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DITERPENOIDS FROM THE STEM BARK OF *AZADIRACHTA INDICA*

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Key Word Index—*Azadirachta indica*, Meliaceae, neem, diterpenoids; nimbonone; nimbonolone

Abstract—Two new isomeric diterpenoids named nimbonone and nimbonolone have been isolated from the neutral fraction of the stem bark of *Azadirachta indica* along with methyl grevillate which has not been reported earlier as a natural product The structures of nimbonone and nimbonolone have been determined as 12-ethyl-13-methoxy podocarpa-8,11,13-trien-7-one and 12-ethyl-13-methoxy podocarpa-8,11,13-trien-3-one, respectively, by spectroscopic methods and chemical transformations.

INTRODUCTION

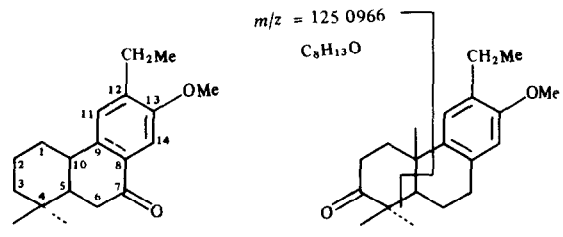
Studies undertaken by several groups of workers on the various parts of *Azadirachta indica* (neem) in view of their enormous importance, have led to the isolation of a series of new constituents [1–3]. In continuation of our investigations on the terpenoidal constituents of the stem bark of neem [4, 5], two new isomeric diterpenoids nimbonone, nimbonolone and methyl ester of grevillic acid have been isolated. The structures of these constituents have been deduced as **1–3**, respectively, through spectral studies and chemical reactions.

RESULTS AND DISCUSSION

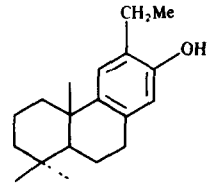
The ethanolic extract of neem bark was separated into acidic and neutral fractions and after usual work-up the neutral fraction was subjected to solvent fractionation followed by purification through repeated preparative TLC on silica gel, ultimately yielding nimbonone (**1**), nimbonolone (**2**) and the methyl derivative of grevillic acid (**3**).

Nimbonone (**1**) has the molecular formula $C_{20}H_{28}O_2$. Its UV spectrum showed absorptions at 206, 230, 278, 310 and 380 nm and the IR spectrum displayed peaks at 2850 (C–H), 1700 (α,β-unsaturated ketone), 1600 (aromatic double bond) and 1360 (C–O) cm^{-1}

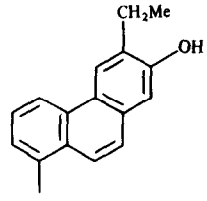
The diterpenoidal nature of **1** was indicated by the molecular formula and presence in the ^1H NMR spectrum of three three-proton singlets at δ0.87, 0.92 and 0.98. The molecular formula showed seven double bond equivalents in the molecule, four of which have been accounted for by the aromatic ring, two by the remaining rings of the skeleton and one by the carbonyl function. The appearance of the aromatic protons as singlets at δ6.88 and 7.49 suggested two substituents at C-12 and C-13. The ^1H NMR spectrum indicated that one of these substituents is a methoxy group ($\delta_H = 3.91$, $\delta_C = 56.0$) while the other is an ethyl group (δ1.25, 3H, *t*, $J = 6.2$, H-16; δ2.78, 2H, *m*, H-15) which was further supported by ^{13}C NMR spectrum (broad band and DEPT; $\delta_{CH_2} = 22.6$ and $\delta_{Me} = 14.0$). The second oxygen was taken as a ketonic function at C-7 in the light of the chemical shifts of H-11 (δ6.88), H-14 (δ7.49) and C-7 (δ195.0) which are comparable with those reported for sugiol [6, 7]. The assignments made so far were confirmed through ^1H–^1H homonuclear decoupling and COSY-45 experiments.



1 **2**



1a



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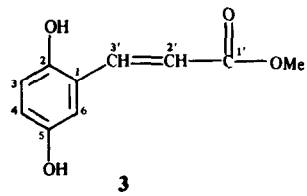

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Table 1 ^{1}H NMR spectral data of nimbonone (**1**), nimbonolone (**2**) and methyl grevillate (**3**)

H	1	2	3	H	1	2	3
1α	1·65 (*ddd*) J_{gem} 14·5 $J_{1\alpha\ 2\beta}$ 11·0 $J_{1\alpha\ 2\alpha}$ 3·0	1·99–2·03 (*m*)		4			7·06 (*dd*) $J_{4\ 3}$ 8·1 $J_{4\ 6}$ 1·8
1β	2·61 (*ddd*) J_{gem} 14·5 $J_{1\beta\ 2\alpha}$ 3·5 $J_{1\beta\ 2\beta}$ 3·5	2·70–2·72 (*m*)		5	1·85 (*dd*) $J_{5\ 6\beta}$ 13·5 $J_{5,6\alpha}$ 5·0	2·28–2·32 (*m*)	
2α	1·78–1·79 (*m*)	2·51–2·52 (*m*)		6α	2·69 (*dd*) J_{gem} 18·0 $J_{6\alpha\ 5}$ 5·0	2·60–2·62 (*m*)	
2β	1·80 (*ddddd*) J_{gem} 15·0 $J_{2\beta\ 1\alpha}$ 11·0 $J_{2\beta\ 3\alpha}$ 11·0 $J_{2\beta\ 1\beta}$ 3·5 $J_{2\beta\ 3\beta}$ 2·0	2·80–2·82 (*m*)		6β	2·58 (*dd*) J_{gem} 18·0 $J_{6\beta\ 5\alpha}$ 13·5	2·70–2·72 (*m*)	
				6			7·02 (*d*) $J_{6\ 4}$ 1·8
2′			6·28 (*d*) $J_{2\ 3}$ 15·8	7		2·70–2·72 (*m*)	
3α	1·60 (*ddd*) J_{gem} 16·0 $J_{3\alpha\ 2\beta}$ 11·0 $J_{3\alpha\ 2\alpha}$ 3·7			11	6·88 (*s*)	6·68 (*s*)	
				14	7·49 (*s*)	6·90 (*s*)	
				15	2·78 (*m*)	2·20 (*m*)	
3β	1·52 (*ddd*) J_{gem} 16·0 $J_{3\beta\ 2\alpha}$ 3·7 $J_{3\beta\ 2\beta}$ 2·0			16	1·25 (*t*) J = 6·2	1·23 (*t*) J = 6·2	
				18	0·87 (*s*)	1·12 (*s*)	
3			6·90 (*d*) $J_{3\ 4}$ 8·1	19	0·92 (*s*)	1·19 (*s*)	
				20	0·98 (*s*)	1·41 (*s*)	
3′			7·60 (*d*) $J_{3\ 2}$ 15·8	OMe	3·91 (*s*)	3·39 (*s*)	3·92 (*s*)

Thus, irradiation at δ1.80 (H-2β) simplified the multiplet at δ1·78–1·79 (H-2α) and collapsed the doublet of double doublets at δ2·61 (H-1β, J = 14·5, 3·5, 3·5 Hz), 1·65 (H-1α, J = 14·5, 11·0, 3·0 Hz), 1·60 (H-3α, J = 16·0, 11·0, 3·7) and 1·52 (H-3β, J = 16.0, 3·7, 2·0 Hz) each into a double doublet with the coupling constant of 14·5, 3·5, 14·5, 3.0; 16·0, 3·7 and 16·0, 3·7, respectively, while irradiation at δ2·58 (H-6β) changed the double doublets at δ2·69 (H-6α, J = 18.0, 5·0 Hz) and δ1·85 (H-5, J = 13·5, 5·0 Hz) each into a doublet with the same coupling constant of 5·0 Hz. When the signal at δ2·69 (H-6α) was irradiated, the sets of double doublets at δ1·85 and 2·58 (H-6β, J = 18.0, 13·5 Hz) collapsed into doublets with the same coupling constant of 13·5 Hz. These correlations were further supported by the proton connectivity pattern observed in a COSY-45 plot which showed connectivity of H-15 with H-16, H-1α with H-2β and H-2β with H-3α

The placement of ethyl and methoxyl groups at C-12 and C-13, respectively, has been established through 2-D NOE (NOESY) spectral analysis which showed spatial connectivity of OMe with H-14, and H-15 with H-11 In the light of these observations the methoxyl and ethyl groups have been placed at C-13 and C-12, respectively

Clemmensen reduction of **1** yielded **1a**, in the ^{1}H NMR spectrum of which the signals of H-11 and H-14 shifted to δ6.82 and 7·07, respectively The acidic conditions of the reaction also caused the hydrolysis of the aromatic OMe to a phenol which was evident from the ^{1}H NMR spectrum of **1a** in which the three-proton methoxy signal was replaced by a broad singlet of the hydroxyl proton at δ5·20 It was further confirmed by the molecular ion peak in the low resolution mass spectrum at m/z 272. In the light of the above facts structure **1** has been assigned to nimbonone.

The molecular formula $C_{20}H_{28}O_2$ of nimbonolone (**2**) was obtained through peak matching of its molecular ion Its UV spectrum has absorptions at 205, 230, 272 and 305 nm and IR spectrum displayed peaks at 2850 (C–H), 1720 (six-membered ring ketone), 1600 (aromatic double bond) and 1100 cm^{-1} (C–O) The chemical shifts of various protons in the ^{1}H NMR spectrum and the above data suggested that **2** has the same skeleton and functional groups as nimbonone (**1**) and they differ only in the position of the ketonic group The appearance of H-11 and H-14 at δ6.68 and 6·90, respectively, in the ^{1}H NMR spectrum showed that the ketonic function is not at C-7 A significant fragment at m/z 125.0966 ($C_8H_{13}O$) in the mass spectrum [8] and the downfield chemical shift of H-20 (δ1·41) [9] suggested that it is located at C-3 These data led to structure **2** for nimbonolone Chemical verification of the structures **1** and **2** and their correlation has been made as follows Clemmensen reduction of **2** gave a product which was identical in all respects with **1a**, which on dehydrogenation with selenium oxide furnished the phenanthrol (**1b**) thus confirming the carbon skeleton Further, the UV absorptions (λ_{max} 255, 292 and 330 nm) which are comparable with those reported for 2-hydroxy phenanthrol [10] and the molecular ion of **2b** (M^+ at m/z

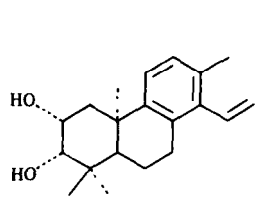
Table 2 ^{13}C NMR spectral data of nimbonone (**1**) and sugiol (**5**) [7]

C	1	5	C	1	5
1	37 9	37 3	12	147.0	160 3
2	18 8	18 4	13	151.0	133 0
3	41.3	40 9	14	108 5	125 6
4	31 8	32.7	15	22 6	26 1
5	49 7	49 1	16	14.0	21 8
6	35 8	35 4	17	—	21 6
7	195 0	199 2	18	21 2	20 7
8	125 2	122 4	19	32 4	31 9
9	145.6	156 3	20	23 1	22 5
10	37 8	37 4	-OMe	56 0	—
11	109 5	109 0			

236) finally established the nature and position of the substituents in ring C of **1** and **2.**

The unique feature of nimbonone and nimbonolone is the unusual presence of the ethyl group at C-12. These diterpenes can be hypothetically derived from the pimarane skeleton by a shift of the ethyl group from C-13 to C-12, degradation of the C-13 methyl group and oxidation of ring-C. A similar biogenesis has been demonstrated by biochemical experiments which involved migration of a C-13 vinylidene group of a pimarane derivative to C-14 resulting in the cleistanthane skeleton as for example cleistanthol (**4**) [11, 12] On the other hand, it may also be regarded as a derivative of dehydroabietane, which has been suggested as the biosynthetic precursor of sugiol (**5**) totarol, ferruginol and sempervirol (**6**) [13] Degradation of the isopropyl side chain of sempervirol (**6**) might result in the ethyl group at C-12

Compound **3** has the molecular formula $C_{10}H_{10}O_4$ Its UV spectrum showed peaks at 204, 230, 280 and 322 nm and its IR spectrum displayed peaks at 3550 and 3400 (OH), 2850 (C–H), 1690 (α,β-unsaturated carbonyl) 1600 (aromatic double bond) and 1100 cm^{-1} (C–O) The molecular formula showed six double bond equivalents, four of which are accounted for by the aromatic ring and two by the α,β-unsaturated carbonyl function of the ester group. Two doublets at δ6.90 (H-3, J = 8.1 Hz), 7.02 (H-6, J = 1.8 Hz) and a double doublet at δ7.06 (H-4, J = 8 1, 1.8 Hz) showed that the aromatic ring is 1,2,5-trisubstituted and the chemical shift of H-6 exhibited that it is flanked by a hydroxyl and an α,β-unsaturated carbonyl system. Two AB doublets at δ7.60 and 6.28 with a coupling constant of 15.8 Hz showed that the double bond of the side chain is *trans*-substituted. The methoxy proton of the ester appeared as a singlet at δ3.92. The two hydroxyl groups were confirmed by methylation of **3** to **3a** (M^+ at m/z 222, δ_H 3 59, 3 77 and 4.02, 3 × OMe). These data established the structure of **3** as methyl grevillate which has not previously been obtained as a natural product, although the isolation of grevillic acid is reported from *Grevillea robusta* [14].



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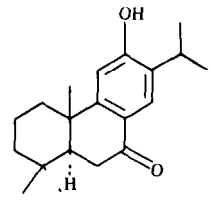

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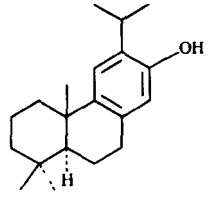

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EXPERIMENTAL

Mps uncorr IR and UV spectra were measured in $CHCl_3$ and MeOH, respectively NMR spectra were recorded in $CDCl_3$, at 300 MHz for ^1H and 75 MHz for ^{13}C nuclei and the chemical shifts are in δ (ppm) The assignments of ^{13}C NMR chemical shifts are based on chemical shift rules [15] and comparison with sugiol and other similar compounds [7] Optical rotations were measured at 24° in $CHCl_3$ Merck Kieselgel 60 PF_{254} coated on glass plates was used for analytical (thin layer) and preparative (thick layer) chromatography Neem stem bark collected in February 1985 from Karachi region was identified by Dr S I. Ali and a voucher specimen (No. NM-1) is deposited in the herbarium of the Botany Department, University of Karachi

Neem stem bark (1 7 kg) was repeatedly percolated with EtOH at room temp and the concd extract partitioned between EtOAc and H_2O, and the former extracted out with 4% Na_2CO_3 soln. to separate the acidic and neutral fractions The neutral fraction, after usual work-up, was divided into *n*-hexane soluble and *n*-hexane insoluble fractions The former was shaken with 90% MeOH and the *n*-hexane phase was subjected to prep TLC (silica gel) in $CHCl_3$–MeOH (97 3) and *n*-hexane–EtOAc (9 1), respectively, as a result of which nimbonone (**1**) and methyl grevillate (**3**) were obtained as pure crystalline constituents The *n*-hexane insoluble fraction was subjected to flash CC [16] (silica gel, $CHCl_3$–MeOH, 97 3) followed by purification of the major fraction on preparative TLC (silica gel, *n*-hexane–EtOAc, 3 1) when nimbonolone (**2**) was obtained as a pure product

Nimbonone (**1**) On crystallization from hot *n*-hexane, formed irregular plates (13 mg) mp 68–69°, $[\alpha]_D$ + 15° ($CHCl_3$, *c* 0.06); EIMS *m/z* (rel int) 300 2076 $[M]^+$, (calc for $C_{20}H_{28}O_2$, 300 2089) (28), 285 1854 $[M-Me]^+$ (26), 125 1330 $[M-C_{11}H_{11}O_2]^+$ (36) and 55 (100)

Nimbonolone (**2**). On crystallization from hot *n*-hexane, formed irregular plates (4 mg) mp 73–74° EIMS *m/z* (rel int) 300 2009 $[M]^+$ (calc for $C_{20}H_{28}O_2$, 300 2089) (4), 285 1854 $[M-Me]^+$ (4), 269 1905 $[M-OMe]^+$ (6), 125 0966 $[M-C_{12}H_{15}O]^+$ and 57(100)

Methyl grevillate (**3**). On crystallization from *n*-hexane, formed rods (4 9 mg) mp 173–174° EIMS *m/z* (rel int) 194 0513 $[M]^+$ (calc for $C_{10}H_{10}O_4$, 194 0579) (69), 177 0551 $[M-OH]^+$ (60), 148.0524 $[M-H_2O-CO]^+$ (40), 137 0602 $[C_8H_9O_2]^+$ (36) and 59 (100)

Reduction of nimbonone (**1**) A mixture of Zn powder (100 mg) and $HgCl_2$ (10 mg) was stirred with conc HCl (2 ml) and H_2O (50 ml), for 10 min The aq soln was decanted and the amalgamated Zn covered with 50 ml of H_2O and 75 ml of conc HCl. A solution of **1** (10 mg) in toluene was added immediately and the reaction mixture refluxed under a slow stream of HCl gas until the Zn amalgam dissolved On usual work-up **1a** was obtained as rods (MeOH) mp 59–60°C UV λ_{max}^{MeOH} nm 206, 230, 280 and 330,

IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 2850, 1600 and 1100. EIMS *m/z* (rel. int.): 272 $[M]^+$ (4). ^{1}H NMR: 7.07 (*s*, H-14), 6.82 (*s*, H-11), 5.20 (*br s*, OH).

Degradation of nimbonone (**1**). SeO_2 (2.5 mg) was added to **1a** (4.0 mg) and the mixture heated on a hot plate for 12–13 hr. On working up the reaction mixture in the usual manner, and purification on prep. TLC in $CHCl_3$, chromatographically pure 2-phenanthrol (**1b**) was obtained. EIMS *m/z* (rel. int.): 236 $[M]^+$ (55), 221(12) and 207(15).

Reduction of nimbonolone (**2**). Compound **2** was reduced in the same manner as described for **1**, yielding **1a**.

Methylation of methyl grevillate (**3**). A soln of **3** (2.5 mg) in Et_2O was treated with freshly prepared CH_2N_2 at room temp for 6 hr. The reaction mixture was dried under red pres when methylated product **3a** was obtained as irregular plates, mp 165–166°. UV λ_{max}^{MeOH} nm: 205, 230, 310 and 325. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2850, 1690, 1600 and 1150. EIMS *m/z* (rel. int.): 222 $[M]^+$ (3). ^{1}H NMR: 3.59, 3.77 and 4.02 (each, 3H, 3 × OMe).

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