra. Assignments of various protons are based on COSY-45 and NOESY as well as HMQC and HMBC specra. The assignments of both ^{1}H and ^{13}C shifts match very well with those of compounds with related partial structures.[7–11] Chemical shifts are recorded in ppm (δ), and coupling constants (J) are in Hz. The purity of compounds was checked on precoated alumina cards (Riedel-de-Haen 37364 DC-cards ALF). Petrol refers to petroleum spirit of distillation range 60–70 °C.

Plant material. The leaves were collected in spring from the Karachi region and identified by Professor Dr S. I. Ali, Department of Botany, University of Karachi. A specimen (voucher specimen No. NM-1) has been deposited in the Herbarium of the Botany Department of the University of Karachi.

Extraction and isolation

The fresh, uncrushed neem leaves (20 kg) were repeatedly (five times) extracted with methanol at room temperature and the solvent of the combined extract was removed under reduced pressure. The methanolic concentrate thereby obtained was partitioned between EtOAc and water. The ethyl acetate layer was washed (water), dried (anhydrous Na_2SO_4), treated with charcoal and filtered. The charcoal bed was successively eluted with ethyl acetate and benzene–methanol (1:1; v/v). The EtOAc and benzene–methanol filtrates were combined and the solvent was removed at reduced pressure. The residue thereby obtained was divided into petrol-soluble and petrol-insoluble fractions. The latter fraction was treated with 4% aq. Na_2CO_3 to separate acidic and neutral fractions. The EtOAc layer containing the neutral fraction was washed with water, dried (anhydrous Na_2SO_4), and evaporated under vacuum. The residue (34.42 g) was successively treated with different percentages of dilute aq. MeOH [10%, 20%, ... 100%]. As a result, several fractions were obtained, and were combined on the basis of their TLC results. The 40%, 50% and 60% fractions were combined (5.74 g) and subjected to vacuum liquid chromatography (VLC) (silica gel-60 GF60-254; $CHCl_3$, $CHCl_3$–MeOH; 5%, 10%, 15%, ... 50% MeOH in $CHCl_3$, 1 l each; MeOH). The $CHCl_3$–MeOH (9.9:0.1 and 9.85:0.15) eluates were combined together on the basis of TLC and freed from solvent to give a fraction A (3.9 g), which was further subjected to VLC (silica gel GF60-254; petrol, petrol–EtOAc; 5%, 10%, 15%, ... 50% EtOAc in petrol, 500 ml each and then $CHCl_3$, $CHCl_3$–MeOH; 10%, 20%, 30%, ... 50% MeOH in $CHCl_3$, 500 ml each; MeOH). The petrol–EtOAc (6:4) eluate furnished fraction B (50 mg) containing two major and three minor spots on TLC. The residue of this eluate was finally subjected to separation on alumina-coated preparative TLC cards (petrol–EtOAc; 5.5:4.5) to afford 23-*O*-methylnimocinolide **1** (15 mg) showing a single spot on TLC. The petrol–EtOAc (7:3) and (6.5:3.5) eluates were combined (139 mg) and subjected to alumina-coated preparative TLC (petrol–EtOAc, 6.5:3.5) to afford a major component (69 mg) showing a single spot on TLC. The ^{1}H NMR spectrum indicated that it was still a mixture of several constituents with one major band, which after a number of trials could ultimately be purified on precoated alumina

cards (Riedel-de-Haen 37364 DC-cards ALF; petrol–EtOAc; 6.5 : 3.5) to afford pure **2** (21 mg).

23-*O*-Methylnimocinolide 1. Prismatic rods (15.0 mg), mp 119–120 °C; $[\alpha]_D^{27}$ +11.23 (*c*, 0.18); λ_{max}/nm 230; ν_{max}/cm^{-1} 3450 (OH), 1760 (α,β-unsaturated γ-lactone), 1680–1720 (α,β-unsaturated ketone and ester), 1600 (C=C), 1375 (geminal methyls), 840 (C–H bending of C=C–H); *m*/*z* (EI) 498 (M^+); HRMS *m*/*z* (rel. int.) 498.2590 [M^+, Calc. for $C_{29}H_{38}O_7$; *M*, 498.2617] (6.2), 259.1313 [$C_{16}H_{19}O_3$] (100), 239.1273 [$C_{13}H_{19}O_4$] (9.4), 225.1174 [$C_{12}H_{17}O_4$] (6.1), 207.0984 [$C_{12}H_{15}O_3$] (4.7), 180.1071 [$C_{11}H_{16}O_2$] (11.4), 137.0966 [**1A**, $C_9H_{13}O$] (45.7), 115.0457 [**1B**, $C_5H_7O_3$] (9.7) and 109.0690 [C_7H_9O] (23.2). ^{1}H and ^{13}C NMR data are listed in Table 1.

Acetylation of 1

To a solution of **1** (10 mg) in pyridine (1 ml) was added Ac_2O (1 ml) and the reaction mixture was kept for two days at room temp. On usual work-up, the acetylated product **1a** was obtained, showing a single spot on TLC; λ_{max}/nm 230; ν_{max}/cm^{-1} 1680–1720 (α,β-unsaturated ketone and ester); *m*/*z* (EI) 540 (M^+); δ 5.42 (dd, *J* 11.78 and 2.94; H-6), 2.05 (3H, s, OAc).

Methylation of nimocinolide

Nimocinolide (10 mg) was taken up in MeOH (1 ml) and a few drops of 5% HCl were added. The contents were left at room temp. for up to 6 days and then refluxed for 1.5 h on a water-bath. The reaction was monitored by TLC. After this time, TLC showed conversion of nimocinolide to **1**, *m*/*z* (EI) 498 (M^+); δ (OCH_3) 3.57.

7-*O*-Deacetyl-23-*O*-methyl-7-*O*-senecioylnimocinolide 2. Prismatic rods (from MeOH) (21 mg), mp 116–118 °C; $[\alpha]_D^{27}$ +48.0 (*c*, 0.12); λ_{max}/nm 233; ν_{max}/cm^{-1} 3450 (OH), 1765 (α,β unsaturated γ-lactone), 1660 (cyclohexenone), 1600 (C=C) and 1375 (geminal methyls); FAB [M + H] *m*/*z* 539; HREIMS *m*/*z* (rel. int) 438.2414 [$C_{27}H_{34}O_5$, $M^+ - C_5H_8O_2$] (17.3), 421.2311 [$C_{27}H_{33}O_4$, $M^+ - C_5H_8O_2 - OH$] (11.8), 407.2145 [$C_{26}H_{31}O_4$, $M^+ - C_5H_8O_2 - OCH_3$] (10.5), 391.1937 [$C_{25}H_{27}O_4$, $M^+ - C_5H_8O_2 - OH - 2 \times CH_3$] (9.3), 137.0931 [$C_9H_{13}O$, **2A**] (78.3), 115.0454 [$C_5H_7O_3$, **2B**] (12.3); ^{1}H and ^{13}C NMR data are listed in Table 2.

Insecticidal activity

Insects. *Aedes aegypti larvae* (P.C.S.I.R. strain) were reared in the laboratory of the Zoology Department, University of Karachi under controlled temperature (28° ± 1 °C). They were fed with sterilized powder of dried prawns.

Biological tests. (Screening procedure).—Ten young-4th instar mosquito larvae were collected in 5 ml of the rearing tap water and transferred in 100 ml glass beakers containing 45 ml of distilled water. The compounds were tested at 28 ± 1 °C at 5 final concentrations. The controls were run in the same way with water containing no sample. Each concentration and control was run as duplicate set and mortality was recorded after 24 h.

Accurate tests. The WHO modified method of application was followed for which a batch of 10 insects (4th instar larvae) was released in a 100 ml beaker containing 50 ml of filtered tap water. The concentrations selected in the preliminary screening of each compound were tested at 28 ± 1 °C. A group of 7 beakers was set up, five for different concentrations and one for each control and check. Each experiment was repeated five times. The experiment was discarded if the mortality was found to be more than 10% of that in control samples. The mortality was recorded after 24 h and readings were subjected to Abbot's formula.[13]

Calculation of LC_{50}. The lethal concentration for 50% mortality (LC_{50}) was calculated using probit analysis[14] taking the average mortalities on the *y*-axis with the dose in ppm on the *x*-axis.

References

1 W. Dymock, C. J. H. Warden and D. Hooper, *Pharmacographia Indica 1890*, The Institute of Health and Tibbi Research, New Delhi, India, republished under the auspices of the Hamdard National Foundation, Karachi, Pakistan, 1977, vol. 1, p. 89.
2 R. N. Chopra, S. L. Nayar and I. C. Chopra, *Glossary of Indian Medicinal Plants*, Council of Scientific and Industrial Research, New Delhi, India, 1956, p. 31.
3 T. Fujiwara, T. Takeda, Y. Ogihara, M. Shimizu, T. Nomura and Y. Tomita, *Chem. Pharm. Bull.*, 1982, **30**, 4025.
4 S. N. Okpanyi and G. C. Ezeukwu, *Planta Med.*, 1981, **41**, 34.
5 W. Kraus, in *Neem Tree, Azadirachta Indica A. Juss and Other Meliaceous Plants*, ed. H. Schmutterer, VCH, Weinheim, Germany, 1995, pp. 35–74, 76–88.
6 S. Siddiqui, B. S. Siddiqui, S. Faizi and T. Mahmood, *J. Nat. Prod.*, 1988, **51**, 30.
7 C. Y. Ragasa, Z. D. Nacpil, G. M. Natividfad and M. Tada, *Phytochemistry*, 1997, **46**, 555, and the references cited therein.
8 D. Lavie, E. C. Levy and M. K. Jain, *Tetrahedron*, 1971, **27**, 3927.
9 S. Siddiqui, B. S. Siddiqui, S. Faizi and T. Mahmood, *Phytochemistry*, 1984, **23**, 2899.
10 S. Siddiqui, S. Faizi, T. Mahmood and B. S. Siddiqui, *J. Chem. Soc., Perkin Trans. 1*, 1986, 1021.
11 W. Kraus and R. Cramer, *Liebigs Ann. Chem.*, 1981, 181.
12 B. S. Siddiqui, Ghiasuddin and S. Faizi, *Phytochemistry*, 1998, **47**, 1631.
13 W. S. Abbot, *J. Econ. Ent.*, 1925, **18**, 265.
14 M. Raymond, G. Proto and D. Ratsira, PROBIT Analysis of Mortality Assays Displaying Quantal Response, version 3.3 Licence L 93019, Praxeme, 34680 St. Georges d'Orques, France, 1993.

Paper 9/02326G