The Structure of Azadirachtin; the Functional Groups

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Azadirachtin ($C_{35}H_{44}O_{16}$), isolated from the seeds of the Neem tree (*Azadirachta indica*) and found to be highly active in inhibiting the feeding response of the desert locust (*Schistocerca gregaria*), has been shown to be a highly oxygenated triterpenoid substance, with fourteen of the oxygen atoms deployed in five ester groups, three free hydroxy-groups and a dihydrofuran ring; the remaining oxygen atoms are tentatively assigned to two ether groups.

A SYSTEMATIC study of the feeding inhibiting effect of the seeds of Neem (*Azadirachta indica* A. Juss) for 5th instar hoppers of the desert locust (*Schistocerca gregaria* Forskal) led to the isolation of azadirachtin, which is active in inhibiting the feeding of locusts on an artificial diet, at concentrations of 10^{-6}% or 10^{-8} mol l^{-1}. Azadirachtin was isolated from the seeds in a yield of 0·7 g per kg by extraction with ethanol, partition between solvents, and chromatography.[1] It was obtained as a microcrystalline powder, $C_{35}H_{44}O_{16}$, m.p. 154—158°, $[\alpha]_D$ −53°. Structural studies have shown that the compound belongs to a new class of hexanor-triterpenoids, probably related biogenetically to the tetranor-triterpenoids nimbin[2] and salannin.[3] Azadirachtin contains two methyl ester groups, two acetate and one tiglate [(*E*)-2-methylcrotonate] ester groups, one secondary and two tertiary hydroxy-groups, and a dihydrofuran ring linked to another ether ring; the remaining oxygen atom is tentatively assigned to another ether ring. The structural fragments so far elucidated are summarized in the partial structure (1).

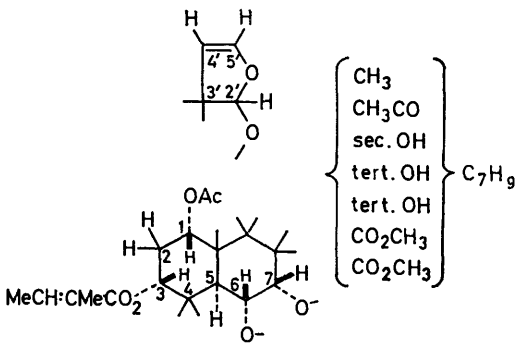
The molecular formula was determined by accurate mass measurements on the molecular ions of a tris-(trimethylsilyl ether) and a bis(trimethylsilyl ether); the parent compound and its derivatives with unprotected hydroxy-groups lost one molecule of water in the mass spectrometer. The molecular ion of the tris-ether corresponded to the formula $C_{44}H_{68}O_{16}Si_3$ and

[1] J. H. Butterworth and E. D. Morgan, *J. Insect Physiol.*, 1971, **17**, 969.

[2] M. Harris, R. Henderson, R. McCrindle, K. H. Overton, and D. W. Turner, *Tetrahedron*, 1968, **24**, 1517.

[3] R. Henderson, R. McCrindle, A. Melera, and K. H. Overton, *Tetrahedron*, 1968, **24**, 1525.

that of the bis-ether to $C_{41}H_{60}O_{16}Si_2$. In a preliminary communication, the molecular formula $C_{29}H_{38}O_{16}$ was given for the ion *m/e* 642;[4] recalculations gave better



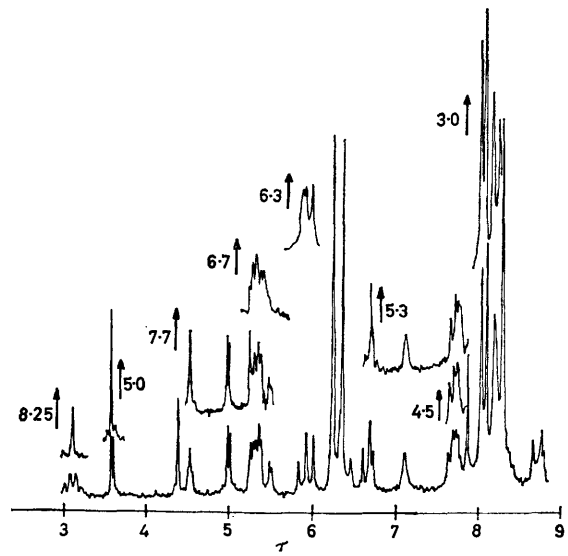
(1)

correspondence with $C_{33}H_{38}O_{13}$ for this ion ($M^+ - H_2O$ — AcOH).

The presence of two acetate ester groups was shown by two methyl singlets (τ 8·00 and 8·08) in the n.m.r. spectra of the parent compound and various derivatives (Figure and Table 1), the successive loss of two molecules of acetic acid in the mass spectrometer and the presence of a prominent peak at *m/e* 43 (CH_3CO^+), and by alkaline hydrolysis, the group at C-1 being the more readily hydrolysed.

The tiglate ester group was recognized by the n.m.r. absorptions at τ 3·08 (1H, m) and at τ 8·16 and 8·22 which on spin decoupling were seen to consist of a broadened singlet for the α-methyl group (τ 8·16, *J ca.* 0·5 Hz) and the β-methyl doublet (τ 8·22, *J* 5 Hz).[5] The mass spectrum showed a prominent ion at *m/e* 83 ($C_5H_7O^+$). The i.r. spectrum showed a carbonyl absorption (1710 cm^{-1}) and a C=C stretching band (1650 cm^{-1}). Alkaline hydrolysis followed by methylation with diazomethane gave methyl tiglate, recognized by g.l.c. (co-chromatography with an authentic specimen). The u.v. spectrum of azadirachtin has only one maximum (217 nm; $\varepsilon_{max.}$ 9200); thus the tiglate ester is the only strong chromophore present.

The presence of two methyl ester groups was recognized from the methyl singlets at τ 6·24 and 6·35 (Table 1),



N.m.r. spectrum (100 MHz) of azadirachtin in [^{2}H]chloroform with spin decoupling

loss of methanol and methoxyl in the mass spectrometer, and formation of acidic derivatives on strong alkaline hydrolysis.

The number of hydroxy-groups was determined from the n.m.r. spectrum of a solution in [^{2}H]dimethyl sulphoxide (Table 2). Three exchangeable proton signals, τ 5·19 (d, *J ca.* 2 Hz), 3·75 (s) and 4·82 (s), were assigned to one secondary and two tertiary hydroxy-groups.[6] Hot acetic anhydride acetylated one of the tertiary hydroxy-groups to give monoacetylazadirachtin. Silylation of azadirachtin with bis(trimethylsilyl)-acetamide in chloroform or dimethylformamide gave *OO*-bis(trimethylsilyl)azadirachtin, in which both tertiary hydroxy-groups were silylated and the secondary group was not. More vigorous silylating con-

TABLE 1

Chemical shifts (τ values) of some groups in azadirachtin and derivatives (in $CDCl_3$)

	5′-H*	4′-H*	2′-H*	*HC*·OAc	*HC*·OTig	*HC*·OH	Methyl groups: OMe	OMe	MeCO	Ac	Ac	Tig	Tig	CMe	Ac
Azadirachtin	3·58	4·95	4·36	4·51	5·23	~5·4	6·24	6·35		8·00	8·08	8·16	8·22	8·25	
Bis(trimethylsilyl ether)	3·58	4·97	4·46	4·54	5·28	~5·5	6·24	6·4		8·10	8·14	8·18	8·26	8·34	
Tris(trimethylsilyl ether)	3·58	4·92	4·44	4·41	4·80	~5·7	6·20	6·40		8·06	8·06	8·18	8·17	8·54	
Mono-*O*-acetylazadirachtin	3·55	4·95	4·38	4·52	4·83	~5·3	6·22	6·33		8·06	8·11	8·20	8·21	8·26	7·96
O-Acetyltrimethylsilyl-deriv.	3·55	4·95	4·45	4·50	4·81	~5·4	6·22	6·32		8·08	8·14	8·18	8·24	8·28	7·97
Dihydroazadirachtin			4·72	4·52	5·25	~5·3	6·22	6·33		8·00	8·07	8·16	8·26	8·18	
Tetrahydroazadirachtin			4·74	4·56	~5·4	~5·4	6·25	6·35		7·82	7·98	8·79	9·10	8·30	
Detigloylpyruvoyldihydro-deriv.			4·70	4·45	4·85	~5·4	6·10	6·15	7·48	7·80	7·82			8·20	
Detigloyldihydro-deriv.			4·65	n.r.	n.r.	n.r.	6·12	6·15		7·85	7·85			8·20	
Deacetylazadirachtin	3·59	4·99	4·36	5·56 †	5·19	~5·3	6·24	6·37		8·00		8·17	8·22	8·26	

* Numbering of partial structure (1). † *HC*·OH. n.r. = Not resolved. Tig = tigloyl.

[4] J. H. Butterworth and E. D. Morgan, *Chem. Comm.*, 1968, 23.

[5] R. R. Frazer, *Canad. J. Chem.*, 1960, **38**, 549.

[6] O. L. Chapman and R. W. King, *J. Amer. Chem. Soc.*, 1964, **86**, 1257.

TABLE 2

Hydroxy-proton resonances (approx. τ values, variable in $CDCl_3$ with concentration)

	Solvent	tert. OH	tert. OH	sec. OH	sec. OH
Azadirachtin	$CDCl_3$	4·95	6·95	7·05	
	$(CD_3)_2SO$	3·75	4·82	5·19	
O-Acetylazadirachtin	$CDCl_3$		6·7	6·8	
	$(CD_3)_2SO$		4·53	5·20	
O-Acetyl-*O*-trimethylsilyl-azadirachtin	$CDCl_3$			7·40	
	$(CD_3)_2SO$			5·10	
Bis(trimethylsilyl ether)	$CDCl_3$			7·20	
	$(CD_3)_2SO$			5·08	
Deacetylazadirachtin	$CDCl_3$	4·99	6·70	6·90	*ca.* 7·6
	$(CD_3)_2SO$	3·82	4·85	5·14	5·35

ditions [7] were necessary to obtain a tris-ether. The resistance of the secondary hydroxy-group to silylation and acetylation, and the failure to oxidize it to a ketone indicated that it must be in a highly hindered position. Deuterium exchange and double irradiation showed that the secondary hydroxy-proton signal was coupled to a one-proton doublet at τ [$(CD_3)_2SO$] 5·75 (J 2 Hz).

The n.m.r. spectrum of azadirachtin also showed a pair of coupled doublets [τ 3·6 (1H) and 4·95 (1H) (J 2·5 Hz)], indicating a dihydro-furan or -pyran unit. The low value of the coupling constant indicated a 2,3-dihydrofuran ring.[8]

Azadirachtin is resistant to hydrogenation at atmospheric pressure under a variety of conditions, but at 50 lb in^{-2} gave dihydroazadirachtin, in which the n.m.r. vinyl ether absorptions had been replaced by unresolved absorptions in the τ 5·9 (CH_2·O) and 7·8 (β-CH_2) regions. The i.r. absorption at 1625 cm^{-1} had disappeared and the mass spectrum showed an increase of two mass units in all the major high-mass peaks. In some experiments, hydrogenation produced some tetrahydroazadirachtin, in which the tiglate ester system was also reduced. The product showed no u.v. absorption, and the conditions for its formation were not reproducible.

Data on dihydrofuran ring systems are scarce; however comparison with chemical shifts given for sterigmatocystin,[9] clerodin,[10] aflatoxins,[11,12] and 2,3-dihydrofuran itself [13] confirm the assignment made but indicate that coupling should be observed between the 3- and 4-protons and between the 2- and 3-protons in a 2,3-dihydrofuran. That there is no further coupling of the β-proton in the enol ether of azadirachtin shows that the adjacent carbon atom is fully substituted. This was confirmed by the identification of the n.m.r. absorption at τ 4·36 (1H, s). This signal was assigned to the 2-proton in the dihydrofuran ring. It was unaffected when azadirachtin was converted into a number of derivatives and was not shifted when in [$^2H_6$]dimethyl sulphoxide. However, in dihydroazadirachtin the signal was shifted to τ 4·7, remaining as a singlet. The lack of coupling confirmed that the 3-position is fully substituted. The chemical shift suggested that another oxygen atom is attached at the 2′-position [see structure (1)], as in clerodin and the aflatoxins, though the τ value is not closely comparable with that of the 2-proton of aflatoxins because the latter are affected by the adjacent benzene ring (see Table 3).

TABLE 3

N.m.r. shifts (τ values) of some dihydrofuran systems

	H-5	H-4	H-2	$J_{4,5}$/Hz	$J_{3,5}$/Hz
Azadirachtin	3·58	4·95	4·36	2·5	
2,3-Dihydrofuran	3·78	5·18	5·8	2·6	2·6
Aflatoxin B_1	3·48	4·47	3·11	2·5	2·5
Aflatoxin M_1	3·17	4·36	3·54	3	
Clerodin	Unresolved	3·61	4·0	2·5	

The chemical shift of the 2-proton in dihydroazadirachtin (τ 4·7) corresponds to that of the 21-proton (τ 4·62) in the hemiacetal system of melianone.[14] The presence of a hemiacetal structure in azadirachtin can be ruled out by a number of factors, including the resistance of the compound to chromic acid oxidation, the lack of coupling shown by the proton at τ 4·4 in [$^2H_6$]dimethyl sulphoxide, and the result of ozonolysis of the dihydrofuran double bond, which gave a stable formate ester [τ 2·2 (1H, s)]. Attempted lactone titration gave a negative result. The second oxygen atom therefore probably forms part of an ether group. No evidence of an epoxide group has been found, either chemical [15,16,17] or spectral. Attempted reduction with chromous acetate [18] left the compound unchanged. The presence of the dihydrofuran ring accounts for the great sensitivity of the compound to acidic reagents.

The remaining oxygen atom is tentatively assigned to another ether ring; there are signals for four protons between τ 5·8 and 6·4, coupled in pairs which must be attached to carbon atoms bearing oxygen. The inability to form a ketone derivative or to reduce azadirachtin with sodium borohydride was taken to indicate the absence of a ketone group, though the c.d. curve showed a weak maximum at 308 nm.

An n.m.r. signal for one angular methyl group is is present at the unusually low field of τ 8·25, partially hidden by the tiglate methyl resonances but revealed on double irradiation. The position of this signal is probably due to the anisotropic effect of one or more of the carbonyl groups present, as in hirtin.[19]

[7] E. M. Chambaz and E. C. Horning, *Analyt. Letters*, 1967, **1**, 201.
[8] L. M. Jackman, 'Applications of Nuclear Magnetic Resonance in Organic Chemistry,' Pergamon, Oxford, 1959, p. 87.
[9] E. Bullock, J. C. Roberts, and J. G. Underwood, *J. Chem. Soc.*, 1967, 4179.
[10] D. H. R. Barton, H. T. Cheung, A. D. Cross, L. M. Jackman, and M. Martin-Smith, *J. Chem. Soc.*, 1961, 5061.
[11] K. J. Van der Merwe, L. Fourie, and de B. Scott, *Chem. and Ind.*, 1963, 1660.
[12] C. W. Holzapel, P. S. Steyn, and I. F. H. Purchase, *Tetrahedron Letters*, 1966, 2799.
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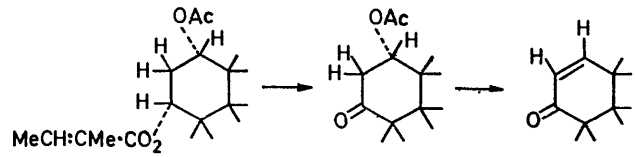
[14] D. Lavie, M. K. Jain, and I. Kirson, *J. Chem. Soc.*, 1967, 1347.
[15] R. Fuchs, R. C. Waters, and C. A. Vander Werf, *Analyt. Chem.*, 1952, **24**, 1514.
[16] J. M. Ross, D. S. Torbell, W. E. Lovett, and A. D. Cross, *J. Amer. Chem. Soc.*, 1956, **78**, 4675.
[17] J. W. Cornforth, R. H. Cornforth, and K. K. Mathew, *J. Chem. Soc.*, 1959, 122.
[18] W. Cole and P. Julian, *J. Org. Chem.*, 1954, **19**, 131.
[19] W. R. Chan and D. R. Taylor, *Chem. Comm.*, 1966, 206.

The presence of a double bond in the tigloyl group made it possible to remove this group selectively. Treatment of dihydroazadirachtin with sodium periodate and potassium permanganate [20] gave detigloylpyruvoyldihydroazadirachtin, in which the n.m.r. absorptions due to the tigloyl group were replaced by a singlet at τ 7·5 attributable to the methyl group of the pyruvate ester. The three-proton singlet at τ 8·25, obscured in the spectrum of azadirachtin, but revealed by double irradiation, was clearly seen. Selective hydrolysis of the pyruvate ester with aqueous methanolic sodium hydrogen carbonate [21] gave detigloyldihydroazadirachtin, which showed only end absorption in the u.v. region. The i.r. carbonyl absorption was much sharper, and lacked the 1710 cm^{-1} peak of the tiglate ester. The molecular ion (*m/e* 640), as with other derivatives in which the tertiary hydroxy-groups are unprotected was not seen.

The secondary hydroxy-group of azadirachtin was resistant to oxidation and was unaffected by Cornforth's reagent [22] (chromium trioxide–pyridine); however the additional hydroxy-group released by removal of the tiglate ester group was slowly attacked by this reagent. Insufficient material was obtained for comcomplete characterization; however the major oxidation product isolated was clearly recognized as an αβ-unsaturated ketone, in which the 1-acetate group had also been lost, by elimination in the mildly basic medium. The u.v. spectrum of the ketone showed $\lambda_{max.}$ 225 nm; and the i.r. spectrum had a new absorption at 1690 cm^{-1} characteristic of a cyclohexenone. The n.m.r. spectrum showed only one acetate group and the vinyl protons [τ 2·9 and 3·8 (J 10 Hz)]. The absence of further coupling indicated the structural fragment (2) in dihydroazadirachtin, and this confirmed double irradiation experiments on azadirachtin and various derivatives of it, in which the proton attached to the carbon atom bearing the tiglate ester and the proton attached to that bearing the acetate group lost in deacetylazadirachtin are both coupled to a signal at τ 7·8 (CH_2). From the width at half height of these single proton peaks (6 Hz), they are assigned an equatorial position,[23] placing both acetate and tiglate groups axial, as is found in the A ring of salannin [3] and other meliacins.[24] Ring A of a triterpenoid is the most probable site of this grouping, and further comparison of the chemical shift of the enone protons with those of known compounds [25,26] and the ready loss of acetic acid from the acetoxy-ketone indicated a 1-en-3-one rather than a 2-en-1-one system.

Dehydrogenation by selenium of the products obtained by lithium aluminium hydride reduction of azadirachtin produced a mixture of di- and tri-methylnaphthalenes and probably tetramethylnaphthalenes, separated and identified by linked g.l.c.–mass spectrometry. No phenanthrenes were detected. Since one acetate and the tiglate group have already been located in ring A,



(2)

rings A and B must be intact, and ring C has been cleaved; this is a characteristic feature of the liminoids (*e.g.* nimbin and salannin) isolated from this plant. The structural features recognized this far clearly relate azadirachtin to these liminoids and show that it is a high oxygenated and altered triterpenoid, probably sharing a common precursor with nimbin and salannin.

The presence of H-5, H-6, and H-7 is revealed by the similarity of their n.m.r. absorptions to those of nimbin, salannin, and nimbidic acid.[27] The 5-proton [axial; τ 6·65 (d, J 12 Hz)] is coupled only to H-6 [axial; τ 5·40 (pair of doublets, partly obscured, J 12 and 3 Hz)], which is also coupled to H-7 [equatorial; τ 5·28 (d, J 3 Hz); this apparently has no further coupling. Both C-6 and C-7 bear oxygen functions. As in nimbin and salannin the protons are assigned as H-5α, H-6β, and H-7β.

The formula $C_{35}H_{44}O_{16}$ contains 14 double bond equivalents, ten of which are accounted for in the partial structure (1). Two more are tentatively assigned to ether rings, leaving two still unrecognized. Further work on the remaining structural fragments is in progress.

EXPERIMENTAL

M.p.s were taken with a Reichert hot-stage microscope. U.v. spectra were obtained with a Unicam SP 800 (ethanol as solvent), i.r. spectra with a Perkin-Elmer Infracord 257 (solutions in chloroform or potassium bromide discs), and n.m.r. spectra with a Perkin-Elmer R10 at 60 MHz and on a Varian H-100D at 100 MHz. Mass spectra were routinely determined on a Hitachi–Perkin-Elmer RMU-6 single-focusing instrument; accurate mass determinations were made with an A.E.I. MS-902 double-focusing spectrometer.

Azadirachtin.—The seeds of *Azadirachta indica* were ground with ethanol, and the ethanolic extract was purified by partitioning between light petroleum and methanol, followed by chromatography on Floridin earth (Florex XXS, B.D.H. Ltd.). The crude active material was then purified by multiple elution preparative layer chromatography (p.l.c.) on 2·5 mm layers of Kieselgel PF_{254} as

[20] E. von Rudloff, *Canad. J. Chem.*, 1956, **34**, 1413.
[21] J. D. Connolly, R. Henderson, R. McCrindle, K. H. Overton, and N. S. Bhacca, *J. Chem. Soc.*, 1965, 6935.
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[23] A. Hassner and C. Heathcock, *J. Org. Chem.*, 1964, **29**, 1350.
[24] J. D. Connolly, K. H. Overton, and J. Polonsky, *Progr. Phytochem.*, 1970, **2**, 385.
[25] W. R. Chan, J. A. Gibbs, and D. R. Taylor, *Chem. Comm.*, 1967, 720.
[26] J. D. Connolly, K. L. Handa, and R. McCrindle, *Tetrahedron Letters*, 1968, 437.
[27] C. R. Mitra, H. S. Garg, and G. N. Pandey, *Tetrahedron Letters*, 1970, 2761.

described earlier,[1] to give azadirachtin, m.p. 154—158° (from CCl_4), $[\alpha]_D$ −53° [*c* 0·5 ($CHCl_3$)], $\lambda_{max.}$ 217 nm (ε 9200); $\nu_{max.}$ (CCl_4) 3460br, 1745s, 1720sh, 1710sh, 1655w, and 1625w cm^{-1} (n.m.r. spectrum in Figure), *m/e* 702 (*M* − H_2O), 688 (*M* − MeOH), 660 (*M* − AcOH), 670 (702 − MeOH or 688 − H_2O), 642 (642·2298. $C_{33}H_{38}O_{13}$ requires 642·2312; 702 − AcOH, 670 − CO or 660 − H_2O), 627 (627·2055. $C_{32}H_{35}O_{13}$ requires 627·2077; 642 − CH_3), 611 (611·2119. $C_{32}H_{35}O_{12}$ requires 611·2128; 642 − CH_3O), 100 (100·0526; $C_5H_8O_2$), 83 (83,0496; C_5H_7O), and 55 (C_4H_7), *m** 36·5 (83 ⟶ 55) (Found: C, 58·8; H, 6·55. $C_{35}H_{44}O_{16}$ requires C, 58·3; H, 6·1), R_F 0·60 (ethyl acetate), 0·50 (ether–acetone, 4 : 1), 0·40 (chloroform–acetone, 7 : 3), and 0·15 (ether–acetone, 49 : 1).

OO-Bis(trimethylsilyl)azadirachtin.— Bis(trimethylsilyl)-acetamide (0·5 ml) was added to a solution of azadirachtin (80 mg) in chloroform (1 ml) and after 10 min solvent and excess of reagent were removed *in vacuo*. The residue was purified by preparative layer chromatography giving *OO-bis(trimethylsilyl)azadirachtin* (42 mg), m.p. 110—112°, R_F 0·66 (ether–acetone, 9 : 1) (Found: C, 57·3; H, 7·4%; M^+, 864·3454, 864·3438. $C_{41}H_{60}O_{16}Si_2$ requires C, 56·9; H, 7·0%; M^+, 864·3420), $\nu_{max.}$ (CCl_4) 3570m, 1750s, 1710s, 1655w, and 1620w cm^{-1}.

OOO-Tris(trimethylsilyl)azadirachtin.—A mixture of azadirachtin (205 mg), pyridine (1·2 ml), bis(trimethylsilyl)-acetamide (1 ml), trimethylsilylimidazole (1 ml), and chlorotrimethylsilane (0·66 ml) kept at room temperature for 47 h gave only the bis-silyl ether, but the same mixture at 60° for 90 h gave, after p.l.c. in benzene–ethyl acetate (85 : 15) and preparative t.l.c. in benzene–acetonitrile (92 : 8), *OOO-tris(trimethylsilyl)azadirachtin* (33 mg), m.p. 151—153° (Found: C, 56·6; H, 7·7%; M^+, 936·3798. $C_{44}H_{68}O_{16}Si_3$ requires C, 56·4; H, 7·3%; M^+, 936·3816), $\nu_{max.}$ (CCl_4) 1745, 1710, 1650, and 1615 cm^{-1}.

O-Acetylazadirachtin.—Azadirachtin (100 mg) was heated under reflux with acetic anhydride (10 ml) for 10 min; excess of reagent was removed under vacuum and the residue was submitted to p.l.c. Elution twice with ether–acetone (9 : 1) yielded azadirachtin (24 mg) and *O-acetylazadirachtin* (35 mg), m.p. 154—156°, R_F 0·40 in the same solvent (Found: C, 58·2; H, 6·45. $C_{37}H_{46}O_{17}$ requires C, 58·2; H, 6·05%), *m/e* 744 (1·3%, *M* − H_2O), 702 (12%, *M* − AcOH), 685 (56%, 744 − CO_2Me) 670 (11%, 702 − MeOH), 661 (25%, 744 − tigloyl), 643 (44%, 702 − CO_2Me), 625 (685 − AcOH), 602 (100%, 685 − tigloyl), and 559 (66%) (abundances relative to *m/e* 602).

Treatment of acetylazadirachtin with bis(trimethylsilyl)-acetamide for 1 h at room temp. and work-up in the usual way gave *O-acetyl-O-trimethylsilylazadirachtin*, m.p. 131—135° (M^+, 834·3134. $C_{40}H_{54}O_{17}Si$ requires 834·3114), R_F 0·55 (ether–acetone, 4 : 1), $\nu_{max.}$ (CCl_4) 3500br, 1745s, 1652w, and 1615w cm^{-1}.

Dihydroazadirachtin.—Azadirachtin (50 mg) in ethyl acetate (50 ml) was shaken with Adams platinum catalyst (20 mg) under hydrogen at 50 lb in^{-2} for 1 h at room temp. Filtration, removal of solvent, and p.l.c. gave *dihydroazadirachtin* (30 mg), m.p. 119—122°, $[\alpha]_D$ −15° [*c* 0·4 ($CHCl_3$)], $\lambda_{max.}$ 219 nm (ε 7300), $\nu_{max.}$ (CCl_4) 3450br, 1740s, 1710s, and 1650w cm^{-1} (Found: C, 57·8; H, 6·4. $C_{35}H_{46}O_{16}$ requires C, 58·1; H, 6·4%), *m/e* 704 (*M* − H_2O), 690 (*M* − MeOH), 672 (704 − MeOH or 690 − H_2O), 662 (690 − CO), 644 (662 − H_2O, 672 − CO, or 704 − AcOH), and 629 (642 − CH_3).

Detigloylpyruvoyldihydroazadirachtin.—The pH of a solution of dihydroazadirachtin (98 mg) in t-butyl alcohol (30 ml) and water (40 ml) was adjusted to 7·7 with sodium carbonate, and a solution of sodium periodate (98·33 mmol) and potassium permanganate (1·67 mmol) in water (30 ml) was added. After 75 min at room temp. the mixture was extracted with chloroform; the extract (190 mg) gave, after p.l.c. in ether–acetone (4 : 1) (two elutions), some starting material and *detigloylpyruvoyldihydroazadirachtin* (21 mg), m.p. 147—150°, R_F 0·19 (ether–acetone, 4 : 1), $\nu_{max.}$ 3440br, 1740s, and 1720sh cm^{-1}, $\lambda_{max.}$ 227 nm (ε 1500) (Found: C, 56·1; H, 6·3. $C_{33}H_{42}O_{17}$ requires C, 55·8; H, 5·9%), *m/e* 692 (1%, *M* − MeOH), 649 (14%, 692 − CH_3CO), 632 (13%, 692 − AcOH), 617 (31%), 605 (10%), 589 (40%), 575 (26%), 561 (16%), 547 (100%), and 537 (34%) (abundances relative to *m/e* 547).

Detigloyldihydroazadirachtin.—The foregoing compound (42 mg) in methanol was treated with saturated aqueous sodium hydrogen carbonate for 70 min at room temp. Extraction of the product with chloroform and p.l.c. in ether–acetone (4 : 1) (two elutions) gave starting material and *detigloyldihydroazadirachtin* (14 mg), m.p. 164—168°, R_F 0·10 (ether–acetone, 4 : 1) and 0·16 (ethyl acetate) (Found: C, 55·7; H, 6·3. $C_{30}H_{40}O_{15}$ requires C, 56·3; H, 6·3%), *m/e* 622 (2%, *M* − H_2O), 604 (2·5%, *M* − $2H_2O$) 590 (4·5%, 622 − MeOH), 580 (<1%, *M* − AcOH), 579 (9%, 622 − CH_3CO), 562 (14%, 622 − AcOH), 547 (7%, 562 − CH_3 or 579 − MeOH), 537 (4·5%, 580 − CH_3CO), 519 (22%), 505 (9%, 537 − MeOH), 477 (45%), 467 (14%), 451 (9%), and 439 (100%) (abundances relative to *m/e* 439).

Oxidation of Detigloyldihydroazadirachtin.—Cornforth's reagent was prepared by adding chromium trioxide (5 g) in water (3 ml) to pyridine (50 ml) cooled in ice. Detigloyldihydroazadirachtin (77 mg) in pyridine (0·25 ml) was treated with Cornforth's reagent (2·0 ml) for 65 h at room temp. Water (15 ml) was then added, and the product was extracted with chloroform. The crude product (66 mg) was subjected to p.l.c. in ether–acetone (85 : 15), giving the starting material (17 mg) and a product (15 mg), R_F 0·18 (ether–acetone, 4 : 1), which was further resolved by p.l.c. in ethyl acetate to give a mixture of compounds (6 mg) and an αβ-unsaturated ketone (9·5 mg), m.p. 145—148°, R_F 0·35 (ethyl acetate), $\lambda_{max.}$ (EtOH) 225 nm ($E^{1\%}_{1cm}$ 2300), $\nu_{max.}$ (CCl_4) 3500br, 1740s, and 1690m cm^{-1}.

Deacetylazadirachtin.—Azadirachtin (200 mg) in methanol (10 ml) was treated with aqueous 2·5% potassium hydroxide solution (10 ml) for 1 h at room temp. The mixture was made just acidic with dil. hydrochloric acid and extracted with ethyl acetate, first by shaking, and then by continuous extraction for 2 h. The extract gave a residue (160 mg) on evaporation which contained free carboxylic acid. This was esterified with diazomethane in chloroform and purified by p.l.c. in ether–acetone (95 : 5) (two elutions) to give *deacetylazadirachtin* (25 mg) as a glass, m.p. 159—164° (Found: C, 57·9; H, 6·7. $C_{33}H_{42}O_{15}$ requires C. 58·4; H, 6·2%), R_F 0·32 (ether–acetone, 4 : 1), $\nu_{max.}$ ($CBCl_3$) 3440br, 1740s, 1655w, and 1630w cm^{-1}, *m/e* 660 (8%, M^+ − H_2O), 646 (7%), 642 (7%), 633 (18%), 628 (14%), 612 (12%), 600 (13%), 585 (19%), 551 (72%), 528 (27%), and 519 (100%) (abundances relative to *m/e* 519).

Dehydrogenation.—Azadirachtin (151 mg) in boiling tetrahydrofuran (10 ml; dried over lithium aluminium hydride) was treated with lithium aluminium hydride (1 g) for 4 h. Excess of reagent was decomposed with water and the product was extracted repeatedly with hot

ethyl acetate to give a yellow oil (89 g), shown to be a mixture of chiefly three components (R_F 0·15, 0·21, and 0·36 on t.l.c. in ether–acetone, 1 : 3).

This material was intimately mixed with selenium powder (146 mg) and heated at 320° for 66 h. The product was

TABLE 4

Peak no.	Retention time (min)	Relative peak area
1	2·9	19
2	3·2	3
3	4·1	6
4	4·8	29
5	6·2	21
6	11·0	100
7	14·6	61

extracted repeatedly with hot ethanol, to give, on evaporation, a residue (11 mg) which was chromatographed on a 9 ft column of 5% SE30 on Chromosorb G (helium as carrier gas, flow rate 17 ml min^{-1}). The retention times and relative peak areas of the major peaks are given in Table 4. Some naphthalene derivatives under the same conditions had the following retention times (min): naphthalene, 2·2; 1-methylnaphthalene, 3·8; 2,3-dimethylnaphthalene, 6·6.

The gas chromatograph was linked directly to the mass spectrometer and the mass spectrum of each peak was determined. Peaks 1—4 were not identifiable aromatic systems. Peak 5 was a dimethylnaphthalene [M^+ 156 (100), *m/e* 155 (30), 141 (64), 119 (16), 115 (18), 105 (42), 91 (13), 77 (16), and 65 (7) (% abundances relative to *m/e* 156]. Peak 6 was a trimethylnaphthalene [M^+ 170 (100), *m/e* 169 (11), 155 (78), 141 (8), 133 (8), 128 (8), 119 (7), 115 (32), 105 (9), 91 (10), and 77 (6) (% abundances relative to *m/e* 170]. Peak 7 was tentatively identified, in the absence of a good reference spectrum, as a tetramethylnaphthalene, M^+ 184 (53), *m/e* 169 (100), 143 (95), 115 (90), 105 (41), 93 (17), 91 (90), and 71 (24) (% abundances relative to *m/e* 169).

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