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ISOLATION OF A TRITERPENOID FROM *AZADIRACHTA INDICA*

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Key Word Index—*Azadirachta indica*; Meliaceae; neem leaves; triterpenoid; nimbocinone.

Abstract—A new triterpenoid named nimbocinone, has been isolated from fresh, undried winter leaves of *Azadirachta indica* along with two sterols identified as sitosterol and stigmasterol. The structure of nimbocinone has been elucidated through spectral and chemical studies. It is the first 26-hydroxytriterpenoid isolated from any part of the neem tree.

INTRODUCTION

In a continuation of studies on the terpenoidal constituents of fruits [1] and leaves [2] of *Azadirachta indica*, a new triterpenoid nimbocinone (**1**) and two sterols, sitosterol and stigmasterol, have been isolated from the leaves.

RESULTS AND DISCUSSION

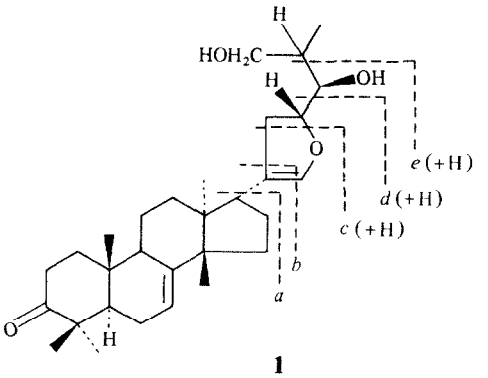
Nimbocinone (**1**) has the molecular formula $C_{30}H_{46}O_4$ (high resolution mass spectrometry). Its IR spectrum showed peaks at 3450 (OH), 1705 (six membered ring ketone), 1620 and 820 cm^{-1} (trisubstituted double bond). It possessed one secondary (δ1.02, *d*, J = 7 Hz) and five tertiary (δ0.87, 0.95, 1.00, 1.05 and 1.25) methyls, a carbonyl (δ216), one primary hydroxyl (δ79), one secondary hydroxyl (δ78.9) and two trisubstituted double bonds [δ5.37 (*d*, $J_{7,6\alpha}$ = 6 Hz, H-7) and 6.08 (*dd*, ^4J = 1.4 Hz, ^4J = 0.7 Hz, H-21); δ118.1 (C-7), 144.8 (C-8), 116.9 (C-20) and 141.1 (C-21)]. Comparison of the ^{1}H NMR and ^{13}C NMR resonances (Table 1) of **1** with those of 20,21-anhydromelianone [3] indicated that both have the same carbocyclic nucleus and the 22,23-dihydrofuran ring system. Support for this suggestion comes from the important fragments in the mass spectrum of **1** at *m/z* 384.2669 ($C_{25}H_{36}O_3$), characteristic of 3-keto triterpenes [4], and *m/z* 245.1895 ($C_{17}H_{25}O$), 257.1892 ($C_{18}H_{25}O$) and 271.2047 ($C_{19}H_{27}O$) observed in compounds with a double bond at C-7 [5]. Dehydrogenation of the diacetyl derivative **2** with mercuric acetate yielded the diene **3** displaying UV absorption bands typical for 7,9(11)-hetroannular dienes in an euphane (tirucallane) skeleton: λ_{max} 232, 238 and 249 nm. These observations disclosed the stereochemistry of the ring system as well as the position of one of the double bonds [6]. Comparison of the spectral data of **1** with those of 20,21-anhydromelianone (**7**) further exhibited that **1** has a modification in the side chain from C-24 to C-27. The appearance of a fragment in the mass spectrum at *m/z* 439.3200 ($C_{29}H_{43}O_3$) resulting from the loss of CH_2OH, and signals for six methyls in the ^{1}H NMR and ^{13}C NMR (Table 1) instead of seven methyls in compounds having related structure [3, 7] located the primary hydroxyl function at C-26. On the other hand, appearance of H-27 as a three-proton doublet (δ1.02, J = 7 Hz) led to the placement of the second hydroxyl group at C-24. All these structural assignments were substantiated by the important fragments *a–e* in the mass spectrum, ^{13}C NMR chemical shifts and chemical reactions. Thus acetylation of **1** afforded the diacetyl product **2** in which the signals for H-24 (δ3.63, *dd*, $J_{24,23}$ = 7.4 Hz, $J_{24,25}$ = 10 Hz) and H-26 (3.20, *m*) shifted to δ4.62 and 5.07 along with the appearance of two three-proton singlets at δ2.00 and 2.15 for the acetoxy methyl functions. Sodium borohydride reduction of **1** gave the alcohol **4** which upon oxidation (CrO_3–pyridine) afforded the 24-ketonimbocinone (**5**), 24-keto-26-nimbocinoic acid (**6**) and nimbocinone.

Table 1. ^{13}C NMR spectral data of nimbocinone (**1**) (25 MHz)

C	δ	C	δ
1	37.5	16	31.8
2	35.5	17	46.2
3	216.0	18	13.1
4	47.5	19	17.5
5	52.3	20	116.9
6	24.3	21	141.1
7	118.1	22	24.9
8	144.8	23	78.7*
9	48.7	24	78.9*
10	35.0	25	30.1
11	27.5	26	79.0
12	36.2	27	19.1
13	44.3	28	22.1
14	51.3	29	23.7
15	34.3	30	21.2

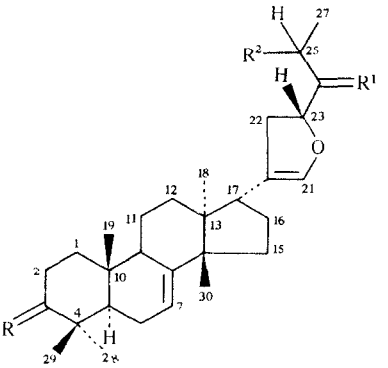
*Assignment may be reversed.

Application of Horeau's method [8] to **1** disclosed the stereochemistry of C-24 as *R* and the coupling constants of H-23 with H-24 ($J_{23,24}$ = 7.4 Hz), H-22α ($J_{23,22\alpha}$ = 1.5 Hz) and H-22β ($J_{23,22\beta}$ = 7.4 Hz) showed that the configuration of C-23 is also *R*. Information regarding the configuration of various centres was obtained through 2D-NOE spectroscopy (NOESY) [9], which showed the interactions of H-18 with H-22α, 26-OH and H-9; H-28

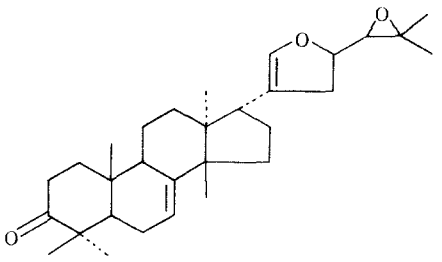


1

$a = C_{22}H_{33}O$
$b = C_{23}H_{33}O$
$c = C_{24}H_{35}O_2$
$d = C_{26}H_{38}O_2$
$e = C_{27}H_{40}O_3$



	R	R^1	R^2
2	O	β-OAc α-H	CH_2OAc
3	O	β-OAc α-H	CH_2OAc, $\Delta^{9(11)}$
4	β-OH α-H	β-OH α-H	CH_2OH
5	O	O	CH_2OH
6	O	O	COOH



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with H-5 and H-6α; H-19 with H-6 β, H-1β and H-11; H-9 with H-1α and 26-OH; and H-7 with H-15α. These observations demonstrated the typical *trans*-A/B ring junction with the C-13 methyl group on the α-side of the molecule. The spatial proximity of H-22α with H-18 showed that the side chain at C-17 had the α-disposition. All these observations led to the structure of nimbocinone as **1**. This is the first 26-hydroxytriterpenoid isolated from any of the various parts of the neem tree.

EXPERIMENTAL

Mps were recorded in glass capillary tubes and are uncorr. MS were recorded on double focussing mass spectrometers connected to a PDP 11/34 computer system. ^{1}H NMR and ^{13}C NMR (broad band and gated spin echo) spectra were recorded in $CDCl_3$ with TMS as internal reference. The NOESY experiments were carried out on a 300 MHz instrument, model Bruker Aspect 3000, with pulse delay 2 sec and mixing time 0.5 sec. The assignments of the chemical shifts in the ^{13}C NMR spectrum of **1** have been made through comparison of chemical shifts of model compounds [3] and the gated spin echo spectrum. The purity of samples was checked on TLC (silica gel SIF-254 precoated aluminium sheets).

The EtOH extract of the undried neem leaves, collected in winter from the Karachi region, was divided into acidic and neutral fractions. The latter was charcoaled and successively eluted with EtOAc and MeOH–C_6H_6 (1:1). The residue obtained on removal of the solvent from the EtOAc eluate was divided into petrol soluble and insoluble fractions, the former of which was partitioned between petrol and 90% MeOH. The residue from the methanolic phase was subjected to preparative TLC (silica gel, C_6H_6–EtOAc, 9:1), as a result of which nimbocinone (**1**) was obtained as whitish crystals along with nimocinol [2]. On recrystallization from EtOAc, **1** formed slender needles (250 mg; yield 0.025% on the wt of neutral fraction), mp 76–78°; $[\alpha]_D^{23}$ + 10° ($CHCl_3$). HRMS *m/z* (rel. int.): 470.3379 $[M]^+$ (calc. for $C_{30}H_{46}O_4$: 470.3396) (14), 439.3200 $[C_{29}H_{43}O_3]^+$ (100), 412 (2), 384.2669 $[C_{25}H_{36}O_3]^+$ (8), 382 (5), 355.2632 $[C_{24}H_{35}O_2]^+$ (36), 325.2535 $[C_{23}H_{33}O]^+$ (15), 313.2535 $[C_{22}H_{33}O]^+$ (4), 271.2047 $[C_{19}H_{27}O]^+$ (4), 257.1892 $[C_{18}H_{25}O]^+$ (4), 245.1895 $[C_{17}H_{25}O]^+$ (5). ^{1}H NMR (300 MHz): 6.08 (1H, *dd*, ^4J = 1.4 Hz, ^4J = 0.7 Hz, H-21), 5.37 (1H, *d*, $J_{6\alpha,7}$ = 6.0 Hz, H-7), 4.17 (1H, *ddd*, $J_{23,24}$ = 7.4 Hz, $J_{23,22\beta}$ = 7.4 Hz, $J_{23,22\alpha}$ = 1.5 Hz, H-23), 3.63 (1H, *dd*, $J_{24,23}$ = 7.4 Hz, $J_{24,25}$ = 10.0 Hz, H-24), 3.20 (2H, *m*, H-26), 2.24 (1H, *ddd*, J_{gem} = 15.0 Hz, $J_{15\alpha,16\alpha}$ = 9.0 Hz, $J_{15\alpha,16\beta}$ = 3.0 Hz, H-15α), 2.21 (1H, *dd*, J_{gem} = 12.0 Hz, $J_{22\alpha,23}$ = 1.5 Hz, H-22α), 2.17

(1H, *m*, 24-OH), 1.99 (1H, *m*, H-6β), 1.97 (1H, *ddd*, J_{gem} = 15.0 Hz, $J_{1\alpha,2\beta}$ = 9.0 Hz, $J_{1\alpha,2\alpha}$ = 3.0 Hz, H-1α), 1.95 (1H, *ddd*, J_{gem} = 15.0 Hz, $J_{1\beta,2\alpha}$ = 3.0 Hz, $J_{1\beta,2\beta}$ = 3.0 Hz, H-1β), 1.85 (1H, *m*, 26-OH), 1.82 (1H, *dd*, $J_{5,6\beta}$ = 12.0 Hz, $J_{5,6\alpha}$ = 3.0 Hz, H-5), 1.75 (1H, *ddd*, J_{gem} = 15.0 Hz, $J_{6\alpha,5}$ = 3.0 Hz, $J_{6\alpha,7}$ = 6.0 Hz, H-6α), 1.42 (1H, *dd*, $J_{9,11\beta}$ = 9.0 Hz, $J_{9,11\alpha}$ = 3.0 Hz, H-9), 1.22 (2H, *m*, H-11), 1.25 (3H, *s*, H-30), 1.05 (3H, *s*, H-28), 1.02 (3H, *d*, *J* = 7.0 Hz, H-27), 1.00 (3H, *s*, H-29), 0.95 (3H, *s*, H-18) and 0.87 (3H, *s*, H-19).

The residues from the petrol phase and the MeOH–C_6H_6 (1:1) eluate of the above work-up afforded two sterols through crystallization from MeOH, which were identified as sitosterol and stigmasterol respectively through comparison of their physical and spectral data with those of the authentic samples.

Acetylation of **1** *to* **2**. To a soln of **1** (75 mg) in pyridine (1 ml), Ac_2O (1 ml) was added and the reaction mixture was kept overnight at room temp. On usual work-up **2** was obtained as a whitish crystalline product which on recrystallization from EtOAc formed prismatic rods, mp 85–86°. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1735 (br), 1702, 1625, 820. EIMS *m/z* (rel. int.): 496 $[M-43-15]^+$ (2), 481.2962 $[M-43-15-15]^+$ (5), 421 $[481-60]^+$ (10), 355 (2), 325 (12), 313 (4), 297 (6), 271 (4), 83 (100). ^{1}H NMR (100 MHz): δ6.05 (1H, *d*, *J* = 1.5 Hz, H-21), 5.32 (1H, *m*, H-7), 5.07 (2H, *d*, *J* = 6.25 Hz, H-26′), 4.62 (1H, *dd*, $J_{24,23}$ = 7.5 Hz, $J_{24,25}$ = 10.0 Hz, H-24), 4.12 (1H, *ddd*, $J_{23,24}$ = 7.5 Hz, $J_{23,22\beta}$ = 7.5 Hz, $J_{23,22\alpha}$ = 1.5 Hz, H-23), 2.15 and 2.00 (each 3H, *s*, 2 × OAc), 1.09 (1H, *d*, *J* = 6.5 Hz, H-27), 1.03, 0.86 (6H), 0.85, 0.80 (*s*, 5 × Me).

Dehydrogenation of **2** *to* **3**. To a soln of **2** (32 mg) in $CHCl_3$ (1 ml), was added a soln of mercuric acetate (70 mg) in glacial HOAc (1 ml). The reaction mixture was kept stirring for 24 hr at room temp., filtered and freed of the solvent, when chromatographically pure **3** was obtained as needles, mp 78–80°. UV λ_{max}^{MeOH} nm: 232, 238 and 249. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730 (br), 1705, 1625 and 825; EIMS *m/z*: 552.3461 $[M]^+$ (calc. for $C_{34}H_{48}O_6$: 552.3451).

$NaBH_4$ *reduction of* **1** *to* **4**. A soln of **1** (25 mg) in MeOH (2 ml) was stirred with $NaBH_4$ (40 mg) at −10° for 30 min. After usual work-up chromatographically pure **4** was obtained as an amorphous residue. UV λ_{max}^{MeOH} nm: 205; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (br), 1605. EIMS *m/z* (rel. int.): 472.3564 $[M]^+$ (calc. for $C_{30}H_{48}O_4$: 472.3552) (2), 441.3380 $[C_{29}H_{45}O_3]^+$ (4), 357 (14), 327 (8) 315 (4), 55 (100). ^{1}H NMR (100 MHz): δ6.07 (1H, *d*, *J* = 1.5 Hz, H-21), 5.30 (1H, *m*, H-7), 4.16 (1H, *m*, H-23), 3.68 (1H, *m*, H-24), 3.45 (1H, *m*, $W_{1/2}$ = 18.0 Hz, H-3α), 3.20 (2H, *m*, H-26), 1.01 (3H, *d*, *J* = 6.5 Hz, H-27), 0.95, 0.88, 0.86, 0.85 and 0.82 (*s*, 5 × Me).

Oxidation of **4**. A soln of **4** (25 mg) in pyridine (1 ml) was added to a slurry of CrO_3 (40 mg) and pyridine (1 ml) and stirred overnight at room temp. On working-up the reaction mixture in the usual manner and preparative TLC (silica gel, C_6H_6–EtOAc, 49:1), three products were obtained which have been characterized as nimbocinone (**1**); 24-ketonimbocinone (**5**): mp 75–76°; UV λ_{max}^{MeOH} nm: 205. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400, 1710–1700 (br), 1600 and 820. EIMS *m/z* 468.3250, $[M]^+$ (calc. for $C_{30}H_{44}O_4$: 468.3240); and 24-keto-26-nimbocinoic acid (**6**): mp 88–90°, UV λ_{max}^{MeOH} nm: 208, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3750–3100, 1725–1705 and 1605. EIMS *m/z*: 482.3005 $[M]^+$ (calc. for $C_{30}H_{42}O_5$: 482.3032) and 467.2792 $[C_{29}H_{39}O_5]^+$.

Application of Horeau's method to nimbocinone (**1**). A soln of **1** (10 mg) and α-phenylbutyric anhydride (13 mg) in dry pyridine (1 ml) was left at room temp. for 24 hr. H_2O (0.5 ml) was added, the reaction mixture left for further 3 hr and worked up employing the modified procedure [10]. $[\alpha]_D$ in 5 ml C_6H_6 (0.5 dm tube) +0.01°. $[\alpha]_D^{23}$ = 14.70°, optical yield = 45.70%; configuration = 24*R*.

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