The Isolation of Avenacins A-1, A-2, B-1, and B-2, Chemical Defences Against Cereal 'Take-All' Disease. Structure of Their 'Aglycones', the Avenestergenins, and their Anhydro dimers

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The isolation is described of the four pre-formed antifungal compounds, the avenacins, from oat roots. These prevent attack by the wheat pathogen *Gaeumannomyces graminis* var. *tritici* (Ggt): the latter is the causative fungus of 'take-all' disease. The four avenacins A-1, A-2, B-1, and B-2 have the same trisaccharide attachment and on deglycosation give as 'aglycones' avenestergenins A-1, A-2, B-1, and B-2. A-1 and B-1 contain an esterifying *N*-methylanthranilate, A-2 and B-2 a benzoate. All four contain a triterpene core, the A-series being related to the B- by possession of an extra 23-hydroxy group. The structure of avenestergenin A-1 is examined in detail using ^{1}H n.m.r. with spin decoupling and Cosy plot, n.O.e. difference spectroscopy and 2D linkage of ^{13}C and ^{1}H resonances. This and mass spectral work, leads to complete structural and stereochemical proposals for the avenestergenins. After considerable difficulties, an *X*-ray structure of avenestergenin A-2 has been achieved.

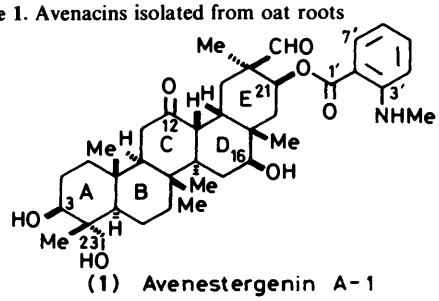
The four avenestergenins form a set of eight anhydro dimers, investigated by n.m.r. and f.a.b. mass spectra. These result from formation of 1,3-dioxane rings using the C-3 and C-23 hydroxy groups of one molecule of the A series with the C-30 aldehyde of another molecule of either the A or the B series.

'Take-all' is one of the most widespread stem-base diseases of cereals, attacking mainly wheat, but also barley and rye.[1] It is caused in wheat by the fungus *Gaeumannomyces graminis* (Sacc.)† Arx and Olivier var. *tritici* Walker, (Ggt) which infects the roots and tiller bases and is extremely difficult to eradicate by application of synthetic fungicides. However, oats are resistant to the fungus and land may be cleansed by planting such a crop because the fungus has low competitive saprophytic ability and does not survive over long periods without a host. The subject was advanced by Goodwin and Pollock[2] who recognised a fluorescent compound referred to as 'root tip glycoside.' This was studied extensively by Turner,[3] and Maizel, Burkhardt, and Mitchell[4] eventually isolated a fungicidal substance named avenacin from oat-root extracts. The compound is a pre-formed inhibitor[5] and is therefore not to be classed as a phytoalexin. Maizel *et al.*[4] recognised avenacin as a triterpene glycoside esterified with *N*-methylanthranilic acid and work was continued by Tschesche and his colleagues.[6] They revised the functional groups showing that an aldehyde but no double bond was present, though the material could not be assigned to a triterpene class. Important progress was made on the nature of the trisaccharide attachment and this is referred to in the following paper. A second compound, claimed to be a disaccharide with one Me replacing CH_2OH in the triterpene section, was also reported by them.[6]

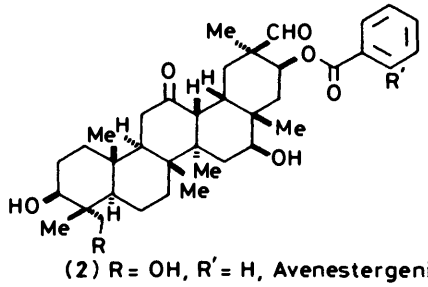
For our investigation oat roots were grown hydroponically as described in the Experimental section, harvested and freeze-dried, and then extracted with methanol–water (4:1) and methanol. Separations involved mainly reversed-phase h.p.l.c. C_{18}-column material, eluting with methanol–water (3:1), and led not to one but to four avenacins as shown in Table 1. The approximate proportion of A-1:A-2 is nearly 2:1 and of A-1 to B-1 10:1. Avenacin A-1 and B-1 had an intense blue fluorescence in methanol, and on methanolysis each produced 1 mol equiv. of methyl *N*-methylanthranilate as shown by g.c.–mass spectroscopy and ^{1}H n.m.r. The non-fluorescent A-2 and B-2 compounds gave 1 mol equiv. of methyl benzoate in place of

† Formerly known as *Ophiobolus graminis*

Table 1. Avenacins isolated from oat roots



(1) Avenestergenin A-1



(2) R = OH, R' = H, Avenestergenin A-2
(3) R = H, R' = NHMe " B-1
(4) R = H, R' = H " B-2

	M.p. (°C)	Mol. formula (*M*)[a]	$\lambda_{max.}$(EtOH)/ nm (ε)
Avenacin A-1	228—233	$C_{55}H_{83}NO_{21}$ (1 093)	223 (25 250), 255 (7 900), 357 (5 500)
Avenacin A-2	237—239	$C_{54}H_{80}O_{21}$ (1 064)	223 (14 700), 274 (1 200), 281 (1 100)
Avenacin B-1	Glass	$C_{55}H_{83}NO_{20}$ (1 077)	223 (23 300), 255 (7 900), 356 (5 300)
Avenacin B-2	Glass	$C_{54}H_{80}O_{20}$ (1 048)	228 (14 600), 274 (1 300), 281 (1 250)

[a] +Ve ion f.a.b. from *M* + 23 and *M* + 1; −ve ion f.a.b. from *M* − 1.

Table 2. Avenestergenins

	M.p. (°C)	Mol. formula (*M*)	λ_{max}(EtOH)/ nm (ε)
Avenestergenin A-1	210—211	$C_{38}H_{55}NO_7$ (637)	224 (23 100), 255 (6 500), 359 (4 600)
Avenestergenin A-2	189—190	$C_{37}H_{52}O_7$ (608)	228 (12 300),[a] 274 (820), 281 (700)
Avenestergenin B-1	186—187	$C_{38}H_{55}NO_6$ (621)	225 (23 400), 255 (6 900), 357 (4 700)
Avenestergenin B-2	177—178	$C_{37}H_{52}O_6$ (592)	229 (12 000),[b] 273 (530), 281 (360)

[a] Also 254 inflexion (1 700). [b] Also 257 inflexion (850).

the *N*-methylanthranilate. Avenacins A-1 and A-2 each contained one extra oxygen atom relative to their B-1 and B-2 counterparts, though all four compounds are trisaccharides of the same type. Hydrolysis (1M-HCl, reflux) gave in each case 1 mol equiv. of arabinose with 2 mol equiv. of glucose, analysed and identified by g.l.c. of the trimethylsilyl (TMS) derivatives. Short-term hydrolysis gave the two sugars in a ratio of 1:13 which suggests that it is the arabinose which is directly attached to the triterpene.

From the acid hydrolysis four crystalline avenestergenins A-1, A-2, B-1, and B-2 were isolated after removal of the carbohydrate section and data are given in Table 2: these are the apparent aglycones, still containing the ester attachments (A-1 and B-1, *N*-methylanthranilate; A-2 and B-2, benzoate). All four have u.v. data similar to their parent glycosides because of the chromophores of the esterifying acids whose presence was

Table 3. Avenestergenins: ^{1}H n.m.r. spectra ($CDCl_3$); δ values (*J* in Hz)

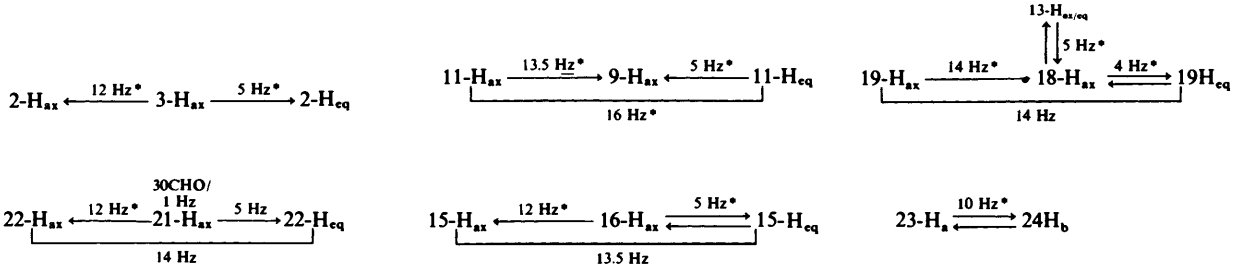
	Tertiary methyls							23-C*H*$_2$OH		3-C*H*OH	16-C*H*OH	30C*H*O
Avenestergenin A-1	0.82 (s, 3 H)	0.92 (s, 3 H)	0.94 (s, 3 H)		1.06 (s, 3 H)	1.15 (s, 3 H)	1.20 (s, 3 H)	3.75 (d, 1 H, *J* 11)	3.39 (d, 1 H, *J* 11)	3.68 (br, m, 1 H)	4.14 (dd, 1 H, *J* 11.5)	9.91 (d, 1 H, *J* 0.7)
Avenestergenin A-2	0.84 (s, 3 H)		0.94 (s, 6 H)		1.06 (s, 3 H)	1.16 (s, 3 H)	1.20 (s, 3 H)	3.74 (d, 1 H, *J* 11)	3.41 (s, 1 H, *J* 10.5)	3.6 (m, 1 H)	4.14 (dd, 1 H, *J* 11, 5)	9.94 (d, 1 H, *J* 0.7)
Avenestergenin B-1	0.80 (s, 3 H)	0.89 (s, 3 H)	0.92 (s, 3 H)	1.01 (s, 3 H)	1.04 (s, 3 H)	1.14 (s, 3 H)	1.19 (s, 3 H)			3.22 (dd, 1 H, *J* 11, 5)	4.20 (dd, 1 H, *J* 11, 5)	9.90 (d, 1 H, *J* 0.7)
Avenestergenin B-2	0.80 (s, 3 H)	0.89 (s, 3 H)	0.93 (s, 3 H)	1.01 (s, 3 H)	1.04 (s, 3 H)	1.16 (s, 3 H)	1.19 (s, 3 H)			3.22 (dd, 1 H, *J* 11, 5)	4.21 (dd, 1 H, *J* 11, 5)	9.94 (d, 1 H, *J* 0.7)

	Aromatic hydrogens								
	7′-H	5′-H	4′-H	6′-H	N*H*	NH*Me*	C*H*OCOAr	13-H$_{ax}$	19-H$_{eq}$
Avenestergenin A-1	7.88 (dd, 1 H, *J* 7.7, 1.5)	7.40 (dt, 1 H, *J* 7.7, 1.5)	6.67 (d, 1 H, *J* 8, 1.5)	6.59 (dt, 1 H, *J* 8, 1.5)	7.52 (br, d,[b] (1 H, *J ca.* 5)	2.92 (d, 3 H, *J* 5)	5.08 (dd, br, 1 H, *J* 12, 4)	2.87 (d, 1 H, *J* 4.5)	2.81 (dd, 1 H, *J* 14, 3.5)
Avenestergenin A-2	8.03 (d,* 2 H, *J* 7.5)	7.59 (t, 1 H, *J* 7.5)	7.45 (t, 1 H, *J* 7.5)	7.45 (t, 1 H, *J* 7.5)			5.14 (dd, 1 H, *J* 12.5, 4)	2.87 (d, 1 H, *J* 4.5)	2.80 (dd, 1 H, *J* 14.5, 3.5)
Avenestergenin B-1[a]	7.91 (dd, 1 H, *J* 8, 1.5)	7.43 (dt, 1 H, *J* 8, 1.5)	6.67 (d, 1 H, *J* 8)	6.60 (dt, 1 H, *J* 7.5)		2.83 (s,[a] 3 H)	5.12 (dd, 1 H, *J* 12.5, 3.5)	2.86 (d, 1 H, *J* 4.5)	2.80 (dd, 1 H, *J* 14.5, 3.5)
Avenestergenin B-2	8.03 (dd,* 2 H, *J* 8.5, 1.5)	7.59 (m, 1 H)	7.45 (t, 1 H, *J* 7.5)	7.45 (t, 1 H, *J* 7.5)			5.19 (dd, 1 H, *J* 12, 4)	2.87 (d, 1 H, *J* 4.5)	2.80 (dd, 1 H, *J* 14.5, 3.5)

* and 3′.

Additional data for Avenestergenin: A-1
2-H (eq and ax) *ca.* 1.7 (m), 9-H (ax), 1.73, 11-H (eq), 2.32, 11-H (ax) 2.21, 18-H (ax) 2.10 (ddd), 19-H (ax) 1.49, 19-H (eq) 2.81 (dd, *J* 14, 3.5), 22-H (eq) 2.32 (dd), 22-H (ax) 1.62, 15-H (eq) 1.36, 15-H (ax) 1.78.

Coupling connectivities for avenestergenin A-2



* Spin decoupled.

[a] D_2O Present. [b] Exchanges with D_2O.

Table 4. Avenestergenin acetates: ^{1}H n.m.r. spectra ($CDCl_3$); δ values (*J* in Hz)

		Tertiary methyls							24-C*H*$_2$OAc		3- and 16-C*H*OAc		30-CHO
Avenestergenin A-1	Triacetate	0.85 (s, 3 H)		0.95 (s, 3 H)	0.99 (s, 3 H)	1.11 (s, 3 H)	1.16 (s, 3 H)	1.20 (s, 3 H)	3.86 (d, 1 H, *J* 11.5)	3.73 (d, 1 H, *J* 11.5)	4.78 (dd, 1 H, *J* 5, 11)	5.40 (dd, 1 H, *J* 5, 11)	9.90 (s, 1 H)
Avenestergenin A-2	Triacetate	0.85 (s, 3 H)		0.95 (s, 3 H)	1.00 (s, 3 H)	1.12 (s, 3 H)	1.19 (s, 3 H)	1.20 (s, 3 H)	3.86 (d, 1 H, *J* 11.5)	3.74 (d, 1 H, *J* 11, 5)	4.78 (dd, 1 H, *J* 5, 11)	5.41 (dd, 1 H, *J* 5, 11)	9.92 (s, 1 H)
Avenestergenin B-1	Diacetate	0.87 (s, 3 H)	0.88 (s, 3 H)	0.92 (s, 3 H)	0.99 (s, 3 H)	1.11 (s, 3 H)	1.16 (s, 3 H)	1.19 (s, 3 H)			4.48 (dd, 1 H, *J* 5, 11)	5.41 (dd, 1 H, *J* 5, 11)	9.89 (s, 1 H)
Avenestergenin B-2	Diacetate	0.88 (s, 3 H)	0.89 (s, 3 H)	0.92 (s, 3 H)	1.00 (s, 3 H)	1.13 (s, 3 H)	1.18 (s, 3 H)	1.20 (s, 3 H)			4.48 (dd, 1 H, *J* 5, 11)	5.41 (dd, 1 H, *J* 5, 11)	9.92 (s, 1 H)

		Aromatic				NH	NH*Me*	CHOCOAr
Avenestergenin A-1	Triacetate	7.85 (dd, 1 H, *J* 8, 1.5)	7.38 (dd, 1 H, *J* 7.7, 1.5)	6.66 (d, 1 H, *J* 8.5)	6.56 (t, 1 H, *J* 7.7)	7.6 (br, 1 H)	2.90 (s, 3 H)	5.04 (dd, 1 H, *J* 12.5, 4)
Avenestergenin A-2	Triacetate	8.00 (m, 2 H)	7.56 (m, 1 H)	7.43 (m, 2 H)				5.05 (dd, 1 H, *J* 12.5, 4)
Avenestergenin B-1	Diacetate	7.85 (dd, 1 H, *J* 8, 1.5)	7.37 (m, 1 H)	6.66 (d, 1 H, *J* 8)	6.57 (t, 1 H, *J* 8)	7.6 (br, m, 1 H)	2.90 (s, 3 H)	5.06 (dd, 1 H, *J* 12.5, 4)
Avenestergenin B-2	Diacetate	8.00 (m, 2 H)	7.56 (m, 1 H)	7.43 (m, 2 H)				5.05 (dd, 1 H, *J* 12.5, 3.5)

Table 5. Avenestergenins: ^{13}C n.m.r. spectra ($CDCl_3$); δ values

	12-C=O	30-CHO	1'-CO_2	C-3'	C-5'	C-7'	C-6' and C-4'	C-2'	C-16	C-3	C-21	C-23	C-20	C-9	C-13
Avenestergenin A-1	211.4s	204.1d	168.8s	152.3s	135.3d	132.1d	114.8d, 111.1d	109.6s	65.3d	73.8d	74.3d	67.4t	50.7s	49.9d	49.3d
Avenestergenin A-2	211.2s	203.7d	166.7s	133.3d	129.8d	(2-C)	128.6 (2-C)	130.1s	65.7d	74.6d	75.0d	67.4t	50.6s	49.9d	49.4d
Avenestergenin B-1	211.4s	204.2d	168.3d	152.1s	135.0d	132.0d	114.7d, 110.9d	109.9s	65.9d	78.7d	73.7d		50.6s	49.8d	49.3d
Avenestergenin B-2	211.3s	203.7d	166.4s	133.2d	129.8d	(2-C)	128.5d (2-C)	130.2s	65.9d	78.8d	74.7d		50.6s	49.8d	49.3d

	C-5	C-14, C-17	C-4	C-8	C-18	C-1	C-11	C-22	C-15	C-10	C-19	C-7	C-8'	C-23	C-2
Avenestergenin A-1	48.2d	44.5s, 42.0s	42.4s	41.0s	39.4d	38.7t	38.1t	36.9t	35.4t	36.9s	32.9t	31.8t	29.6q		27.0t
Avenestergenin A-2	48.8d	44.7s, 42.0s	42.3s	40.9s	39.3d	38.7t	38.0t	37.0t	35.4t	36.8s	32.9t	31.8t			26.8t
Avenestergenin B-1	55.3d	44.6s, 42.0s	38.9s	40.7s	39.4d	38.6t	38.2t	37.1t	35.3t	36.8s	32.8t	32.0t	29.6q	28.1q	27.1t
Avenestergenin B-2	55.3d	44.7s, 42.0s	38.9s	40.8s	39.3d	38.7t	38.2t	37.1t	35.3t	36.8s	32.8t	32.1t		28.1q	27.1t

	C-29	C-28	C-27	C-6	C-26	C-25	C-24
Avenestergenin A-1	22.7q	21.3q	20.8q	18.1t	16.2q	15.8q	11.7q
Avenestergenin A-2	22.5q	21.4q	20.7q	18.3t	16.3q	15.8q	11.6q
Avenestergenin B-1	22.3q	21.3q	20.6q	18.4t	16.2q	15.4q	15.3q
Avenestergenin B-2	22.3q	21.3q	20.7q	18.4t	16.3q	15.3q	15.4q

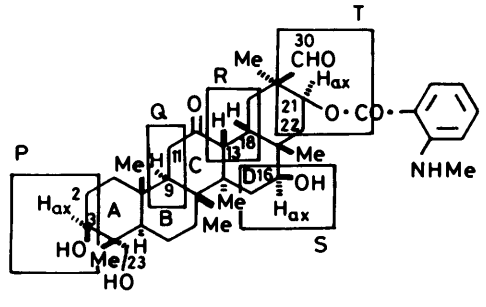


Figure 1. Patch diagram of ^{1}H connectivities for avenestergenin A-1

confirmed by methanolysis. Avenestergenin A-1* contained three free hydroxy groups giving a triacetate and a tri-TMS derivative: from the ^{1}H n.m.r. spectra and characteristic shifts on acetylation (see Table 4), two were secondary and one primary. A fourth hydroxy group was esterified with *N*-methylanthranilic acid. The presence of an aldehyde group was confirmed by ^{1}H n.m.r. [δ 9.91 and $\nu_{max.}$($CHCl_3$) 1 722 cm^{-1}], as was a ketonic

* This is probably similar to the avenamine of Maizel *et al.*[4] and the avenamine-A of Tschesche *et al.*[6] but now we know that 'aglycones' without nitrogen are present, the amine ending is no longer appropriate, and we refer to the group as avenestergenins.

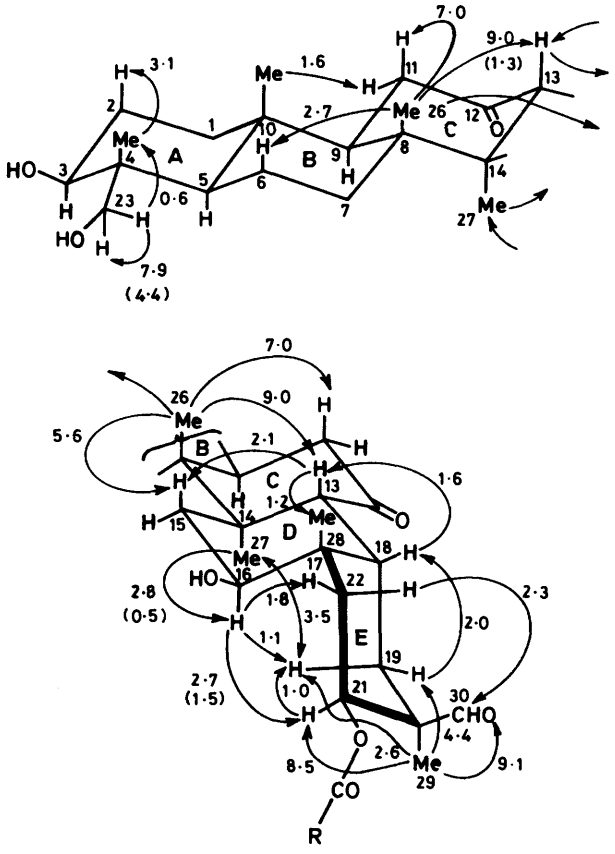


Figure 2. N.O.e. difference spectrum for avenestergenin A-1

carbonyl in a 6-membered ring (^{13}C n.m.r. δ 211.4, $\nu_{max.}$ 1 695 cm^{-1}). Considering these functionalities and their effect on n.m.r. data which shows that there are 6 tertiary methyls along with two oxidised methyls (CHO and CH_2OH), together with molecular formulae data, led us to the view that we were probably dealing with an oleanane framework. Spectroscopic investigation was pursued with this in mind.

Table 3 gives a comparison of some of the main ^{1}H n.m.r. assignments for the four avenestergenins. Additional data are included for avenestergenin A-1 which is considered more specifically in this discussion. With the aid of specific proton decoupling, coupling connectivities were established as indicated in Table 3 and the connexions are shown as a ^{1}H 'patch' diagram in Figure 1. Within each local 'patch' connectivity is established. A point of interest is the small W coupling (1.0 Hz) between the 30-C*H*O and the C-21$_{ax}$ proton, the latter carbon carrying the aryl ester, thus relating functionalities within block T. Figure 1 is further supported by a ^{1}H–^{1}H Cosy plot to reveal couplings.

The results of nuclear Overhauser difference (n.O.e.) spectroscopy were particularly revealing and the molecule is shown in two parts for clarity in Figure 2. Although P (Figure 1) remains an isolated block, connectivities are established *via* the n.O.e. enhancements for Q-S, Q-R, R-S, S-T, R-T, and Q-T. These relationships may be direct, as in the case S-T (16-H$_{ax}$ ⟶ 21-H$_{ax}$ or 19-H$_{ax}$ or 22-H$_{eq}$), or indirect as for Q-S (11-H$_{ax}$ ⟵ 26-Me$_{ax}$ ⟶ 15-H$_{ax}$). Apart from the interconnection of the ^{1}H-coupled blocks, other significant points are revealed. Thus in ring A the 24-Me is axial with the ·CH_2OH-α. The *cis*-D/E fusion is verified (n.O.e. of 27-Me$_{ax}$ with 19-H$_{ax}$, or16-H$_{ax}$ with 21-H$_{ax}$). The latter n.O.e. also shows that the C-16 hydroxy is equatorial (β) whilst the ester at C-21 is also equatorial (β) supplementing evidence derived from ^{1}H n.m.r. coupling constants. From its n.O.e. with 22-H$_{ax}$, the 30-CHO must be axial whilst the 29-Me$_{eq}$ shows n.O. effects with 19- and 21-H$_{ax}$.

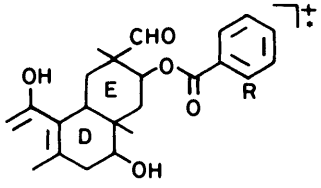
^{13}C N.m.r. assignments are shown in Table 5 and the data in the Table are supported by a 2D ^{13}C–^{1}H correlation diagram for avenestergenin A-1. They are fully consistent with structure (**1**) and the majority of the signals are securely assignable. Excellent analogies for the A/B systems of both the avenestergenin A and B series are to be found in the literature[7] and this makes the absence of an n.O.e. overlap between patches P and Q of little disadvantage (Figures 1 and 2). However, because of the unusual functionalities of the C/D/E system (12-carbonyl, 16-hydroxy, 30-aldehyde, and 21-ester), existing comparative triterpene data are of less direct value and recourse needs to be had to more general ^{13}C n.m.r. assignments and the 2D linkage with ^{1}H data.

Electron impact mass spectral data[8] add some useful confirmatory evidence for the distribution of functional groups in the avenestergenins. Both avenestergenins A and B give the expected ester fragmentation, *N*-methylanthranilic acid being a prominent elimination product, and the well recognised fragmentation (**6**) leads to (**5b**) in the A-1 case and (**5a**) in the A-2 case.

These lead on to the group of D/E fragments (**7**)—(**9**): mass measurements are recorded for the A-2 example. A cleavage such as (**10**) leading to (**11**) distinguishes the A from the B series, mass measurements for (**12**) and (**13**) relating the avenestergenins A-2 and B-2. Formula (**14**), leading to (**15**) represents the origins of the tetracyclic fragment (**16**) measured as (**16a**) in the spectrum of A-2 and as (**16b**) in the spectrum of B-2. A further mode of cleavage producing the similar tetracyclic A–D fragment is apparently prefaced by elimination of oxygenated substituents at C-20 and C-21 to form (**17**) followed by a retro-Diels–Alder reaction to give (**18**). The measurements were for A-2 but the B series shows a similar fragmentation. Finally an A–C fragment was recognised, mass measured as (**21a**) from avenestergenin B-2 and as (**21b**) from avenestergenin A-2. Its origins may be rationalised as in (**19**) and (**20**).

Reduction of avenestergenin A-1 with lithium aluminium hydride strips the ester function and reduces the aldehyde and ketone giving a hexaol, avenagenol (**22**; R = H) characterised as its crystalline hexa-acetate (**22**; R = Ac). Some avenagenol 21-*N*-methylanthranilate was also produced as a side-product. Whilst the hydroxy orientation at C-12 in avenagenol is not rigorously established, it is probably β and on this basis avenagenol is written as (**22**).

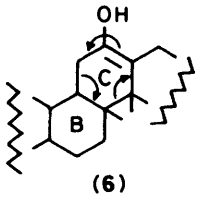
During chromatographic isolation of the avenestergenins formed by acid hydrolysis of the avenacins an interesting discovery was made. Reversed-phase h.p.l.c. columns sometimes blocked, and on stripping the column with 100% methanol higher molecular weight material was encountered. This was found to consist mainly of anhydro dimers (and possibly higher 'oligomers' in some cases) apparently formed on acid treatment. Avenestergenin A-1 (**1**) for example anhydro dimerises to form A-1/A-1 (**23a**), isolated as a high-melting colourless powder which gave no M^+ using electron impact or chemical ionisation mass spectrometry techniques. Using f.a.b. methods for ionization however [matrix: Carbowax 200 or diamylphenol (DAP)–benzonitrile] the compound gave a strong, clear M^+ at *m/z* 1 257, *i.e.* $[637 + 637 - H_2O + 1]^+$. Only one aldehyde group was present in the structure (δ 9.91) and the new acetal ring could be recognised among other expected spectral features by the single acetal proton located at δ 4.98 (see Table 6). Part structure (**24**) shows the conformation around the new dioxane ring and in the case of the A1/A1 series the anhydro dimer possesses the necessary 1,3-diol system and aldehyde functions for anhydro dimerisation to continue. The A-2/A-2 anhydro isomer (**23b**) has similar characteristics to the A1/A1 and formed the expected tetra-acetyl derivative.



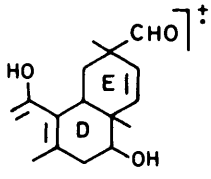
(5)

a; R = H, $C_{23}H_{26}O_5$

b; R = NHMe, $C_{24}H_{29}NO_5$



(6)

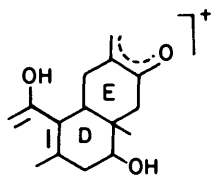


(7)

$C_{16}H_{22}O_3$

Requires: 262·1569

(Found : 262·1564)

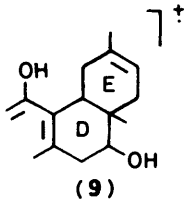


(8)

$C_{15}H_{21}O_3$

249·1490

(249·1523)

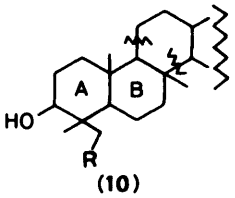


(9)

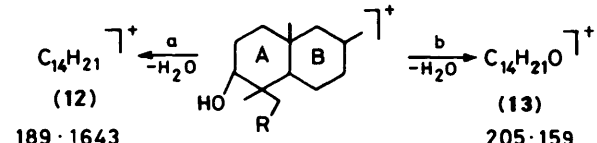
$C_{15}H_{22}O_2$

234·1620

(234·1626)



(10)



$C_{14}H_{21}$ (12)

189·1643

(189·1633)

(11)

$C_{14}H_{23}O$

a; R = H 207·1749

(207·1748)

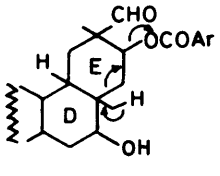
b; R = OH 223·1698

(223·1693)

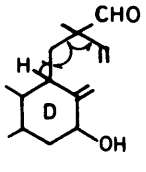
$C_{14}H_{21}O$ (13)

205·159

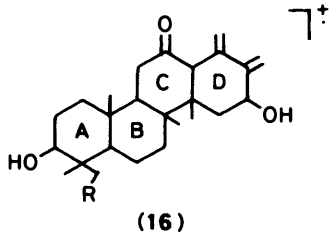
(205·158)



(14)



(15)



(16)

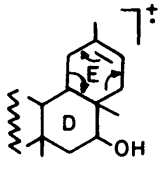
$C_{25}H_{38}O_4$

a; R = OH 402·2770

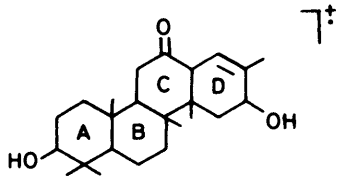
(402·2798)

b; R = H 386·2821

(386·2815)



(17)

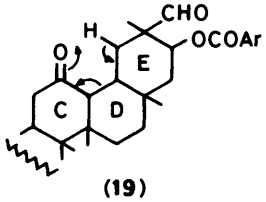


(18)

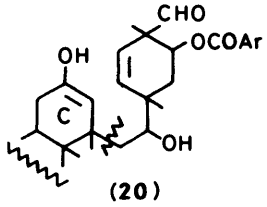
$C_{24}H_{38}O_4$

390·2770

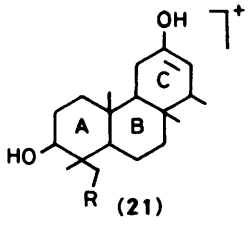
(390·2752)



(19)



(20)



(21)

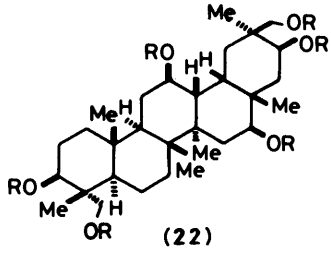
$C_{19}H_{31}O_2$

a; R = H 291·2324

(291·2242)

b; R = OH 307·2273

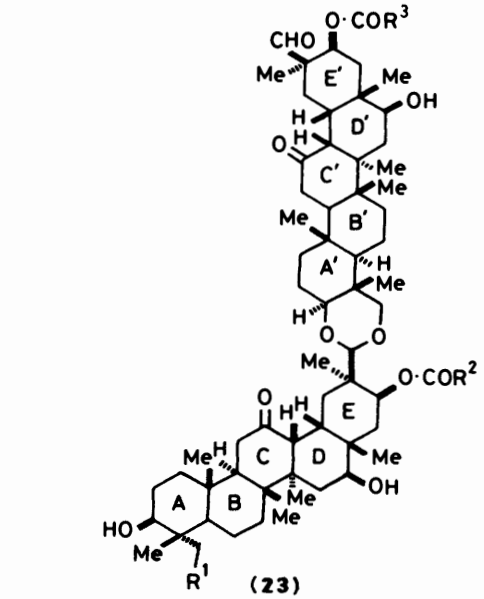
(307·2265)



(22)

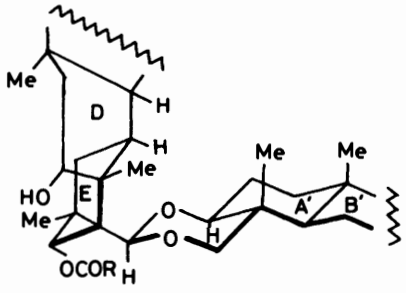
Crossed dimers were also found. Thus avenestergenin A-1 anhydro dimerised with A-2 to give a pair of products A-1/A-2 (**23c**) and A-2/A-1 (**23d**), recognisable in admixture by their ^1H n.m.r. spectra. When avenestergenins of the B series are involved, anhydro dimers can only form with the B component providing the aldehyde part of the dioxane ring, *i.e.* B-1/A-1 (**23f**). The crossed product B-2/A-2 compound (**23e**) was isolated and gave a f.a.b. M^+ (from DAP–benzonitrile) cluster, m/z 1 183/1 184 due to $[608 + 592 - H_2O + 1]^+$. A pair of products B-1/A-2 (**23g**) and B-2/A-1 (**23h**) was also isolated as a mixture.

Early in this investigation attempts were made to solve the structure and stereochemistry of an avenestergenin by *X*-ray methods. Over a period of time no less than 17 samples or derivatives were examined but none proved acceptable or soluble and consequently the above mainly spectroscopic



(23)

a; R^1 = OH, R^2 = R^3 = o-$MeHNC_6H_4$
b; R^1 = OH, R^2 = R^3 = Ph
c; R^1 = OH, R^2 = o-$MeHNC_6H_4$, R^3 = Ph
d; R^1 = OH, R^2 = Ph, R^3 = o-$MeHNC_6H_4$
e; R^1 = H, R^2 = R^3 = Ph
f; R^1 = H, R^2 = R^3 = o-$MeHNC_6H_4$
g; R^1 = H, R^2 = o-$MeHNC_6H_4$, R^3 = Ph
h; R^1 = H, R^2 = Ph, R^3 = o-$MeHNC_6H_4$



(24)

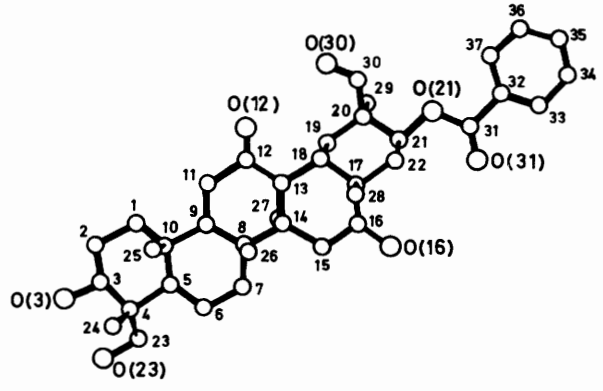


Figure 3. The avenestergenin A-2 molecule including crystallographic numbering

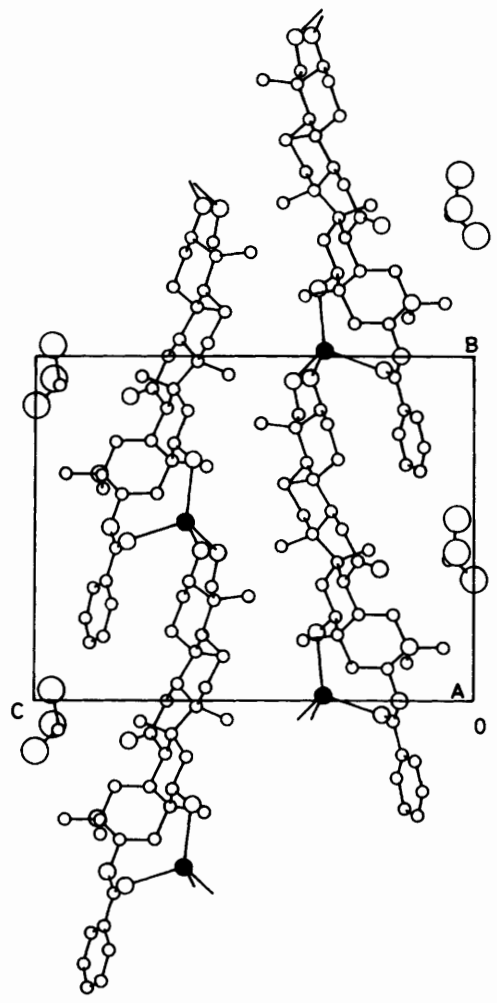


Figure 4. Projection of the crystal structure down the *x* axis including the hydrogen bonds. The water molecule is shown in black

approach was employed. Only one crystal of underivatised material proved suitable for data collection and this was obtained by accident. After shaking a specimen of avenestergenin A-2 in deuteriochloroform with D_2O in an n.m.r. tube during spectroscopic examination, and setting aside, a single crystal grew at the D_2O–$CDCl_3$ interface. *X*-Ray data were collected but the structure remained insoluble by direct methods despite numerous attempts over a period of some years. Success was not achieved until March 1985, some 18 months after our structures (**1**)—(**4**) were published as a preliminary communication.[9] Our original structure (**2**) is confirmed in all details.

Least-squares refinement of the diffractometer produced data converged to *R* 8.98% over 2 092 independent observed reflections. The structure and stereochemistry of the molecule are shown in Figure 3. Bond lengths and bond angles are listed in Tables 7 and 8 respectively together with their standard deviations. Both a [$^2H_2$]water and a deuteriochloroform molecule are incorporated into the crystal structure. The chloroform molecule is disordered, accounting for the relatively high final *R* value and poor bond length and angle data for this molecule. It is not tightly bound in the crystal structure but simply loosely occupies a hole, as can be seen from Figure 4 showing a projection of the crystal structure down the short *x* axis. On the other hand the water molecule forms four strong intermolecular hydrogen bonds to two different avenestergenin

Table 6. Avenestergenin anhydro dimers: ^{1}H n.m.r. spectra ($CDCl_3$); δ values (*J* in Hz)

	Tertiary methyls							23-CH_2OH	23′ Acetal CH_2O
Compound 154 (A-1/A-1) (**23a**)	0.79 (s, 3 H)	0.91 (s, 6 H)	0.93 (s, 3 H)	0.95 (s, 6 H)	1.05 (s, 6 H)	1.14 (s, 6 H)	1.18 (s, 6 H)	3.7 (m)	3.38 (br) (4 H)
Compound 112 (A-2/A-2) (**23b**)	0.82 (s, 3 H)	0.93 (s, 6 H)	0.94 (s, 6 H)	1.04 (s, 3 H)	1.06 (s, 6 H)	1.16 (s, 6 H)	1.18 (s, 6 H)	3.72 (br)	3.42 (m) (4 H)
Compound Q (B-1/A-1) (**23f**)	0.80 (s, 3 H)	0.89 (s, 3 H)	0.92 (s, 3 H)	0.94 (s, 3 H)	1.01 (s, 3 H)	1.03 (s, 3 H)	1.04 (s, 6 H)	1.14 (s, 6 H)	1.16 (s, 3 H)	1.18 (s, 6 H)	3.94 (d, 1 H, *J* 10 Hz)	3.35 (d, 1 H, *J* 10 Hz)
Compound Z (B-2/A-2) (**23e**)	0.80 (s, 3 H)	0.88 (s, 3 H)	0.94 (s, 6 H)	1.01 (s, 3 H)	1.04 (s, 6 H)	1.05 (s, 3 H)	1.10 (s, 3 H)	1.14 (s, 3 H)	1.16 (s, 3 H)	1.18 (s, 6 H)	3.95 (d, 1 H, *J* 10 Hz)	3.35 (d, 1 H, *J* 10 Hz)

	3-$CHOH$	3-Acetal CHO	16-$CHOH$	30-CHO	Aromatic hydrogens					NH*Me*	CHOCOAr	
Compound 154 (A-1/A-1) (**23a**)	~3.7 (2 H)	4.98 (s, 1 H)	4.16 (br, 2 H)	9.91 (s, 1 H)	7.99 (br d, 1 H)	7.90 (dd, 1 H)	7.53 (m, 2 H)	6.68 (cd, 2 H)	6.60 (cd, 2 H)	2.91 (m, 6 H)	5.15 (br d) (2 H)	5.05 (br
Compound 112 (A-2/A-2) (**23b**)	~3.7 (2 H)	4.99 (s, 1 H)	4.2 (br, ~2 H)	9.94 (s, 1 H)	8.09 (d, 2 H)	8.03 (d, 2 H)	~7 6 (m, 2 H)	~7.45 (m, 4 H)			5.19 m, (2 H)	
Compound Q (B-1/A-1) (**23f**)	3.2 (m)	4.92 (s, 1 H)	4.2 (br, 2 H)	9.92 (s, 1 H)	7.8—7.9 (m, 2 H)	~7.6 (m, 2 H)	6.67 (d, 2 H)	~6.6 (m, 2 H)		2.9 (m, 6 H)	5.10 (m, 2 H)	
Compound Z (B-2/A-2) (**23e**)	3.2 (m)	4.94 (s, 1 H)	4.19 (br, 2 H)	9.95 (s, 1 H)	8.05 (m, 4 H)		7.59 (m, 2 H)	7.45 (m, 4 H)			5.16 (m, 2 H)	

Table 7. Bond lengths in Å with standard deviations in parentheses

C(1)–C(2)	1.55(1)	C(16)–C(17)	1.51(1)
C(1)–C(10)	1.52(1)	C(16)–O(16)	1.41(1)
C(2)–C(3)	1.49(1)	C(17)–C(18)	1.56(1)
C(3)–C(4)	1.56(1)	C(17)–C(22)	1.53(1)
C(3)–O(3)	1.44(1)	C(17)–C(28)	1.55(1)
C(4)–C(5)	1.57(1)	C(18)–C(19)	1.53(1)
C(4)–C(23)	1.51(1)	C(19)–C(20)	1.55(1)
C(4)–C(24)	1.54(1)	C(20)–C(21)	1.52(2)
C(5)–C(6)	1.53(1)	C(20)–C(29)	1.54(2)
C(5)–C(10)	1.56(1)	C(20)–C(30)	1.49(2)
C(6)–C(7)	1.55(1)	C(21)–C(22)	1.51(2)
C(7)–C(8)	1.51(1)	C(21)–O(21)	1.48(1)
C(8)–C(9)	1.57(1)	C(23)–O(23)	1.45(1)
C(8)–C(14)	1.61(1)	C(30)–O(30)	1.22(2)
C(8)–C(26)	1.53(1)	C(31)–C(32)	1.50(2)
C(9)–C(10)	1.60(1)	C(31)–O(21)	1.35(1)
C(9)–C(11)	1.51(1)	C(31)–O(31)	1.19(2)
C(10)–C(25)	1.52(1)	C(32)–C(33)	1.39(2)
C(11)–C(12)	1.54(1)	C(32)–C(37)	1.38(2)
C(12)–C(13)	1.49(1)	C(33)–C(34)	1.42(3)
C(12)–O(12)	1.20(1)	C(34)–C(35)	1.30(3)
C(13)–C(14)	1.59(1)	C(35)–C(36)	1.36(3)
C(13)–C(18)	1.55(1)	C(36)–C(37)	1.36(2)
C(14)–C(15)	1.54(1)	C(60)–Cl(61)	1.78(3)
C(14)–C(27)	1.52(1)	C(60)–Cl(62)	1.49(3)
C(15)–C(16)	1.50(1)	C(60)–Cl(63)	1.74(4)

molecules and is thus tightly bound, holding the crystal structure together. The hydrogen bonds are illustrated in Figure 4 and tabulated in Table 9. There is also an intramolecular hydrogen bond within the avenacin molecule. The avenacin molecule adopts the expected conformation with all rings as nearly perfect chairs.

Experimental

Unless otherwise stated n.m.r. data refer to $CDCl_3$ solutions.

Growth and Extraction of Oat Roots.—Oat seeds which had not been pre-treated with fungicide (in early work the variety Victory was used but most of our work was done with Peniarth) were germinated on gauze mats placed on expanded aluminium mesh over water-filled 9 in × 14 in plastic trays in a greenhouse. In a normal run 64 such trays were used, each being enclosed with aluminium foil in the early stages. Each tray was aerated continuously by a pump system and growth of batches continued throughout the year. After 23 days roots were cut away and excess of water was removed by centrifugation in a spin-drier. The material was freeze-dried and then deep-frozen until extraction.

Freeze-dried material from at least two growth periods was combined (the weight varied with season and variety of oat from 300—700 g). In a typical extraction Peniarth oat roots (dry wt. 400 g) were extracted twice with 80% methanol (8 l and then 3 l), followed by twice with 100% methanol (2 × 3 l). The combined extracts were evaporated at <40 °C, redissolved in methanol (200 ml), and filtered: ether (450 ml) was added to the filtrate and the precipitated material was collected. The latter was dissolved in water (120 ml) and ether (210 ml) was added and the mixture left at 0 °C for 2—3 h. The fine precipitate was collected by cold centrifugation (10 000 r.p.m.), followed by freeze drying. Crude avenacin (2.3 g, 0.58%) was thus obtained. Crude yields varied with season and oat variety being between 0.12 and 0.8% based on dry root.

Chromatographic Isolation of the Avenacins.—In this second isolation method the crude methanol extract from freeze-dried oat roots (800 g) was adsorbed onto silica and packed on a silica column (10 × 22 cm, containing 800 g of adsorbent). Elution was as follows: (1) 4% methanol in chloroform (2.5 l), (2) chloroform–methanol (2:1; 2.5 l), (3) chloroform–methanol–water (63:32:5; 1.5 l), (4) chloroform–methanol–water (53:38:9; 1.6 l), (5) chloroform–methanol–water 45:44:22; 2.6 l) (6) chloroform–methanol–water (30:55:15; 1.5 l), (7) methanol–water (4:1; 1.5 l). Crude avenacins (4.69 g, 0.59% of

Table 8. Bond angles in degrees with standard deviations in parentheses

C(2)–C(1)–C(10)	112.2(8)	C(15)–C(14)–C(27)	108.8(7)
C(1)–C(2)–C(3)	110.8(8)	C(14)–C(15)–C(16)	116.3(8)
C(2)–C(3)–C(4)	114.4(7)	C(15)–C(16)–C(17)	114.7(8)
C(2)–C(3)–O(3)	104.6(7)	C(15)–C(16)–O(16)	104.3(8)
C(4)–C(3)–O(3)	111.3(8)	C(17)–C(16)–O(16)	112.4(9)
C(3)–C(4)–C(5)	106.3(7)	C(16)–C(17)–C(18)	108.8(8)
C(3)–C(4)–C(23)	106.7(8)	C(16)–C(17)–C(22)	114.1(9)
C(3)–C(4)–C(24)	112.0(7)	C(16)–C(17)–C(28)	110.0(8)
C(5)–C(4)–C(23)	106.3(7)	C(18)–C(17)–C(22)	107.5(8)
C(5)–C(4)–C(24)	114.6(8)	C(18)–C(17)–C(28)	111.6(9)
C(23)–C(4)–C(24)	110.5(8)	C(22)–C(17)–C(28)	104.9(8)
C(4)–C(5)–C(6)	113.3(7)	C(13)–C(18)–C(17)	109.1(1)
C(4)–C(5)–C(10)	115.4(7)	C(13)–C(18)–C(19)	115.7(8)
C(6)–C(5)–C(10)	111.0(7)	C(17)–C(18)–C(19)	112.3(8)
C(5)–C(6)–C(7)	110.3(7)	C(18)–C(19)–C(20)	111.5(8)
C(6)–C(7)–C(8)	114.3(7)	C(19)–C(20)–C(21)	107.0(8)
C(7)–C(8)–C(9)	109.4(7)	C(19)–C(20)–C(29)	109.3(9)
C(7)–C(8)–C(14)	110.2(7)	C(19)–C(20)–C(30)	111.0(10)
C(7)–C(8)–C(26)	106.5(8)	C(21)–C(20)–C(29)	110.4(11)
C(9)–C(8)–C(14)	106.5(7)	C(21)–C(20)–C(30)	113.0(10)
C(9)–C(8)–C(26)	113.5(7)	C(29)–C(20)–C(30)	106.2(10)
C(14)–C(8)–C(26)	110.8(7)	C(20)–C(21)–C(22)	114.4(10)
C(8)–C(9)–C(10)	114.7(7)	C(20)–C(21)–O(21)	105.1(8)
C(8)–C(9)–C(11)	112.0(8)	C(22)–C(21)–O(21)	108.8(9)
C(10)–C(9)–C(11)	112.1(7)	C(17)–C(22)–C(21)	116.0(8)
C(1)–C(10)–C(5)	107.7(7)	C(4)–C(23)–O(23)	108.9(8)
C(1)–C(10)–C(9)	106.3(7)	C(20)–C(30)–O(30)	126.1(12)
C(1)–C(10)–C(25)	109.6(8)	C(32)–C(31)–O(21)	111.3(12)
C(5)–C(10)–C(9)	105.5(7)	C(32)–C(31)–O(31)	124.3(13)
C(5)–C(10)–C(25)	114.4(7)	O(21)–C(31)–O(31)	124.4(11)
C(9)–C(10)–C(25)	112.9(7)	C(31)–C(32)–C(33)	116.2(14)
C(9)–C(11)–C(12)	115.8(8)	C(31)–C(32)–C(37)	121.8(13)
C(11)–C(12)–C(13)	115.8(7)	C(33)–C(32)–C(37)	122.0(14)
C(11)–C(12)–O(12)	119.4(9)	C(32)–C(33)–C(34)	115.4(17)
C(13)–C(12)–O(12)	124.6(9)	C(33)–C(34)–C(35)	123.4(17)
C(12)–C(13)–C(14)	111.3(7)	C(34)–C(35)–C(36)	118.5(16)
C(12)–C(13)–C(18)	115.0(7)	C(35)–C(36)–C(37)	123.8(19)
C(14)–C(13)–C(18)	116.0(7)	C(32)–C(37)–C(36)	116.8(17)
C(8)–C(14)–C(13)	106.1(6)	C(21)–O(21)–C(31)	117.7(9)
C(8)–C(14)–C(15)	111.5(7)	Cl(61)–C(60)–Cl(62)	116.9(23)
C(8)–C(14)–C(27)	111.6(7)	Cl(61)–C(60)–Cl(63)	89.4(16)
C(13)–C(14)–C(15)	109.4(7)	Cl(62)–C(60)–Cl(63)	111.8(24)
C(13)–C(14)–C(27)	109.4(7)		

Table 9. Hydrogen bonds

O(3)–H(03)–O(23)		2.64(1)
O(16)–H(016)–O(50)	($x-1, y, z$)	2.83(1)
O(23)–H(023)–O(50)	($x-1, y+1, z$)	2.82(1)
O(50)–H(050a)–O(3)	($x, y-1, z$)	2.72(1)
O(50)–H(050b)–O(31)	($x+1, y, z$)	2.78(1)

Table 10. Atomic co-ordinates

Atom	*x/a*	*y/b*	*z/c*
C(1)	0.440(1)	0.7050	0.312 3(6)
C(2)	0.473(1)	0.805 8(7)	0.338 3(6)
C(3)	0.289(1)	0.848 3(6)	0.352 0(5)
C(4)	0.187(1)	0.796 2(6)	0.408 6(5)
C(5)	0.164(1)	0.694 1(6)	0.381 5(5)
C(6)	0.056(1)	0.633 6(7)	0.429 0(6)
C(7)	−0.002(1)	0.541 9(6)	0.390 1(5)
C(8)	0.165(1)	0.488 6(7)	0.368 8(5)
C(9)	0.289(1)	0.553 1(6)	0.326 3(5)
C(10)	0.355(1)	0.646 8(6)	0.366 9(5)
C(11)	0.455(1)	0.503 1(7)	0.301 7(6)
C(12)	0.407(1)	0.409 8(7)	0.265 4(5)
C(13)	0.272(1)	0.351 3(6)	0.299 5(5)
C(14)	0.087(1)	0.407 3(6)	0.313 7(5)
C(15)	−0.051(1)	0.343 1(7)	0.347 2(5)
C(16)	−0.076(2)	0.248 6(7)	0.315 4(6)
C(17)	0.110(2)	0.198 2(7)	0.311 4(5)
C(18)	0.233(1)	0.255 7(7)	0.264 5(6)
C(19)	0.148(2)	0.255 2(7)	0.183 8(6)
C(20)	0.138(2)	0.157 0(7)	0.151 8(6)
C(21)	0.016(2)	0.100 5(7)	0.195 9(7)
C(22)	0.082(2)	0.102 7(7)	0.276 9(6)
C(23)	−0.016(1)	0.834 6(7)	0.402 0(6)
C(24)	0.294(1)	0.807 9(7)	0.486 8(6)
C(25)	0.504(1)	0.633 0(7)	0.434 6(6)
C(26)	0.278(1)	0.450 2(7)	0.439 3(5)
C(27)	−0.018(1)	0.444 1(7)	0.242 0(5)
C(28)	0.220(2)	0.181 1(8)	0.389 2(6)
C(29)	0.044(2)	0.160 2(10)	0.071 2(7)
C(30)	0.336(2)	0.118 5(9)	0.152 8(7)
C(31)	−0.107(2)	−0.054 6(9)	0.182 7(6)
C(32)	−0.058(3)	−0.150 4(9)	0.161 7(6)
C(33)	−0.188(3)	−0.218 4(10)	0.174 1(8)
C(34)	−0.137(4)	−0.308 5(12)	0.156 4(9)
C(35)	0.012(4)	−0.327 4(12)	0.125 6(10)
C(36)	0.135(3)	−0.258 4(13)	0.115 4(10)
C(37)	0.108(3)	−0.169 9(10)	0.133 6(8)
O(3)	0.342(1)	0.939 9(5)	0.376 5(5)
O(12)	0.485(1)	0.386 9(6)	0.215 5(4)
O(16)	−0.193(1)	0.202 8(5)	0.360 0(5)
O(21)	0.030(1)	0.005 3(5)	0.169 8(4)
O(23)	−0.004(1)	0.932 6(5)	0.416 2(5)
O(30)	0.482(2)	0.162 2(10)	0.147 3(10)
O(31)	−0.245(2)	−0.034 9(7)	0.209 0(6)
O(50)	0.664(1)	0.021 4(5)	0.342 6(5)
C(60)	0.490(5)	0.918 6(24)	0.944 4(14)
Cl(61)	0.247(2)	0.936 5(10)	0.957 7(5)
Cl(62)	0.611(4)	0.859 6(18)	0.998 5(8)
Cl(63)	0.536(4)	1.034 3(15)	0.960 8(15)

dried roots) were eluted in fractions (4) and (5). After h.p.l.c. purification as described below the yield of pure avenacin A-1 was 0.03% of dried oat roots, together with smaller amounts of the other avenacins. This alternative method was less preferred to that above: it was also expensive in terms of silica and solvents. Our first method was modified from the work of Maizel *et al.*,[4] and this from the work of Tschesche *et al.*[6]

Separation and Purification of the Avenacins.—In a typical example crude avenacin (2.3 g, originating from the first extraction method) in methanol (44 ml) and water (4 ml) was injected onto a Waters Pre Pak-500/C_{18}-reversed phase column (5.7 × 30 cm) and eluted with 75% methanol in water at 150 ml/min: detection was by a refractive index recorder. Three main peaks were collected: fraction 1 (1.70 g), dark colour, containing no avenacin; fraction 2 (0.21 g) contained mainly the benzoate esters, avenacins A-2 and B-2; fraction 3 (0.25 g) contained mainly *N*-methylanthranilate esters, avenacins A-1 and B-1.

Material from fraction 2 (720 mg: from several separations) was further purified by reversed-phase h.p.l.c. on a 1 in. diam. Waters C_{18}-preparative column (55—100 μm particles) eluting with 70% methanol in water at 50 ml/min to give avenacins A-2 plus B-2 (100 mg, 0.01% dry root wt.) free from *N*-methylanthranilate esters. Material of fraction 3 was treated in a similar way, eluting with 75% methanol in water to give avenacins A-1 plus B1 (0.015% of dry root wt).

H.p.l.c. of the products from these two separations was continued using a similar C_{18} reversed phase column (Partisil M9 ODS-2), eluting with 70 or 80% methanol at 1 ml/min and monitoring by u.v. absorption (245 mm) and R.I. simul-

taneously. This separated each of the two products into a major and a minor component, the minor component being *ca.* 10% of the major. Thus the product from fraction 2 gave avenacin A-2 as the major and B-2 as the minor component. The product from fraction 3 gave avenacin A-1 as the major and B-1 as the minor. These products at this stage were seldom pure and rechromatography (C_{18}-reversed phase) was continued until purity was attained. Physical data for the four avenacins is mainly tabulated in this and the following paper.

Avenacin A-1. This formed microneedles from methanol. Mass spectrum (f.a.b., +ve ion): 1 116 (*M* + 23, sodium, 22%), 1 095 (*M* + 2, 57%), 1 094 (*M* + 1, 100%), 1 093 (*M*, 27%), 932 [(*M* + 1) −162 (hexose), 19%], 770 [(*M* + 1) −324 (2 hexoses), 5%], 638 [(*M* + 1) −324 and −132 (2 hexoses and one pentose), 78%]; f.a.b. (−ve ion) 1 092 (*M* − 1).

Avenacin A-2. This was microcrystalline. Mass spectrum (f.a.b., +ve ion) 1 087 (*M* + 23); f.a.b. (−ve ion) 1 065 (*M* + 1, 23%), 1 064 (*M*, 58%), 1 063 (*M* − 1, 100%), 901 (−162, 14%), 739 (−324, 3%), 607 (−324 and −132, 1.5%).

Avenacin B-1. This was a powder—probably a glass. Mass spectrum (f.a.b., +ve ion) 1 102 (*M* + 24, 100%), 1 100 (*M* + 22, 96%), 1 078 (*M* + 1, 80%), 916 [(*M* + 1) −162, 14%], 622 [(*M* + 1 −324 and −132, 44%].

Avenacin B-2. This was a powder, probably a glass. Mass spectrum (f.a.b., +ve ion) 1 073 (39%), 1072 (60%), 1 071 (*M* + 23, 100%), 594 (21%), 593 [(*M* + *1*) −324 and −132, 44%]; f.a.b. (−ve ion) 1 049 (37%), 1 048 (*M*) (62%), 1 047 (*M* − 1, 100%), 885 [(*M* − 1) −162, 19%].

Formation and Purification of the Avenestergenins.—Crude avenacin (2.75 g: after precipitation and freeze drying) was heated with M-hydrochloric acid (220 ml) under reflux (2.5 h). After cooling the precipitate was filtered off, well washed with dilute aqueous sodium carbonate and dried *in vacuo* at 40 °C (0.46 g, 17%). Extraction of the aqueous layer with chloroform yielded further material (0.01 g). The total sample was added to silica and packed on a dry silica column in nylon tubing (1 cm × 30 cm) and eluted (ethyl acetate). Ten bands were cut off and stripped with chloroform. After investigation by t.l.c. (silica, eluant ethyl acetate), these were appropriately combined to give fraction 1 (40 mg, mainly avenestergenins B-1/B-2), fraction 2 (20 mg, avenestergenins A-1/A-2/B-1/B-2), and fraction 3 (165 mg, mainly avenestergenins A-1/A-2 and fraction 4 (110 mg, containing little avenestergenins).

Fractions 2 and 3 were united (450 mg from combined samples) and packed on a dry column of (1 × 6 cm) of Woelm grade IV alumina and eluted successively with ether, 5% methanol in ether, 15% methanol in ether, 30% methanol in ether, and 20% methanol in chloroform. Fractions (80 mg) eluting with 5% methanol in ether were mainly avenestergenins B-1/B-2. Avenestergenins A-1/A-2 (170 mg) eluted mainly in 15% methanol in ether. Fraction 1 above (130 mg from combined samples) was separated on a similar alumina column, eluting with benzene, 20% ether in benzene, 40% ether in benzene, 70% ether in benzene, 100% ether, and 4% methanol in ether increasing to 20% methanol in ether, Avenestergenins B-1/B-2 (60 mg) eluted in 20—40% ether in benzene. Small amounts of avenestergenins A-1/A-2 eluted in 4% methanol in ether.

Separation of the Avenestergenins A-1 *and* A-2.—The A-1/A-2 mixture (170 mg) resulting from alumina chromatography was dissolved in a minimum of chloroform and injected (5 mg in 50 or 100 μl at a time) onto a reversed phase h.p.l.c. column (Partisil M9 10/50 ODS-2) and eluted with methanol–water (9:1) at 1.5 ml/min using either i.r. or u.v. (at the less sensitive λ 243 nm wavelength). Two main peaks were eluted in 90% methanol, avenestergenin A-2 (20 mg, R_t 24 min) and avenestergenin A-1 (20 mg, R_t 31 min). Further elution with 100% methanol gave compound 112 (5 mg), compound 131 (9 mg) and compound 154 (5 mg), anhydro dimers having high and indeterminate m.p.s.

Avenestergenin A-1. This formed microneedles from methanol–water, m.p. 210—211 °C; M^+, 637.3960 ($C_{38}H_{55}NO_7$ requires *M*, 637.3978): on trimethylsilylation, three hydroxy groups were derivatised (M^+, 853), $\nu_{max.}$($CHCl_3$) (high resolution F.T. instrument) 1 722 (CHO), 1 695 (C=O) and 1 676 cm^{-1} (ester); δ[$^2H_5$-pyridine) 10.26 (1 H, d, *J ca.* 1 Hz), 8.22 (1 H, dd, *J* 1.6, 8.2 Hz), 7.91 (1 H, br d, *J ca.* 4 Hz, D_2O exchg.), 7.45 (1 H, dt), 6.65 (1 H, dd, *J* 0.7 and 9 Hz), 6.66 (1 H, dt, *J* 7.5 and 0.6), 6.40 (1 H, br, D_2O exchg.), 6.14 (1 H, br, D_2O exchg.), 5.71 (1 H, dd, *J* 4.2, and 12.4 Hz), 5.10 (1 H, br, D_2O exchg.), 4.51 (1 H, dd, *J* 4.9 and 10.7 Hz), 4.21 (1 H, d, *J* 10.5 Hz), *ca.* 4.2 (1 H, dd, *J ca.* 10, *ca.* 5 Hz), 3.72 (1 H, d, *J* 10 Hz), 3.11 (1 H, dd, *J* 3.5 and 14.1 Hz), *ca.* 3.02 (2 H, m) [after D_2O clarifies into 3.01 (1 H, d, *J* 4.4 Hz), and 2.93 (1 H, dd, *J* 4.8, 13.1 Hz)], 2.79 (3 H, d, *J* 3 Hz), 2.55—2.3 (3 H, m), 2.1—1.84 (4 H, m), 1.86—1.64 (3 H, m) [including 1.74 (1 H, d, *J* 5 Hz), and 1.64 (1 H, dd, *J* 5 and 13.5 Hz)], 1.64—1.41 (5 H, m), 1.35 (1 H), 1.18 (3 H, s), 1.16 (3 H, s), 1.15 (3 H, s), 1.06 (3 H, s), 1.05 (3 H, s), and 0.95 (3 H, s). The compound formed (pyridine–acetic anhydride) a triacetate m.p. 168—170 °C (Found: M^+, 763. $C_{44}H_{61}NO_{10}$ requires *M*, 763) and also a non-fluorescent tetra-acetate (Found: M^+, 805. $C_{46}H_{63}NO_{11}$ requires *M*, 805). For other data see Tables.

Avenestergenin A-2. This crystallised as fine needles, m.p. 189—190 °C from methanol–water. It had M^+, 608.3740 ($C_{37}H_{52}O_7$ requires *M*, 608.3713). Its triacetate had m.p. 142—143 °C (Found: M^+, 734. $C_{43}H_{58}O_{10}$ requires *M*, 734). For other data see Tables.

Separation of the Avenestergenins B-1 *and* B-2.—This was effected on the same type of column as for the A compounds, using elution of the B-1/B-2 mixture above (60 mg) with 100% methanol for these less polar compounds. Avenestergenin B-2 eluted first (8 mg, R_t 19 min), followed by B-1 (10 mg, R_t 22 min). The later peaks, compound Z (1 mg), compound P (1.5 mg), and compound Q (2 mg) proved to be dimers of avenestergenins A and B of similar high and indeterminate m.p.

Avenestergenin B-1 formed very fine needles from chloroform–methanol, m.p. 186—187 °C and had M^+, 621.4016. ($C_{38}H_{55}NO_6$ requires *M*, 621.4029). A diacetate m.p. 108 °C was obtained on acetylation with pyridine–acetic anhydride: it had M^+, 705); o.r.d. data (dioxane); $\lambda_{max.\ or\ min.}$ 342 (−759), 309 (+660), 273 (−660), 257 (+1 420), and 244 nm (−1 680). For other data see Tables.

Avenestergenin B-2. This also formed very fine needles from chloroform and methanol, m.p. 177—178 °C. It had M^+, 592.3781 ($C_{37}H_{52}O_6$ requires 592.3764). The diacetate was a glass, M^+, 676 ($C_{41}H_{56}O_8$ requires *M*, 676). For other data see Tables.

Methanolysis of Avenestergenins.—Avenestergenin A-1 (80 mg) was kept overnight at 20 °C with dry methanol (5 ml) in which a small piece of sodium had been dissolved. It was extracted with ether and the extract was separated by p.l.c. on two 20 × 20 cm HF 254 preparative silica plates, eluting with ethyl acetate. The uppermost fluorescent band (R_f 0.93) (20 mg) was identical with an authentic sample of methyl *N*-methylanthranilate. It had M^+, 165.0787 (Calc. for $C_9H_{11}NO_2$: 165.0790) and an identical ^1H n.m.r. spectrum. Avenestergenin A-2 similarly gave methyl benzoate. Mixed A-1/A-2 and B-1/B-2 samples similarly gave methyl *N*-methylanthranilate and methyl benzoate separated and identified by g.l.c. (OV17 at 150 °C)/m.s.

Avenestergenin anhydro dimers. For isolation see above. Compound 154, avenestergenin anhydro dimer A-1/A-2 (**23a**), was a white powder, probably a glass, with a very high indefinite m.p. A mass spectrum could not be obtained by electron impact or chemical ionization but the f.a.b. method (Carbowax 200 matrix) gave a strong clear $(M + 1)^+$ at 1 257 [(637 + 637 − H_2O) + 1]$^+$ ($C_{76}H_{108}N_2O_{13}$ requires *M*, 1 256.7): a second f.a.b. measurement (diamylphenol + benzonitrile) also gave $(M + 1)^+$ 1 257 with cleavage to an avenestergenin A-1 unit $(M + 1)^+$ 638. For ^{1}H n.m.r. data see Table 6.

Compound 112, avenestergenin anhydro dimer A-2/A-2 (**23b**) (see Table 6) was an amorphous powder, had $\lambda_{max.}$ 227 nm (ε 22 000) and gave a tetra-acetate [acetates ^{1}H δ: 2.15 (3 H, s), 2.09 (6 H, s), and 2.03 (3 H, s)].

Compound Z, avenestergenin anhydro dimer B-1/A-2 (**23e**), was a high m.p. (indefinite) powder; it also had a molecular ion cluster (matrix diamylphenol and benzonitrile) at 1 183/1 184 [(608 + 592 − H_2O) + 1]$^+$ ($C_{70}H_{102}O_{12}$ requires *M*, 1 182.7). For ^{1}H n.m.r. data on this, and compound Q, avenestergenin anhydro dimer B-1/A-1 (**23f**) see Table 6.

Compound 131 was not homogeneous but consisted of a mixture of two avenestergenin anhydro dimers, A-1/A-2 (**23c**) and A-2/A-1 (**23d**) involving the aldehyde of A-1 with the diol of A-2 or the aldehyde of A-2 with the diol of A-1. The f.a.b. spectrum (glycerol–thioglycerol) showed ions at *m/z* 1 242 (1 227 + 15)$^+$ 1 210 (very strong) (1 227 − 18 + 1)$^+$, and fragments 638 [(A-1) + 1] and 609 [(A-2) + 1] ($C_{75}H_{105}NO_{13}$ requires *M*, 1 227.7). The ^{1}H n.m.r. spectrum was complex but contained the following: 9.93 (0.5 H, s, CHO of one compound), 9.90 (0.5 H, s, CHO of other compound), 8.05 (2 H, m, benzoate aromatics), 7.88 (0.5 H, dd, *N*-methylanthraniliate aromatics), 7.4—7.6 (4.5 H, m, mixed benzoate and anthranilate aromatics), 6.6—6.7 (2 H, m, *N*-methylanthranilate protons), 4.98 (1 H, s, 3-acetal proton), 2.91 (1.5 H, s, NH*Me*).

Compound P also was a mixture, this time of anhydro dimers B-1/A-2 (23 g) and B-2/A-1 (23 h). The ^{1}H spectrum was again complex but contained: 9.95 (0.5 H, s, aldehyde), 9.92 (0.5 H, s, aldehyde), 8.05 (2 H, m), 7.9 (0.5 H, dd), 7.3—7.7 (4.5 H, m), 6.6—6.67 (2 H, m) (total 9 ArH), 4.94 (0.5 H, s, 3-acetal H), 4.92 (0.5 H, s, 3-acetal H), 2.91 (1.5 H, s), and 2.93 (1.5 H, s) (anthranilate *N*-methyls).

Reduction of Avenestergenin A-1 *with Lithium Aluminium Hydride* [*Avenagenol Hexa-acetate* (**22**; R = Ac)].—Avenestergenin A-1 (37 mg) was dissolved in dry tetrahydrofuran (3 ml) and added with stirring to lithium aluminium hydride (20 mg) in dry THF. Stirring was continued (3.5 h) and the product was worked up and chromatographed on neutral alumina grade IV eluting first with carbon tetrachloride, through ether to ethyl acetate, and finally 50% methanol in ethyl acetate. Fractions 11—19, eluting with 10% methanol in ethyl acetate, were combined and further purified on a C_{18} Microbondapak semi-preparative column, eluting with 80% methanol in water at 2.5 ml/min. Three bands were collected. The minor two (112 and 113) were still anthranilate esters with characteristic blue fluorescence). The major, non-fluorescent compound (111) was acetylated with pyridine–acetic anhydride for 2 days. Preparative layer chromatography on silica G HF254 in ethyl acetate–petroleum (b.p. 60—80 °C) (1 : 1) gave *avenagenol hexa-acetate*, m.p. 220—223 °C. Under electron impact it showed a M^+, 760 ($C_{42}H_{64}O_{12}$ requires M^+, 760). The sample was re-acetylated and M^+ remained unchanged. In the ^{1}H n.m.r. spectrum there were 6 acetate resonances at: 2.06 (9 H), 2.02 (3 H), and 2.00 (6 H): there was no aldehyde proton.

Compound 112 had M^+, 641 ($C_{38}H_{59}NO_7$ requires *M*, 641). N.m.r. data show that the *N*-methylanthranilate residue is still present whereas the aldehyde proton has disappeared. It is avenagenol 21-*N*-methylanthranilate.

Crystallographic Analysis of Avenestergenin.—A suitable sample fortuitously crystallised in an n.m.r. tube containing $CDCl_3$ and D_2O after more conventional recrystallisations had failed. The only crystal produced had dimensions 0.7 × 0.5 × 0.25 mm^3 and space group and preliminary cell parameters were initially determined photographically. For intensity measurement the crystal was mounted on a Hilger and Watts Y290 four-circle diffractometer. Accurate lattice parameters were obtained by least-squares refinement of the positions of 23 reflections measured on the diffractometer with θ *ca.* 12°. Intensity data were collected with Mo-K_α radiation using an ω/2θ scan for 1° < θ < 25°. A total of 3 422 independent reflections was measured of which 2 092 had $I > 5\delta(I)$ and were considered observed and used in the subsequent refinement. The data were corrected for Lorentz and polarisation factors but no absorption corrections were applied. Data reduction and subsequent crystallographic calculations were performed using the CRYSTALS[10] system of programs.

Crystal data. $C_{37}H_{52}O_7 \cdot D_2O \cdot CDCl_3$, *M* = 749.21, Monoclinic, *a* = 7.015(2), *b* = 14.608(2), *c* = 18.582(4) Å, β = 98.96(2)°, *U* = 1 880.96 Å^3, *Z* = 2, D_c = 1.32 g cm^{-3}, *F*(000) = 796, space group $P2_1$, Mo-K_α radiation, λ = 0.710 69 Å, μ(Mo-K_α) = 3.0 cm^{-1}.

Structure solution and refinement. Innumerable attempts to solve the structure by direct methods were made over a period of more than 5 years without success. False solutions typically showed only small ring systems or infinite hexagons ('chicken wire') but attempts to develop the more promising trial structures were all unsuccessful. Eventually another run of the MULTAN program[11] with a different variation of starting parameters produced a set of phases with by no means the best figures of merit, whose *E* map showed a different and more promising ring system. 28 Atomic positions were selected from this solution which gave an *R* value of only 46%. A succession of least-squares calculations and difference-Fourier maps was used to slowly locate the remaining atomic positions a few at a time. Ten difference-map calculations were required to locate all 49 non-hydrogen atoms (including solvent molecules). Full-matrix isotropic least-squares refinement of these positions gave a value for *R* of only 17.4%.

Refinement was continued with anisotropic thermal parameters but blocked least-squares was necessary. A difference map revealed the approximate positions of many of the hydrogen atoms. Geometric considerations were then used to calculate the accurate positions of those hydrogen atoms that could be fixed in this way. The remaining hydrogen atom positions were taken directly from the peaks in the difference map. The hydrogen atoms were then included in the calculations but without refinement. Other major features in the difference map were peaks in the neighbourhood of the chloroform molecule. This is clearly disordered, merely loosely filling a hole in the crystal structure. Attempts were made to allow for this with multiple chlorine atom positions with occupation fractions without success. A weighting scheme based on a Chebyshev polynomial was adopted. Refinement finally converged with the largest parameter shift 0.1σ after more than 150 cycles of least-squares refinement. The final *R* value at convergence was 8.98% with R_w 11.83%. A final difference map was calculated which showed peaks of 0.6 e Å^{-3} in the vicinity of the chloroform molecule but no features > 0.3 e Å^{-3} elsewhere. The poor agreement is thus largely due to the disordered chloroform solvent molecule. Final atomic co-ordinates are listed in Table 10, and calculated hydrogen positions and temperature factors are listed as a Supplementary publication [Sup. No. 56624 (4 pp.)].*

* Structure factors are available from the Editorial office on request.

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