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STRUCTURE OF NIMONOL FROM FRESH WHOLE GREEN LEAVES OF *AZADIRACHTA INDICA*

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Key Word Index—*Azadirachta indica*; nimonol; nimocinol.

Abstract—Nimonol was obtained from the fresh green whole leaves of *Azadirachta indica* and its structure has been reassigned on the basis of COSY, NOESY and ^{1}H NMR spectral data. 6-Acetylnimonol was identified as 6-acetoxyazadirone and dihydronimonol was shown to be identical to isomeldenin. The mp and ^{1}H NMR spectra of 6-acetylnimocinol were markedly different from those of 6-acetoxyazadirone showing that nimocinol and nimonol have different structures.

INTRODUCTION

Different parts of the Indian neem tree, *Azadirachta indica* A. Juss, have been used in traditional medicine [1] and in agriculture [2] in India. It was observed that swarms of the desert locust *Schistocerca americana gregaria*, would repeatedly settle on neem trees, but would fly away without feeding on the leaves [3]. Presumably the neem leaves contained repellent and antifeedant compounds. Although a number of compounds have been isolated from neem leaves [4], little is known of their bioactivities. As we had a ready access to neem leaves, we undertook a systematic investigation of the neem leaf chemistry.

RESULTS AND DISCUSSION

Soaking of the fresh, green, uncrushed whole leaves of *Azadirachta indica* in *n*-hexane for 18 hr provided a mixture of compounds in the hexane extract. Solvent partitioning and chromatography of the hexane extract yielded nimonol (**1**), $C_{28}H_{36}O_5$, and isomeldenin [5].

The ^{1}H NMR signals of nimonol were at δ 7.39 *m*, 7.26 *m*, 6.29 *m* (β-substituted furan), 7.12 *d*, J = 10.06 Hz; 5.90 *d*, J = 10.06 Hz (-CH = CH-CO-), 5.42 *dd*, J = 1.82, 2.84 Hz (C = CH-CH_2), 2.05 (CH_3CO) 1.41, 1.31, 1.27, 1.14, 0.82 (five Mes). The ^{1}H NMR spectrum also had three methine proton signals at δ 2.21 *d*, J = 11.65 Hz (-CH-); 4.38 *dd*, J = 11.65 Hz and 2.37 (-CHOH) and 5.36 *d*, J = 2.37 Hz (-CHOAc-). The coupling connectivity of these methine protons was seen in the COSY spectrum, where the proton at δ 2.21 had cross peaks with that at δ 4.38, the proton at δ 4.38 with those at δ 2.21 and δ 5.3 and the proton at δ 5.36 with that at δ 4.38. The COSY spectrum thus indicated the presence of the group -CH-CHOH-CHOAc (-CH-CHOH-*trans*, -CHOH-CHOAc *cis* on a cyclohexane ring system). The molecular formula and the spectral data then indicated that nimonol was a tetranortriterpenoid. From an NOE experiment it was observed that in the NOESY spectrum, the H on the carbon bearing a secondary alcohol had NOE interaction with three Me groups at δ 1.41, 1.27 and 1.14. This was possible only in structure **1**, where the H at C6, which had β-configuration, was in close proximity to methyl groups located at C-4, C-8 and C-10.

In several limonoids, e.g. azadirone [6], dihydroazadirone [6] and 6-acetoxyazadirone [7], which had (i) intact ABCD-rings; (ii) no substituent in the C-ring; and (iii) an α-oriented furan ring at C-17 and also in 4α,6α-dihydroxy A-homoazadirone [8] which had an unsubstituted C-ring and an α-oriented furan ring at C-17, the most shielded methyl group was around δ 0.8. This signal has indeed been assigned [8] in 4α,6α-dihydroxy-A-homoazadirone to the methyl C-13, which was in the α-orientation and *cis* to the furan ring at C-17. Since compound **1** also had a signal at δ 0.82, it was surmised that the methyl at C-13 and the furan at C-17 were also in the *cis*-orientation. The NOE was in agreement with this. Thus, the methyl at C-13 had an NOE interaction with the β-proton (at δ 6.29) of the furan ring, but no interaction with the proton at C-17 having the β-configuration (at δ 2.83 *dd*, unresolved *dd*; benzylic type; most deshielded of the methine protons; in 1,3-diacetyl vilasinin [9] it is at δ 2.83 *dd*, J = 11.5, 7.5 Hz and in 4α,6α-dihydroxy

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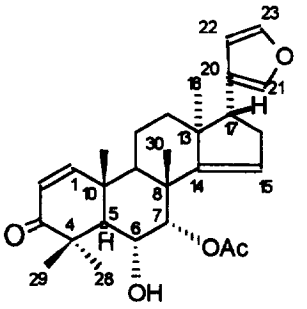

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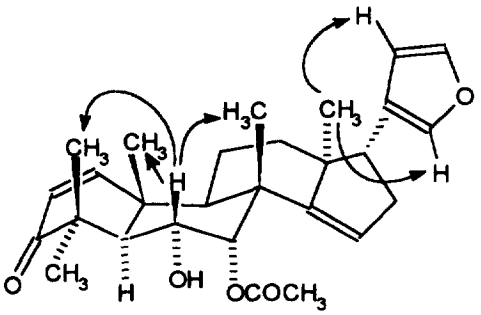

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A-homoazadirone [8] it is at δ 2.85 *dd*, J = 9.0, 9.5 Hz). Nimonol was then assigned the complete structure as in **1**. The stereostructure is as in **2**, which also shows the NOE interactions (NOESY).

The structure corresponding to **1** has been assigned by Siddiqui *et al.* [10] to nimocinol, the tetranortriterpenoid from the fruits of *Melia indica*. There were, however, differences in the ^{1}H NMR and ^{13}C NMR spectra of nimonol and nimocinol (Tables 1 and 2). In particular, nimonol had, in the ^{1}H NMR, the most shielded methyl group protons at δ 0.82, while in nimocinol this was at δ 1.07. In the ^{13}C NMR spectrum nimonol had a methyl signal at δ 31.87 while nimocinol had one at δ 14.04. The two compounds were therefore different.

Siddiqui *et al.* [10] deduced the structure of nimocinol on the basis of a comparison of the NMR spectrum of the acetate of nimocinol with that of 6-acetoxy azadirone [7] and it was claimed that they were identical. However, the reported mp of 6-acetoxyazadirone was 186–188° [7], while acetyl nimocinol had mp 107–108° [10]. Furthermore, when the chemical shift values, as reported by the authors, were checked, it was noticed that there was a serious difference. Thus, the acetate of nimocinol had the most shielded methyl signal at δ 1.12, while for 6-acetoxy azadirone it was at δ 0.81. On the other hand, when nimonol was converted to its acetate, the product had mp. 182–184° and the most shielded methyl signal at δ 0.81, as in 6-acetoxyazadirone. The other ^{1}H NMR signals of the acetate (Table 3) of nimonol also matched with those of 6-acetoxy azadirone. This conclusively established that it was the acetate of nimonol, which was identical with 6-acetoxyazadirone and not the acetate of nimocinol. Structure **1**, then represented nimonol and not nimocinol. This was further confirmed when nimonol, on hydrogenation, gave a product which had a ^{1}H NMR spectrum identical with that of isomeldenin.

It is possible that nimocinol has the furan at C-17 in the β-orientation and that it is 17-*epi*-nimonol. This, if proved correct, would be of great interest since the

Table 1. ^{1}H NMR spectral data of nimonol (**1**) and nimocinol [10]

Proton	Nimonol	Nimocinol [10]
H-1	7.12, *d*, J = 10.06	7.06, *d*, J = 10.0
H-2	5.90, *d*, J = 10.06	5.82, *d*, J = 10.0
H-5	2.21, *d*, J = 11.65	2.17, *d*, J = 11.25
H-6	4.38, *dd*, J = 11.65, 2.37	4.30, *dd*
H-7	5.36, *d*, J = 2.37	5.30, *d*, J 6β7β = 2.5
H-15	5.42, *dd*, J = 1.82, 2.84	5.37, *m*
H-17	2.83, unresolved *dd*	Not indicated
H-21*	7.26, *m*	7.30, *m*
H-22	6.29, *m*	6.22, *m*
H-23	7.39, *m*	7.18, *m*
OAc	2.05	1.97, *s*
OH	Not identified	2.50, *br m*
C-methyls	0.82	1.07
	1.14	1.18
	1.27	1.21
	1.31	1.25
	1.41	1.35

* NOE interaction is between Me at 13 and furan proton at δ 7.26. The signal at δ 7.26 is then assigned to H-21.

Table 2. ^{13}C NMR spectral data of nimonol (1) and nimocinol [10]

Carbon	Nimonol	Nimocinol [10]
C-1	157.40	157.30
C-2	126.14	126.10
C-3	205.96	205.90
C-4	40.51	40.50
C-5	49.88	49.80
C-6	68.08	68.00
C-7	79.08	79.00
C-8	45.43	45.43
C-9	37.13	37.15
C-10	43.11	43.11
C-11	16.39	16.30
C-12	32.72	33.60
C-13	47.08	47.08
C-14	158.53	158.00
C-15	119.55	119.55
C-16	34.30	34.32
C-17	51.62	51.64
C-20	124.37	124.36
C-21	142.57	142.55
C-22	110.94	110.93
C-23	139.64	139.63
$CH_3\underline{C}O$	172.02	171.97
$\underline{C}H_3CO$	21.16	21.20
C-Me	31.87	
	27.08	27.07
	20.84	20.79
	20.79	20.22
	20.23	19.64
		14.04

occurrence of a furan in the β-orientation is unknown, except when a carbonyl group is present at C-16. It is also possible that the hydroxyl and acetyoxyl substitution pattern is different in nimocinol.

EXPERIMENTAL

Fresh, uncrushed, green neem leaves (1 kg) were dipped in *n*-hexane (15 l) for 18 hr and the decanted *n*-hexane extract concd to 1 l *in vacuo*. The hexane extract was partitioned with 95% MeOH. The MeOH extract was concd to dryness *in vacuo* leaving a residue (6 g). CC of the MeOH residue on silica (70–300 mesh), with 15% EtOAc in *n*-hexane yielded, in frs 17–23, nimonol (1.727 g), $C_{28}H_{36}O_5$, mp 174°; $[\alpha]_D + 60.78°$ (*c* 1.02; $CHCl_3$); ^1H NMR and ^{13}C NMR: Tables 1 and 2; Found C 73.78, H 8.07; HRMS 452.25688; $C_{28}H_{36}O_5$ requires C 73.34, H 7.96; HRMS 452.25628.

Frs 24–30 yielded isomeldenin (247 mg) mp 152° (lit. 148–152°) [5]; $[\alpha]_D + 90°$ (*c* 1, $CHCl_3$) (lit + 100); ^1H NMR ($CDCl_3$) 7.30, *m* (H-21), 7.17, *m* (H-23), 6.20, *m* (H-22), 5.34, *m* (H-15), 5.26, *d*, $J = 2.93$ Hz (H-7), 4.16, *dd*, $J = 2.93$, 11.23 Hz (H-6), 2.05, *d*, $J = 11.23$ Hz (H-5), 2.00 (OAc), 1.26, 1.22, 1.13, 0.77, 0.74 (five methyls), 2.63–2.74 (*m*), 2.19–2.39 (*m*), 1.45–1.85 (*m*); ^{13}C NMR ($CDCl_3$) 219.35(C-3), 172.28 ($CH_3\underline{C}O$), 158.88(C-14), 142.78(C-21), 139.89(C-23), 124.78(C-20), 119.75(C-15), 111.27(C-22), 79.47(C-7), 68.39(C-6), 51.79(C-17), 47.37, 47.02, 43.09, 41.56, 38.63, 37.98, 34.53, 33.09, 32.98, 31.86, 31.81, 26.39, 21.56, 20.82, 19.52, 16.86, 16.54.

Acetylation of nimonol. A soln of nimonol (100 mg) in pyridine (1 ml) and Ac_2O (1 ml) was kept at room temp. overnight. Usual work-up, followed by chromatography (silica gel, 10% EtOAC in *n*-hexane as eluent), gave nimonol acetate (60 mg, mp 182–184° (lit. [7] mp 186–88°). ^1H NMR was identical with that of 6-acetoxyazadirone.

Hydrogenation of nimonol. A soln of nimonol (50 mg) in MeOH (3 ml) was stirred in a hydrogen atmosphere at room temp and atmos. pres. in the presence of 10% Pd/C (5 mg) for about 20 min. Filtration

Table 3. ^1H NMR spectral data of nimonol acetate, 6-acetoxyazadirone and nimocinol acetate [10]

Proton	Nimonol acetate	6-Acetoxyazadirone	Nimocinol acetate [10]
H-1	7.14, *d*, $J = 9.5$	7.14, *d*	7.10, *d*, $J = 10.0$
H-2	5.92, *d*, $J = 9.5$	5.93, *d*	5.95, *d*, $J = 10.0$
H-5	Not resolved	Not indicated	2.25, *d*, $J = 11.25$
H-6	5.40, *bs* overlapping	5.42, *dd*	5.41, *dd*
H-15	5.40, *bs*	5.40, *d*	5.38, *m*
H-7	5.47, *bs*	5.47, *d*	5.45, *m*
H-21*	7.38, *m*	7.38, *m*	7.32, *m*
H-22	6.28, *m*	6.28, *m*	6.20, *m*
H-23*	7.24, *m*	7.25, *m*	7.11, *m*
OAc	2.01, *s*	2.00, *s*	2.00, *s*
OAc	2.05, *s*	2.04, *s*	2.04, *s*
Me	0.81, *s*	0.81, *s*	1.12, *s*
2 × Me	1.19, *s*	1.18, *s*	1.17, *s*
Me	1.27, *s*	1.26, *s*	1.25, *s*
Me	1.34, *s*	1.33, *s*	1.31, *s*

* The assignments may be reversed.

and removal of solvent, followed by chromatography (silica gel, 20% EtOAc in *n*-hexane as eluent) gave dihydronimonol (21 mg), mp 150–152° (lit. mp 148–152°). ^{1}H NMR was identical with that of isomeldenin [5].

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