Studies on the Chemical Constituents of Azadirachta indica A.Juss (Meliaceae) Part-VI [1]

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Summary: A new triterpenoid, nimolinone (I) has been isolated from the fresh, uncrushed, ripe fruits (nimoli) of Azadirachta indica and its structure elucidated through spectral and chemical studies.

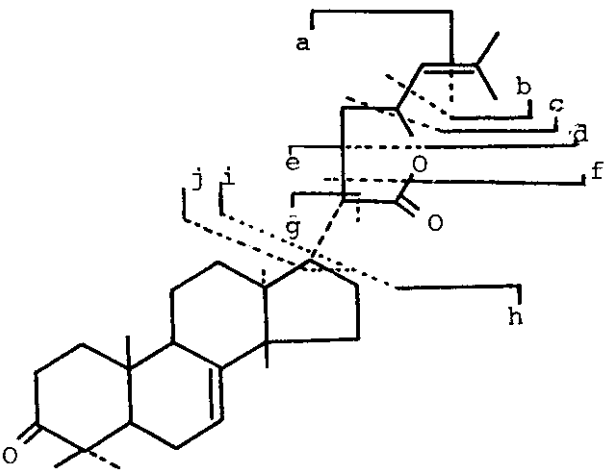
Introduction

Azadirachta indica (neem) is widely distributed in Asia and Africa and almost every part of the tree has long been used for the treatment of a variety of human ailments [2-6]. More recent studies have shown that some of the constituents of neem have pronounced pesticidal activity [7].

Previous investigations of the chemical constituents of the fresh, undried, uncrushed fruits and leaves carried out by us have resulted in the isolation and structure elucidation of nimolicinol [8], azadirachtol [9], nimocinol [10], nimbocinone [11], nimbochalcin and nimbocetin [12] nimocinolide, isonimocinolide and nimocin [1]. The present paper deals with the isolation and structure elucidation of a new triterpenoid nimolinone (I) from fresh, ripe fruits.

Results and Discussion

Nimolinone (I) has been obtained as needles (0.15% on the wt. of neutral fraction) from the ethanolic extract of neem fruits. It has molecular formula $C_{30}H_{44}O_3$ (high resolution mass). Its UV spectrum showed maxima at 210 nm while IR spectrum showed peaks at 1770 (γ-lactone), 1700 (six membered ring ketone), 1620 (trisubstituted double bonds), 1375 and 1370



I: Nimolinone

II: $\Delta^{9,11}$Nimolinone

cm^{-1} (gem dimethyl). The ^{1}H-NMR spectrum of I (Table-1) showed five methyl groups (δ 0.96, 1.02, 1.05, 1.10 and 1.25) located on quaternary carbons and two vinylic methyls(δ 1.62 and 1.68) indicating its

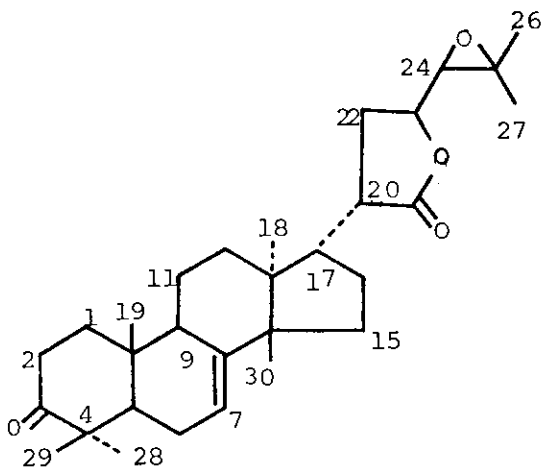
Table-1: ^{1}H-NMR spectral data ($_{H}$ and J/Hz)

Assignment	Nimolinone (I)	Assignment	Nimolinone (I)
H-1 α	1.98, ddd, J_{gem}=15.3	H-16 α	2.30, dddd, J_{gem}=18.0
	$J_{1\alpha,2\alpha}$=5.4, $J_{1\alpha,2\beta}$=10.3		$J_{16\alpha,15\alpha}$=10.3, $J_{16\alpha,15\beta}$=4 $J_{16\alpha,17}$,=1.0
H-1 β	1.95, m	H-16 β	2.20, m
H-2 α	2.60, m	H-17	2.07, ddd, $J_{17,16\alpha}$=1.0
			$J_{17,16\beta}$=6.0, $J_{17,20}$=14.5
H-2 β	2.12, ddd, J_{gem}=14.0	H-20	2.70, ddd, $J_{20,17}$=14.5
	$J_{2\beta,1\alpha}$=10.3, $J_{2\beta,1\beta}$=5.4		$J_{20,22\alpha}$=5.5, $J_{20,22\beta}$=14.5
H-5	1.80, dd, $J_{5,6\alpha}$=2.6	H-22α	2.01, m
	$J_{5,6\beta}$=9.2		
H-6 α	2.60, m	H-22	2.20, m
H-6 β	2.09, dd, J_{gem}=15.6	H-23	5.10, m
	$J_{6\beta,5}$=9.2		
H-7	5.34, d, $J_{7,6\alpha}$=2.7	H-24	5.05, m
H-9	1.76, dd, $J_{9,11}$ =4.4	H-18	0.96, s
	$J_{9,11\beta}$=11.6		
H-11 α	1.40, m	H-19	1.10,s
H-11 β	1.70, m	H-26	1.68, br.s
H-12 α	1.65,m	H-27	1.62, br.s
H-12 β	1.70,m	H-28	1.05 s
H-15 α	2.15, m	H-29	1.02, s
H-15 β	2.44, ddd, J_{gem}=12.5	H-30	1.25, s
	$J_{15\beta,16\alpha}$=4.0, $J_{15\beta,16\beta}$=8.3		

All values are in (ppm) relative to TMS=0

triterpenoidal nature. The presence of the latter two along with the two olefinic proton signals at δ 5.34 (H-7, d, J=2.70 Hz) and 5.05 (H-24,m) showed that (I) has two double bonds.The molecular formula of I indicated nine double bond equivalents, three of which have been accounted for by the two double bonds and a carbonyl function while the remaining six by the four rings of carbocyclic nucleus and γ-lactone in the side chain. The carbonyl function placed at C-3 on biogenetic grounds, was supported by an important fragment at m/z 366.2545 ($C_{25}H_{34}O_2$) in the mass spectrum of I due to the loss of $C_5H_{10}O$ in the 3-keto triterpenoids [13]. One of the trisubstituted double bonds could be at either C-7 or C-14 on biogenetic considerations. However, the mass spectrum of I showed significant fragments at m/z 245.1848 ($C_{17}H_{25}O$), 257.1874 ($C_{18}H_{25}O$) and 271.2007 ($C_{19}H_{27}O$) resulting from the characteristic cleavages in compounds with a double bond at C-7 [14]. This was further confirmed through dehydrogenation of I with mercuric acetate which yielded the diene II displaying UV absorption bands characteristic of 7,9 (11)-heteroannular dienes in an euphane (tirucallane) skeleton: λ_{max} 230, 238 and 248 nm [15]. These observations disclosed the position of one of the double bonds as well as the stereochemistry of the ring system. The diagnostic ions at m/z 313.2496 ($C_{22}H_{33}O$, fragment 'h') and 139.0738 ($C_8H_{11}O_2$, fragment 'i') indicated that the side chain consists of $C_8H_{11}O_2$. The presence of two vinylic methyl signals in the ^{1}H-NMR spectrum and the down field resonance of H-23 (δ 5.10,m) led to the location of the second double bond at C-24, which was corroborated by the significant ions at m/z 409.2747 ($C_{27}H_{37}O_3$, fragment 'a') resulting from the loss of isopropylidene group and 56.0625 (fragment 'b') corresponding to the isobutenyl group.

The structure of nimolinone was finally confirmed through high resolution mass spectrum which showed important fragments at m/z 69.0704 (C_5H_9, fragment 'c'), 82.0784 (C_6H_{10}, fragment 'd'), 370.2462 ($C_{24}H_{30}O_3$, fragment 'e'), 98.0720 ($C_6H_{10}O$, fragment 'f'), 325.2484 ($C_{23}H_{33}O$, fragment 'g') and 151.0746 ($C_9H_{11}O_2$,



III

fragment 'j') resulting from various cleavages of side chain and ring D. Further, the ^{13}C-NMR chemical shifts observed in the broad band spectrum and the polarization transfer (DEPT) experiments (45°, 90° and 135°) (Table-2) are in agreement with the assigned structure.

Table-2: ^{13}C-NMR Spectral data

C.No.	Nimolinone	III [17]	C.No.	Nimolinone	III [17]
1	38.3	38.5	16	28.8[b]	30.0[e]
2	35.8[a]	35.1	17	58.3	47.1
3	216.2	216.7	18	12.5	12.7
4	46.6	47.8	19	17.9	17.6
5	52.7	52.4	20	45.5	40.4
6	24.4	24.4	21	180.5	177.6
7	118.6	118.2	22	24.4	23.5
8	143.6	145.3	23	82.4	77.9
9	48.0	48.4	24	123.5	64.5
10	35.5	34.9	25	132.7	57.2
11	26.1	27.4	26	25.7[c]	23.5[f]
12	29.3[b]	33.8[e]	27	12.5[c]	19.4[f]
13	39.6	43.7	28	21.6[d]	23.0
14	55.2	50.5	29	24.5	24.4
15	34.8[a]	31.0[e]	30	21.5[d]	21.5

a-f: Assignments may be reversed.

All values are in (ppm) relative to TMS=0

The assignments of all the protons tabulated in Table-1 has been possible through two dimensional NMR experiments COSY, NOESY, J.resolved and the multiplicities observed in the ^{1}H-NMR spectrum. The stereochemistry of various centres as drawn in the structure has been determined through 2-D NOE (NOESY) experiment, which showed the spatial connectivities of various protons as depicted in Table-3. Apart from other centres, the stereochemistry of C-17 and C-23 could also be conclusively established. Thus the spatial connectivity of H-18 with H-9, H-15α , H-16α , H-22α and H-24; and of H-17 with H-30 showed that the side chain at C-17

Table-3: Spatial connectivities of various protons observed in the NOESY spectrum of Nimolinone (I)

Proton signal	Connected with	Proton signal	connected with
0.96 (H-18)	1.76(H-9)	1.25 (H-30)	1.70 (H-11 β and H-12β)
	2.01 (H-22α)		2.07 (H-17)
	2.15 (H-15α)	1.40 (H-11α)	1.65 (H-12α)
	2.30 (H-16α)		1.98 (H-1α)
	5.05 (H-24)	1.62 (H-27)	2.30 (H-16α)
1.02 (H-29)	2.09 (H-6β)	1.68 (H-26)	2.15 (H-15α)
1.05 (H-28)	1.80 (H-5)		5.05 (H-24)
1.10 (H-19)	1.70 (H-11 β) and H-12β)	1.76 (H-9)	1.05 (H-28)
	2.09 (H-6β)	2.20 (H-16β)	2.44 (H-15β)
	2.12 (H-2β)	2.15 (H-15α)	2.01 (H-22α)
			5.34 (H-7)
		5.05 (H-24)	5.34 (H-7)

All values are in (ppm) relative to TMS=0

is located on the α-side of the molecule. Moreover, the spatial connectivity of H-24 with H-7 and H-18 exhibited that the isobutenyl side chain at C-23 is located on the α-side of the γ-lactone ring i.e. the configurtion of C-23 is 'R'.The spatial connectivity of H-20 with H-22 β and H-18 further showed that the configuration of C-20 is S i.e. it is a tirucallane derivative. It may be noted in this context, that this is the first report of the isolation of **I** from a natural source, which has previously been prepared through oxidation of flindissol [16]. Further, the paper describes the stereochemistry of C-17 and C-23, which could not be determined in the case of flindissol, as well as of the various other centres proposed tentatively earlier [16].

Experimental

Mps were recorded in glass capillary tubes and are uncorrected. IR (in $CHCl_3$) and UV (in MeOH) spectra were measured on a JASCO IRA-I spectrometer and a Pye-unicam SP-800 spectrometer respectively. Mass spectra were recorded on Finnigan MAT 112 and 312 double focussing mass spectrometers. ^1H and ^{13}C-NMR spectra were recorded on a Bruker Aspect 3000 spectrometer operating at 300 MHz for ^1H and 75 MHz for ^{13}C nuclei. TMS was used as internal reference. The ^{13}C-NMR spectral assignments have been made through comparison with published data for similar compounds [11,17] and DEPT experiments. The purity of samples was checked on TLC (silica gel SIF-254 precoated aluminium cards).

The ethanolic extract of fresh, uncrushed, undried, ripe fruits (nimoli,20 Kg) of neem was divided into acidic and neutral fractions. The darkish neutral ethyl acetate layer after usual workup was charcoaled and freed of the solvent. The residue was repeatedly extracted out with 50% ethanol, which was partitioned with a

mixture of benzene and hexane (2:1, v/v). The residue obtained on usual workup of the benzene-hexane phase was subjected to a great deal of experimentation following classical methods of isolation. As a result, three new triterpenoids namely, nimolicinol [8], azadirachtol [9] and azadirachnol [18] were isolated and their structures elucidated. The mother liquors obtained from these workings were combined and their residue subjected to column chromatography (silica gel 40, 70-230 mesh). The column was successively eluted with benzene and benzene-ethyl acetate mixtures in the order of increasing polarity. The benzene eluate afforded two new triterpenoids nimocin [1] and nimolinone (I) along with azadirone, β-sitosterol, gedunin, epoxyazadiradione, 7-deacetyl-7-benzoylazadiradione, azadiradione and 17-hydroxyazadiradione [1 and references cited therein]. On recrystallization from methanol **I** formed needles (0.14 g, 0.15% on the wt. of neutral fraction), m.p. 186-190° $[\alpha]_D^{24}$ - 24° (c 0.25 in $CHCl_3$). High resolution MS m/z (rel.int.): 452.3267 $[M]^+$ calc. for $C_{30}H_{44}O_3$:452.3290) (2), 437.3023 ($C_{29}H_{41}O_3$) (10), 409.2747 ($C_{27}H_{37}O_3$) (1), 384.2669 ($C_{25}H_{36}O_3$) (2), 370.2462 ($C_{24}H_{34}O_3$) (3), 352.2396 ($C_{24}H_{32}O_2$) (1), 325.2484 ($C_{23}H_{33}O$) (1), 313.2496 ($C_{22}H_{33}O$) (12), 271.2007 ($C_{19}H_{27}O$) (8), 257.1874 ($C_{18}H_{25}O$) (7), 245.1848 ($C_{17}H_{25}O$) (2), 163.1123 ($C_{11}H_{15}O$) (3), 151.0746 ($C_9H_{11}O_2$) (2), 149.0965 ($C_{10}H_{13}O$) (3), 140.0826 ($C_8H_{12}O_2$) (9), 139.0738 ($C_8H_{11}O_2$) (3), 133.0672 (C_9H_9O) (2), 125.0608 ($C_7H_9O_2$) (2), 122.0737 ($C_8H_{10}O$) (4), 98.0720 ($C_6H_{10}O$) (4), 82.0784 (C_6H_{10}) (98), 79.0564 (C_6H_7) (35), 69.0704 (C_5H_9) (100) and 56.0625 (C_4H_8) (7)

Dehydrogenation of I to II

To a solution of **I** (15 mg) in $CHCl_3$ (2 ml), was added a solution of mercuric acetate (35 mg) in glacial acetic acid (1 ml). The reaction mixture was kept stirring for 24 hours at room temperature, filtered and freed of the solvent, when chromatographically pure **II** was obtained as rods on recrystallization from methanol, m.p. 160°C, UV λ_{max} nm: 230, 238, 248, EIMS m/z (rel.int.): 450 $[M]^+$ (1), 435.2963 ($[M-15]^+$, calcd. for $C_{29}H_{39}O_3$: 435.2899) (2).

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