ISOLATION OF MELIACIN CINNAMATES FROM THE ROOT BARK OF AZADIRACHTA INDICA A.JUSS (MELIACEAE)

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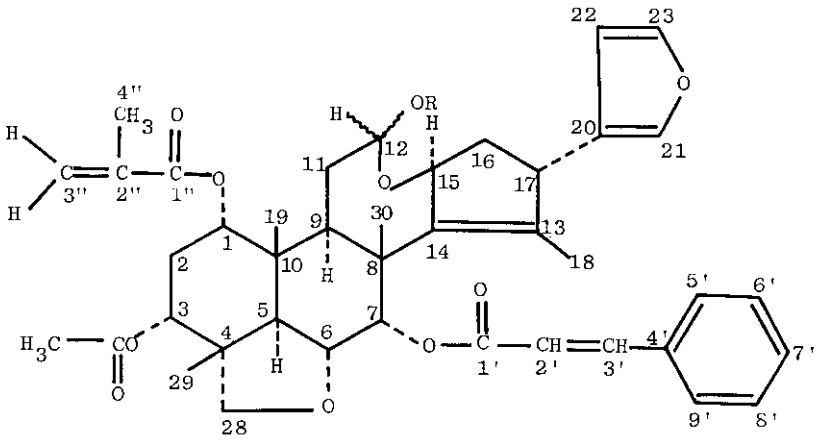
Abstract — A new tetranortriterpenoid named as nimbolicin has been isolated from the neutral fraction of root bark of Azadirachta indica A.Juss (neem) besides nimbolin B previously reported from the trunk wood. The structure elucidation of nimbolicin through chemical and spectroscopic methods along with the hitherto unreported ^{13}C-nmr and 2D spectral studies (NOESY, COSY, heterocosy) of nimbolin B form the subject of present communication.

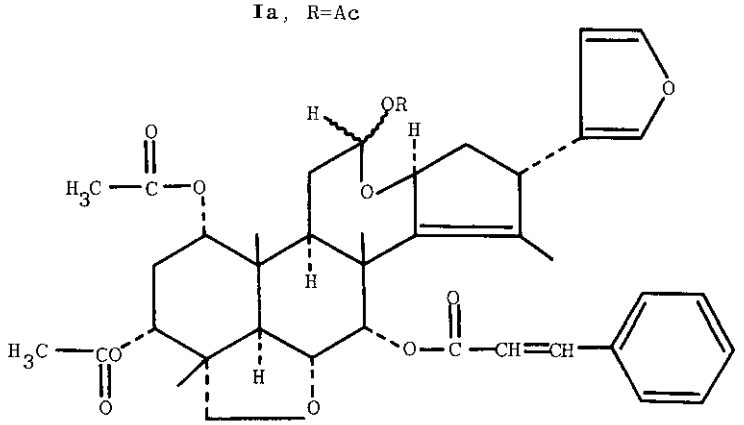
In pursuance of studies in the constituents of fruits, leaves, twigs[1] and stem bark of Azadirachta indica[2], a new meliacin nimbolicin along with nimbolin B has been isolated from the neutral fraction of neem roots. The structure of the new terpenoid has been determined through spectral and chemical studies while nimbolin B has been identified through comparison of its spectral data with those reported in literature.[3] Further, ^{13}C-nmr and 2D spectral studies of nimbolin B have also been carried out which have not been reported earlier.

Nimbolicin (**I**) was obtained from the neutral fraction of the methylene chloride extract of neem roots, following the course of isolation procedure recorded in the experimental. It has molecular formula $C_{41}H_{48}O_{10}$ and showed maxima at 205 and 280 nm in uv spectrum. Its ir spectrum showed peaks at 3400 (hydroxy group), 1720 (ester carbonyl function) and 1120 and 980 cm^{-1} (ether linkage). The ^{1}H-nmr spectrum showed the presence of a β-substituted furan ring (δ 7.20, 7.27, 6.34; H-21, H-23 and H-22 respectively), a cinnamoyloxyl group (δ 7.73, 6.39, each 1H, d, J=15.96 Hz, H-3' and H-2' respectively; 7.52, 2H, m, H-5', H-9', 7.38, 2H, m, H-6', H-8' and 7.39, 1H, m, H-7'), an acetoxyl group (δ 2.03, 3H, s), a hemiacetal moiety (δ 5.30, 1H, m, H-12; 5.26, dd, J=8.00, 2.80 Hz, H-15) and an ether linkage between C-6 and C-28 (δ 3.72, 3.78, each 1H, d, J=7.04 Hz, H-28a and H-28b). The hydroxyl group of the hemiacetal was also confirmed by acetylation of **I** to **Ia**. The data so far recorded showed that nimbolicin (**I**) is closely related with nimbolin B.[3] The chemical shifts and coupling constants of H-5 (δ 2.78, d, J=12.80 Hz), H-6 (δ 4.19, dd, J=12.80, 2.80 Hz) and H-7 (δ 5.77, d, J=2.8 Hz) as well as H-9, H-11α, H-11β, H-16α, H-16β and H-17 (vide table) further showed that the part of the skeleton discussed so far has the identical



I, R=H

Ia, R=Ac



II, R=H

IIa, R=Ac

substitution pattern as that of nimbolin B, and they differ in the substituents of ring A only. Justification of the functionalities noted above left a $C_4H_5O_2$ unit of the moelcular formula to be explained. The nmr spectral data (δ 1.93, H-4", δ 7.43, 7.44, each 1H d, J=3.50 Hz, H-3"a, H-3"b; δ C-1" 170.4, δ C-2" 128.9, δ C-3" 108.4 and δ C-4" 12.1) and a mass fragment at m/z 614.2870 ($M-C_4H_6O_2$) conclusively established the identity of $C_4H_5O_2$ unit as a 2-methylpropenoate function. The proton at δ 4.94 which is comparable with that of H-3 of nimbolin B suggested that the acetoxy function is at C-3 while the 2-methylpropenoate function is at C-1 and thus H-1 appears at δ 5.00 as against δ 4.83 in nimbolin B. The position

Table I ^{1}H-Nmr Spectral Data of Nimbolicin (**I**) and Nimbolin B (**II**)

Assignments	I	Ia	II	IIa
H-1	5.00(t) J=3.08	5.00(m)	4.82(t) J=2.88	4.81(t) J=2.84
H-2α	2.08(m)	2.08(m)	2.21(m)	2.22(m)
H-2β	2.18(m)	2.18(m)	2.26(m)	2.26(m)
H-3	4.94(t) J=3.08	4.93(m)	4.93(t) J=2.88	4.93(t) J=2.84
H-5	2.78(d) $J_{5,6}$=12.80	2.78(m)	2.82(d) $J_{5,6}$=12.76	2.84(d) $J_{5,6}$=12.76
H-6	4.19(dd) $J_{6,5}$=12.80 $J_{6,7}$=2.80	4.16(dd) $J_{6,5}$=12.80 $J_{6,7}$=2.80	4.14(dd) $J_{6,5}$=12.76 $J_{6,7}$=2.92	4.12(dd) $J_{6,5}$=12.76 $J_{6,7}$=2.76
H-7	5.77(d) $J_{7,6}$=2.80	5.77(d) $J_{7,6}$=2.80	5.85(d) $J_{7,6}$=2.92	5.85(d) $J_{7,6}$=2.76
H-9	3.49(dd) $J_{9,11\alpha}$=7.00 $J_{9,11\beta}$=2.60	3.47(m)	3.45(dd) $H_{9,11\alpha}$=7.50 $H_{9,11\beta}$=2.50	3.25(m)
H-11α	2.04(m)	2.04(m)	1.64(m)	1.64(m)
H-11β	2.21(m)	2.21(m)	2.27(m)	2.26(m)
H-12	5.30(m)	6.36(m)	5.26(m)	6.37(m)
H-15	5.26(dd) $H_{15,16\alpha}$=8.00 $H_{15,16\beta}$=2.80	5.25(m)	5.25(dd) $J_{15,16\alpha}$=9.50 $J_{15,16\beta}$=3.00	5.12(m)
H-16α	2.20(ddd) $J_{16\alpha,16\beta}$=13.32 $J_{16\alpha,17}$=9.00 $J_{16\alpha,15}$=8.00	2.20(m)	2.28(ddd) $J_{16\alpha,16\beta}$=14.00 $J_{16\alpha,17}$=9.80 $J_{16\alpha,15}$=9.50	2.28(m)
H-16β	1.49(dd) $J_{16\beta,16\alpha}$=13.32 $J_{16\beta,15}$=2.80	1.49(m)	1.42(dd) $J_{16\beta,16\alpha}$=14.00 $J_{16\beta,15}$=3.00	1.41(m)
H-17	3.25(d) J = 9.00	3.25(m)	3.26(d) J = 9.80	3.26(d) J = 9.60
CH_3-18	1.78(s)	1.78(s)	1.78(s)	1.78(s)
CH_3-19	0.94(s)	0.93(s)	1.01(s)	1.01(s)
H-21	7.20(m)	7.17(m)	7.20(dd) $J_{21,23}$=1.60 $J_{21,22}$=1.60	7.20(m)
H-22	6.34(m)	6.34(m)	6.32(dd) $J_{22,23}$=1.60 $J_{22,21}$=1.60	6.32(dd) $J_{22,23}$=1.60 $J_{22,21}$=1.60
H-23	7.27(m)	7.28(m)	7.27(dd) $J_{23,22}$=1.60 $J_{23,21}$=1.60	7.27(dd) $J_{23,22}$=1.60 $J_{23,21}$=1.60
H-28a	3.72(d) J_{gem}=7.04	3.63(d) J_{gem}=7.20	3.45(d) J_{gem}=7.64	3.47(d) J_{gem}=7.76
H-28b	3.78(d) J_{gem}=7.04	3.77(d) J_{gem}=7.20	3.51(d) J_{gem}=7.64	3.53(d) J_{gem}=7.76
CH_3-29	1.15(s)	1.17(s)	1.18(s)	1.18(s)

Contd..

Table I (Contd.)

Assignments	I	Ia	II	IIa
CH_3-30	1.47(s)	1.45(s)	1.47(s)	1.47(s)
H-2'	6.39(d) $J_{2',3'}$=15.96	6.39(m)	6.39(d) $J_{2',3'}$=15.96	6.39(d) $J_{2',3'}$=16.00
H-3'	7.73(d) $J_{3',2'}$=15.96	7.73(d) $J_{3',2'}$=15.08	7.70(d) $J_{3',2'}$=15.96	7.70(d) $J_{3',2'}$=16.00
H-5' H-9'	7.52(m)	7.52(m)	7.47(m)	7.47(m)
H-6' H-8'	7.38(m)	7.38(m)	7.38(m)	7.38(m)
H-7'	7.39(m)	7.38(m)	7.39(m)	7.39(m)
H-3"a	7.43(d) J_{gem}=3.50	7.43(m)	-	-
H-3"b	7.44 J_{gem}=3.50	7.43(m)	-	-
H-4"	1.93(s)	1.93(s)	-	-
$CO\underline{CH}_3$	2.03(s)	2.03,2.11(2xs)	1.89,2.18(2xs)	1.87,2.19,2.07(3xs)
	-			
	-	-	-	

Table II ^{13}C-Nmr Spectral Data of Nimbolicin (I) and Nimbolin B (II)

Carbon Nos.	(I)	(II)	Carbon Nos.	(I)	(II)
1	69.5	71.2	28	77.9	78.0
2	29.3	28.0	29	19.2	19.2
3	71.2	71.6	30	21.0	21.9
4	42.8	42.5	1'	165.3	165.1
5	41.0	40.3	2'	118.1	118.1
6	72.5	72.3	3'	146.5	143.8
7	75.0	75.0	4'	134.4	134.5
8	45.7	45.4	5'	130.4	127.7
9	35.9	35.7	9'		
10	42.4	40.3	6'	128.2	129.0
11	32.0	31.7	8'		
12	91.8	91.8	7'	144.6	130.4
13	142.7	141.2	1"	170.4	-
14	144.7	142.8	2"	128.9	-
15	77.2	77.4	3"	108.4	-
16	38.1	38.3	4"	12.1	-
17	46.7	46.6	$CO\underline{CH}_3$	20.6	20.0,22.5
18	16.3	16.2	$\underline{CO}CH_3$	172.9	170.3,169.8
19	16.2	16.2			
20	118.7	128.3			
21	139.7	139.0			
22	110.4	110.2			
23	142.6	142.8			

of propenoate function at C-1 and acetoxy group at C-3 has also been supported in the NOESY spectrum which showed the interaction of H-4'' with H-11α; H-1 with H-11β and the acetoxy methyl protons with H-5. The coupling constants of H-1 and H-3 (vide table I) further showed that both the ester functions at C-1 and C-3 are α-oriented. In the light of above facts structure (**I**) has been assigned to nimbolicin. Nimbolin B obtained in the present working has mp 241 - 243°C, $[\alpha]_D$ - 93.3° which are identical with those reported.[3] A complete assignment of the protons has been made (vide table) in the present working through 2D-nmr (2D J resolved, COSY-45, NOESY) and homonuclear decoupling experiments as well as ^1H-^{13}C hetero-COSY which also enabled to exactly assign various protons and carbons noted in table I and II respectively. On reaction with acetic anhydride/pyridine **II** formed the monoacetyl derivative (**IIa**) which was characterized through mass and ^1H-nmr spectral data (table I).

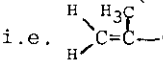
The stereochemistry of various centres of nimbolin B has been confirmed through 2D-NOE (NOESY) spectrum which showed the spatial connectivities of H-21 with H-2'; H-23 with H-18; H-7 with H-6, H-18 and H-30; and H-6 with H-30 and H-7. Spatial connectivity of H-21 with H-2' showed that cinnamate group and furan ring both have α disposition. Further the interaction of H-30 with H-19 and H-19 with H-29 were also observed along with that of H-5 and H-9 thus conforming to the B/C trans ring junction.

EXPERIMENTAL

Melting points were recorded in air-bath melting point apparatus and are uncorrected. Ir and uv spectra were measured on JASCO-IRA-I and pye unicam sp-800 spectrophotometers respectively. Mass spectra were recorded on Finnigan MAT 112 and 312 double focussing mass spectrometers; exact masses have been measured through peak matching; ^1H-nmr spectrum and NOESY experiment (pulse delay 2 sec. mixing time 0.5 sec) were run on Bruker AM 300 NMR spectrometer. ^{13}C-Nmr spectra were recorded in $CDCl_3$ at 75 MHz and assignments are based on DEPT experiment and comparison with similar compounds.[4] Chemical shifts are reported in ppm (δ) and delay time for heterocosy was 36 m sec. Optical rotations were measured at 24°C in chloroform. Merck Kieselgel 60 PF_{254} coated on glass plates was used for analytical (thin layer) and preparative (thick layer) chromatography.

Extraction and Fractionation: Neem roots (28 kg) (collected from Karachi region) were repeatedly extracted with methylene chloride at room temperature. The combined

extracts were freed of the solvent under reduced pressure and partitioned between ethyl acetate and water. The former was shaken out with 4% Na_2CO_3 solution to separate the acidic from neutral constituents. The residue obtained on usual work up of the ethyl acetate layer containing the neutral fraction was treated with petroleum ether to separate petroleum ether soluble (A) and insoluble fractions. The latter fraction was successively treated with ether and ethyl acetate to give ether and ethyl acetate soluble fractions. Each of these was concentrated and treated with an excess of petroleum ether ultimately affording ether-petroleum ether soluble (B) and insoluble fractions; and ethyl acetate petroleum ether soluble (C) and insoluble fractions respectively. Fraction A, B and C were combined on the basis of tlc, freed of the solvent and partitioned between 90% methanol and petroleum ether. The 90% methanolic phase, after addition of NaCl, was shaken out with ethyl acetate which was concentrated and again treated with an excess of petroleum ether. The insoluble fraction (3.6 g) thus obtained was subjected to flash column chromatography[5] (silica gel, E.Merck 9385). The column was successively eluted with petroleum ether and ethyl acetate in the order of increasing polarity. Nimbolicin was obtained with some allied impurities in the fractions eluted with the solvent system, petroleum ether-ethyl acetate 7.5:2.5 while the less polar constituents obtained with the same solvent system gave nimbolin B on repeated purification through flash column chromatography and final cyrstallization with moist methanol. Nimbolicin was finally purified by repetition of flash column chromatography using solvent system petroleum ether-ethyl acetate 7:3.

Nimbolicin: Nimbolicin (16.3 mg) (I) was obtained as irregular plates, mp 121-122°C, $[\alpha]_D^{24}$ = -33.3 ($CHCl_3$). EIMS m/z (%): 700.3249 (calc. for $C_{41}H_{48}O_{10}$, 700.3243) (M^+) (3), 614.2870 ($C_{37}H_{42}O_8$) ($M^+-C_4H_6O_2$ i.e. ) (4.5), 596.2768 ($C_{37}H_{40}O_7$) ($M^+-C_4H_6O_2-H_2O$) (5), 550.2889 ($C_{33}H_{42}O_7$) (2) and 95.0495 (C_6H_7O) (100).

Acetylation of I to **Ia**: To a solution of **I** (4 mg) in pyridine (2 ml), acetic anhydride (4 ml) was added and the reaction mixture kept overnight at room temperature. On usual work up **Ia** was obtained as plates (3.1 mg), mp 100-101°C, uv λ_{max}(MeOH) nm 203 and 270; ir ν_{max}($CHCl_3$) cm^{-1} 2900 (C-H), 1720 (br., ester carbonyls) 1360 and 1080 (ether linkage). EIMS m/z (%): 701 (M^+-41) (1), 684 (M^+-41-17) (2), 675 (M^+-64) (6), 615 (M^+-60) (30), 449 (M^+-cinnamic acid - 2 methylpropenoic acid - $OCOCH_3$) (10) and 131 (100).

Nimbolin B: Nimbolin B (553 mg) (II) was obtained as white crystalline powder, mp 140-142°C, $[\alpha]_D^{24}$ = -93.3 ($CHCl_3$), EIMS m/z (%): 674.3100 (calc. for $C_{39}H_{46}O_{10}$, 674.3100) (M^+) (1.7), 596.2969 ($C_{34}H_{44}O_9$) ($M^+-C_5H_2O$) (5.8), 508.2459 ($C_{30}H_{36}O_7$) ($M^+-C_9H_8O_2$ - H_2O) (6) and 450.3134 ($C_{30}H_{42}O_3$) (100).

Acetylation of nimbolin B: To a solution of nimbolin B (10 mg) in pyridine (2 ml), acetic anhydride (4 ml) was added and reaction mixture kept overnight at room temperature. The acetylated product obtained after usual work up crystallized from petroleum ether as irregular plates (7.8 mg), mp 162-128°C; uv λ_{max}(MeOH) nm. 205, 218, 223 and 278, ir ν_{max}($CHCl_3$) cm^{-1} 2850 (C-H), 1720 (br., ester carbonyls), 1640 (trisubstituted double bond), 1160 and 1020 (ether linkage). EIMS (%): 656 (M^+-60) (8), 638(8), 596(7), 147(12), 131(100), 103(22).

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