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TERPENOIDS FROM THE STEM BARK OF *AZADIRACHTA INDICA*

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Key Word Index—*Azadirachta indica*; Meliaceae; neem; stem bark; diterpenoids; ring C-seco tetranortriterpenoid.

Abstract—Two new isomeric diterpenoids nimbione and nimbinone and a new ring C-*seco*-tetranortriterpenoid isonimbinolide have been isolated from the stem bark of *Azadirachta indica*. Their structures were elucidated by spectroscopic methods and chemical transformations.

INTRODUCTION

Azadirachta indica A. Juss is indigenous to the Indo-Pakistan subcontinent and its various parts are reputed as therapeutic agents [1, 2]. Recently it has been found that polysaccharides isolated from the neem bark have strong antitumour and anti-inflammatory action [3, 4]. More recently an antineoplastic drug was obtained from the bark [5]. As a result of present studies in the constituents of neem stem bark two new diterpenoids nimbione, nimbinone and a new ring C-*seco*-tetranortriterpenoid have been isolated and their structures elucidated as 13-hydroxy-12-methylpodocarpa-8,11,13-trien-3,7-dione **(1)**,12-hydroxy-13-methylpodocarpa-8,11,13-trien-3,7-dione **(2)** and isonimbinolide **(3)**, respectively, through spectral and chemical studies. Compounds **1** and **2** are of potential biological significance since various other diterpenes have been reported to possess diverse medicinal and biological properties [6–13]. The importance of compound **3** is reflected from the fact that other γ-hydroxy-butenolides and ring C-*seco*-limonoids possess insect growth regulating and antifeeding properties [14–16]. Regarding the isolation of isonimbinolide it may be noted that an α,β-unsaturated γ-lactone of nimbin was reported [17] from neem oil but it was considered to be an artefact since it was not detected in fresh neem oil. As a result of our studies several α,β-unsaturated γ-hydroxy butenolides have been isolated from various parts of neem, most of which are iso-compounds [21-hydroxy-20(22)-butene 21,23-γ-lactone] [14,18] including isonimbinolide **(3)**. These are, however, regarded as genuine natural products since they were detected in fresh plant extracts.

RESULTS AND DISCUSSION

Nimbione **(1)** had the molecular formula $C_{18}H_{22}O_3$. Its UV spectrum showed absorptions at 205, 225, 279 and 304 nm and the IR spectrum showed peaks at 3400 (OH), 2850 (C-H), 1710–1680 (six membered and α,β-unsaturated carbonyls) and 1400–1610 cm^{-1} (4 peaks, aromatic ring). The molecular formula showed eight double bond equivalents, two of which were considered to be carbonyl functions, three were in rings and the remaining three represented double bonds of an aromatic ring.

The ^1H NMR spectrum of **1** (Table 1) showed three three-proton singlets at δ1.13 (H-18), 1.19 (H-19) and 1.42 (H-20). Two singlets of aromatic protons were observed at δ6.71 and 7.84 related to H-11 and H-14, respectively, wlile a three-proton singlet at δ2.25 was attributed to an aromatic methyl group. The presence of a hydroxyl function was indicated by the IR spectrum (3400 cm^{-1}) and the diagnostic fragment in the mass spectrum at *m/z* 253 $[M-Me-H_2O]^+$. The aromatic nature of the compound was demonstrated by the formation of the methyl derivative (**1a**, OMe δ3.99; M,$^+$at *m/z* 300) on reaction with diazomethane. One of the carbonyl functions was placed at C-3 keeping in view the chemical shift rules [19] and the down field chemical shifts of the quaternary methyl groups as compared to those of nimbiol (Table 1) which lacks the 3-keto functionality [20, 21]. The position of the second carbonyl at C-7 was supported by the chemical shifts of H-11, H-14 and C-7 (δ197.1).

The configuration of various centres and placement of functional groups was finally decided through NOE difference spectra of compound **1**. Irradiation of H-14 gave the signal of the hydroxyl proton, while irradiation of H-11 gave the signals of 12-methyl protons and H-20. On irradiation of H-18 (δ1.13) a signal of only H-19 (δ1.19) was obtained while irradiation of H-19 gave the signals of both H-18 (δ1.13) and H-20 (δ1.42). These observations led to the assignment of structure **1** for nimbione which was finally confirmed by the ^{13}C NMR chemical shifts.

The molecular formula ($C_{18}H_{22}O_3$), UV (205,227,280 and 310 nm) and IR (3420, 2850, 1710–1680, 1420–1620 cm^{-1}) spectra of compound **2** suggested that it is closely related to **1**. The chemical shifts of various protons in the ^1H NMR spectrum and the formation of the methyl derivative **(2a)** on reaction with diazomethane further showed that compound **2** had the same skeleton and functional groups as **1** and they differed only in position of the aromatic substituents. The appearance of H-11 and H-14 as singlets at δ6.70 and 7.85 respectively in the ^1H NMR spectrum further exhibited that the substituents were located at C-12 and C-13 and thus compound **2** is the positional isomer of **1**. This was further confirmed through acetylation (Ac_2O–pyridine) when **2** afforded the acetyl derivative **(2b)** in the ^1H NMR spectrum of which H-11 shifted to δ6.97, with the appearance of the acetoxy methyl protons at δ2.16.

In the NOE difference spectra of compound **2** some

Table 1. ^{1}H NMR spectral data of compounds **1** and **2***

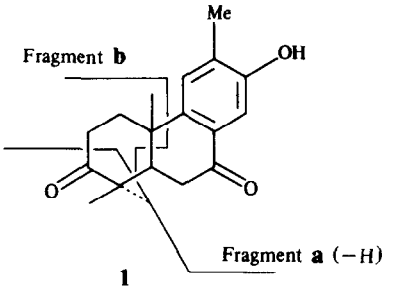
Assignment	Nimbione (**1**)	**1a**	Nimbinone (**2**)	**2a**	**2b**
H-1α	2.03 (*ddd*) J_{gem} = 14.40 $J_{1\alpha,2\beta}$ = 14.40 $J_{1\alpha,2\alpha}$ = 5.50	2.01 (*m*)	2.01 (*ddd*) J_{gem} = 14.60 $J_{1\alpha,2\beta}$ = 14.40 $J_{1\alpha,2\alpha}$ = 5.50	2.01 (*m*)	2.03 (*m*)
H-1β	2.72 (*ddd*) J_{gem} = 14.40 $J_{1\beta,2\beta}$ = 7.50 $J_{1\beta,2\alpha}$ = 3.76	2.72 (*m*)	2.72 (*ddd*) J_{gem} = 14.60 $J_{1\beta,2\beta}$ = 7.50 $J_{1\beta,2\alpha}$ = 3.76	2.72 (*m*)	2.72 (*m*)
H-2α	2.53 (*ddd*) J_{gem} = 13.60 $J_{2\alpha,1\alpha}$ = 5.50 $J_{2\alpha,1\beta}$ = 3.76	2.53 (*ddd*) J_{gem} = 13.96 $J_{2\alpha,1\alpha}$ = 6.36 $J_{2\alpha,1\beta}$ = 3.58	2.53 (*ddd*) J_{gem} = 14.70 $J_{2\alpha,1\alpha}$ = 5.50 $J_{2\alpha,1B}$ = 3.76	2.53 (*m*)	2.53 (*m*)
H-2β	2.85 (*ddd*) J_{gem} = 13.60 $J_{2\beta,1\alpha}$ = 14.40 $J_{2\beta,1\beta}$ = 7.50	2.85 (*ddd*) J_{gem} = 13.96 $J_{2\beta,1\alpha}$ = 14.00 $J_{2\beta,1\beta}$ = 6.68	2.85 (*ddd*) J_{gem} = 14.70 $J_{2\beta,1\alpha}$ = 14.40 $J_{2\beta,1\beta}$ = 7.50	2.85 (*m*)	2.85 (*m*)
H-5	2.30 (*dd*) $J_{5,6\beta}$ = 13.50 $J_{5,6\alpha}$ = 3.80	2.30 (*m*)	2.30 (*dd*) $J_{5,6\beta}$ = 13.80 $J_{5,6\alpha}$ = 3.80	2.30 (*m*)	2.30 (*m*)
H-6 α	2.62 (*dd*) J_{gem} = 18.00 $J_{6\alpha,5}$ = 3.80	2.62 (*dd*) J_{gem} = 18.00 $J_{6\alpha,5}$ = 4.36	2.62 (*dd*) J_{gem} = 18.00 $J_{6\alpha,5}$ = 3.80	2.62(*m*)	2.62 (*m*)
H-6 β	2.75 (*dd*) J_{gem} = 18.00 $J_{6\beta,5}$ = 13.50	2.75 (*dd*) J_{gem} = 18.00 $J_{6\beta,5}$ = 13.60	2.75 (*dd*) J_{gem} = 18.00 $J_{6\beta,5}$ = 13.80	2.75 (*m*)	2.75 (*dd*) J_{gem} = 18.20 $J_{6\beta,5}$ = 13.40
H-11	6.71 (*s*)	6.80 (*s*)	6.70 (*s*)	6.67 (*s*)	6.97 (*s*)
H-14	7.84 (*s*)	7.84 (*s*)	7.85 (*s*)	7.83 (*s*)	7.84 (*s*)
H-18	1.13 (*s*)	1.13 (*s*)	1.13 (*s*)	1.13 (*s*)	1.13 (*s*)
H-19	1.19 (*s*)	1.19 (*s*)	1.19 (*s*)	1.19 (*s*)	1.19 (*s*)
H-20	1.42 (*s*)	1.42 (*s*)	1.42 (*s*)	1.42 (*s*)	1.42 (*s*)
Me-12	2.25 (*s*)	2.24 (*s*)			
Me-13			2.24 (*s*)	2.24 (*s*)	2.33 (*s*)
OAc					2.16 (*s*)
OMe		3.99 (*s*)		3.88 (*s*)	

*See ref. [21] for the spectrum of nimbiol.

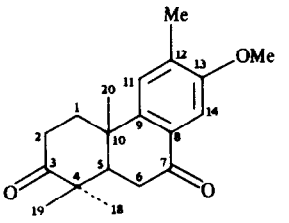
interactions between the quaternary methyl groups were observed as in **1**. However, those of the aromatic protons were reversed; thus irradiation of H-14 gave the signal of the aromatic methyl protons whereas on irradiation of H-11 the signals of H-20 and a hydroxyl proton were obtained. In the light of these data structure **2** has been ascribed to nimbinone.

Isonimbinolide **(3)** had the molecular formula $C_{30}H_{36}O_{11}$ (through peak matching of the molecular ion). Its IR spectrum showed peaks at 3400 (OH), 2900 (C-H), 1620–1735 (C=C; α,β-unsaturated and ester carbonyls), 1760 (α,β-unsaturated γ-lactone) and 1250 cm^{-1} (C-O).

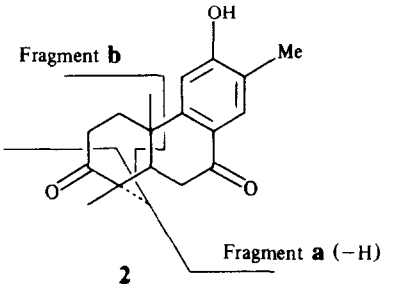
The ^{1}H NMR spectrum of compound **3** showed the presence of three quaternary methyls at δ1.19, 1.20 and 1.28 and a vinylic methyl at δ1.73. Two one-proton singlets were observed at δ5.93 and 5.75 which correspond to H-22 and H-21, respectively. A pair of doublets appeared at δ5.86 (J = 10.08 Hz) and 6.36 (J = 10.08 Hz) related to H-2 and H-3, respectively, whereas two one-proton doublets at δ3.34 (J = 12.00 Hz) and 4.10 (J = 3.28 Hz) were attributable to H-5 and H-7, respectively. These values are in agreement with those reported for the same protons in desacetylisonimbinolide [22]. However, the signal of H-6 at δ3.93 in the spectrum of desacetylisonimbinolide was replaced by a double doublet (J = 12.00, 3.28 Hz) at δ5.19 in that of compound **3**, as observed in the case of nimbin [23], showing the presence of an α-oriented acetoxy function at C-6. The latter was also indicated by a three-proton singlet at δ2.03. The assignments of various protons and location of functional groups was further demonstrated by ^{1}H–^{1}H homonuclear decoupling and COSY-45 experiments. Thus irradiation at δ4.10 (H-7) collapsed the double doublet at



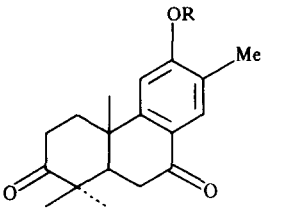
1



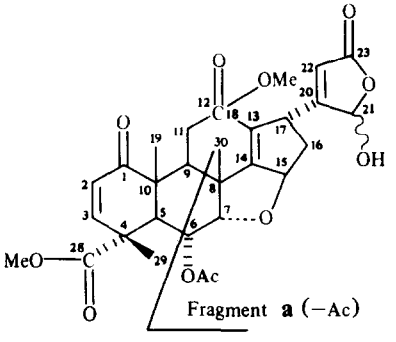
1a



2



2a R = Me
2b R = Ac



3

δ5.19 (H-6) into a doublet (J=12.00 Hz) while irradiation at δ5.19 converted the doublets of H-5 and H-7 into singlets. Irradiation at δ5.86 (H-2) collapsed the doublet at δ6.36 (H-3) into a singlet and vice versa. In the COSY-45 spectrum through-bond connectivities were observed between H-3 and H-2; H-21 and OH; H-5 and H-6; and H-9 and H-11.

These structural features were confirmed through peak matching of significant ions in the mass spectrum at m/z 405.1702 ($C_{25}H_{25}O_5$) [M − OAc − COOMe − OMe $-H_2O$]$^+$ and 263.0920 ($C_{14}H_{15}O_5$, fragment **a**) resulting from the cleavage of ring B. These observations showed that it has an identical skeleton as that of desacetylisonimbinolide [22] and differs only in the acetyl substituent at C-6.

The stereochemistry of various centres of compound **3** has been established through 2D NOE (NOESY) which showed spatial connectivities of H-3 with H-2; H-7 with H-18; H-9 with 12-OMe and H-3; and H-18 with 12-OMe.

EXPERIMENTAL

Mps: uncorr. ^{1}H NMR: ($CDCl_3$) 400 MHz. ^{13}C NMR: (broad band and DEPT, $CDCl_3$) 75 MHz. Chemical shifts are reported in ppm (δ) and coupling constants (J) are in Hz. The assignments of ^{13}C NMR chemical shifts are based on chemical shift rules [24] and comparison with similar compounds [25]. IR spectra (in $CHCl_3$) were recorded on a Jasco IRA-I and UV spectra (in MeOH) were measured on Pye Unicam SP-800 spectrometer. Merck Kieselgel 60 PF 254 coated on glass plates was used for analytical and prep. TLC.

Extraction of plant material. An ethanolic extract of neem stem bark (1.7 kg) collected in Feb. 1985 from the Karachi region was divided into acidic and neutral fractions. The residue obtained on removal of the solvent from the former was divided into petrol soluble and insoluble portions. The petrol insoluble portion was subjected to prep. TLC (silica gel; $CHCl_3$–MeOH, 97.5:2.5) as a result of which **1** and **2** were obtained with some allied impurities. These were further purified on TLC yielding nimbione (**1**) and nimbinone (**2**). Residue obtained on removal of the solvent from the neutral fraction was also divided into petrol soluble and insoluble fractions and the latter, on solvent fractionation, gave the EtOAc soluble portion which was purified by prep. TLC (silica gel; $CHCl_3$–MeOH; 39:1) yielding isonimbinolide (**3**) as an uniform constituent.

Nimbione (**1**) on recrystallization from MeOH formed irregular plates mp 102–103° EIMS m/z rel. int. 286.1550 [M]$^+$ (calc. for $C_{18}H_{22}O_3$, 286.1568) (50); 253, 215.1071 [M − C_4H_7O, fragment **a**]$^+$ (33), 125.0966 [M − $C_{10}H_9O_2$, fragment **b**]$^+$ (100) and 97.1017 [fragment **b** − CO]$^+$ (83). ^{13}C NMR: 37.3 (C-1), 34.6 (C-2), 214.8 (C-3), 47.3 (C-4), 49.5 (C-5), 37.4 (C-6), 197.1 (C-7), 123.4 (C-8), 154.1 (C-9), 37.1 (C-10), 109.9 (C-11), 131.5 (C-12), 160.3 (C-13), 130.8 (C-14), 25.3 (C-18), 28.6 (C-19), 21.0 (C-20) and 15.3 (Me).

Methylation of compound **1** *to* **1a**. To a soln of nimbione (1.2 mg) in MeOH was added an ethereal soln of freshly prepared CH_2N_2 and the reaction mixture was kept for 3–4 hr at room temp. It was worked-up in the usual manner, when chromatographically pure **1a** was obtained as sharp needles mp

95–96°. UV λ_{max} (nm): 208, 280 and 322; IR (cm^{-1}): 2900(C-H) 1700–1680 (six membered and α,β-unsaturated ketone), 1430–1640 (aromatic ring) and 1100 (ether linkage). EIMS *m/z* (rel. int.): 300 $[M]^+$ (2), 269 $[M-31]^+$ (1), 203 $[M-97]^+$ (1), 125 $[M-175]^+$ (3) and 58(100).

Nimbinone **(2)** on recrystallization from MeOH formed irregular plates mp 124–125°. EIMS *m/z* (rel.int.): 286.1558 $[M^+]$ (calc. for $C_{18}H_{22}O_3$, 286.1568) (33); 215.1072 $[M-C_4H_7O$, fragment **a**$]^+$ (13), 125.0966 $[M-C_{10}H_9O_2$, fragment **b**$]^+$ (67), and 97.1017 [fragment **b**$-CO]^+$ (100).

Methylation of compound **2** *to* **2a.** Compound **2** was taken in MeOH and treated with an ethereal soln. of freshly prepared CH_2N_2 and kept for 3–4 hr at room temp. On usual work-up pure **2a** was obtained as irregular plates mp 106–107°; UV λ_{max} (nm): 205, 235 and 320 nm; IR ν_{max} (cm^{-1}): 2900, (C-H), 1700–1680 (six membered and α,β-unsaturated ketone and ester carbonyls), 1400–1600 (aromatic ring) and 1100 (ether linkage). EIMS *m/z* (rel. int.): 300 $[M^+]$ (4), 269 $[M-31]^+$ (2), 203 $[M-97]^+$ (1) 125 $[M-175]^+$ (3) and 58 (100).

Acetylation of compound **2** to **2b**. To a soln. of nimbinone in pyridine, Ac_2O was added and the reaction mixture was kept overnight at room temp. On usual work-up it yielded pure irregular plates mp 89–90°. IR ν_{max} (cm^{-1}): 2850 (C-H), 1705–1720 (carbonyl of six-membered and α,β-unsaturated ketone and ester function), 1400–1600 (aromatic double bond) and 1150 cm^1 (C–O). EIMS *m/z* (rel.int.): 328 $[M^+]$ (18), 313 $[M-Me]^+$ (16), 298 $[M-2Me]^+$ (24), 285 $[M-Ac]^+$ (100), 270 $[M-Ac-Me]^+$ (15), 214 $[M-Ac-C_4H_7O]^+$ (14) and 125 $[C_8H_{13}O]^+$ (88).

Isonimbinolide **(3)** on recrystallization from MeOH formed needles mp 172–173°. EIMS *m/z* (rel.int): 572.2280 $[M]^+$ (calc. for $C_{30}H_{36}O_{11}$, 572.2257) (7); 405.1702 $[M-OAc-COOMe-OMe-H_2O]^+$ (2), 263.0920 $[C_{14}H_{15}O_5$, fragment **a**$]^+$ (16), 107.0860 $[C_8H_{11}]^+$ and 93.0704 $[C_7H_9]^+$. ^1H NMR: δ 1.19 (3H, *s*, H-19), 1.20 (3H, *s*, H-29), 1.28 (3H, *s*, H-30), 1.73 (3H, *d*, $J_{18,15}$ =1.5, H-18), 2.03 (3H, *s*, OAc), 2.05 (2H, *m*, H-16), 2.50–2.94 (3H, *m*, H-9, H-11), 3.34 (1H, *d*, $J_{5,6}$=12.0, H-5), 3.59 (1H, *d*, $J_{17,16\beta}$=7.17, H-17), 3.73 (3H, *s*, OMe), 3.80 (3H, *s*, OMe), 4.10 (1H, *d*, $J_{7,6}$=3.28, H-7), 5.19 (1H, *dd*, $J_{6,5}$=12.0, $J_{6,7}$=3.28, H-6), 5.65 (1H, *m*, H-15), 5.75 (1H, *br s*, H-21), 5.86 (1H, *d*, $J_{2,3}$ = 10.08, H-2), 5.93 (1H, *br s*, H-22) and 6.36 (1H, *d*, $J_{3,2}$=10.08, H-3).

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