Crystal and molecular structure of Azadirachtin-A

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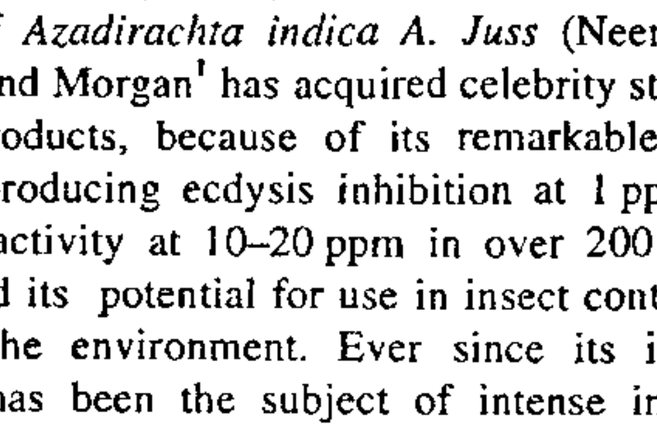
Azadirachtin-A isolated from the seed kernels of *Azadirachta indica A. Juss* has been crystallized and the structure has been studied through X-ray diffraction techniques Mr = 720.72, tetragonal, P41 $a = b = 20.960(5)$, $c = 9.415(4)$ Å, $V = 4136(2)$ Å^3, $Z = 4$, $d_{cal} = 1.157$ gm c^{-3}, λ (CuKα) = 1.5418 Å, $\mu = 0.74$ mm^{-1} (F000) = 1528, $T = 295$°K final $R = 0.071$ for 2139 reflections. $I > 3\sigma(I)$. The rotation of the two bulky groups about the C8–C14 bond is – 162.4(7)° in Azadirachtin-A and is different from that in detigloyl-22,23-dihydroazadirachtin. Also there is no hydrogen bond that connects the two halves as in detigloyl-22,23-dihydroazadirachtin. The intra and inter- molecular hydrogen bondings are also quite different in these two structures.

AZADIRACHTIN-A (Aza-A), first isolated from the seed kernels of *Azadirachta indica A. Juss* (Neem) by Butterworth and Morgan[1] has acquired celebrity status among natural products, because of its remarkable biological activity, producing ecdysis inhibition at 1 ppm and antifeedant activity at 10–20 ppm in over 200 species of insects and its potential for use in insect control without harming the environment. Ever since its isolation in 1968, it has been the subject of intense investigation in many leading laboratories in UK, Europe and USA by chemists and entomologists. Its structure could not be solved by X-ray diffraction, since it was not obtained in crystalline form, suitable for such studies. The structure (**1**) was solved by extensive NMR studies extending over 17 years, which have been summarized in a special series of articles[2]. The structure was confirmed by X-ray diffraction analysis of detigloyl-22,23-dihydroazadirachtin (DTA) obtained from Aza-A[3].



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In a recent communication[4] in this Journal the successful crystallization of Aza-A was reported and the crystal parameters were defined. The elucidation of structure of Aza-A by X-ray crystallography is reported in this paper.

Experimental

The needle-shaped crystals obtained by crystallization from ethyl acetate-hexane (see cover) were employed for securing X-ray diffraction data. Similar crystals were also obtained by crystallization from carbon tetrachloride but these did not give satisfactory X-ray diffraction patterns, due to occlusion of solvent molecules.

A crystal of $0.5 \times 0.05 \times 0.1$ mm was mounted along the needle axis and used for data collection on an Enraf-Nonius CAD4 computer-controlled diffractometer equipped with graphite monochromator. Cell constants were obtained from the setting angles of 15 reflections with $15° < \theta < 25°$. Intensity data were collected using w/2 θ scan mode for 3062 reflections with $0 < h < 24$, $0 < k < 24$, $0 < l < 10$ of which 2139 reflections $I > 3\sigma(I)$ were considered and used for refinement. The intensities were corrected for Lorentz and polarization and not for absorption. The structure was solved using SHELX86 programme[5]. Most of the hydrogens were located from a difference electron density map and others were fixed stereochemically. Full matrix least squares refinement of anisotropic thermal parameters for all non-hydrogen atoms and isotropic for hydrogens obtained from ΔF map gave a final R factor of 0.071 and $R_w = 0.071$ where $w = 1.0000/(\sigma^2(F) + 0.015611 \times F^2)$. The $\Delta\rho$ excursions were from – 0.25 to 0.3 e Å^{-3} in the final difference Fourier map. The atomic scattering factors used for all atoms were as provided by the SHELX76[6] for refinement and PARST[7] for calculations of geometric parameters. All calculations were performed on MicroVax-II computer.

Discussion

Atomic coordinates of all non-hydrogen atoms and their equivalent temperature factors are given in Table 1. Some important torsional angles are given in Table 2. Figure 1 gives the atomic numbering of the molecule.

Bond lengths and angles are normal and similar to those observed in DTA[3]. The Aza-A molecule consists

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Table 1. Positional parameters of nonhydrogen atoms

Atom	X	Y	Z	U_{eq} Å^2
01	0.6093(3)	0 8281(3)	0 2468(9)	0.047(3)
03	0 5389(3)	0.9388(3)	0.1676(9)	0.048(3)
06	0 6418(3)	1 0342(3)	0 4247(10)	0.051(3)
07	0 6343(3)	0 9083(3)	0 5682(10)	0.053(3)
011	0.8394(3)	0 8138(3)	0.3975(9)	0.052(3)
013	0.7407(3)	0 8806(3)	0.7806(9)	0.060(4)
015	0.7345(3)	0 7374(3)	0.5647(9)	0 052(4)
019	0.7991(3)	0.8382(3)	0 1749(9)	0 045(3)
020	0 8753(3)	0.7233(4)	0 7861(10)	0 072(4)
021	0.7758(5)	0 6384(4)	0 6044(10)	0.096(6)
024	0.4833(3)	1.0205(4)	0 0793(10)	0 075(5)
026	0 6904(4)	1.0225(4)	−0 0388(10)	0.067(4)
029	0.7409(4)	1 0496(4)	0.1564(10)	0 075(5)
031	0.5738(4)	0.7539(4)	0 0951(10)	0.088(6)
012	0.8133(3)	0.7013(3)	0.2818(10)	0.066(4)
027	0.7122(3)	0 7286(3)	0 2486(10)	0.064(4)
C1	0 6573(4)	0.8518(4)	0.1512(10)	0.038(4)
C2	0 6249(4)	0 8936(4)	0 0360(10)	0 046(5)
C3	0.5960(4)	0.9553(4)	0 0880(10)	0.049(5)
C4	0 6428(4)	0.9938(4)	0 1817(10)	0.045(5)
C5	0 6669(3)	0 9469(4)	0.2910(10)	0 040(4)
C6	0 6900(4)	0 9846(4)	0.4158(10)	0 040(4)
C7	0 6925(4)	0 9406(4)	0.5435(10)	0 043(5)
C8	0 7459(4)	0 8905(4)	0.5150(10)	0.044(4)
C9	0.7308(4)	0.8513(4)	0.3790(10)	0.039(4)
C10	0 7060(4)	0 8888(4)	0 2428(10)	0.042(5)
C11	0.7853(4)	0.8110(4)	0.3138(10)	0.045(5)
C13	0 7929(4)	0 8349(5)	0.7647(10)	0 052(5)
C14	0 7465(4)	0.8455(4)	0.6458(10)	0.048(5)
C15	0.7034(4)	0.7850(4)	0.6479(10)	0 047(5)
C16	0 7033(5)	0 7646(5)	0 8046(10)	0.059(6)
C17	0.7733(5)	0.7729(5)	0.8389(10)	0 058(6)
C18	0.8568(6)	0.8640(5)	0.7857(10)	0.073(7)
C19	0.7672(4)	0 8968(4)	0.1610(10)	0 047(5)
C20	0 8094(5)	0.7155(5)	0.7760(10)	0 057(6)
C21	0.7879(5)	0 7030(5)	0.6203(10)	0.057(6)
C22	0 7914(5)	0 6507(6)	0 8378(10)	0 071(7)
C23	0.7748(7)	0 6124(6)	0.7371(20)	0.093(10)
C24	0 4854(4)	0 9731(5)	0.1507(10)	0 048(5)
C25	0 4336(5)	0 9454(6)	0.2358(10)	0.077(8)
C26	0.7405(7)	1.0495(6)	−0.1239(10)	0.089(9)
C28	0 6130(5)	1 0441(4)	0.2873(10)	0 059(6)
C29	0 6961(4)	1 0252(4)	0.1006(10)	0 046(5)
C30	0 8107(4)	0.9289(4)	0.5072(10)	0.049(5)
C31	0 5736(5)	0.7793(5)	0 2093(10)	0.066(7)
C32	0 5318(6)	0 7548(7)	0 3320(20)	0 086(9)
C33	0 4999(8)	0.6918(8)	0 2992(20)	0.136(13)
C34	0 5267(8)	0 7897(10)	0 4477(20)	0 135(15)
C35	0.4833(10)	0 7842(20)	0.5692(30)	0 135(26)
C12	0 7725(5)	0.7402(4)	0.2805(10)	0 050(5)
C27	0 6967(7)	0 6639(6)	0 2230(20)	0 090(9)

$U_{eq} = (U_{11} + U_{22} + U_{33})/3$.

Table 2. Selected torsions in degrees

Atoms				Angle
C28	–C4	–C29	–O29	57.2(10)
C28	–C4	–C29	–O26	–124 3(9)
O6	–C6	–C7	–O7	59.2(10)
C5	–C6	–C7	–O7	–53.1(10)
O7	–C7	–C8	–C14	–56 2(9)
O7	–C7	–C8	–C9	62.6(9)
C7	–C8	–C14	–O13	–42.9(10)
C7	–C8	–C14	–C13	–110.6(10)
C7	–C8	–C14	–C15	89.3(10)
C9	–C8	–C14	–C13	129 9(10)
C9	–C8	–C14	–O13	–162.4(7)
C9	–C8	–C14	–C15	–30 1(10)
C10	–C9	–C11	–O11	134 1(8)
C8	–C9	–C11	–O11	4.1(10)
C9	–C11	–C12	–O27	29 8(10)
C9	–C11	–C12	–O12	–151 3(10)
C16	–C17	–C20	–O20	172 1(9)
C13	–C17	–C20	–O20	57 6(10)
C19	–O19	–C11	–O11	–109.4(8)
O20	–C20	–C22	–C23	–108 3(10)
O20	–C20	–C21	–O21	102 1(10)
O20	–C20	–C21	–O15	–133.7(10)

Table 3. Hydrogen bond geometry (in Å)

Type	D . . A	Symmetry
O7 . . . O6	2.969(10)	X, Y, Z
O11 . . . O12	2.654(10)	X, Y, Z
O11 . . . O29	2.785(10)	$-Y+2, X, Z+¼$
O7 . . . O24	2.883(9)	$-X+1, -Y+2, Z+½$
O20 . . . O6	2.760(10)	$-Y+2, X, Z+¼$

of two bulky groups, the first with rings A to D and their substituents at different positions and the other consisting of rings A′ to C′ and their associated side groups (Figure 1). The two groups are connected through C8–C14 bond. The rotation of these two groups about C8–C14 in Aza-A is −162.4(7)° whereas in DTA it is 27° which shows that removal of the tigloyl group in Aza-A completely changes the conformation. The effect of this conversion is to change the pattern of inter and intra-molecular hydrogen bonding. In Aza-A the two halves are not connected by any intra-molecular hydrogen bonds whereas in DTA 011 hydrogen bonds with epoxy oxygen 013. In Azadirachtin- H (to be published) 011 does not involve itself in any intra- molecular hydrogen bonding.

The overall conformations of the rings A to D and A′ to C′ are similar in both Aza-A and DTA. Ring A adopts sofa conformation[8] ($\Delta C_s = 1.4°$) whereas ring B is in half chair confirmation ($\Delta C_2 = 2.8°$). Ring D is in envelope conformation ($\Delta C_s = 4.5°$) and C is in half chair conformation ($\Delta C_2 = 3.1°$). The only difference in Aza-A is that the two-fold passes through C11 whereas in DTA it passes through 019[8]. A' and B' are in sofa ($\Delta C_s = 5.2°$, $\Delta C_s = 8.1°$) and C' is in envelope form ($\Delta C_s = 0.71°$). The acetoxy group at C3 is planar and the relative orientation of this with respect to ring A, defined by the dihedral angle C4–C3–03–C24 is 101.5° in Aza-A and 95.0° in DTA. There is not much change of the orientation of all the hydroxyl groups in these two structures.

The stereoview of the molecule[9] is given in Figure 2. The molecules in the unit cell are stabilized by inter- and intra-molecular hydrogen bonding. In this structure 011 forms a hydrogen bond with 012 and 07 with 06

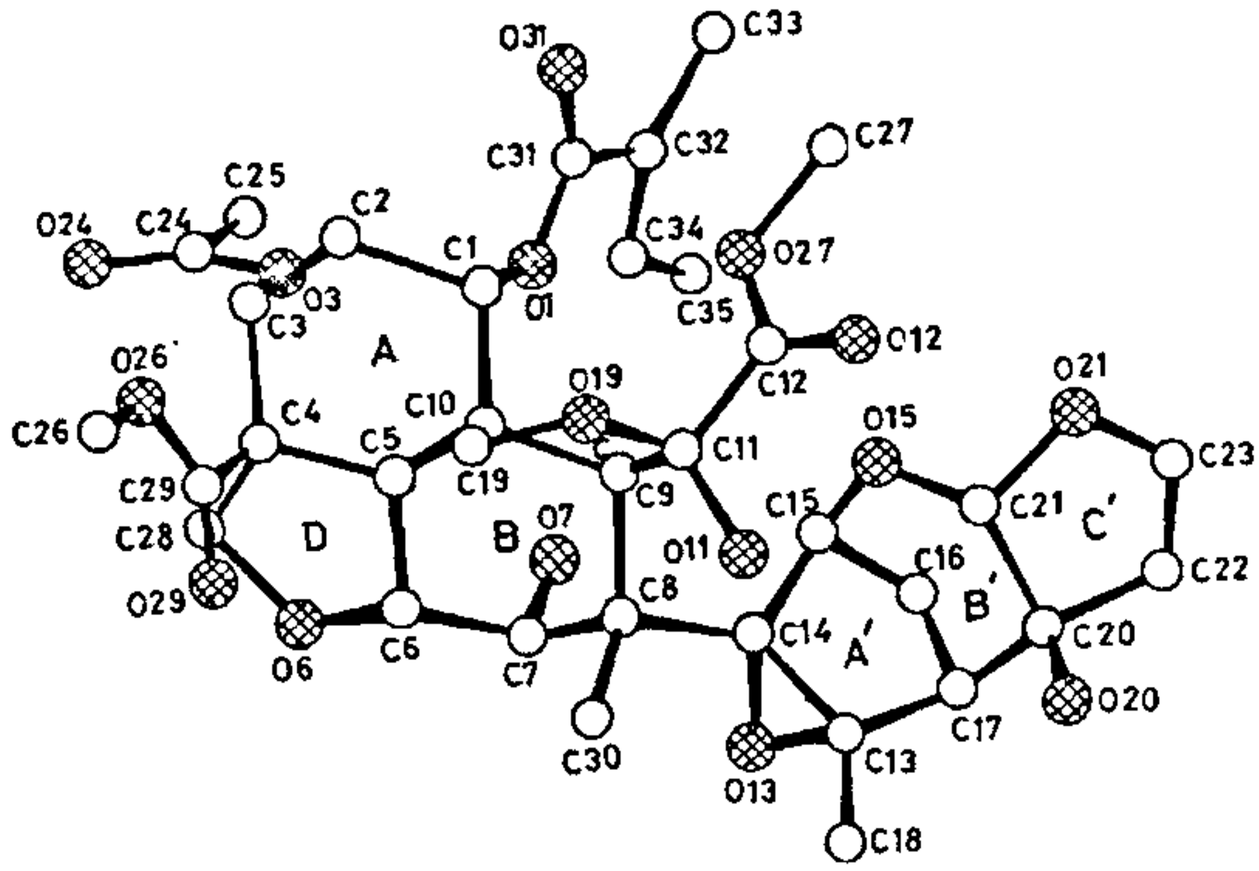

Figure 1. A perspective view of Azadirachtin-A

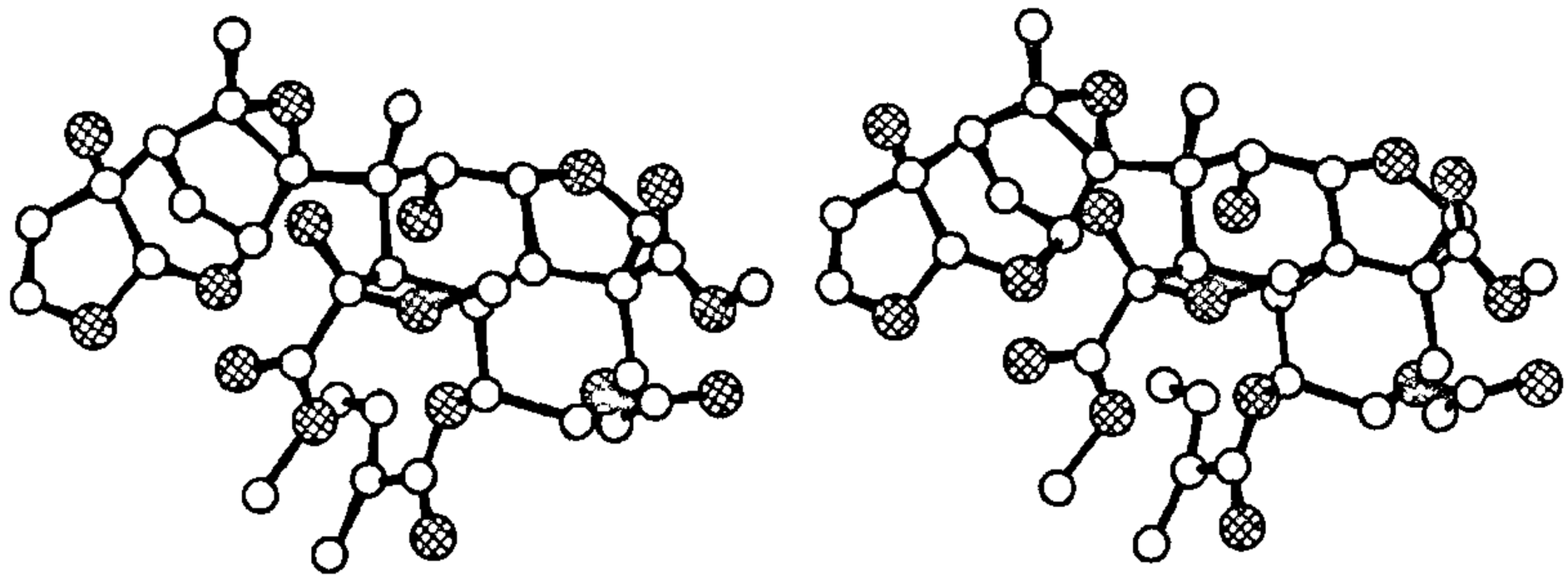

Figure 2. Stereo diagram of the molecule.

and there is no hydrogen bonding that connects the two halves whereas in DTA 011 is hydrogen-bonded with the epoxy oxygen 013. All the hydroxyl oxygens in Aza-A are involved in inter-molecular hydrogen bonding. The hydrogen bonding geometry is given in Table 3. The distance 011–015 in Aza-A is 3.14(10) Å and is not a hydrogen bond. Also the epoxy oxygen is not involved in any hydrogen bond. Thus the two halves are free to rotate about C8–C14 but stabilized in the crystal structure by inter-molecular hydrogen bonds.

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