Phytochemistry, Vol. 23, No. 12, pp. 2899–2901, 1984.
Printed in Great Britain.

0031–9422/84 $3.00+0.00
Pergamon Press Ltd.

ISOLATION OF A TETRANORTRITERPENOID FROM *AZADIRACHTA INDICA*

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(*Revised received* 1 *May* 1984)

Key Word Index—*Azadirachta indica*; Meliaceae; neem leaves; tetranortriterpenoid; nimocinol; 6α-hydroxyazadirone; ^{13}C NMR.

Abstract—A new tetranortriterpenoid, nimocinol has been isolated from the undried winter leaves of *Azadirachta indica*. Its structure has been determined as 6α-hydroxyazadirone through comparison of its spectral data with those of related meliacins and chemical studies.

INTRODUCTION

Azadirachta indica (Meliaceae) is indigenous to the Indo-Pakistan subcontinent. Its various parts are reputed as therapeutic agents and the leaves have been used in the treatment of ulcer, eczema, jaundice and liver complaints [1–4]. As a result of studies in the constituents of neem leaves, a new bitter tetranortriterpenoid provisionally named nimocinol has been isolated and its structure elucidated as 6α-hydroxyazadirone (**1**) through spectral and chemical studies. Compound **1** is of potential pharmacological importance as 6β-acetoxyazadirone (dysobinin) [5] has been reported to possess the general CNS-depressant action.

RESULTS AND DISCUSSION

Nimocinol (**1**) has molecular formula $C_{28}H_{36}O_5$ (high resolution mass). Its UV spectrum showed absorption at 210 nm, and the IR spectrum showed peaks at 3480 cm^{-1} (–OH), 1720 cm^{-1} (ester carbonyl), 1665 cm^{-1} (cyclohexenone), 1650 and 820 cm^{-1} (trisubstituted double bond), 3100, 1505 and 875 cm^{-1} (furan ring).

The ^{1}H NMR spectrum of **1** (Table 1) showed a pair of doublets at δ7.06 and 5.82 (J = 10.0 Hz) related to H-1 and H-2 respectively, three one-proton multiplets at δ7.30 (H-21), 7.18 (H-23) and 6.22 (H-22) and a narrow multiplet at δ5.37 (H-15). These values are in agreement with those reported for the same protons in azadirone [6]. However,

Table 1. ^{1}H NMR spectral data of tetranortriterpenoids

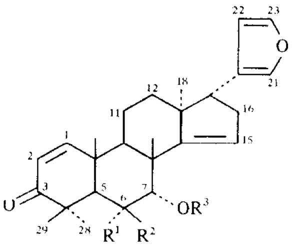
Assignment	**1**	**2**	**3**	**4**	6α-Acetoxy azadirone [8]
H-1	7.06 (*d*)*	7.10 (*d*)*	7.08 (*d*)*	6.96 (*d*)*	7.14 (*d*)†
H-2	5.82 (*d*)*	5.95 (*d*)*	5.87 (*d*)*	5.77 (*d*)*	5.93 (*d*)†
H-5	2.17 (*d*)*	2.25 (*d*)*	2.27 (*d*)*	3.40 (*s*)	NI
H-6	4.30 (*dd*)*	5.41 (*dd*)*	4.17 (*dd*)*	—	5.42 (*dd*)†
H-7	5.30 (*d*)*	5.45 (*d*)*	4.00 (*d*)*	4.91 (*s*)	5.47 (*d*)†
H-15	5.37 (*m*)	5.38 (*m*)	5.60 (*m*)	5.35 (*m*)	5.40 (*d*)†
H-21	7.30 (*m*)	7.32 (*m*)	7.39 (*m*)	7.27–7.40‡	7.38 (*m*)
H-22	6.22 (*m*)	6.20 (*m*)	6.29 (*m*)	6.22 (*m*)	6.28 (*m*)
H-23	7.18 (*m*)	7.11 (*m*)	7.26 (*m*)	7.27–7.40‡	7.25 (*m*)
OAc	1.97 (*s*)	2.00, 2.04 (*s*)	—	1.95 (*s*)	2.00, 2.04 (*s*)
OH	2.50 (*br m*)	—	3.50 (2H, *br m*)	—	—
C-methyls	1.07 (*s*)	1.12 (*s*)	1.08 (*s*)	0.82 (*s*)	0.81 (*s*)
	1.18 (*s*)	1.17 (6H, *s*)	1.16 (*s*)	1.07 (*s*)	1.18 (6H, *s*)
	1.21 (*s*)	1.25 (*s*)	1.18 (*s*)	1.14 (*s*)	1.26 (*s*)
	1.25 (*s*)	1.31 (*s*)	1.23 (*s*)	1.17 (6H, *s*)	1.33 (*s*)
	1.35 (*s*)		1.35 (*s*)		

*Multiplicities: $J_{1,2}$ = 10.0 Hz, $J_{5\alpha,6\beta}$ = 11.25 Hz, $J_{6\beta,7\beta}$ = 2.5 Hz.

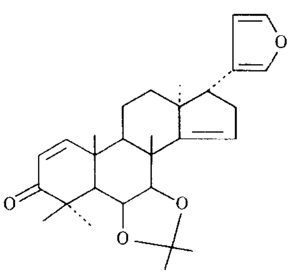
†Multiplicities: $J_{1,2}$ = 10.0 Hz, $J_{5\alpha,6\beta}$ = 12.0 Hz, $J_{6\beta,7\beta}$ = 2.0 Hz.

‡Two-protons broad multiplet.

NI, Not indicated; all values are in δ (ppm) relative to TMS.



Azadirone, R^1, R^2 = H, R^3 = Ac
1 R^1 = OH, R^2 = H, R^3 = Ac
2 R^1 = OAc, R^2 = H, R^3 = Ac
3 R^1 = OH, R^2 = H, R^3 = H
4 R^1, R^2 = O, R^3 = Ac
Dysobinin, R^1 = H, R^2 = OAc, R^3 = Ac
Dysobindiol, R^1 = H, R^2 = OH, R^3 = H



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in the ^{1}H NMR spectrum of azadirone, the 7β-proton geminal to the acetoxy group is a triplet at δ5.35 whereas the signal corresponding to the same proton in nimocinol (**1**) is a doublet at δ5.30 (J = 2.50 Hz) indicating the presence of a substituent at C-6. The hydroxyl nature of this substituent was indicated by IR, ^{1}H (Table 1) and ^{13}C NMR (Table 2) spectra, formation of an acetate (**2**) and oxidation (CrO$_3$–pyridine) [7] to a ketone (**4**). The ^{1}H NMR spectrum of **1** showed a doublet at δ2.17 (J = 11.25 Hz) and a doublet of doublets at δ4.30 (J = 11.25, 2.50 Hz) attributable to H-5 and H-6 respectively. The latter signal was fully resolved on addition of D$_2$O while the signal of the hydroxyl proton at δ2.50 disappeared. The location of the hydroxyl function at C-6 was conclusively shown by the ^{1}H NMR spectrum of **4** (Table 1) in which the signals of H-5 and H-7 appeared as one proton singlets at δ3.40 and 4.91 respectively. The orientation of the hydroxyl function was arrived at through a comparison of the coupling constants of H-6 of nimocinol with those of 6α-acetoxyazadirone (Table 1), isolated earlier from the fruits of *Chisocheton paniculatus* [8], and other 6α-substituted meliacins [9, 10] which led to the conclusion that the hydroxyl function at C-6 in **1** is also equatorial and α-oriented.

Further, acetylation (Ac$_2$O–pyridine) of **1** gave the corresponding acetate (**2**) which has been identified as 6α-acetoxyazadirone through its ^{1}H NMR spectrum which showed close similarity in the coupling constants of H-6 and H-7 with those reported for the latter (loc. cit.). On the other hand, a direct comparison of the coupling constants of H-6 and H-7 of nimocinol acetate (**2**) and dysobinin, isolated earlier from the fruits of *Dysoxylum binectariferum* [5], could not be made since they are not reported in the latter case. However, the small coupling constants reported for the carbinylic protons (H-6 and H-7) in dysobindiol (J = 3.0 and 4.0 Hz) and its failure to form the isopropylidine derivative [5] showed that in dysobindiol the hydroxyl function at C-6 is β-oriented and is *trans* to the hydroxyl group at C-7. In contrast to this observation, the present diol (**3**) obtained through mild alkaline hydrolysis of **1**, formed the isopropylidine derivative (**5**), in analogy to the behaviour of the known diol obtained through the alkaline hydrolysis of 6α-acetoxyazadirone (loc. cit.). Further, the ^{1}H NMR of **3** exhibited larger coupling constant for H-6 ($J_{6\beta,5\alpha}$ = 11.25 Hz) showing that the hydroxyl group at C-6 is α-oriented and *cis* to the hydroxyl function located at C-7. The diol (**3**) on reacetylation (Ac$_2$O–p-toluenesulphonic acid) [11] gave nimocinol acetate (**2**), showing that no change in configuration had taken place during alkaline hydrolysis of nimocinol.

In the light of the above facts it is evident that nimocinol acetate (**2**) is identical with 6α-acetoxyazadirone and is the C-6 epimer of dysobinin (loc. cit.).

Table 2. ^{13}C NMR spectral data of tetranortriterpenoid **1**

C	δ	C	δ
1	157.30	15	119.55
2	126.10	16	34.32*
3	205.90	17	51.64
4	40.50	20	124.36
5	49.80	21	142.55
6	68.00	22	110.93
7	79.00	23	139.63
8	45.43	$\mathrm{O{-}\overset{\overset{O}{\|}}{\underline{C}}{-}CH_3}$	171.97
9	37.15	$\mathrm{O{-}\overset{\overset{O}{\|}}{C}{-}\underline{C}H_3}$	21.20†
10	43.11	CMe	27.07
11	16.30		20.79†
12	33.60*		20.22†
13	47.08		19.64
14	158.00		14.04

All values are in δ (ppm) relative to TMS.
*†Assignments may be reversed.

EXPERIMENTAL

Mps were recorded in glass capillary tubes and are uncorrected. Mass spectra were recorded on double focussing mass spectrometers connected to PDP 11/34 computer system. ^{1}H and ^{13}C NMR (broad band and gated spin echo) spectra were recorded in CDCl$_3$ with TMS as internal reference. ^{13}C NMR spectral assignments have been made partly through the appearance of signals in the spin-echo spectrum and partly through a comparison with published data for similar compounds [12, 13]. The purity of samples was checked on TLC (silica gel SIF-254 precoated aluminium cards).

The ethanolic extract of the undried neem leaves, collected in winter from Karachi region, was divided into acidic and neutral fractions. The petroleum soluble portion of the latter fraction was partitioned between petroleum and 90% MeOH and the residue, obtained from the methanolic phase after usual working, was subjected to prep. TLC (silica gel, C$_6$H$_6$–EtOAc, 17:3). As a result, **1** has been isolated as a crystalline solid which on recrystallization from MeOH formed needles, mp 130°, $[\alpha]_D^{24}$ +24.4° (CHCl$_3$). EIMS m/z (rel. int.): 452.2585 ($[M]^+$, calc. for C$_{28}$H$_{36}$O$_5$: 452.2563) (80), 392.2347 $[M-AcOH]^+$ (22), 359.2032 $[M-AcOH-H_2O-Me]^+$ (13), 137.0981 $[C_9H_{13}O]^+$ (100) and 81.0341 $[C_5H_5O]^+$ (65).

Acetylation of **1** *to* **2**. To a soln of **1** (25 mg) in pyridine (1 ml), Ac$_2$O (1 ml) was added and the reaction mixture was kept for 4 hr at room temp. On usual work-up chromatographically pure **2** was obtained, mp 107–108°. $[\alpha]_D^{27}$ +75° (CHCl$_3$).

IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3100 (furan), 1735 (*br s*, ester carbonyls), 1665 (cyclohexenone), 1505 and 875 (furan). EIMS *m/z* (rel. int.): 494 $[M]^+$ (10), 374 $[M-60-60]^+$ (8), 359 $[374-15]^+$ (8), 43 (100).

Hydrolysis of **1** *to* **3**. Compound **1** (25 mg) was taken in 5% methanolic KOH and refluxed at 100° for 1.5 hr. On usual work-up of the reaction mixture, **3** was obtained as a crystalline solid which on recrystallization from MeOH formed rods, mp 100–102°, $[\alpha]_D^{23}$ +29.12° ($CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH), 3100 (furan), 1665 (cyclohexenone), 1505 and 875 (furan). EIMS *m/z* (rel. int.): 410 $[M]^+$ (40), 395 $[M-15]^+$ (12), 392 $[M-18]^+$ (14), 359 $[392-15-18]^+$ (10), 315 $[M-95]^+$ (100).

Oxidation of **1** *to* **4**. A soln of **1** (260 mg) in pyridine (1 ml) was added to a slurry of CrO_3 (300 mg) and pyridine (1.5 ml) and stirred for 4 hr at room temp. The product **4** was obtained as a crystalline solid which recrystallized from MeOH in fine needles mp 227°, $[\alpha]_D^{27} = +27.7$ ($CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3150 (furan), 1740 (ester carbonyl), 1730 (cyclohexanone), 1665 (cyclohexenone), 1502 and 875 (furan). EIMS *m/z* (rel. int.): 450 $[M]^+$ (60), 435 $[M-15]^+$ (3), 407 $[M-43]^+$ (5), 390 $[M-60]^+$ (6) and 137 (100).

Isopropylidine derivative (**5**) *of the diol* (**3**). To a soln of 30 mg of the diol (**3**) in dry Me_2CO (5 ml) was added 0.4 g anhydrous $CuSO_4$ and one drop of conc. H_2SO_4. Reaction mixture was stirred at room temp. for 4 hr and filtered. The filtrate was ammoniated, the resulting $(NH_4)_2SO_4$ was filtered off and the product was taken into EtOAc. Fine needles of the isopropylidine derivative (**5**) were obtained, mp 79–80°, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3150 (furan), 1662 (cyclohexenone), 1500 and 875 (furan). EIMS *m/z* (rel. int.): 450 $[M]^+$ (2), 435 $[M-15]^+$ (2), 392 $[M-58]^+$ (3), 137 (20), 95 (42), 81 (50) and 55 (100).

Acknowledgement—One of us (Tariq Mahmood) wishes to express grateful thanks to the Hamdard Foundation of Pakistan for provision of a Research Fellowship in the course of this work.

REFERENCES

1. Dymock, W. (1890) *Pharmacographia Indica*, p. 89. The Institute of Health and Tibbi Research, republished under the auspices of Hamdard National Foundation of Pakistan.
2. Chopra, R. N., Nayar, S. L. and Chopra, I. C. (1956) *Glossary of Indian Medicinal Plants*, p. 31. C.S.I.R. Publications, New Delhi.
3. Fujiwara, T., Takeda, T., Ogihara, Y., Shimizu, M., Nomura, T. and Tomita, Y. (1982) *Chem. Pharm. Bull.* **30**, 4025.
4. Okpanyi, S. N. and Ezeukwu, G. C. (1981) *Planta Med.* **41**, 34.
5. Singh, S., Garg, H. S. and Khanna, N. M. (1976) *Phytochemistry* **15**, 2001.
6. Lavie, D., Levy, E. C. and Jain, M. K. (1971) *Tetrahedron* **27**, 3927.
7. Poos, G. I., Arth, G. E., Beyler, R. E. and Sarett, L. H. (1953) *J. Am. Chem. Soc.* **75**, 422.
8. Saikia, B., Kataky, J. C. S., Mathur, R. K. and Baruah, J. N. (1978) *Indian J. Chem.* **16B**, 1042.
9. Connolly, J. D., Handa, K. L. and McCrindle, R. (1968) *Tetrahedron Letters* 437.
10. Lavie, D., Levy, E. C. and Zelnik, R. (1972) *Bioorg. Chem.* **2**, 59.
11. Akisanya, A., Bevan, C. W. L., Halsall, T. G., Powell, J. W. and Taylor, D. A. H. (1961) *J. Chem. Soc.* (*C*) 3705.
12. Connolly, J. D., Labbé, C., Rycroft, D. S. and Taylor, D. A. H. (1979) *J. Chem. Soc. Perkin Trans.* 1, 2959.
13. Ayafor, J. F., Sondengam, B. L., Connolly, J. D., Rycroft, D. S. and Okogun, J. I. (1981) *J. Chem. Soc. Perkin Trans.* 1, 1750.