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NIMBIDIOL, A MODIFIED DITERPENOID OF THE ROOT-BARK OF *AZADIRACHTA INDICA*

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Key Word Index—*Azadirachta indica*; Meliaceae; Indian 'neem'; modified diterpene; nimbidiol.

Abstract—Nimbidiol, a modified diterpenoid, isolated from the root-bark of *Azadirachta indica* (Indian 'neem'), was characterized by spectroscopic method.

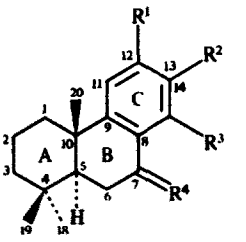
INTRODUCTION

Azadirachta indica A. Juss, popularly known as 'neem' in India, has been the subject of extensive chemical investigation by several groups of workers [1–14] because of its varied biological activities [15–20]. Of particular importance is the isolation of the antifeedant azadirachtin [8] and several of its structural analogues [10, 11] from the leaves and fruits of this plant. There are, however, few reports of the chemical investigation of its root. This has prompted us to undertake a systematic chemical investigation on the root of *A. indica*, the root-bark of which has yielded a new modified diterpenoid (**1a**), which we have named nimbidiol.

RESULTS AND DISCUSSION

Nimbidiol (**1a**), $C_{17}H_{22}O_3$ ($[M^+] = m/z$ 274), showed UV absorptions [λ_{max}^{EtOH} 210, 238, 282 and 323 nm (log ε4.19, 4.12, 3.91 and 3.78)] strikingly similar to those of nimbiol (**1e**) [3] and sujiol (**1f**) [3] indicating the presence of a *p*-hydroxybenzoyl chromophore. Its phenolic nature was indicated by its characteristic colour reactions, by the alkali-induced bathchromic shift of its UV maxima [$\lambda_{max}^{EtOH-0.1\,N\,NaOH}$ 211, 256 and 353 nm (log ε4.40, 3.99 and 4.23)] and by the presence of bands at 3500, 3420 and 3230 cm^{-1} in its IR spectrum. The presence of these three bands, particularly the last one, suggested that the hydroxyl groups were partially hydrogen-bonded. The IR spectrum of nimbidiol also revealed the presence of an aromatic keto-carbonyl function (ν_{max} 1650 cm^{-1}). The absorption of this carbonyl group at a relatively high wavelength may be attributed to the presence of a hydroxyl function *para* to the carbonyl group. The presence of two phenolic hydroxyl groups in nimbidiol was confirmed by the formation of a monoacetate, $C_{19}H_{24}O_4$ ($[M]^+ = m/z$ 316), a diacetate, $C_{21}H_{26}O_5$ ($[M]^+ = m/z$ 358), and a dimethyl ether derivative, $C_{19}H_{26}O_3$ ($[M]^+ = m/z$ 302).

The ^{1}H NMR spectrum of nimbidiol showed a two-proton broad signal (disappeared on deuterium exchange) at δ9.22 corroborating the presence of two phenolic hydroxyl groups in the compound. The downfield position of this signal also indicated the involvement of these hydroxyl functions in intramolecular hydrogen bonding. The spectrum of nimbidiol exhibited signals for only two aromatic protons at δ6.85 and 7.39 (each 1H, *s*) indicating the tetrasubstituted nature of its benzenoid ring. The chemical shift of the proton corresponding to the signal at δ7.39 was consistent with its being flanked by a carbonyl and a hydroxyl group, and the multiplicity of the two aromatic proton signals suggested that the two aromatic protons in nimbidiol were *para* to each other and were each *ortho* to a hydroxyl group. This was borne out by the fact that the signals at δ6.85 and 7.39 of nimbidiol were shifted downfield to δ7.19 and 7.80 respectively in the ^{1}H NMR spectrum of nimbidiol diacetate. In the ^{1}H NMR spectrum of the monoacetate only the signal at δ7.39 was shifted downfield to δ7.67, the signal for the other aromatic proton remaining essentially unchanged



	R^1	R^2	R^3	R^4
1a	OH	OH	H	O
1b	OH	OAc	H	O
1c	OAc	OAc	H	O
1d	OMe	OMe	H	O
1e	OH	Me	H	O
1f	OH	—CH(Me)$_2$	H	O
1g	H	H	—CH(Me)$_2$	O
1h	OMe	H	H	H_2
1i	OMe	—CH(Me)$_2$	H	H_2
1j	—CH(Me)$_2$	OMe	H	H_2
1k	OAc	—CH(Me)$_2$	H	H_2
1l	H	OAc	—CH(Me)$_2$	H_2

(δ6.89). This suggested that in nimbidiol monoacetate only the hydroxyl group *ortho* to the aromatic proton corresponding to the signal at δ7.39 of nimbidiol had undergone acetylation. The ^{1}H NMR spectrum of nimbidiol also displayed three three-proton singlets at δ0.93, 1.00 and 1.21 for three methyl groups attached to sp^3 carbon atoms, and a two-proton multiplet centred at δ2.54 for the protons of a keto-methylene group of the type >CH–CH_2–CO–C<. The above spectral features were also discernible in the ^{1}H NMR spectra of all the derivatives of nimbidiol. Based on the foregoing spectral data nimbidiol was assigned the structure **1a**, and its mono and diacetyl and dimethyl ether derivatives structures **1b**, **1c** and **1d** respectively.

More compelling evidence in support of the above structure of nimbidiol was provided by the ^{13}C NMR spectral analysis of its more soluble monoacetyl (**1b**), diacetyl (**1c**) and dimethyl ether (**1d**) derivatives. The degree of protonation of the carbon atoms in each compound was determined by DEPT experiments and the assignments of the carbon chemical shifts (Table 1) were made by comparison with the δ_c values of sujiol (**1f**) [21, 23] and those of its structural analogues **1g–1l** [21–23] taking into consideration of the additive parameters of the functional groups. Thus the δ_c values of C-1, C-7, C-10, C-18, C-19 and C-20 of **1b**, **1c** and **1d** were almost identical with those of the corresponding carbon atoms of sujiol (**1f**) [21, 23] and (**1g**) [21, 23] confirming the structural identity of the rings A and B part of their molecules. The presence of a conjugated keto-carbonyl function in all the compounds was indicated by the signals at δ_c 198.40, 197.42 and 198.12 and its location at C-7 was corroborated by the downfield shift of C-6 of all the compounds by ~ 17 ppm and a concomitant upfield shift of C-9 by ~ 5–6 ppm compared to the corresponding carbon atoms of **1h–1l** having a methylene group at C-7. The appearance of the methoxyl carbon resonance of **1d** at δ_c 55.90 indicated that each methoxyl group had at least one *ortho* hydrogen atom, and the upfield shifts of C-8, C-9, C-11 and C-14 of **1d** compared to those of the corresponding carbon atoms of **1c** confirmed the placement of the two hydroxyl groups of nimbidiol at C-12 and C-13. The difference in the δ_c values of C-8 and C-11 of the mono and diacetyl derivatives of nimbidiol was consistent with the placement of the hydroxyl group in the mono acetyl derivative at C-12.

Nimbidiol is thus a unique modified diterpenoid of the abiatane skeleton lacking an isopropyl group at C-13. Biogenetically it may be assumed to be formed from an abiatane skeleton by an unknown oxidative process.

Table 1. ^{13}C NMR spectral data of monoacetyl nimbidiol (**1b**), diacetyl nimbidiol (**1c**) and dimethyl ether of nimbidiol (**1d**)

	(δ values)		
	1b	**1c**	**1d**
1	37.80*	37.84	38.04
2	18.75	18.68	18.84
3	41.20	41.15	41.28
4	33.20	33.24	33.21
5	49.29	49.13	49.90
6	35.70	35.86	35.85
7	198.40	197.42	198.12
8	124.45	129.50	124.20
9	152.85	154.9	147.34
10	38.03	38.14	38.04
11	112.40	119.16	105.64
12	156.50	146.42	153.84
13	136.90	140.4	151.27
14	121.90	122.30	108.67
18	32.45	32.44	32.40
19	21.28	21.23	21.25
20	23.16	23.31	23.16
–OCOMe	169.35, 20.80	168.09, 167.63 20.63, 20.49	—
–OMe	—	—	56.90

* Values are in ppm downfield from TMS: $\delta_{(TMS)} = \delta_{(CDCl_3)} + 76.9$ ppm.

EXPERIMENTAL

Mps: uncorr; CC: silica gel (60–120 mesh); TLC: silica gel G; UV: 95% aldehyde-free EtOH; IR: KBr; ^{1}H NMR: 80 MHz, $CDCl_3$, TMS as int. standard; ^{13}C NMR: 62.5 MHz, $CDCl_3$, TMS; MS: direct inlet, 70 eV. All the analytical samples were routinely dried over P_2O_5 for 24 hr *in vacuo* and were tested for purity by TLC and mass spectrometry. Na_2SO_4 was used for drying organic solvents and the petrol used had bp 60–80°.

Isolation of nimbidiol (**1a**). Air-dried powdered root-bark (2 kg) was extracted with $CHCl_3$ in a Soxhlet apparatus for 72 hr. The $CHCl_3$ extract was concd (150 ml) and subjected to CC. The petrol–EtOAc (5:1) eluate afforded a gummy residue containing mainly **1a**. Repeated chromatography of the above material gave pure **1a** (0.2 g), crystallized from a mixture of petrol, EtOAc and C_6H_6, mp 226°, $[\alpha]_D$ + 3.4° ($CHCl_3$), (Found: C, 74.39; H, 8.10; $C_{17}H_{22}O_3$ requires: C, 74.45; H, 8.03%). MS: *m/z* 274 $[M]^+$ (100%), 259 (97), 245 (5), 217 (23), 203 (13), 191 (70), 189 (71), 177 (44), 163 (19), 151 (6), 137 (6), 115 (8), 83 (6), 77 (6), 69 (27), 55 (11), and 41 (20).

Nimbidiol monoacetate (**1b**), *nimbidiol diacetate* (**1c**) *and nimbidiol dimethyl ether* (**1d**). Nimbidiol (0.15 g) was acetylated with Ac_2O and C_5H_5N to give a mixture of **1b** and **1c**. On CC of the mixture the petrol–EtOAc (30:1) eluate gave **1c** (0.1 g), crystallized from petrol–EtOAc, mp 160°, $[\alpha]_D$ + 2.2° ($CHCl_3$), (Found: C, 70.41; H, 7.20; $C_{21}H_{26}O_5$ requires: C, 70.39; H, 7.26%). UV λ_{max} nm: 210, 253, 290 (log ε 4.32, 4.09 and 3.46); IR ν_{max} cm^{-1}: 1775 and 1260 (OAc), 1680 (conjugated C=O), 1600, 880, 860 and 820 (phenyl nucleus); ^{1}H NMR: δ7.80 (1H, *s*, H-14), 7.19 (1H, *s*, H-11), 2.60 (2H, *m*, H_2-6), 2.29 and 2.28 (each 3H, *s*, 2 × OAc), 1.25, 0.99 and 0.94 (each 3H, *s*, 3 × C-Me); MS: *m/z* 358 $[M]^+$ (18%), 316 (12), 274 (100), 259 (41), 217 (6), 191 (15), 189 (18), 177 (14), 163 (6), 91 (5), 84 (8), 69 (15), 55 (13), 49 (35) and 43 (62).

The petrol–EtOAc (20:1) eluate gave **1b** (0.045 g), crystallized from petrol–EtOAc, mp 220°. (Found: C, 71.99; H, 7.62. $C_{19}H_{24}O_4$ requires: C, 72.15; H, 7.59%). UV λ_{max} nm: 210, 254 and 295 (log ε 4.27, 4.03 and 3.30); IR ν_{max} cm^{-1}: 3400 (OH), 1770 and 1265 (OAc), 1655 (conjugated C=O), 1590, 885, 870 and 820 (phenyl nucleus); ^{1}H NMR: δ7.67 (1H, *s*, H-14), 6.89 (1H, *s*, H-11), 2.69 (2H, *m*, H_2-6), 2.31 (3H, *s*, OAc), 1.20, 0.97 and 0.92 (each 3H, *s*, 3 × C-Me); MS: *m/z* 316 $[M]^+$ (18%), 274 (100), 259(63), 217 (13), 203 (9), 191 (38), 189 (44), 177 (10), 151 (6), 137 (6), 113 (9), 91 (6), 83 (10), 77 (9), 69 (36), 55 (31), 49 (10), 43 (64) and 41 (37).

A soln of **1a** (0.05 g) in MeOH (25 ml) was treated with an excess of an ethereal soln. of CH_2N_2 in the cold. The reaction mixture was kept overnight and then the solvent was removed under red. pres. On CC of the residue, the petrol–EtOAc (30:1)

eluate gave **1d** (0.045 g), crystallized from petrol–EtOAc, mp 112° (Found: C, 75.61; H, 8.55. $C_{19}H_{26}O_3$ requires: C, 75.50; H, 8.61%). ^{1}H NMR: δ7.41 (1H, *s*, H-14), 6.73 (1H, *s*, H-11), 3.87 and 3.84 (each 3H, *s*, 2 × OMe), 2.58 (2H, *m*, H_2-6), 1.20, 0.96 and 0.88 (each 3H, *s*, 3 × C-Me); MS: *m/z* 302 $[M]^+$ (17%), 287 (69), 259 (6), 245 (30), 231 (19), 219 (100), 217 (91), 205 (48), 203 (16), 191 (33), 189 (15), 165 (14), 163 (6), 145 (11), 130 (12), 128 (14), 115 (24), 93 (31), 82 (18), 71 (13), 69 (31), 67 (47), 55 (31), 49 (18) and 43 (46).

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