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J. Nat. Prod., **1992**, 55 (5), 596-601 • DOI:
10.1021/np50083a006 • Publication Date (Web): 01 July 2004

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DC 20036

AZADIRACHTINS H AND I: TWO NEW TETRANORTRITERPENOIDS
FROM AZADIRACHTA INDICA

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ABSTRACT.—Two new tetranortriterpenoids, azadirachtin H [3] and azadirachtin I [4], have been isolated from neem kernels. Their structures were determined by spectral analyses and comparison to known compounds.

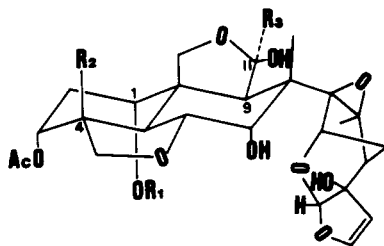
Azadirachtin (1), the potent insect antifeedant and ecdysis inhibitor, is one among several compounds having the same skeletal structure with minor variations in the periphery, isolated from the kernels of neem, *Azadirachta indica* A. Juss. (Meliaceae). The principal compound for which structural and biological studies have been conducted, which occurs to the extent of 0.3–0.6% in neem kernels, should be termed azadirachtin A [1], according to Rembold (2), who has isolated six related compounds (azadirachtins B–G). Azadirachtin B (3-rigloylazadirachtol) has also been isolated by Klenk *et al.* (3). Deacetylazadirachtinol, isolated by Kubo *et al.* (4), appears to be the 1-rigloyl isomer of azadirachtin B (5,6).

The structure of azadirachtin A was elucidated by extensive analysis of nmr spectra (6–8) and finally by X-ray crystallography of a derived product (9). Structures for azadirachtins B–G were assigned on the basis of spectral data.

Two recent publications have described procedures for the isolation of azadirachtin A (10) and the isolation of azadirachtins A, B, and D [2] and two new azadirachtins H [3], and I [4] from neem kernel extract by preparative hplc (11). Here we provide evidence for the structures proposed for the new azadirachtins H and I.

RESULTS AND DISCUSSION

The novel azadirachtins H and I were assigned structures 3 and 4 on the basis of ^1H -nmr (Table 1) and ^{13}C -nmr data (Table 2). Multiplicities of all carbon signals were assigned by DEPT experiments. The proton connectivities were established by ^1H - ^1H COSY spectra (Figures 1 and 2) and decoupling experiments. It is obvious from the nmr data that azadirachtin H differs from A by substitution of a hydrogen in place of the carbomethoxy group at C-11. H-11, appearing at δ 5.41 (d, $J = 4.4$ Hz), was found coupled to H-9 at δ 3.19 (d, $J = 4.4$ Hz) (COSY). The carbomethoxy group linked to C-11 in A, appearing at δ 3.68 (s), was absent in the spectrum of H. H-1 appeared at δ



- 1 R = tig, $R_2 = R_3 = \text{CO}_2\text{Me}$
- 2 $R_1 = \text{tig}$, $R_2 = \text{Me}$, $R_3 = \text{CO}_2\text{Me}$
- 3 $R_1 = \text{tig}$, $R_2 = \text{CO}_2\text{Me}$, $R_3 = \text{H}$
- 4 $R_1 = \text{tig}$, $R_2 = \text{Me}$, $R_3 = \text{H}$

TABLE 1. ^1H -nmr Data of Compounds 1-4 [δ H m, J (Hz)] in CDCl_3/TMS .

Proton	Compound			
	1	2	3	4
H-1	4.75 (dd, 2.9, 3.1)	4.91 (dd, 2.9, 2.4)	5.36 (dd, 2.9, 2.4)	5.01 (dd, 3.4, 2.4)
H-2	2.34 (ddd, 16.7, 2.9, 2.7)	2.30 (ddd, 16.6, 2.9, 2.4)	2.42 (ddd, 16.6, 2.9, 2.4)	2.21 (ddd, 18.0, 3.4, 2.9)
H-2	2.13 (ddd, 16.7, 3.1, 2.9)	2.13 (ddd, 16.6, 2.9, 2.4)	2.33 (ddd, 16.6, 2.9, 2.4)	2.26 (ddd, 18.0, 2.9, 2.4)
H-3	5.50 (dd, 2.7, 2.9)	5.15 (dd, 2.9, 2.4)	5.53 (dd, 2.4, 2.9)	5.41 (dd, 2.9, 2.4)
H-5	3.35 (d, 12.5)	3.16 (d, 12.7)	3.37 (d, 12.2)	3.17 (d, 12.7)
H-6	4.60 (dd, 12.5, 2.7)	4.69 (dd, 12.7, 2.0)	4.46 (dd, 12.2, 2.9)	4.11 (dd, 12.7, 2.9)
H-7	4.75 (d, 2.7)	4.71 (d, 2.0)	4.65 (d, 2.9)	4.66 (d, 2.9)
H-9	3.34 (s)	3.34 (s)	3.1 (d, 4.4)	3.21 (d, 4.4)
H-11	—	—	5.41 (d, 4.4)	5.41 (d, 4.4)
H-15	4.67 (d, 3.4)	4.67 (d, 3.4)	4.58 (d, 3.4)	4.63 (d, 3.4)
H _a -16	1.73 (ddd, 13.0, 3.4, 5.1)	1.70 (ddd, 13.0, 3.4, 5.4)	1.70 (ddd, 13.0, 3.4, 5.4)	1.73 (ddd, 13.0, 3.4, 5.4)
H _b -16	1.31 (d, 13.0)	1.33 (d, 13.0)	1.32 (d, 13.0)	1.31 (d, 13.0)
H-17	2.38 (d, 5.1)	2.34 (d, 5.4)	2.37 (d, 5.4)	2.40 (d, 5.4)
H-18	2.01 (s)	2.05 (s)	1.99 (s)	2.03 (s)
H _a -19	3.63 (d, 9.6)	3.85 (d, 9.3)	3.75 (d, 8.8)	3.71 (d, 9.0)
H _b -19	4.15 (d, 9.6)	4.21 (d, 9.3)	4.09 (d, 8.8)	3.75 (d, 9.0)
H-21	5.65 (s)	5.71 (s)	5.66 (s)	5.72 (s)
H-22	5.05 (d, 2.9)	5.03 (d, 2.9)	5.05 (d, 2.9)	5.10 (d, 2.9)
H-23	6.46 (d, 2.9)	6.44 (d, 2.9)	6.45 (d, 2.9)	6.50 (d, 2.9)
H-28	4.08 (d, 9.0)	4.16 (d, 10.3)	4.09 (d, 8.8)	3.75 (d, 9.0)
H-28	3.76 (d, 9.0)	3.78 (d, 10.3)	3.75 (d, 8.8)	3.71 (d, 9.0)
H-29	—	1.94 (s)	—	2.03 (s)
H-30	1.74 (s)	1.73 (s)	1.64 (s)	1.32 (s)
7-OH	2.89 (br. s)	2.80 (s)	2.64 (s)	2.68 (s)
11-OH	5.05 (s)	5.00 (s)	2.98 (s)	3.84 (s)
20-OH	2.92 (br. s)	3.24 (s)	2.75 (s)	3.06 (s)
12-OMe	3.68 (s)	3.68 (s)	—	—
29-OMe	3.76 (s)	—	3.79 (s)	—
OAc	1.95 (s)	1.94 (s)	1.86 (s)	1.93 (s)
Tigloyl				
H-3'	6.93 (qq, 7.0, 1.5)	6.92 (qq, 7.3, 1.5)	6.98 (qq, 7.3, 1.5)	7.01 (qq, 7.3, 1.5)
H-4'	1.78 (dq, 7.0, 1.1)	1.78 (dq, 7.3, 1.0)	1.77 (dq, 7.3, 1.1)	1.82 (dq, 7.3, 1.0)
H-5'	1.85 (dq, 1.5, 1.1)	1.85 (dq, 1.5, 1.0)	1.84 (dq, 1.5, 1.1)	1.89 (dq, 1.5, 1.0)

5.36 (dd, $J = 2.9, 2.4$ Hz), a more normal position than in azadirachtin A at δ 4.75 (dd, $J = 2.9, 3.1$ Hz), where it is apparently shielded due to conformational factors imposed by the presence of the carbomethoxy function at C-11. In the ^{13}C spectrum of **3**, C-11 appeared as a doublet (DEPT) at 99.57 ppm. The assignment of the C-3 acetate group was confirmed by a low power, selective frequency proton decoupling experiment. The acetate carbonyl, an octet at 168.84 ppm in the uncoupled spectrum, collapsed to a quartet on low power irradiation of H-3 at δ 5.53 (dd, $J = 2.4, 2.9$ Hz). The C-4 carbomethoxy carbonyl at 172.88 ppm, appearing as a broad multiplet due to a weak three-bond coupling with H-3, also simplifies, thereby confirming the assignment of the acetate group.

Azadirachtin I differs from H by the further replacement of the carbomethoxy group at C-4 by a methyl group. This methyl group was assigned the shift of 18.91 ppm, comparable to C-29 (methyl) in azadirachtin D occurring at 18.95 ppm. H-29 occurred as a singlet integrating for three protons at δ 2.03. As in azadirachtin H, the carbomethoxy group linked to C-11 was replaced by a hydrogen. Thus C-11 appeared as a doublet at 100.51 ppm (DEPT). H-11 at δ 5.41 (d, $J = 4.4$ Hz) was found coupled to H-9 at δ 3.21 (d, $J = 4.4$ Hz) (COSY). H-3 coupled to H _{α} -2 at δ 2.21 (ddd, $J = 8.0, 3.4, 2.9$ Hz) and H _{β} -2 at δ 2.26 (ddd, $J = 18.0, 2.9, 2.4$ Hz) (COSY) was also centered at δ 5.41 (dd, $J = 2.9, 2.4$ Hz). As in azadirachtin H, the point of attachment of the acetate group was confirmed by a decoupling experiment. In the uncoupled spectrum, the acetate carbonyl appeared as an octet centered at 170.21 ppm. It collapsed to a quartet when H-3 at δ 5.41 was selectively irradiated at room temperature in

TABLE 2. ^{13}C -nmr Data of Compounds 1-4.

Carbon	Compound			
	1 (CDCl_3)	2 (CDCl_3)	3 [$\text{CDCl}_3/(\text{CD}_3)_2\text{SO}$]	4 (CDCl_3)
C-1	70.51 d	70.90 d	71.58 d	72.29 d
C-2	29.75 t	27.99 t	28.95 t	28.94 t
C-3	66.96 d	70.32 t	66.22 d	71.31 d
C-4	52.49 s	42.44 s	51.49 s	43.18 s
C-5	36.99 d	35.47 d	35.81 d	35.29 d
C-6	73.86 d	72.81 d	73.29 d	73.33 d
C-7	74.31 d	74.87 d	75.04 d	73.66 d
C-8	45.45 s	45.08 s	42.75 s	42.30 s
C-9	44.66 d	44.71 d	46.97 d	47.78 d
C-10	50.18 s	49.96 s	47.32 s	48.78 s
C-11	104.19 s	103.99 s	99.57 d	100.51 d
C-12	171.83 s	171.76 s	—	—
C-13	68.62 s	68.50 s	66.69 s	66.63 s
C-14	69.96 s	69.90 s	69.39 s	69.65 s
C-15	76.45 d	76.38 d	76.02 d	76.80 d
C-16	25.02 t	24.97 t	24.37 t	25.18 t
C-17	48.66 d	48.98 d	47.11 d	48.36 d
C-18	18.44 q	18.42 q	17.77 q	18.76 q
C-19	69.05 t	69.97 t	68.18 t	71.20 t
C-20	83.58 s	83.62 s	81.47 s	83.51 s
C-21	108.67 d	108.72 d	107.54 d	108.80 d
C-22	107.44 d	107.33 d	107.08 d	107.54 d
C-23	146.99 d	146.82 d	144.88 d	146.90 d
C-28	71.15 t	76.88 t	71.50 t	76.38 t
C-29	173.20 s	18.95 q	172.88 s	18.91 q
C-30	21.33 q	21.14 q	19.85 q	20.99 q
COOMe	53.28 q	53.20 q	51.13 q	—
COOMe	52.78 q	—	—	—
OAc	169.78 s	70.09 s	168.84 s	170.21 s
OAc	20.89 q	20.88 q	19.57 q	20.93 q
Tigloyl				
C-1'	166.22 s	166.34 s	165.22 s	166.75 s
C-2'	128.60 s	128.56 s	127.37 s	128.74 s
C-3'	137.50 d	137.59 d	136.98 d	137.96 d
C-4'	14.29 q	14.34 q	13.38 q	14.37 q
C-5'	11.94 q	11.96 q	10.86 q	11.94 q

CDCl_3 . Similarly the C-29 (methyl) signal at 18.91 ppm showing a weak three-bond coupling with H-3 also simplifies. Thus the acetoxy function was unambiguously assigned to C-3. Incidentally the C-11 signal centered at 100.51 ppm in the uncoupled spectrum also simplifies during the irradiation experiment, thereby confirming the assignment of H-11 at δ 5.41.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.— ^1H - and ^{13}C -nmr spectra were recorded on a JEOL 400 spectrometer in CDCl_3 for compounds 1, 2, and 4 and $\text{CDCl}_3/(\text{CD}_3)_2\text{SO}$ for 3. ^1H -nmr spectra were recorded at 400 MHz and the ^{13}C -nmr spectra at 100 MHz. In all cases TMS was used as the internal standard. It was measured in a Perkin-Elmer 598 model spectrometer. Optical rotations were measured on a Rudolph Polarimeter. Analytical hplc studies were carried out on a Shimadzu Model LC-8A system linked to a C-R4A data processor. The peaks were detected with a Shimadzu SPD-6AV detector at 215 nm. An analytical Varian RP-18 column (30 cm \times 4.6 mm i.d.) using $\text{MeOH-H}_2\text{O}$ (60:40) as eluent or an analytical Supelcosil RP-8 column (15 cm \times 4.6 mm i.d.) using $\text{MeCN-H}_2\text{O}$ (28:72) as eluent was employed.

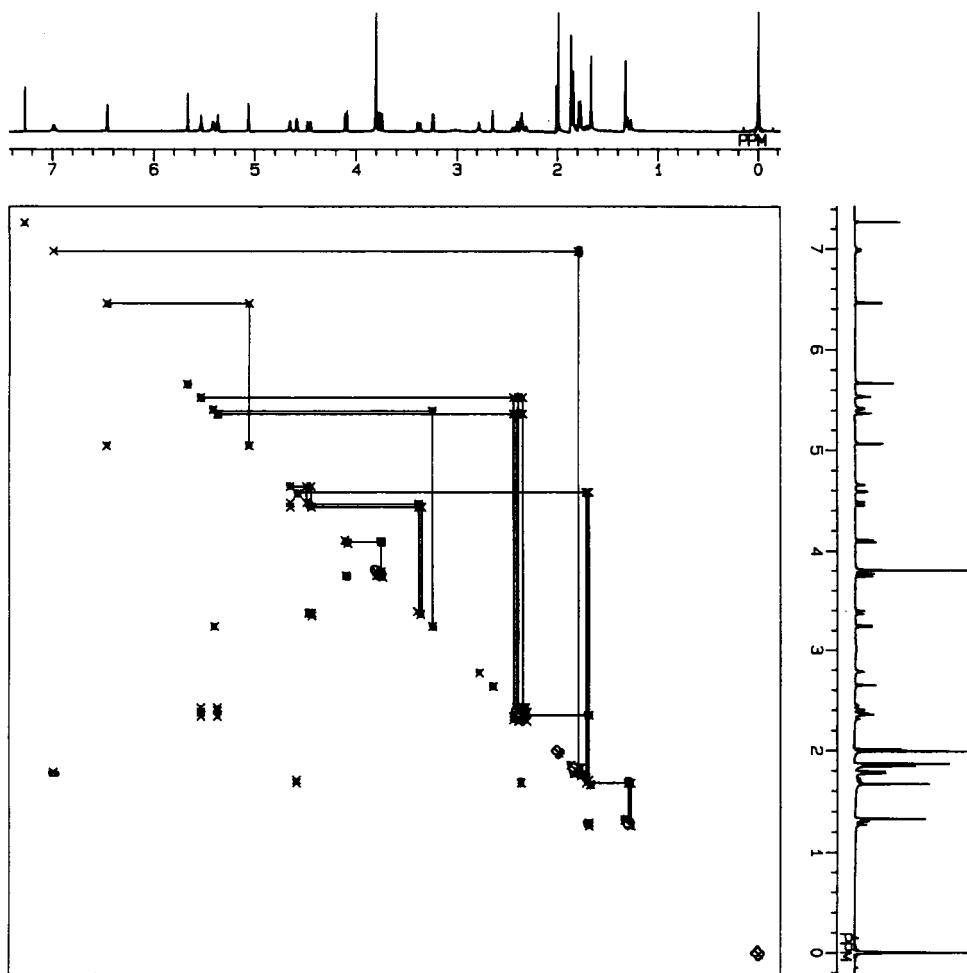


FIGURE 1. 400 MHz proton-correlated 2D nmr spectrum (COSY) of azadirachtin H in CDCl_3 .

Preparative hplc was carried out on a Shimpack RP-18 column (25 cm \times 3 cm i.d.) using $\text{MeOH-H}_2\text{O}$ (60:40) for the preliminary separation of the azadirachtins and a preparative Shimpack RP-8 column (25 cm \times 2 cm i.d.) for separating azadirachtins A, D, H, and I, using $\text{MeCN-H}_2\text{O}$ (28:72) as eluent. All solvents were redistilled and filtered through a Millipore filter (0.45 μm), and solutions for hplc were filtered through a Millipore filter (0.25 μm). Preparative hplc procedures have been described in an earlier publication (11).

From an azadirachtin-enriched MeOH extract (11) of neem kernels (4 g), azadirachtins A (160 mg), B (55 mg), D (21 mg), H (10 mg), and I (3 mg) were obtained. Ten such preparatory hplc runs yielded sufficient material for nmr studies.

Azadirachtin H [3].—Compound **3**, recovered from the appropriate hplc peak after drying at 75° in vacuo for 8 h: mp 248° [α] $^{25}_{\text{D}}$ -33.3° (CHCl_3 , c = 0.6). Found C 56.90, H 6.28%. $\text{C}_{33}\text{H}_{42}\text{O}_{14} \cdot 2\text{H}_2\text{O}$ requires C 56.92, H 6.05%. When crystallized from EtOAc and dried at 110° in vacuo, **3** had mp 258–261°; ir ν max (KBr) 1730, 1710, 1690 (C=O), 1640, 1600 (C=C) cm^{-1} ; uv λ max (MeOH) 221 nm (log ϵ = 3.86); ^1H nmr see Table 1; ^{13}C nmr see Table 2. Found C 59.67, H 6.60%. $\text{C}_{33}\text{H}_{42}\text{O}_{14}$ requires C 59.79, H 6.40%; hrfabms $[\text{M} - \text{H}_2\text{O}]^+$ 644.6822 ($\text{C}_{33}\text{H}_{40}\text{O}_{13}$ requires 644.6712).

Azadirachtin I [4].—Compound **4**, recovered from the appropriate hplc fraction after drying at 67° in vacuo: mp 192°; [α] $^{25}_{\text{D}}$ -21.8° (CHCl_3 , c = 0.8). Found C 58.31, H 6.54%; $\text{C}_{32}\text{H}_{42}\text{O}_{12} \cdot 2\text{H}_2\text{O}$ requires C 58.70, H 6.46%. When crystallized from EtOAc and dried at 110° in vacuo, **4** showed mp 198–200°; ir ν max (KBr) 1725, 1690 (C=O), 1635, 1600 (C=C) cm^{-1} ; uv λ max (MeOH) 220.5 nm (log ϵ = 3.82); ^1H nmr see Table 1; ^{13}C nmr see Table 2. Found C 61.68, H 7.0%. $\text{C}_{32}\text{H}_{42}\text{O}_{12}$ requires C 62.12, H 6.84%; hrfabms $[\text{M} - \text{H}_2\text{O}]^+$ 600.6614 ($\text{C}_{32}\text{H}_{40}\text{O}_{11}$ requires 600.6619).

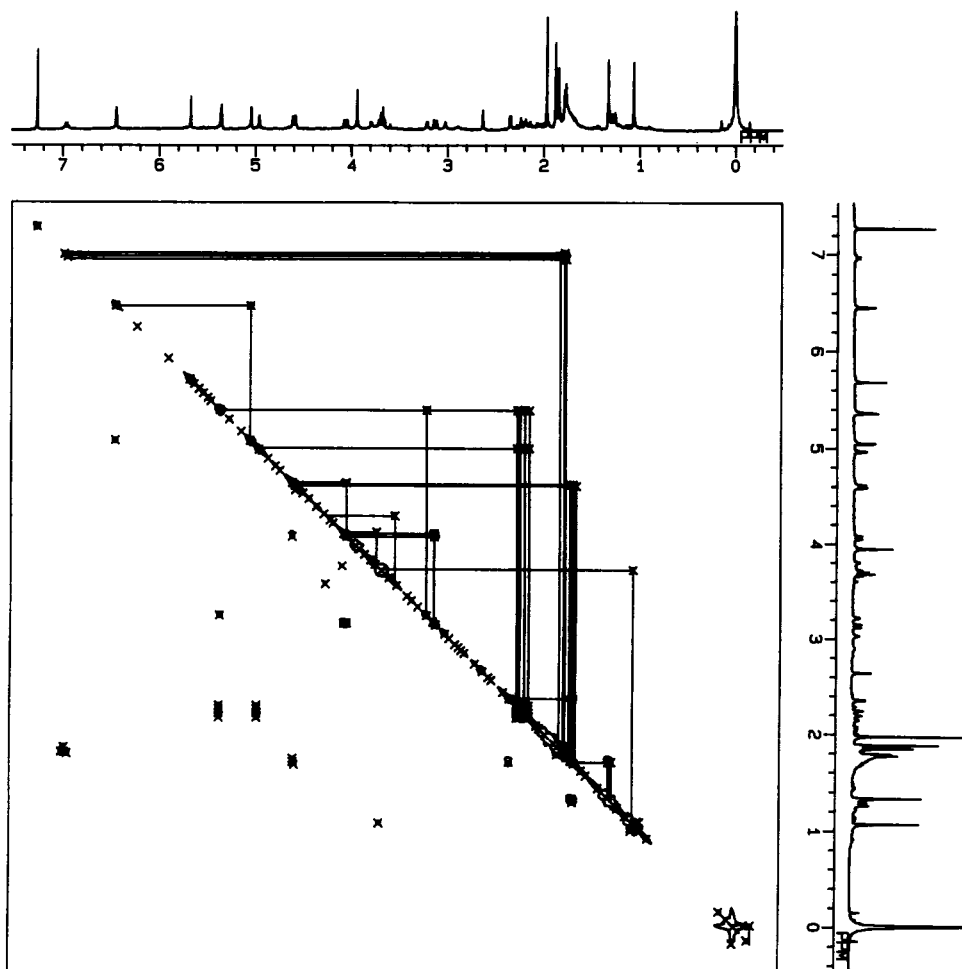


FIGURE 2. 400 MHz proton-correlated 2D nmr spectrum (COSY) of azadirachtin I in $CDCl_3$.

ACKNOWLEDGMENTS

We thank Professor S. Subramanian and Mr. M.S. Moni of the Regional Sophisticated Instrumentation Centre, Indian Institute of Technology, Madras, for all nmr measurements; Professor Kenneth L. Rinehart, School of Chemical Sciences, University of Illinois and Mr. Furong Sun, Mass Spectrometry Laboratory, School of Chemical Sciences, University of Illinois for fabhrms measurements; Dr. R. Balasubramanian, Department of Organic Chemistry, University of Madras for the C, H analyses; Dr. T. Rajamannar for helpful discussions; and Mr. C. Arumugam for technical assistance.

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Received 8 July 1991