*Tetrahedron* Vol. 43, No. 12, pp. 2805 to 2815, 1987
Printed in Great Britain.

0040-4020/87 $3.00 + .00


# AN X-RAY CRYSTALLOGRAPHIC, MASS SPECTROSCOPIC, AND NMR STUDY OF THE LIMONOID INSECT ANTIFEEDANT AZADIRACHTIN AND RELATED DERIVATIVES

John N. Bilton[a], Howard B. Broughton,[a] Philip S. Jones,[a]
Steven V. Ley,[a*] Zev Lidert,[b] E.David Morgan,[c] Henry S. Rzepa,[a]
Richard N. Sheppard,[a] Alexandra M.Z. Slawin,[a], and David J. Williams.[a]

a) Department of Chemistry, Imperial College, London SW7 2AY, UK.
b) Research Laboratories, Rohm and Haas Company, Spring House, Pennsylvania, U.S.A.
c) Department of Chemistry, University of Keele, Keele, Staffordshire, ST5 5BG, U.K.

(*Received in UK* 23 *February* 1987)

*Abstract: The limonoid insect antifeedant azadirachtin (1) may be partially hydrogenated at the C22-C23 position and subsequently treated with an excess of sodium periodate and potassium permanganate in the presence of a base to give detigloyldihydroazadirachtin (4). This compound was examined by X-ray crystallographic techniques which revealed key structural fragments and together with detailed n.m.r. and mass spectroscopic studies allowed the complete unambiguous structure assignment to the parent azadirachtin molecule. Two further compounds, 3-deacetyl-11-desoxyazadirachtin (2), and 3-acetoxy-7-tigloyl-vilasinin lactone (3) were also isolated and characterised from a Senegal sample of neem seed.*

It has been known for centuries that many trees and plants have evolved elaborate defence mechanisms against predatory insect attack. However, it is only relatively recently that scientists have begun to properly characterise and study some of the chemical substances involved in these processes. The neem tree, Azadirachta indica A. Juss, has long been credited with insecticidal or insect repellant properties in the Indian sub-continent and it was noticed that the Desert locust avoided the leaves. From the 1930's onwards crude extracts from this tree were shown to have repellant and insecticidal properties but it was not until 1968 the first pure active component was obtained. One of these compounds, named azadirachtin[1], has been the centre of interest ever since and three international conferences[2] on neem extracts have now been held. From the early biological studies to the more recent work azadirachtin has proved to be an important and exciting pesticidal substance which might eventually lead to new methods of insect pest control[3].

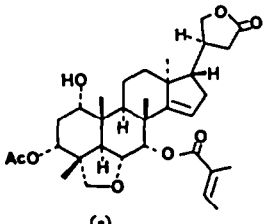
The large number of functional groups and the sensitivity of azadirachtin to acids and bases made conventional structural analysis difficult. Although initial structural studies by Morgan[4] revealed key molecular fragments, it was not until 1975 that a complete structural assignment was made by Nakanishi[5]. Our interest in the synthesis of insect antifeedant compounds[6] and particularly our recent synthetic studies on azadirachtin and related systems, led us to question this original assignment. Based upon high field n.m.r. techniques we proposed an alternative interim sturcture for azadirachtin[7] but felt that the only unambiguous method that could be applied to this difficult system was the use of x-ray crystallography. Here we discuss our studies which unequivocally allow us to assign the structure (1) to azadirachtin[8]. Kraus, by

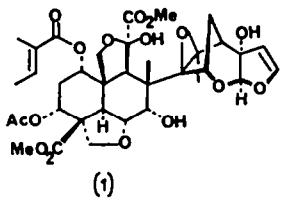
n.m.r. methods,[9] and Nakanishi by n.m.r. methods on a trimethylether derivative[10] have independently reached identical conclusions. During the work two further natural products (2) and (3) were also isolated and characterised from a Senegal sample of neem seeds.

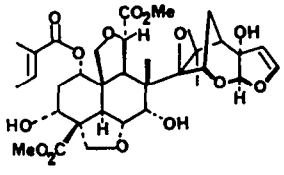
Azadirachtin

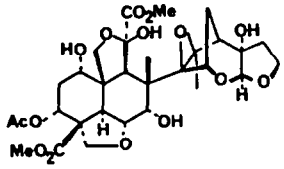
Since considerable discussion of the high field n.m.r. features of azadirachtin and derivatives are included in the accompanying papers only necessary comment is presented here.

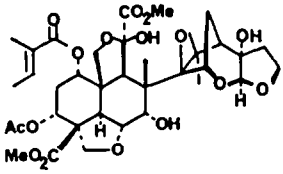
Azadirachtin is a microcrystalline solid which in spite of extensive attempts failed to afford crystals of sufficient quality for x-ray analysis. Several derivatives were also studied but attempts at crystallisation were equally unsuccessful. Finally a suitable crystalline product, detigloyldihydroazadirachtin (4) was obtained by chemical degradation of (1). This was achieved by partial hydrogenation of (1) in methanol to afford dihydroazadirachtin (5) in 70% yield using 10% Pd/C as catalyst. The tigloyl group in (5) was removed in 41% yield to give (4) with an excess of sodium periodate and potassium permanganate in aqueous t-butyl alcohol containing a little sodium carbonate.[4] Recrystallisation of (4) from diethyl ether and ethyl acetate afforded crystals for the x-ray diffraction study (Figure 1). This analysis immediately revealed the presence of the hydrofuran bridge from C-19 to C-11 and other key stereochemical features. It also clearly showed the structure to contain the novel epoxide ring arrangement at C-14 - C-13 and two stabilizing intramolecular hydrogen bonds in the crystalline state. These hydrogen bonds occur between the epoxy oxygen atom and the hydrogen atom of the C-11 hydroxyl group and between C-20 and C-7 hydroxyl oxygen atoms. From this structural determination one can then confidently assign the structure (1) to azadirachtin.

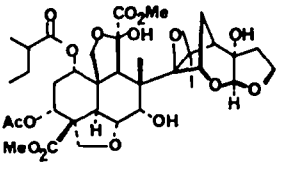

(3)


(1)


(2)

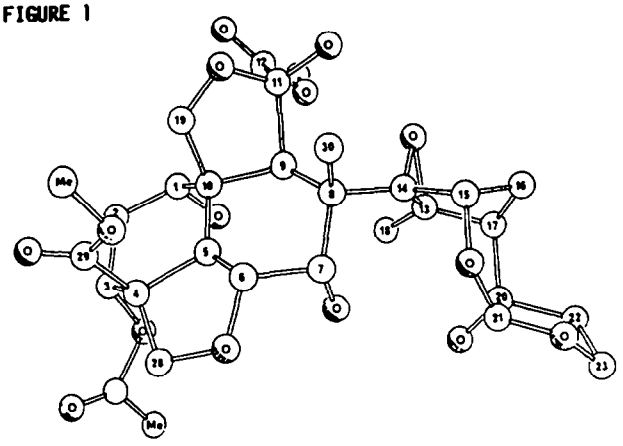

(4)


(5)


(6)

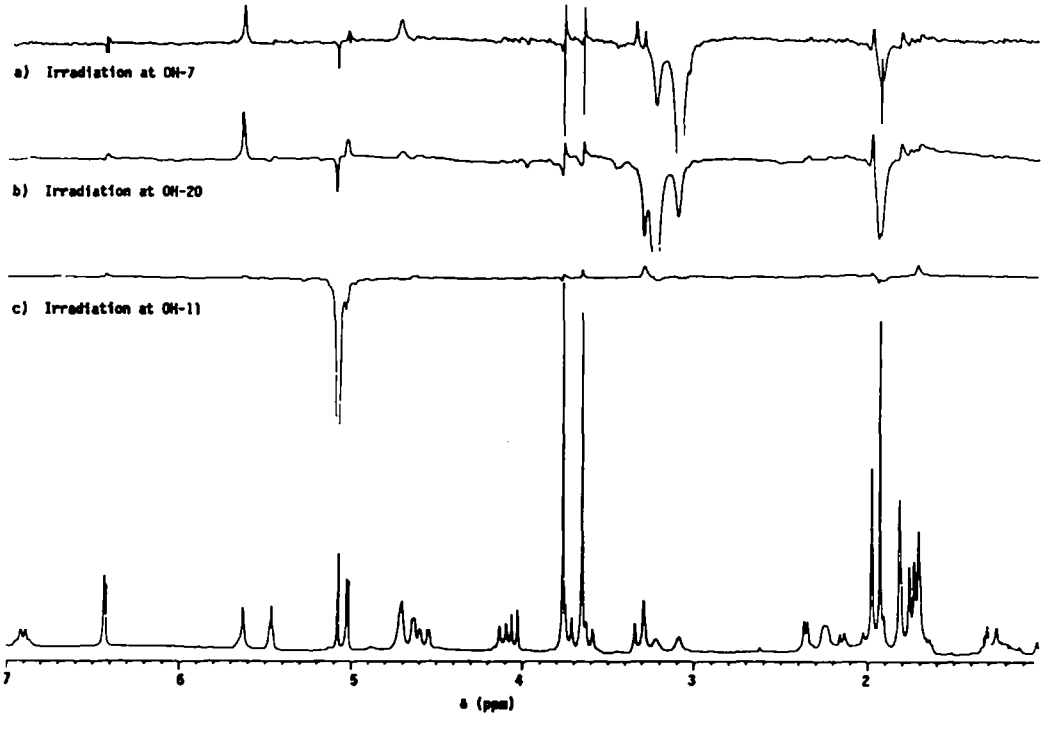
Confirmation that the skeleton of azadirachtin had not undergone rearrangment in the transformation to the detigloyldihydro derivative (4) was provided by nOe experiments on (1) similar to those described in the accompanying papers and by evidence obtained from a homonuclear $^1$H COSY experiment. Although not immediately apparent from a contour plot, small couplings, such as the H-19 methylene to H-1, H-5 and H-9 methine protons, were observable by examination of appropriate sections through the two-dimensional spectrum. Despite the broad nature of the signals due to H-7 and OH-7, it was also possible to observe this 3-bond coupling in the COSY

FIGURE 1



spectrum, thereby allowing direct assignment of this hydroxylic proton in $CDCl_3$. The remaining hydroxylic protons were assigned on the basis of nOe experiments[11] conducted at low temperature (270K); at this temperature saturation transfer effects were virtually eliminated, allowing clear interpretations of the nOe effects observed. Thus irradiation of OH-7 (δ 3.11) produced approximately equal enhancement of H-7 and H-21 and little or no response at H-22 (Fig. 2a) while irradiation of OH-20 (δ 3.25) produced a much larger enhancement of H-21 and H-22 and a smaller enhancement of H-7 (Fig. 2b). By contrast, OH-11 produced an enhancement only at H-9; from this spectrum it was clear that OH-11 took part to little or no extent in spin-exchange with the other hydroxyl groups, from which it may be inferred that the strong hydrogen bond observed in the x-ray structure determination was largely preserved in the solution state. (Fig. 2c).

FIGURE 2 nOe Difference Spectra of Azadirachtin (1) in $CDCl_3$ at 270K with Irradiation of the Hydroxylic Protons



This is further confirmed by examination of the temperature dependency of the chemical shifts of the three hydroxylic protons. The variation of chemical shifts with temperature of these three protons is in each case close to linear over the range 270 to 300K; the relevant equations and regression coefficients are tabulated in Table 1.

TABLE 1

| | $\delta + \delta_0 + kT$ | | $r^2$ |
|---|---|---|---|
| | $\delta_0$ | k | |
| OH-20 | 5.47 | $8.3 \times 10^{-1}$ | 0.998 |
| OH-11 | 5.57 | $1.7 \times 10^{-3}$ | 0.996 |
| OH-7 | 4.78 | $6.2 \times 10^{-1}$ | 0.997 |

The 11-OH function is clearly less exposed than the other two hydroxylic groups, an observation consistent with the hydrogen bond being intact. A mixed - solvent experiment also provided evidence for the degree of involvement in intramolecular hydrogen bonds for each hydroxylic proton. Thus, as DMSO-$d^6$ was added to a $CDCl_3$ solution of azadirachtin, OH-11 was found to have a chemical shift essentially independent of the concentration of DMSO, while OH-7 showed a larger dependency and the chemical shift of OH-20 was dramatically varied by the introduction of even very small amounts of DMSO. Once again it is clear that the exposure to solvent of the three hydroxylic protons lies in the order; OH-11, OH-7, OH-20, with this latter the most exposed and hence, by inferrence, least intramolecularly hydrogen bonded. This may be relevant in interpreting the unusual chemistry of azadirachtin, in particular the ease with which the tertiary hydroxyl group OH-20 is acylated in comparison to the other two hydroxyl groups.

In addition to these methods, we employed the difference decoupling technique[12] to expose partially obscured signals such as those due to H-16a and the H-2 methylene group. From the difference decoupled spectra we were able to determine a number of small (< 0.5 Hz) coupling constants by comparison with computer simulated difference decoupled spectra (Figure 3). By treating the n.m.r. spectrum of azadirachtin as the linear sum of eleven spin systems each containing less than eight protons, we were able to simulate the entire spectrum of the 44-spin system (Figure 4). Such discrepancies as exist between the observed and simulated spectrum may readily be accounted for by long range interactions between spin systems which for the purposes of the simulation were taken as independent. The information obtained from these comparisons may be of value in assessing the preferred conformation of (1).

From mass spectroscopic analysis a few substituent losses in azadirachtin (1) and its derivatives have been reported,[4] but no skeletal fragmentations have been confirmed which would lend support to structural assignments.

We have obtained more extensive accurate mass data (see table 2) in an attempt to identify the latter processes and thus to lend support to nmr analysis. FAB results confirm that azadirachtin has a molecular weight of 720.

The thermal sensitivity of azadirachtin was evident when probe samples that had been heated above 200°C were examined. Thermolysis undoubtedly contributes to the complexity of the mass spectra in this and related compounds.

The problem of assignment of oxygen in the azadirachtin molecule to an epoxide as distinct from a cyclic ether has been longstanding and is crucial to the structural analysis, but it did not yield to chemical methods of confirmation.

However, the mass spectrum revealed ions which strongly suggested the presence of an epoxide; the fragment m/z 195 ($C_{10}H_{11}O_4$) results from cleavage of the C8-C14 bond with charge retention on the epoxide oxygen. This ion is also present in the spectrum of 3-deacetyl-11-desoxyazadirachtin (2).

FIGURE 3 Observed and Simulated Difference Decoupled $^1H$ NMR Spectra of the H-16 Methylene group of (1) with Irradiation at H-15 (δ 4.67 ppm)

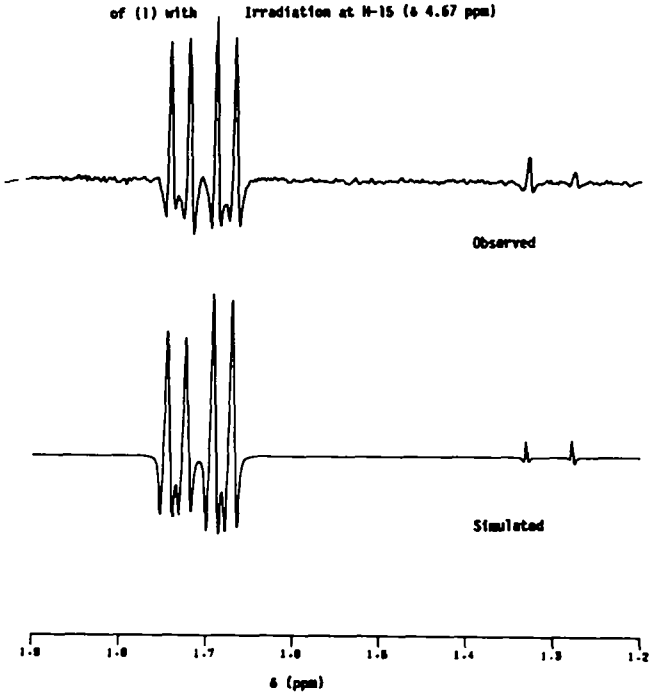
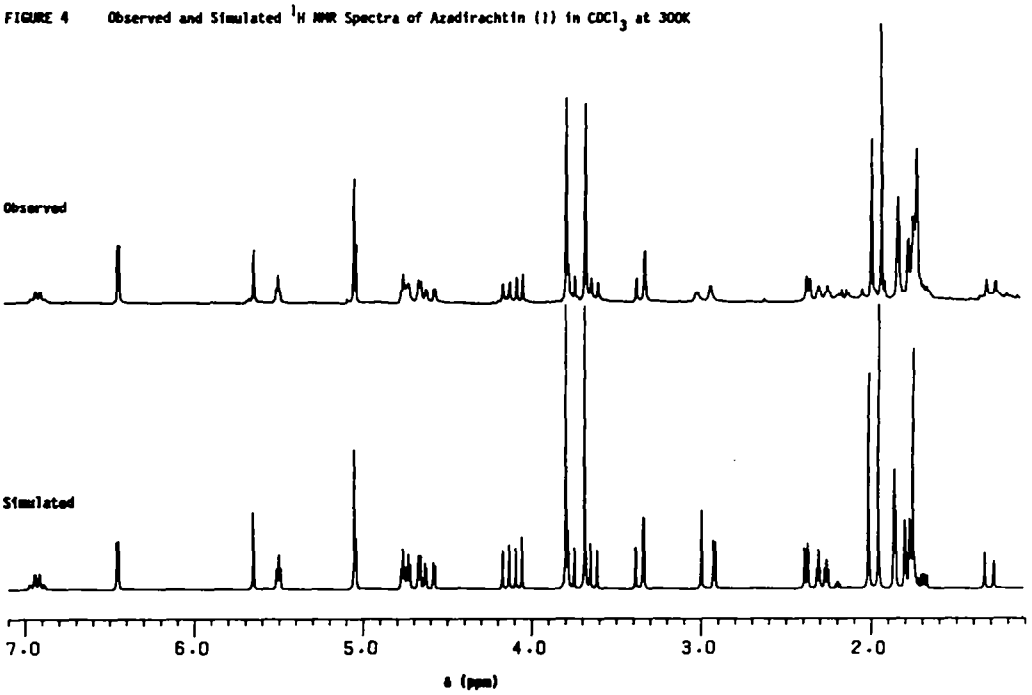



FIGURE 4 Observed and Simulated $^1H$ NMR Spectra of Azadirachtin (1) in $CDCl_3$ at 300K



In the reduced derivatives (4),(5) and (6) this fragment is shifted to m/z 197, of lower abundance, with a related stronger peak occurring at m/z 179 ($C_{10}H_{11}O_3 = C_{10}H_{11}O_4+H_2-H_2O$).

The significance of these ions became apparent once the low ev spectra were examined, for under these conditions the relative intensities of the peaks were enhanced.

Two related fragmentation pathways give rise to prominent ions in the high mass region of the spectra of (1),(4),(5) and (6); the processes correspond to composite losses of water, methanol or acetic acid and the elements of $C_4H_3O_2$ (for 1) or $C_4H_5O_2$ (for (4),(5) and (6)). The $C_4$ unit is lost from the terminal hydroxylated ring of the parent. The facility with which the latter rearrangement occurs must be associated with the strong directing influence of the acetal functionality, since it competes effectively with McLafferty rearrangements at the C-1 ester site.

Strong fragment ions corresponding to ($M-C_8H_{13}O_4$) and ($M-C_9H_{13}O_5$) occur in the reduced compounds (4),(5) and (6); the latter loss may arise in (4) by McLafferty rearrangment of the C-3 acetate followed by Retro Diels Alder fragmentation of ring A with cleavage of the $C_{28}$-$O_6$ bond. However, the observation of identical losses in the C-1 esters (5) and (6) would necessitate C-1 ester migration.

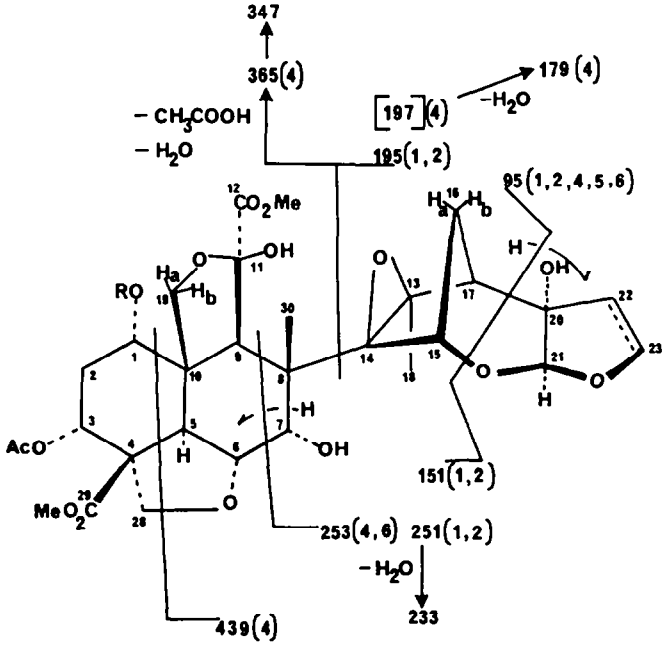
It had been expected that the fragmentation of azadirachtin would be directed by the loss of the bulky C-1 ester substituent to a major extent, but this was shown not to be the case from the accurate mass data; in addition it was noted that the mass losses in (4) and (6), which differ only in C-1 substitution, showed a striking correspondance down to m/z 400.

The ion m/z 251 ($C_{13}H_{15}O_5$) which was shifted to m/z 253 in (4),(5) and (6) results from scission of the C6-C7 and C8-C9 bonds with hydrogen rearrangment away from the charge retaining fragment; further water loss gives rise to m/z 233 in (1) and 235 in (4),(5) and (6).

Most of the strong peaks in the low mass region of the low ev spectra can be assigned to fragments derived from the epoxide containing half of the molecule; m/z 151 ($C_9H_{11}O_2$) and 95 ($C_6H_7O$), which are common to all the spectra, are derived by loss of the $C_4$ unit from ions m/z 251 and 195. The oxygen of the epoxide is retained in these fragments, and m/z 95 becomes the strongest ion at low ev.

The ion m/z 365 ($C_{18}H_{21}O_8$) and 347 ($C_{18}H_{19}O_7$) in (4) arise by composite losses of the epoxide fragment (C13-C23), water and acetic acid.

The additional loss of tiglic acid gives rise to m/z 347 in azadirachtin. The skeletal fragmentations so identified have been summarized in scheme 1.




SCHEME 1

## Other Neem Oil Derivatives

During the structural studies reported above we have also isolated two further components from our supply of neem seeds.

The first, 3-deacetyl-11-desoxyazadirachtin (2) was isolated by reverse phase HPLC of methanolic neem seed extracts (Senegal) which had been previously chromatographed on "Floridin Earth" to remove azadirachtin. The compound was slightly slower running than azadirachtin on ODS stationary phase. Compound (2) had a molecular formula of $C_{33}H_{42}O_{14}$ determined by elemental analysis and high resolution mass masurement. The FAB spectrum of (2) was run in 2,2'-thiodiethanol and gave a sodium attachment ion at m/z 685 = $(M+Na)^+$. Other ions resulting from protonation and dehydration were present at m/z 627 and 645. The EI spectrum was noticeably simpler to analyse than that of azadirachtin. Mass measurements on ions above 400 amu indicated that the fragmentation pattern could be largely explained by combination of four basic processes: water loss, loss of (COOMe)· loss of $(C_4H_3O_2)$· and loss of tiglic acid. The ion m/z 579 consisted predominantly of $(M-C_4H_3O_2)$ with a small contribution from $(M-C_5H_7O)$ representing loss of the tigloyl moiety. The epoxide derived fragment ions at m/z 195, 151 and 95 were dominant in the low ev spectra, with m/z 95 as the base peak.

The IR spectrum showed absorptions at 3446, 2953, 2919, 2848, 1724, 1646 and 1617 $cm^{-1}$. The $^1H$ NMR spectrum (see table 3) proved to be very similar to that of deacetylazadirachtinol described by Kubo[13] and also to that quoted in the published reappraisal of this structure by Kraus.[14] From these data it can be seen that the structure is very closely related to azadirachtin, the notable differences being the lack of acetate signal at δ 1.95 with the expected upfield shift of the 3-H proton to δ 3.52 and more importantly the increased complexity of the δ 4.0 to δ 4.5 region associated with structural change at C-11. Thus the hydroxyl singlet at δ 5.05 is replaced by a broadened singlet at δ 4.47 which can be seen to couple with the C-9 proton in the COSY 2-D spectrum. A more diagnostic picture emerges from the $^{13}C$ spectrum where the signal for C-11 the hemiacetal carbon in compound (1), is replaced by a signal at δ 79.4. The $^{13}C$ spectrum obtained for (2) proved to be identical to that obtained by Kubo but there were small but consistent differences in shift relative to the Kraus data, particularly in the signals due to the tigloyl group.

The question arises as to the position of the tigloyl group. The experiments of Kraus prove conclusively that in the compound he obtained (m.p. 208°C $[\alpha]_D$ = -69°; c = 0.1 $CHCl_3$) the tigloyl group was in the C-3 position. In our compound ($[\alpha]_D^{20}$ = -40.8°; c = 0.36 $CHCl_3$ m.p. 149-151°C c.f. Kubo 3-deacetyl azadirachtinol m.p. 148°C) the diagnostic 5% nOe reported by Kraus from the C-19 methylene protons to H-1 was clearly not present.

It is on the basis of the above evidence and in view of the close similarity of the spectra to (1) that we wish to assign the structure (2) to the compound and suggest that this is in fact the structure of Kubo's deacetylazadirachtinol.

The second compound isolated at longer retention times in the same HPLC separation which gave (2) is 3-acetoxy-7-tigloyl-vilasinin lactone (3). This compound (m.p. 242-3°C, $[\alpha]_D^{20}$ -22.4° C = 1.6 ($CHCl_3$)) had a molecular formula of $C_{33}H_{46}O_8$ indicated by elemental analysis and high resolution mass measurement. The IR spectrum showed significant absorption at 3400, 2997, 2848, 1775, 1735, 1719, 1700 and 1649 $cm^{-1}$. The $^1H$ nmr data are displayed in table 4. On the basis of the above data the carbon skeleton was proposed but the problem of the positions of the esters remained to be resolved. In this instance, the material was crystalline and the structure was determined by x-ray analysis confirming the carbon skeleton proposed and giving an unambiguous assignment of the position of the esters. (Fig. 6)

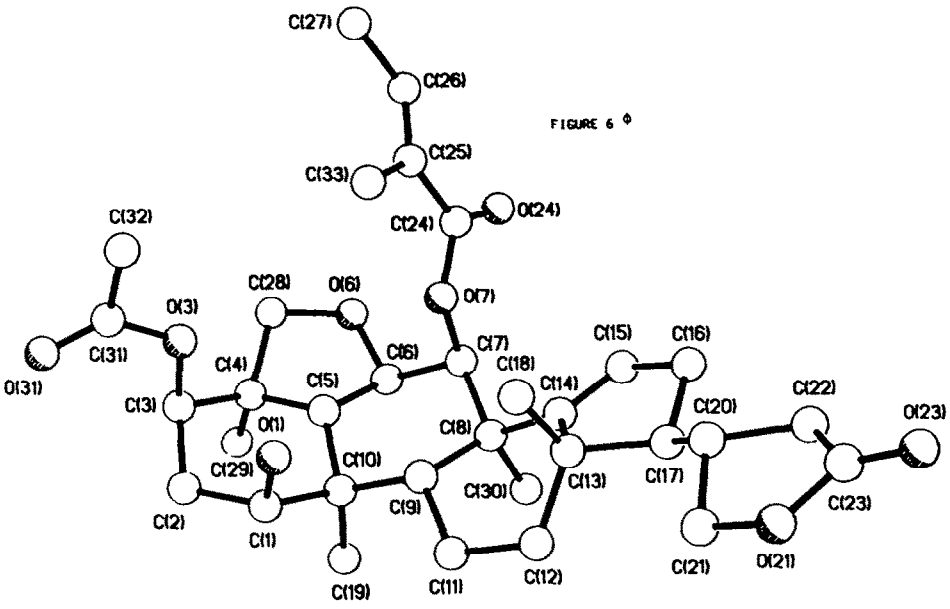



FIGURE 6 ◆

## Experimental Section

$^1$H nmr spectra were recorded on Bruker WH-400 or WM-250 spectrometer; $^{13}$C nmr spectra were recorded on a JEOL FX-90Q (at 22.51 MHz). All spectra were recorded in deuteriochlorform unless otherwise stated. Infra-red spectra were recorded on a Perkin-Elmer 983G or Matteson Instruments Sirus 100 as thin films. Mass spectra were recorded on a VG-7070E instrument. Data for crystal structures were collected on a Nicolet R3m diffractometer. In both cases, the net count of two relections, measured as references every 50 reflections did not alter significantly during data collection indicating that deterioation of the crystal had occurred. The data were brought to a uniform arbitrary scale by these reflections and Lorentz and polarisation corrections were applied. Computations were carried out on the Eclipse SI40 using the SHELXTL program system.[15] Melting points were determined on a Kofler hot stage instrument and are uncorrected.

## Preparation of 22,23-Dihydroazadirachtin (5)

Azadirachtin (1.00g, 1.34 mmol) in methanol (40 ml) was stirred with 10% palladium on charcoal (200 mg) under an atmosphere of hydrogen. After 2.5 hours the suspension was filtered through celite (2 g) and solvent removed from the filtrate under reduced pressure to leave a white foam which was purified by flash chromatography (eluant gradient 50% v/v ethyl acetate: dichloromethane up to 100% ethyl acetate) to give the dihydro derivative (5) (0.74 g, 76%), m.p. 157-60°C (from ethyl acetate); $\nu_{max}$ (film) 3432, 2954, 2910, 1733, 1703, and 1646 cm$^{-1}$; $\delta_H$ see table 6; $[\alpha]_D^{20}$ -27.8° (c = 0.37 in chloroform).

## Preparation of 1-Detigloyl-22,23-dihydroazadirachtin (4)

To the dihydro derivative (5) (300 mg, 0.41 mmol) in water (20 ml) and $^t$Butanol (30 ml) was added sufficient solid sodium carbonate to give pH 8. Sodium periodate (2.63 g, 12.3 mmol( in water (20 ml) was added to the stirring solution followed by potassium permanganate (0.33 g, 2.1 mmol) in water (10 ml). The reaction mixture was stirred at room temperature for 30 hours and then poured into water (100 ml) and extracted with dichloromethane (4 x 100 ml). The organic phases were combined, washed with water (50 ml) and brine (50 ml), dried ($Na_2SO_4$) filtered and concentrated under reduced pressure. The resultant residue was purified by flash chromatography (eluant gradient 50% v/v dichloromethane: ethyl acetate up to 100% ethyl acetate) to give (4) (95 mg, 36%); m.p. 155-7°C $\nu_{max}$ (thin film) 3441, 3060, 2985, 2954, 2920, 2854, and 1733 cm$^{-1}$; $\delta_H$ see table 7; $[\alpha]_D^{20}$ -17.4°(c = 0.46 in chloroform).

## Isolation of 3-Deacetyl-11-desoxyazadirachtin (2) and 3-Acetyl-7-tigloylvilasinin lactone (3)

Neem seeds from Senegal were finely ground in ethanol and extracted by the method of Morgan. Azadirachtin was isolated by chromatography on Florex XXS (Florida Earth Co.) eluting with ether-acetone (95:5). Fractions obtained just prior to those containing azadirachtin were subjected to reverse phase HPLC (DuPont ODS 21.2 x 250 mm) in 70% v/v methanol water; the title compounds being the major products: 500 mg of mixture gave (2) (68 mg), m.p. 149-51°C (from methanol); $\nu_{max}$ (thin film) 3446, 2953, 2549, 1727, 1646, and 1617 cm$^{-1}$; $\delta_H$ see table 3;

(Found: $M^+$, 662.2574. $C_{33}H_{42}O_{14}$ requires 622.2574) (Found: C, 59.60; H, 6.24. $C_{33}H_{42}O_{14}$ requires C, 59.82, H, 6.34%). $[\alpha]_D^{20}$ -40.8°C (C = 0.36 in chloroform) and next was obtained (3)[4] (48 mg), m.p. 242-3°C (from methanol/water); $\nu_{max}$ (thin film) 3400, 2917, 2848, 1775, 1700, and 1649 $cm^{-1}$; $\delta_H$ see table 4; (Found: $M^+$, 570.3200. $C_{32}H_{46}O_8$ requires 570.3192). (Found: C, 69.07; H, 8.30. $C_{33}H_{46}O_8$ requires C, 69.47; H, 8.07%). $[\alpha]_D^{20}$ -22.4°C (C = 1.6 in chloroform).

TABLE 2

Accurate Mass Data

| Azadirachtin | | Detigloyldihydroazadirachtin | |
|---|---|---|---|
| m/z | Formula | m/z | Formula |
| 588 | $C_{30}H_{36}O_{12}$ | 519 | $C_{26}H_{31}O_{11}$ |
| 587 | $C_{30}H_{35}O_{12}$ | 505 | $C_{25}H_{29}O_{11}$ |
| 559 | $C_{29}H_{35}O_{11}$ | 477 | $C_{24}H_{29}O_{10}$ |
| 407 | $C_{24}H_{23}O_6$ | 467 | $C_{22}H_{27}O_{11}$ |
| 347 | $C_{18}H_{19}O_7$ | 439 | $C_{21}H_{27}O_{10}$ |
| 339 | $C_{19}H_{15}O_6$ 2<br>$C_{20}H_{19}O_5$ 1 | 365 | $C_{18}H_{21}O_8$ |
| 277 | $C_{14}H_{13}O_6$ 3<br>$C_{15}H_{17}O_5$ 1 | 347 | $C_{18}H_{19}O_7$ |
| 251 | $C_{13}H_{15}O_5$ | 277 | $C_{14}H_{13}O_6$ 3<br>$C_{15}H_{17}O_5$ 1 |
| 233 | $C_{13}H_{13}O_4$ | 253 | $C_{13}H_{17}O_5$ |
| 195 | $C_{10}H_{11}O_4$ | 179 | $C_{10}H_{11}O_3$ |
| 151 | $C_9H_{11}O_2$ | 151 | $C_9H_{11}O_2$ |
| 95 | $C_6H_7O$ | 125 | $C_7H_9O_2$ |
| | | 111 | $C_6H_7O_2$ |
| | | 109 | $C_7H_9O$ |

TABLE 3

$^1H$ ASSIGNMENTS FOR COMPOUND (2) IN $CDCl_3$ AT 400 MHz

| δ | Assignment | Multiplicity | J(Hz) |
|---|---|---|---|
| 6.95 | 3' | qq | 7.0, 1.2 |
| 6.43 | 23 | d | 3.0 |
| 5.66 | 21 | s | - |
| 5.53 | 1 | t | 2.75 |
| 5.04 | 22 | d | 3.0 |
| 4.73 | 7 | d | 2.8 |
| 4.58 | 15 | d | 3.2 |
| 4.55 | 6 | dd | 12.6, 2.8 |
| 4.47 | 11 | s | - |
| 4.04 | 28 | d | 9.0 |
| 3.95 | 19 | d | 9.2 |
| 3.83 | 28 | d | 9.0 |
| 3.77 | OMe | s | - |
| 3.77 | OMe | s | - |
| 3.52 | 3 | m | - |
| 3.49 | 19 | d | 9.2 |
| 3.33 | s | d | 12.6 |
| 3.21 | 9 | s | - |
| 2.36 | 17 | d | 5.0 |
| 2.31 | 2 | dt | 16.0, 2.5 |
| 2.11-2.07 | 2 | m | - |
| 2.04 | 18 | s | - |
| 1.84 | 5' | m | - |
| 1.79 | 4' | dq | 7.0, 1.0 |
| 1.66 | 16 | ddd | 13.5, 5.0, 4.0 |
| 1.46 | 30 | s | - |
| 1.33 | 16 | d | 13.5 |

## X-Ray Crystal Structure Determinations

Details of the analysis of detigloyldihydroazadirachtin have already been reported.[8] Structure (3) was solved by direct methods. An E-map computed for the phase solution with highest "figure of merit" gave the positions of all the non-hydrogen atoms. Further $\Delta F$ maps revealed rotational disorder about C(24)-C(25) bond, resulting in two discrete positions for C(27), of occupancies 0.8 and 0.2. The minor occupancy carbon atom was refined isotropically. All other non-hydrogen atoms were refined anisotropically. The hydroxy proton on O(1) was revealed in a $\Delta F$ map, and refined isotropically. The remaining hydrogen atoms, with the exception of the methyl groups, which were refined as rigid bodies, were placed at calculated positions (C-H=0.96Å), assigned isotropic thermal parameters $U(H)=1.2U_{eq}(C)$, and allowed to ride on their parent C atoms. Refinement was by block-cascade full-matrix least-squares.

Final atomic coordinates,† including those of the hydrogen atoms, and thermal parameters have been deposited with the Editors as supplementary data.§

## Footnotes

† The atomic coordinates for this work are available on request from the Director of the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. Any request should be accompanied by the full citation for this paper.

§ Supplementary data and structural factors are available. See notice to Authors Tetrahedron, 40 (2), ii (1984)

♦ Fig. (6) molecular drawing of (3). There is an intermolecular hydrogen bond between O(1) and O(6) (O...O, 2.83Å, H...O, 1.95Å, O-H....O angle 148°).

TABLE 4

$^1$H Assignments For Compound (3) in $CDCl_3$ at 400 MHz

| δ | Assignment | Multiplicity | J(Hz) |
|---|---|---|---|
| 6.85 | 3' | qq | 7, 1.2 |
| 5.63 | 7 | d | 3 |
| 5.43 | 15 | dd | 3.5, 1.5 |
| 5.10 | 3 | t | 3 |
| 4.45 | 21 | t | 9 |
| 4.18 | 6 | dd | 12,3 |
| 3.91 | 21 | t | 9 |
| 3.57 | 1 | t | 3 |
| 3.50 | 28 | d | 7.5 |
| 3.27 | 28 | d | 7.5 |
| 2.79 | 9 | dd | 12,6 |
| 2.75-2.63 | 20 | m | - |
| 2.50 | 22 | dd | 17,8 |
| 2.41 | 5 | d | 12 |
| 2.33 | 2 | dt | 16,3 |
| 2.20 | 22 | dd | 17,11 |
| 2.13-2.07 | 2x16 | m | - |
| 2.05 | OAc | s | - |
| 2.01-1.99 | 2 | m | - |
| 1.84 | 5' | m | - |
| 1.80-1.55 | 2x11, 2x12 | m | - |
| 1.77 | 4' | dq | 7,1.2 |
| 1.19 | 29 | s | - |
| 1.13 | 18 | s | - |
| 1.02 | 30 | s | - |
| 0.96 | 19 | s | - |

TABLE 5

Crystallographic Data

| Formula | Compound (4) | Compound (3) |
|---|---|---|
| | $C_{30}H_{40}O_{15}.0.5(C_4H_{10}O)$ | $C_{33}H_{46}O_8$ |
| Molecular Weight | 677.7 | 570.7 |
| Space group, Z | $P22_12_1$,4 | $P1$,1 |
| a/A | 8.485(2) | 7.380(1) |
| b/A | 14.711(4) | 9.888(1) |
| c/A | 25.684(8) | 11.397(2) |
| α/° | 90 | 80.60(1) |
| β/° | 90 | 85.47(1) |
| γ/° | 90 | 67.85(1) |
| Volume/$A^3$ | 3206 | 766 |
| Density ($gcm^{-3}$, calcd) | 1.41 | 1.24 |
| Radiation/A | Cu-$K_\alpha$, 1.54 | Cu-$K_\alpha$, 1.54 |
| Scan mode | ω | ω |
| 2θ max/° | 116 | 116 |
| no. unique reflections | 2490 | 2036 |
| observed reactions $[\|F_o\|>3\sigma\|F_o\|]$ | 2177 | 1967 |
| R | 0.056 | 0.033 |
| $R_w$,g $(w^{-1}=\sigma^2(F)+g(F_o^2))$ | 0.063, 0.00235 | 0.037, 0.0012I |
| no. parameters refined | 468 | 404 |
| Crystal dimensions/mm | 0.27x0.30x0.20 | 0.47x0.22x0.33 |
| Residual electron density/$eA^{-3}$* | 0.27 | 0.16 |
| μ/$cm^{-1}$ | 9 | 7 |

* Calculated from observed data.

TABLE 6

$^1$H Assignments For Compound (5) in $CDCl_3$ at 400 MHz

| δ | Assignment | Multiplicity | J (Hz) |
|---|---|---|---|
| 6.89 | 3' | qq | 7.0, 1.5 |
| 5.50 | 3 | t | 2.9 |
| 5.28 | 21 | s | - |
| 5.05 | 11-OH | s | - |
| 4.75 | 1, 7 | m | - |
| 4.68 | 15 | d | 3.3 |
| 4.60 | 6 | dd | 12.5, 2.7 |
| 4.16 | 19 | d | 9.5 |
| 4.08 | 28 | d | 8.9 |
| 4.05-4.01 | 23 | m | - |
| 3.96-3.88 | 23 | m | - |
| 3.79 | OMe | s | - |
| 3.76 | 28 | d | 8.9 |
| 3.70 | OMe | s | - |
| 3.64 | 19 | d | 9.5 |
| 3.32 | 9 | s | - |
| 3.27 | 5 | d | 12.5 |
| 2.48 | 17 | d | 4.75 |
| 2.37-1.94 | 2x2,2x22 | m | - |
| 2.04 | 18 | s | - |
| 1.95 | OAc | s | - |
| 1.86 | 5' | bs | - |
| 1.78 | 4' | dq | 7.0, 1.0 |
| 1.74 | 30 | s | - |
| 1.70 | 16 | m | - |
| 1.55 | 16 | d | 12 |

TABLE 7

$^1$H Assignments For Compound (4) in $CDCl_3$ at 400 MHz

| δ | Assignment | Multiplicity | J (Hz) |
|---|---|---|---|
| 5.45 | 3 | t | 1.8 |
| 5.21 | 21 | s | - |
| 4.73-4.67 | 7, 11-OH | m | - |
| 4.66 | 15 | d | 3.2 |
| 4.58 | 6 | dd | 12.4, 3.2 |
| 4.17 | 19 | d | 8.8 |
| 4.14-3.72 | 2x23 | m | - |
| 4.03 | 28 | d | 8.4 |
| 3.87 | 28 | d | 8.4 |
| 3.80 | OMe | s | - |
| 3.77 | OMe | s | - |
| 3.75 | 19 | d | 8.8 |
| 3.45 | 1 | bs | - |
| 3.30 | 9 | s | - |
| 3.14 | 5 | d | 12.4 |
| 2.49 | 17 | d | 4 |
| 2.31 | 2 | dt | 16, 1.8 |
| 2.20-2.00 | 2,2x22 | m | - |
| 2.15 | 18 | s | - |
| 2.14 | OAc | s | - |
| 1.78 | 30 | s | - |
| 1.70-1.60 | 2x16 | m | - |

*We acknowledge financial support for this work from the SERC and Rohm and Haas Co., Spring House, Pennsylvania, U.S.A. We thank V.S. Bhatnagar, P.D. Manser, and M. Dreyer for generous supplies of neem seed.*

## References

1. J.H. Butterworth and E.D. Morgan, J. Chem. Soc., Chem. Commun., 1968, 23.
2. First International Neem Conference, Rottach-Egern, Germany, June 1980; Second International Neem Conference, Rauisch Holzhausen Castle, Germany, May 1983; Third International Neem Conference, Nairobi, Kenya, 1986.
3. For recent references, see H. Rembold and E de Souza Garcia, J. Insect Physiol., 1984, 30, 939; A.J. Mordue, P.K. Cottee and K.A. Evans, Physiol. Entomol., 1985, 10, 431; K. Feuerhake and H. Schmutterrer, Z. Pflanzenkrankh. Pflanzenschutz, 1985, 92, 643.
4. J.H. Butterworth, E.D. Morgan and G.R. Percy, J. Chem. Soc., Perkin Trans. 1, 1972, 2445.
5. P.R. Zanno, I. Miura, K. Nakanishi, and D.L. Elder, J. Am. Chem. Soc., 1975, 97, 1975.
6. S.V. Ley, M. Mahon, S.C. Howell, N.M. Ratcliffe and P.A. Worthington, J.Chem. Soc. Perkin Trans. 1, 1983, 1579; S.V. Ley, N.S. Simpkins and A.J. Whittle, J. Chem. Soc. Chem Commun., 1983, 503; S.V. Ley in Recent Advances in the Chemistry of Insect Control, Special Publication n. 53, The Royal Society of Chemistry, ed. N.F. James, 1985, 307.
7. J.N. Bilton, H.B. Broughton, S.V. Ley, Z. Lidert, E.D. Morgan, H.S. Rzepa and R.N. Sheppard. J. Chem. Soc., Chem. Commun., 1985, 968.
8. H.B. Broughton, S.V. Ley, A.M.Z. Slawin, D.J. Williams and E.D. Morgan, J. Chem. Soc., Chem. Commun, 1986, 46.
9. W. Kraus, M. Bokel, A. Klenk and H. Pöhnl, Tetrahedron Lett., 1985, 26, 6435, and accompanying paper.
10. See accompanying paper.
11. D. Neuhaus, R.N. Sheppard and I.R.C. Bick J. Amer. Chem. Soc., 1983, 105, 5996.
12. L.D. Hall and J.K.M Sanders, J. Am. Chem. Soc., 1980, 102, 5703.
13. I Kubo, A. Matsumoto, T. Matsumoto, J.A. Koke, Tetrahedron, 1986, 42, 489.
14. A. Klenk, M. Bokel and W. Kraus, J. Chem. Soc., Chem. Commun., 1986, 523.
15. G.M. Sheldrick, SHELXTL, An Integrated System for Solving, Refining, and Displaying Crystal Structures from Diffraction Data, University of Göttingen, F.R.G. (1982).