Structural Reappraisal of the Limonoid Insect Antifeedant Azadirachtin

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Based upon a detailed 250 MHz ^{1}H n.m.r. study an alternative structure for the limonoid insect antifeedant azadirachtin has been determined.

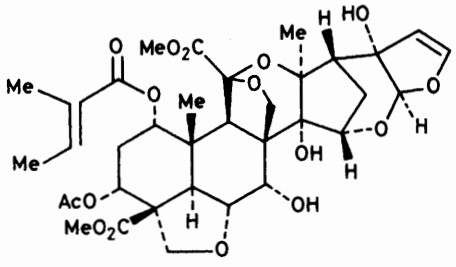
Owing to the biological activity associated with several natural products isolated from the neem tree, *Azadirachta indica* A. Juss and the related china berry tree *Melia azedarach* L., two international conferences have now been held.[1] One of these natural compounds, known as azadirachtin, has been the centre of interest because of its potent physiological effects on a wide range of insect species.[2]

Initial structural studies revealed key molecular fragments[3] while further work by Nakanishi[4] led to a complete structural assignment for azadirachtin. Since we have become interested in synthesis in this area we have had cause to re-examine the Nakanishi structure (**1**) and here present a revised molecular arrangement, compound (**2**), which is more consistent with the spectroscopic parameters.

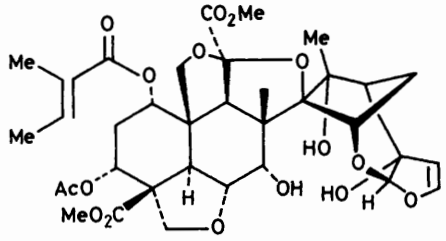
Table 1. ^{1}H N.m.r. assignments for compound (**2**).[d]

δ	Assignment	Multiplicity	Coupled to	*J* (Hz)	δ	Assignment	Multiplicity	Coupled to	*J* (Hz)
6.93	3′	qq	4′,5′	7.1,1.5	3.37	5	d	6	12.5
6.45	23	d	22	2.9	3.34	9	s	—	—
5.65	21	s	—	—	2.36	17	dd	16	5.4,0.4
5.50	3	dd	2	2.6,3.5	2.331	2a	ddd	2b,1,3	−16.9, 2.6,2.5
5.05	22	d	23	2.9	2.238	2b	ddd	2a,1,3	−16.9, 2.7,3.5
4.75	1	dd	2	2.5,2.7	2.00	18	s	—	—
4.73	7	br.s	6	—	1.95	Acetate	s	—	—
4.67	15	dd	16	3.4,0.3	1.85	5′	dq	3′,4′	1.5,1.2
4.60	6	dd	5,7	12.5,2.7	1.78	4′	dq	3′,5′	7.1,1.2
4.15	19b	d	19a	−9.8	1.75	30	s	—	—
4.07	28b	d	28a	−9.0	1.707	16a	ddd	16b,15,17	−13.2, 3.4,5.4
3.80	29-ester	s	—	—	1.306	16b	ddd	16a,15,17	−13.2 0.3,0.4
3.77	28a	d	28b	−9.0	5.07	OH-13	s[a]	—	—
3.68	12-ester	s	—	—	3.10	OH-20	br.s[b]	—	—
3.63	19a	d	19b	−9.8	3.00	OH-7	br.s[c]	—	—

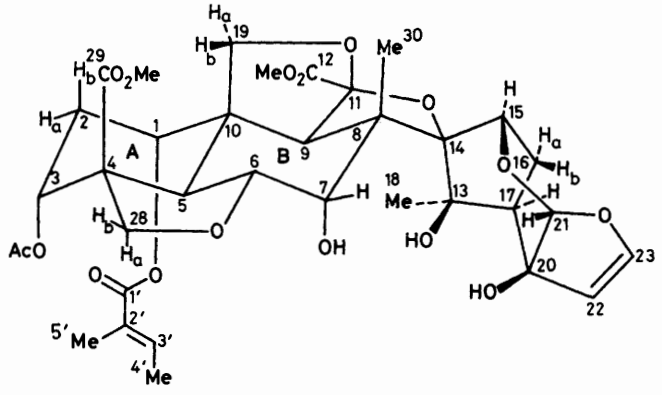
[a] Temperature coefficient -1.73×10^{-3} p.p.m. K^{-1}; δ (Me_2SO) 6.35. [b] Temperature coefficient -8.25×10^{-3} p.p.m. K^{-1}; δ (Me_2SO) 5.23. [c] Temperature coefficient -6.21×10^{-3} p.p.m. K^{-1}; δ (Me_2SO) 4.88, d, *J* 2.9 Hz. [d] Some small couplings of <0.5 Hz are not included in the table.



(1)



(2)



The assignment of the ^{1}H n.m.r. spectrum in $CDCl_3$ (66 mM solution) at 295 ± 1 K was rigorously established by a series of experiments including difference decoupling, saturation transfer, and variable temperature studies and application of the COSY technique. The COSY spectrum is shown in Figure 1 (lower triangle). Our assignments differ from the gross assignments given by Nakanishi in only one respect, H-15 being assigned to a sharp doublet at δ 4.67 and not at δ 4.62. The spectral parameters of the partially obscured H-2 and H-16 protons were established by the difference decoupling experiments. These results are summarized in Table 1. The relative stereochemistry of azadirachtin was established by a two-dimensional NOESY experiment (Figure 1, upper triangle) and by one-dimensional nuclear Overhauser effect (n.O.e.) difference spectra. Of the Overhauser effects observed, five, between H-16a and Me-18, H-19a and H-1, H-7 and Me-30, H-15 and Me-30, and that between H-7 and H-21, are crucial to our proposal for the structure of azadirachtin. The existence of a strong n.O.e. from H-16a to Me-18 is clearly indicative of a *syn* relationship between the bridge methylene at C-16 and the C-18 methyl group, directly contradicting the *anti* arrangement of structure (**1**). Consideration of models also indicated that other observed Overhauser enhancements could not be accounted for merely by assigning azadirachtin a structure epimeric at C-13 with respect to (**1**). Of a considerable number of models we examined whose structures were consistent with the COSY spectrum, only the structure (**2**) satisfied all the available data, in particular the strong H-7/H-21 n.O.e. mentioned above.

The absence of an n.O.e. between H-19b and H-16a and/or H-15 was also inconsistent with structure (**1**). This, taken together with the H-1/H-19a, H-15/Me-30, and H-7/Me-30 n.O.e. enhancements confirmed the revised arrangement about the B ring mentioned above. Further, smaller enhancements which support our view include that from H-19a to H-2b and from Me-30 to H-21 and negative enhancements due to 3-spin effects[5] from H-19b to H-1 and from H-19a to Me-30.

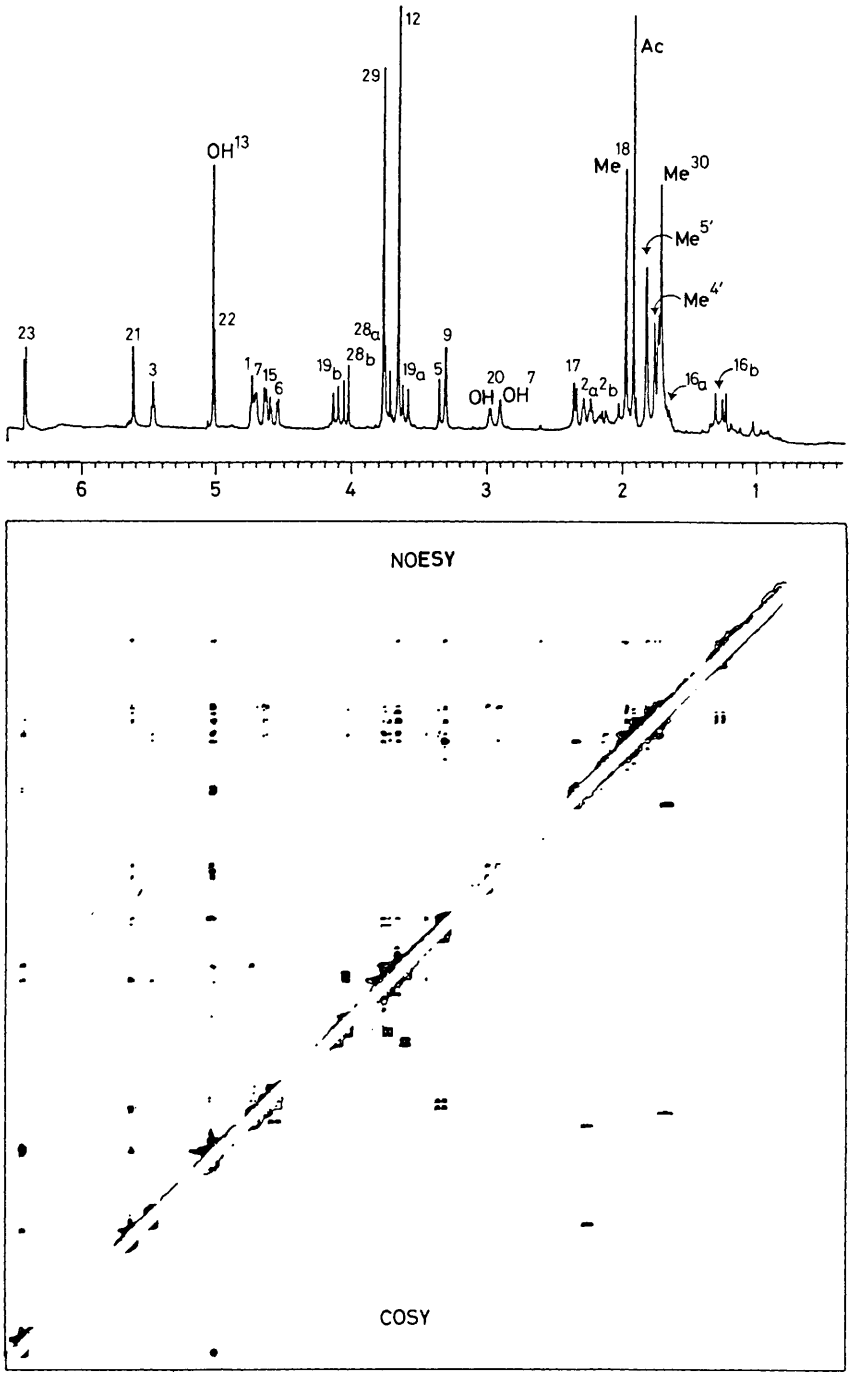


Figure 1

These 3-spin effects, which show that H-1, H-19a, H-19b, and Me-30 comprise a mutually relaxing system, account also for the absence of certain n.O.e's in the NOESY spectrum (Figure 1, upper triangle), which was recorded using a mixing time of 2 s. Protons with a spin–spin relaxation time (T_s) significantly less than this should show greatly reduced intensities, which was confirmed in the projection of the 2-D spectrum for the signals due to H-19a, H-19b, and Me-30.

Assignments of the hydroxy protons were based on n.O.e. measurements conducted at 270 K (to reduce complications due to spin exchange). Enhancements of H-9, H-5, and H-21 were attributed to proximity of OH-13, OH-7, and OH-20 respectively when these hydroxy groups were irradiated. A variable temperature experiment allowed correlation of the signals observed at low temperature with those seen at 295 K, and, following the observation that OH-7 demonstrated a 2.9 Hz coupling to H-7 in $[^2H_6]Me_2SO$ solution, mixed-solvent spectra have permitted absolute assignment of that proton in the $CDCl_3$ spectrum. The observation that the chemical shift of OH-13 has a significantly smaller temperature and solvent dependence than the OH-7 and OH-20 protons accords with the greater hindrance to solvation of this proton due to Me-18. N.O.e. difference spectra recorded in $[^2H_6]Me_2SO$ using irradiation of protons assigned by difference decoupling and

saturation transfer experiments support our proposal that azadirachtin possesses the structure (**2**). The spectra in Me_2SO also reduce the possibility of intermolecular n.O.e. phenomena.

Our initial findings with regard to the mass spectrum of azadirachtin provide further support for our structural assignment.

Fast atom bombardment (FAB) spectra were run in glycerol, *o*-nitrophenyl octyl ether and, most revealingly, in 2,2′-thiodiethanol. Ions observed included the cationized molecular ion at *m/z* 743 (*M* + Na) [or 727 (*M* + Li) when samples were made up in the presence of LiCl] and various ions due to composite losses of water, acetic acid, and tiglic acid. Structurally significant ions at *m/z* 151/2 and 347 were common to both the FAB and electron impact (EI) spectra.

The EI spectrum (run on a glass probe at <200 °C) showed fragmentation consistent with the presence of a monoacetate monotiglate and (at least) one alcohol/methyl ester functionality. The nature and fusion of the ring systems may be indicated by skeletal fragmentations at *m/z* 151($C_9H_{11}O_2$), 347($C_{18}H_{19}O_7$), and 407($C_{24}H_{23}O_6$).[6—8] In particular the ion at mass 347 might arise by direction of fragmentation by the central acetal ring,[7,8] such that a neutral loss of 212 a.m.u. is produced (Scheme 1). This neutral fragment may represent the C-13 to C-23 fragment and the oxygen of the central acetal.

In the case of structure (**1**) any retro Diels–Alder fragmentation[6] in a six ring acetal would give rise to charge retaining fragments containing an extra oxygen atom and this is not in agreement with the mass differences observed.

The spectrum is weak in the high mass region, but is reasonably reproducible with ions of <1% relative abundance. At 70 eV the base peak in the region 300—800 a.m.u. is *m/z* 559, with *m/z* 347 (60%). At low eV peaks become more prominent at *m/z* 587, 560, 527, 475, and 339.

Finally the structural revision reported above also calls into question the assignment for the related deacetylazadirachtinol

$$720^{+\cdot} \longrightarrow 559^{+} \longrightarrow 347^{+} + (212)$$

Scheme 1

molecule[9] where it would appear that an additional, detailed n.O.e. study is now warranted.

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