Structures of the Oat Root Resistance Factors to 'Take-All' Disease, Avenancins A-1, A-2, B-1 and B-2 and Their Companion Substances

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It is shown that avenestergenins, having a 12-oxo group, are not true aglycones of the avenacin series: the latter are 12, 13β-epoxides. Acid hydrolysis would be expected to lead to a 13α, 12-ketone, not the 13β, 12-ketone of the avenestergenins, and the chemistry of the process is modelled using the 12α, 13α- and 12β, 13β-epoxides from 3β-benzoyloxyolean-12-ene and isolating the 13α, and 13β, 12-ketones. The former is readily converted into the latter under acid conditions similar to those employed for hydrolysis of the avenacins. Search of the oat extractives has resulted in isolation of the true free aglycone of the avenacin A-1 series, named epoxyavenagenin A-1.

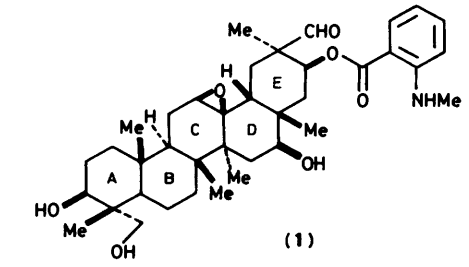
By combination of f.a.b. m.s., methylation, ^{13}C and ^{1}H n.m.r. techniques, the trisaccharide chain of all four avenacines is shown to be [β-D-glucopyranosyl(1 → 4)]-[β-D-glucopyranosyl(1 → 2)]-α-L-arabinopyranosyl attached at the triterpene 3-β-hydroxy group. This completes structural and stereochemical details for avenacins A-1, A-2, B-1, and B-2.

As minor components of healthy oat root extract, two compounds formulated as glucoavenacin A-1 and deglucoavenacin A-1 have been isolated. The latter is of particular interest as, along with bis-deglucoavenacin A-1, they are detoxified products of avenacin A-1 formed by the highly virulent *Gaeumannomyces graminis* var. *avena* which can attack oat roots.

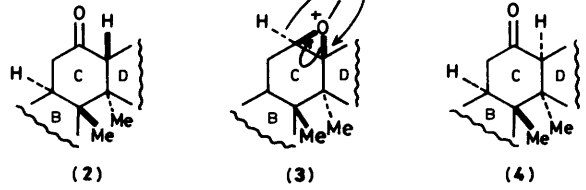
The structures of the four avenestergenins A-1, A-2, B-1, and B-2, formed by acid hydrolysis of the trisaccharide-containing glycosides the avenacins from oat roots, are described in the previous paper.[1] However, it soon emerged that they are not the true aglycones. Thus, although i.r. absorption due to the 30-aldehyde is present in the avenacins [$\nu_{max.}$(KBr) 1 721 cm^{-1}], as is that due to the C-21 ester linkage (1 677 cm^{-1}), that assigned to the 12-oxo carbonyl in the avenestergenins (near 1 695 cm^{-1}) could not be located. Furthermore, the ^{13}C n.m.r. carbonyl at δ 211.4 p.p.m. in avenestergenin A-1 is lacking in avenacin A-1. However, in its place a new carbon doublet resonance has appeared at δ 54.2. Avenestergenins have the molecular formulae one would expect from the corresponding deglycosated avenacins so we were led to the view that the avenacins and their true aglycones contain 12,13-epoxides which rearrange to 12-carbonyls during treatment with acid.

This viewpoint was greatly strengthened as a result of a search in the original oat-root extracts instituted, to see if free aglycone could be found. A 12,13-epoxy compound of the avenestergenin A-1 series, $C_{38}H_{55}NO_7$, m.p. 218—221 °C, isomeric with avenestergenin A-1 itself was found. It was named epoxyavenagenin A-1. The compound contained an *N*-methylanthranilic acid residue and its ^{1}H n.m.r. spectrum in C_5D_5N (Table) or $CDCl_3$ was very similar to avenestergenin A-1 except in the C ring region. The epoxy aglycone had $\nu_{max.}$($CHCl_3$) 1 725 and 1 675 cm^{-1} with no trace of a 12-oxo carbonyl near 1 695 cm^{-1} even when examined under high resolution by Fourier transform i.r. spectroscopy. Acid treatment converted it into the familiar 12-ketone avenestergenin A-1, described in the previous paper. So far as the orientation of the epoxide is concerned, comparative ^{13}C n.m.r. evidence strongly favoured a β-arrangement. Thus, avenacin A-1 had ^{13}C n.m.r. resonances for the epoxide carbons δ 54.2 (C-12) and 66.0 p.p.m. (C-13), whilst for comparison the 12β,13β-carbons in 3β-acetoxy-12β-epoxyoleanane resonate at δ 53.8 and 66.3 p.p.m. and the corresponding carbons of the 12α,13α-epoxide resonate at δ 65.5 and 63.1.[2] The true aglycone of avenacin A-1 isolated in this work is thus formulated (**1**).

However, the assignment of the β-epoxy orientation meant that the acid rearrangement to the ketone (**2**) would probably not be straightforward. There is ample evidence in the literature of the stereospecificity of the rearrangement of an epoxide (**3**) to a ketone.[3] Inversion at the 13-epoxy centre means that the first product should be the *cis*-C/D ketone with a 13α-hydrogen (**4**). This might then be expected to undergo acid-catalysed epimerisation at C-13 to give the *trans*-C/D compound (**2**). We wished



(1)



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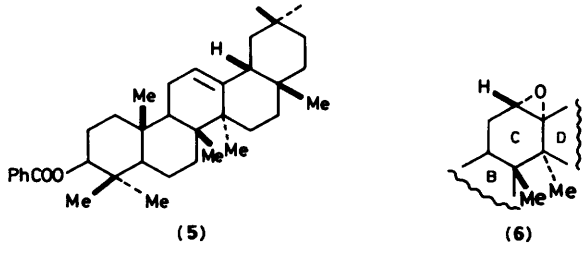


(5) (6)

Table. Avenacins and the 12,13-epoxide (1) ^{1}H n.m.r. spectra ([$^2H_5$]pyridine + D_2O) (*J* in Hz)

		Tertiary methyls							24-CH_2OH		3-CHOH	16-CHOH	30-CHO
Avenacin	A-1	0.88 (s, 3 H)	0.96 (s, 3 H)	1.18 (s, 3 H)	1.25 (s, 3 H)		1.28 (s, 3 H)	1.45 (s, 3 H)	3.6 (d,[a] 1 H, *J* 11)		4.58 (t, 1 H, *J* 7)	4.64 (dd, 1 H, *J* 10.5, 4)	10.20 (s, 1 H)
Avenacin	A-2	0.85 (s, 3 H)	0.94 (s, 3 H)	1.23 (s, 3 H)	1.25 (s, 3 H)		1.33 (s, 3 H)	1.47 (s, 3 H)	Obscured		Obscured	Obscured	10.19 (s, 1 H)
Avenacin	B-1	0.80 (s, 3 H)	1.01 (s, 3 H)	1.16 (s, 3 H)	1.21 (s, 3 H)	1.23 (s, 3 H)	1.35 (s, 3 H)	1.47 (s, 3 H)			4.60 (t, 1 H, *J* 7)	4.70 (dd, 1 H, *J* 4, 10.5)	10.22 (s, 1 H)
Avenacin	B-2	0.80 (s, 3 H)	1.01 (s, 3 H)	1.17 (s, 3 H)	1.22 (s, 3 H)	1.24 (s, 3 H)	1.36 (s, 3 H)	1.47 (s, 3 H)			Obscured	4.71 (dd, 1 H, *J* 10.5, 4)	10.26 (s, 1 H)
Avenestergenin	A-1	0.92 (s, 3 H)	1.01 (s, 3 H)	1.05 (s, 3 H)	1.15 (s, 3 H)		1.16 (s, 3 H)	1.19 (s, 3 H)	4.2 (d, 1 H, *J* 10.5)	3.72 (d, 1 H, *J* 10.5)	~4.2 (dd, 1 H, *J* 10, 3)	4.51 (dd, 1 H, *J* 10.5, 4)	10.26 (d, 1 H, *J* 1)
12,13-Epoxide	(1)	0.90 (s, 3 H)	1.01 (s, 3 H)	1.21 (s, 3 H)	1.26 (s, 3 H)		1.31 (s, 3 H)	1.47 (s, 3 H)	4.22 (d, 1 H, *J* 10.5)	3.71 (d, 1 H, *J* 10.5)	~4.2 (m, 1 H)	4.69 (dd, 1 H, *J* 10.5, 4)	10.20 (s, 1 H)
Avenacin	A-1*	0.71 (s, 3 H)	0.93 (s, 3 H)	1.01 (s, 3 H)	1.10 (s, 3 H)		1.11 (s, 3 H)	1.34 (s, 3 H)	Obscured		4.13 (dd, 1 H, *J* 12, 4)	4.27 (dd, 1 H, *J* 10.5, 4)	9.89 (s, 1 H)

		Aromatic H				NH	NH*Me*	CHO-COAr	13-H_{ax}	22-H_{eq}	Anomeric H's		
Avenacin	A-1	8.16 (dd, 1 H, *J* 8, 1.5)	7.49 (dt, 1 H, *J* 8, 1.5)	6.74 m, 2 H		7.94 (br d,[b] 1 H, *J* 5)	2.78 (d, 3 H, *J* 5)	5.71 (dd, 1 H, *J* 12, 4)	Obscured	Obscured	5.18 (2 × d, 2 H, *J* 7.3, 5.5)	5.10 (d, 1 H, *J* 7.8)	
Avenacin	A-2	8.20 (d, 2 H, *J* 7.5)	7.79 (t, 1 H, *J* 7.5)	7.64 (t, 2 H, *J* 7.5)		—	—	5.80 (dd, 1 H, *J* 12, 4)	Obscured	Obscured	5.31 (d, 1 H, *J* 7.7)	5.18 (d, 1 H, *J* 5.5)	5.08 (d, 1 H, *J* 7.7)
Avenacin	B-1	8.17 (d, 1 H, *J* 8)	7.50 (t, 1 H, *J* 7.5)	6.75 m, 2 H)		7.94 m,[b] 1 H)	2.91 (d, 3 H, *J* 4.5)	5.78 (dd, 1 H)	3.01 (d, 1 H)	3.12 (dd, 1 H)	5.15 (d, 1 H, *J* ~8)	5.10 (d, 1 H, *J* ~8)	4.95 (d, 1 H, *J* 4.7)
Avenacin	B-2	8.24 (d, 2 H, *J* 7)	~7.65 Obsc.	7.54 (t, 2 H, *J* 7.5)				5.73 (dd, 1 H, *J* 12.5, 4)	3.02 (d, 1 H)	3.15 (dd, 1 H, *J* 11.5, 3.5)	5.19 (1 H, *J* 7.6)	5.14 (d, 1 H, *J* 7.6)	4.96 (d, 1 H, *J* 5.1)
Avenestergenin	A-1	8.22 (dd, 1 H, *J* 8, 1.5)	7.45 (dt, 1 H, *J* 8, 1.5)	6.66 (dt, 1 H, *J* 7.5, 1)	6.65 (dd, 1 H, *J* 9, 1)	7.91 (br d, [b] 1 H, *J* 4)	2.79 (d, 3 H, *J* 3)	5.71 (dd, 1 H, *J* 12.5, 4)	3.01 (d, 1 H, *J* 4.5)	2.93 (dd, 1 H, *J* 13, 4.5)			
12,13-Epoxide	(1)	8.19 (dd, 1 H, *J* 8, 1.5)	7.45 (br t, 1 H)	6.75 (m, 2 H)		7.9 (br,[b] 1 H)	2.78 (d, 3 H, *J* 4.5)	5.75 (dd, 1 H, *J* 13, 4.5)	3.00 (d, 1 H, *J* 3.5)	3.07 (dd, 1 H, *J* 13.5, 4.5)			
Avenacin	A-1*	7.84 (dd, 1 H, *J* 7.8, 1.5)	7.34 (dt, 1 H, *J* 7.7, 1.5)	6.73 (d, 1 H, *J* 8)	6.58 (dt, 1 H, *J* 7.5, 0.7)	—	2.91 (s, 3 H)	5.31 (dd, 1 H, *J* 12, 4)	Obscured		4.63 (d, 1 H, *J* 7.8)	4.53 (d, 1 H, *J* 4.7)	4.47 (d, 1 H, *J* 7.5)

* In CD_3OD. [a] One proton observed. [b] Exchanged by D_2O.

to reassure ourselves by using a suitable triterpene model that, under the acid catalysed conditions employed in our deglycosation procedure, such a sequence of events provided an acceptable explanation in the avenacin series.

β-Amyrin benzoate (**5**) was epoxidised by *m*-chloroperbenzoic acid to give the 12α,13α-epoxide (**6**). Ozonisation, in agreement with the literature for the corresponding acetate,[2] gave a mixture of 12α,13α- and 12β,13β-epoxides and these were separated employing a C_{18}-reversed phase column, eluting with 95% methanol. Under acid treatment similar to that used for our deglycosation procedure the expected ketone (**2**) was formed from the 12α,13α-epoxide (**6**) without any signs of a second ketone being involved: this is in accord with the expected stereochemistry of the process, and authentic (**2**)[4] was made for comparison by an independent procedure. The same ketone (**2**) was similarly obtained from the 12β,13β-epoxide indicating that if the stereochemistry of the rearrangement is also as expected in this case, the first-formed ketone (**4**) is very acid labile, epimerising to give (**2**). At this stage the existence of (**4**) was hypothetical, but further cogent evidence for its existence was obtained from the other products formed in the ozonolysis reaction mentioned above. Besides the 12α,13α- and 12β,13β-epoxides, two ketones were also isolated by h.p.l.c. One of these [R_f (h.p.l.c. reversed phase) 12.00 min; δ^{13}C=O 212.2s p.p.m.] was (**2**) whilst the second [R_f 11.3 min; δ^{13}C=O 214.9s] was a labile ketone easily converted into (**2**) by acid treatment. It is therefore identified as (**4**), the missing first-formed rearrangement ketone of the 12β,13β-epoxide (**3**). This excursion into the chemistry of model systems based on (**5**) therefore underpins our suggestion for the formation of avenestergenin A-1 from avenacin A-1 *via* glucoside hydrolysis involving at the same time 12β,13β-epoxide rearrangement to a labile 13α-ketone which is then epimerised to a 13β.

As indicated in the preceding paper the trisaccharide component which is common to all four avenacins is built from two glucose and one arabinose molecules: its attachment was located at C-3 by the deglucosation shift[5] at C-3 and C-2 in the ^{13}C n.m.r. spectrum. In C_5D_5N solution the C-3 and C-2 resonances at δ 82.2 and 25.8 p.p.m. in avenacin A-1 move to δ 72.9 and 27.6 respectively in avenestergenin A-1. Fast atom bombardment (f.a.b.) in the positive ion mode with glycerol/thioglycerol matrix showed that the *M* + 1 parent ion of avenacin A-1 at *m/z* 1 094 loses one hexose (−162), two hexoses (−324), and then two hexoses and one pentose (−456). There is no loss of pentose prior to the last loss, demonstrating, in agreement with the partial hydrolysis evidence of the previous paper, that arabinose is the sugar directly attached to the C-3 oxygen of the triterpene. The location of the two glucose units

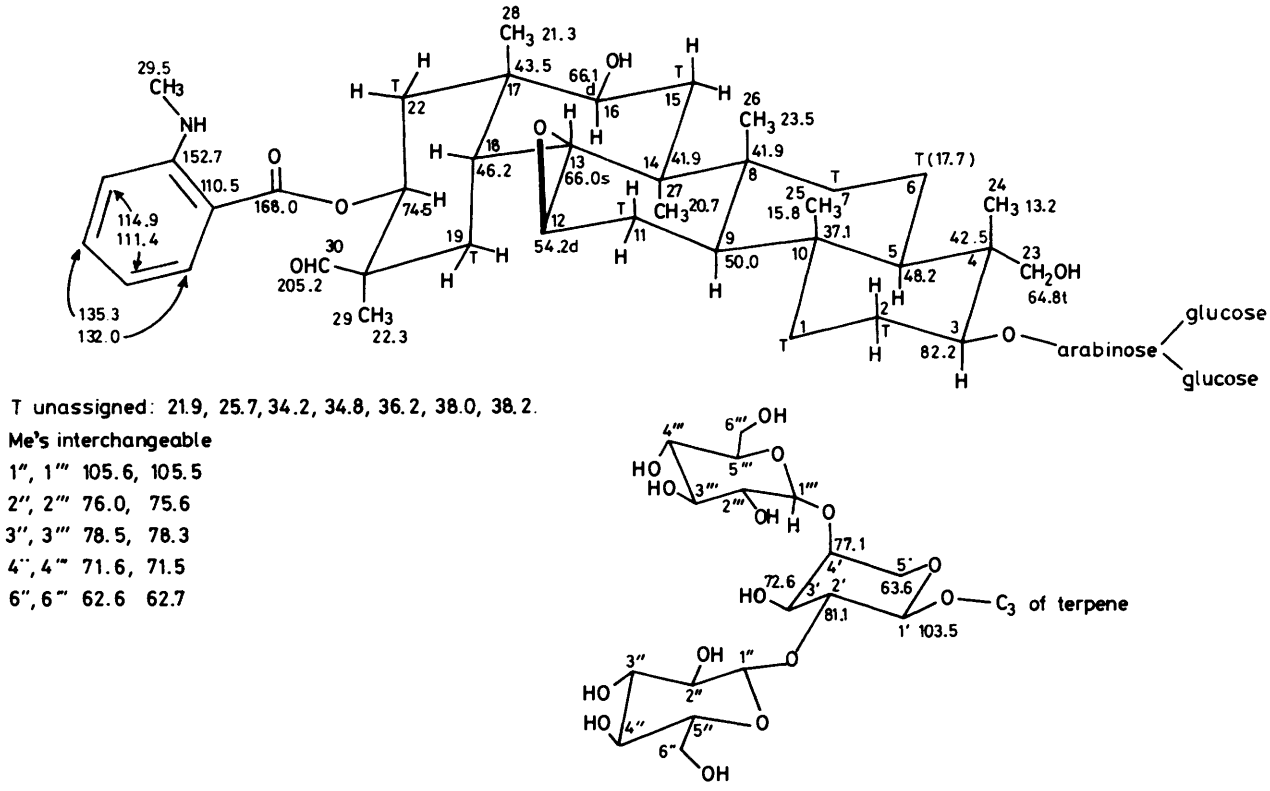
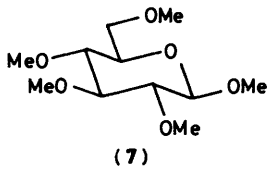
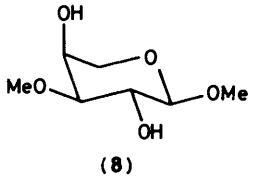


Figure. Proposed ^{13}C n.m.r. assignments ([$^2H_5$]pyridine) for avenacin A-1

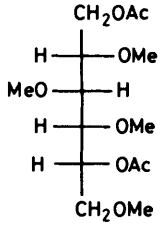
on the arabinose was shown by exhaustive methylation of avenacin A-1 using Ag_2O/MeI/DMF, followed by methanolysis to give the methyl glycosides of 2,3,4,6-tetra-*O*-methyl-D-glucose (**7**) and 3-*O*-methylarabinose (**8**) which were identified



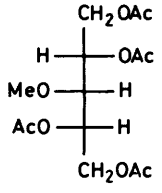
(**7**)



(**8**)



(**9**)



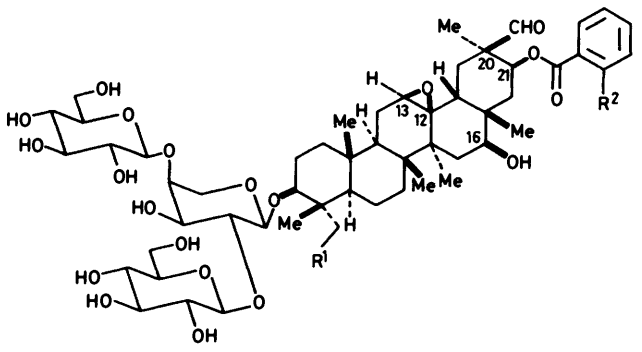
(**10**)

by direct comparison with authentic specimens by t.l.c. and by g.l.c. at 155 °C on 5% neopentyl glycol succinate. Confirmation of these results was obtained by treatment of the methyl glycosides with trifluoroacetic acid, followed by reduction with sodium borohydride, and then acetylation. The products were identified as 2,3,4,6-tetra-*O*-methylglucitol diacetate (**9**), and 3-*O*-methylarabinitol tetra-acetate (**10**) by comparison with authentic specimens using g.c.-m.s. on a 3% ECNS column[6] at 185 °C. Experiments with avenacin A-2 gave similar results. These findings are in accord with the work done by Tschesche[7] on his 'avenacin A' specimen: he identified 2,3,4,6-tetra-*O*-methyl-D-glucose and 3-*O*-methyl-L-arabinose.

^{13}C N.m.r. data allowed further progress to be made now that it was known that two glucopyranose residues were attached to the 2- and 4-positions of arabinose. Assignments (C_5D_5N) (see Figure) could readily be made for the two glucose residues and the C-1 resonances at δ 105.6 and 105.5 p.p.m. showed that they were both β-pyranoses.[8,9] This is supported by ^1H n.m.r. data where the 1-H resonates at δ 5.31 (d) and 5.08 (d) in the two glucoses, both with $J_{1,2}$ 7.7 Hz, again indicating a β-linkage.[10] The remaining unassigned ^{13}C n.m.r. resonances for the carbohydrate section (δ 103.5, 81.1, 72.6, 77.1, and 63.6 p.p.m.) are in good agreement with those calculated[11] (102.0, 79.8, 72.3, 77.8, and 64.6 p.p.m.) for an α-arabinopyranoside having 2- and 4-β-glucopyranoside attachments. Furanoside forms are ruled out, and fits are distinctly less satisfactory for a β-linked arabinopyranoside. The anomeric arabinose proton resonates at δ 5.8 (d, *J* 5.5 Hz), a coupling accommodated by the α-arabinopyranose form.

This work thus establishes the complete structures of the four avenacins as shown in (**11**)—(**14**).

During the work-up of large batches of oat roots two further very minor compounds of the avenacin series were isolated and their structures can be largely, though not completely, defined. The first of these was a bright blue fluorescing compound, and its ^1H n.m.r. spectrum (see Experimental section) left no doubt that it was likely to be identical with avenacin A-1 so far as the avenestergenin A-1 section of the molecular was concerned. It was however clearly lacking the necessary ^1H signals to be a trisaccharide. F.a.b. mass spectral data threw further light on the structure. It had an $M + 1$ ion at 932 showing a loss of 162 to give *m/z* 770, and then a further loss of 132 to give *m/z* 638 which corresponds to the $M + 1$ ion of avenestergenin A-1. The losses correspond to a glucose residue, and after this the loss of a

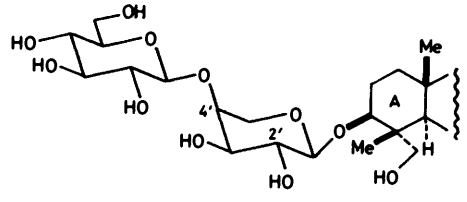


(11) Avenacin A-1 R^1 = OH, R^2 = NHMe
(12) Avenacin A-2 R^1 = OH, R^2 = H
(13) Avenacin B-1 R^1 = H, R^2 = NHMe
(14) Avenacin B-2 R^1 = H R^2 = H

pentose which must be directly linked to the aglycone. This makes a deglucoavenacin A-1 structure highly probable though whether the glucose lost is at the arabinose 2'- or 4'-position, *i.e.* (**15**) or (**16**), remains undefined.

The second compound was extremely scarce (under 1 mg from 600 g of dry roots). It too showed a bright blue fluorescence and its u.v. spectrum is in agreement with an *N*-methylanthranilate ester forming part of the structure. In the f.a.b. mass spectrum there was an $M + 1$ at 1 256.5 ($C_{60}H_{93}NO_{26}$) and successive losses of three hexoses and finally one pentose as indicated by the sequence 1 094.8, 932.1, 770, and 638. The constitution is thus likely to be glucoavenacin A-1 though we have no information on the placing of the extra glucose.

The 'take-all' fungus of wheat *Gaeumannomyces graminis* var. *tritici* (Ggt) does not attack oat roots[12] and it appears to be unable to detoxify avenacins adequately to continue growth: only traces of mono-deglucoavenacin A-1 are formed from avenacin A-1 (the most fungicidal of the avenacins).[13] The var. *avenae* (Gga) however can attack oat roots and in a recent study



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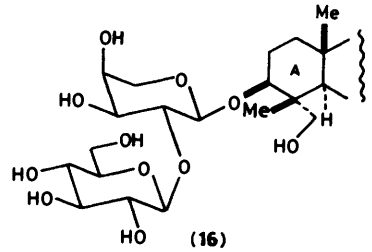

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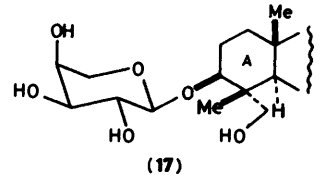

(17)

we have shown that this type degrades avenacin A-1 to mono-(**15**/**16**) and bis-(**17**) degluco A-1.[13] The former is identical with that isolated above from uninfected oat roots as a minor component. Both the mono- and the bis-degluco A-1 compounds are less toxic (and less soluble) than avenacin A-1[13] and it appears that the possession of these sugar-hydrolysing enzymes is a very important factor in enabling the Gga strain to attack oats as well as wheat. Full details are reported elsewhere.[13,14]

Experimental

N.m.r. spectra are for $CDCl_3$ solutions unless stated otherwise.

Epoxyavenagenin A-1.—Fractions from silica column chromatography of the original methanol extract of oat roots, eluted with chloroform–methanol (2:1) and forming a thick brown oil (20.1 g) showed a blue fluorescence in u.v. light and were adsorbed on silica and packed onto a dry silica column (6 × 14 cm). The elution sequence was light petroleum (b.p. 60—80 °C)→ethyl acetate→20% methanol in ethyl acetate. Fractions eluted in the sequence light petroleum (b.p. 60—80 °C)–ethyl acetate (2:1)→ethyl acetate (100%) showed a blue fluorescence (0.18 g, oil). Further purification by h.p.l.c. reversed-phase chromatography on a C_{18} column, eluting with 85% methanol in water at 2 ml/min yielded a few mg of a glassy powder softening at 202 °C and melting at 218—221 °C. It had M^+ 637.3971 ($C_{38}H_{55}NO_7$ requires 637.3978) and was named *epoxyavenagenin* A-1. It had $\nu_{max.}$($CHCl_3$: f.t. spectrum under high resolution) 1 725 (CHO) and 1 675 (ester CO) cm^{-1}: no ketonic carbonyl at 1 695 cm^{-1} as in avenestergenin A-1 could be found. The compound had ^1H n.m.r. data as follows: δ($CDCl_3$) 9.99 (1 H, s), 7.84 (1 H, dd, *J*, 8, 1.5 Hz), 7.63 (1 H, br m, exchangeable with D_2O), 7.40 (1 H, dt, *J* 8, 1.5 Hz), 6.68 (1 H, d, *J* 8 Hz), 6.59 (1 H, t, *J* 8 Hz), 5.18 (1 H, dd, *J* 12.5, 4 Hz), 4.29 (1 H, dd, *J* 11.5 Hz), 3.72 (1 H, d, *J* 10.5 Hz), 3.62 (1 H, br t, *J ca.* 9.5 Hz), 3.39 (1 H, d, *J* 10.5 Hz), 2.92 (3 H, br s), 2.45 (1 H, dd, *J* 14, 4.5), 2.09 (1 H, dd, *J* 14, 4.5), 1.9—0.8 (*ca.* 18 H m) including 1.28 (3 H, s), 1.13 (3 H, s), 1.11 (3 H, s), 1.05 (3 H, s), 0.92 (3 H, s), and 0.85 (3 H, s). In C_5D_5N it had δ 9.57 (1 H, s), 8.19 (1 H, dd, *J* 8, 1.5 Hz), *ca.* 7.9 (1 H, br, exchangeable with D_2O), 7.45 (1 H, br t), 6.73 (2 H, m), 5.75 (1 H, dd, *J* 13, 4.5 Hz), 4.69 (1 H, dd, *J* 4, 11 Hz), *ca.* 4.22 (2 H, m including a 1 H d, *J* 10.5 Hz), 3.71 (1 H, d, *J* 10.5 Hz), 3.07 (1 H, dd, *J* 14, 4.5 Hz), 3.00 (1 H, d, *J* 3.5 Hz), 2.78 (3 H, d, *J* 4.5 Hz), 2.4—0.8 (m) including 1.48 (3 H, s), 1.32 (3 H, s), 1.30 (3 H, s), 1.18 (3 H, s), 1.07 (3 H, s), and 0.96 (3 H, s). In the mass spectrum there was a large ion for elimination of *N*-methylanthranilic acid (*m*/*z* 151).

Conversion of Epoxyavenagenin A-1 *into Avenestergenin* A-1.—The epoxy compound (1 mg) was heated with M-hydrochloric acid for 1.5 h and then extracted with chloroform and the extract washed, dried, and evaporated. T.l.c. on silica eluting with ethyl acetate (twice) gave a spot R_F 0.30 (epoxyavenagenin A-1 had R_F 0.45 and avenestergenin A-1 0.30). Comparison was then made by reversed-phase chromatography using a C-18 column and eluting with 80% methanol in water at 1.5 ml/min. The acid-isomerised product had R_t 5.8 min (epoxyavenagenin A-1 had R_t 7.3 min and avenestergenin A-1 R_t 5.8 min).

3β-*Benzoyloxy*-12α,13-*epoxyoleananes*.—3β-Benzoyloxyolean-12-ene (β-amyrin benzoate) (530 mg) was epoxidised with *m*-chloroperbenzoic acid (220 mg, 85%) in chloroform containing a drop of water and a trace of sodium hydrogen carbonate. After 4 h further *m*-chloroperbenzoic acid was added (110 mg) and the mixture was stirred (10 days). Sodium sulphite solution (10%) was added until starch–iodide paper gave no colour. Work-up of the chloroform layer gave the 12α,13-

epoxide (340 mg), m.p. 236—237 °C from chloroform–methanol (Found: M^+ 546.4066. $C_{37}H_{54}O_3$ requires *M*, 546.4073). The 12,13-epoxide carbons had ^{13}C n.m.r. δ 66.2 (d) and 64.1 (s).

Ozonolysis[2] *of β-Amyrin Benzoate.*—A solution of the benzoate (370 mg) in acid-free carbon tetrachloride (7 ml) was cooled in ice–water and ozone was passed through it for 5 min whilst the temperature was allowed to rise to ambient. T.l.c. on silica (eluting with ether–hexane, 1:1) showed that reaction was nearly complete and the mixture was separated by C_{18}-reversed phase h.p.l.c. in methanol on a preparative column to give two main products. The first (200 mg) was a mixture of ketones. The early fractions of the second peak (53 mg) were almost pure 12α,13-epoxide whilst the later were a mixture of 12α,13- and 12β,13-epoxides. This mixed epoxide fraction was further separated on a reversed-phase C_{18} column, eluting with methanol at 1 ml/min. The first fraction was 12α epoxide. The second fraction after further h.p.l.c. purification gave the 12β,13-*epoxide* (18 mg), crystallised from methanol–water (Found: M^+, 546.4060. $C_{37}H_{54}O_3$ requires *M*, 546.4073). The 12,13-epoxide carbons had ^{13}C n.m.r. δ 56.7 (d) and 65.1 (s).

The mixed ketone fraction (above) was separated on a semi-preparative C_{18}-reversed phase h.p.l.c. column eluting with 95% methanol in water at 2 ml/min. The first fraction was identical with an authentic specimen of 3β-benzoyloxy-13β-oleanan-12-one, m.p. 252—253 °C made according to the literature method.[4] It had M^+ 546.4060. (Calc. for $C_{37}H_{54}O_3$: *M*, 546.4073). The ^{13}C n.m.r. spectrum showed a 12-carbonyl resonance at δ 212.2 (s) and an ester carbonyl at 166.0 (s). The second ketone, 3β-*benzoyloxy*-13α-*oleanan*-12-*one* (19 mg after further h.p.l.c. purification) crystallised as a white solid from methanol–water and had M^+, 546.4075 ($C_{37}H_{54}O_3$ requires *M*, 546.4073). The ^{13}C n.m.r. spectrum showed a 12-carbonyl resonance at 214.9 (s) and an ester carbonyl resonance at δ 166.2 (s).

The above ozonolysis was repeated a number of times and is difficult to control: if ozone is passed for too short a time much remaining starting material causes difficulty. If the optimum time is exceeded most epoxide is converted into ketone.

Acid Treatment of the 3β-*Benzoyloxy*-12α,13- *and* -12β,13-*epoxides and of* 3β-*Benzoyloxy*-13α- *and* 13β-*oleanan*-12-*ones.*—A few mg of each sample was hydrolysed by heating with M-hydrochloric acid for 2 h under reflux. A little tetrahydrofuran was added to aid solubility. Products were extracted into dichloromethane and identified by t.l.c. [silica, elutant hexane–ether (3:1)] and C_{18}-reversed phase h.p.l.c. eluting with methanol at 1 ml/min. A ^1H n.m.r. spectrum was obtained on the acid-treated 13α-ketone above. Although it contained interfering peaks this showed clearly that the 13β 12-ketone had been obtained from the 13α 12-ketone. A significant peak was δ($CDCl_3$) 2.79 (1 H, d, *J* 4.2 Hz, hydrogen adjacent to ketone). This appears at δ 1.94 (1 H, d, *J* 4.3 Hz) in the ^1H spectrum of the 13α 12-ketone and 2.79 (1 H, d, *J* 4.2 Hz) in the spectrum of authentic 13β 12-ketone. From the table above, 12α 13- and 12β 13-epoxides and 13α 12-ketones are all converted into 13β 12-ketone under acid conditions.

Substrate	R_F on t.l.c.	R_t on h.p.l.c. (min)
12α-Epoxide	0.75	14.8
12β-Epoxide	0.73	17.7
12α- and 12β-Ketones (mixture *ex.* ozonolysis)	0.60	11.3 and 12.0
13β-Ketone	0.60	12.0
13α-Ketone	0.60	11.3
Acid-treated 12α-epoxide	0.60	12.0
Acid-treated 12β-epoxide	0.60	12.0
Acid-treated 13α-ketone		12.0

Isolation and Identification of the Constituent Sugars.—Avenacin A-1 was hydrolysed in 1M-aqueous hydrochloric acid under reflux for 2.5 h. The precipitate was filtered off and washed with water. The aqueous extract was neutralised by using the hydroxy form of Amberlite IR 45 ion exchange resin, freeze dried, and dissolved in pyridine. Avenacin A-2 was similarly treated.

Samples of the sugar solutions were applied to Whatman No. 1 (10 × 20 cm) paper sheets and eluted with ethyl acetate–pyridine–water (12:5:4). After drying, the sheet was sprayed with *p*-anisidine hydrochloride reagent and heated for 10 min at 130 °C. Both A-1 and A-2 extracts showed glucose (R_F 0.15, yellow-brown) and arabinose (R_F 0.22, red) present. Results were confirmed using a different elution system butanol–ethanol–water (50:10:40) (upper layer).

Sugars were determined quantitatively after silylation with Trisil-Z by g.l.c. (2% OV-17, using a 5 ft column at 180 °C). Quantitation was attained by making up standard ratios of glucose:arabinose and subjecting these to the hydrolysis and work-up above. The mean ratio of glucose:arabinose was 1.98:1 for A-1, and 2.10:1 for A-2. In experiments using 0.8M-hydrochloric acid and shorter hydrolysis time or lower temperatures, the ratio rose to 13:1 indicating very incomplete removal of arabinose and suggesting that this is the sugar residue directly linked to the triterpene.

Methylation and Methanolysis of the Sugar Residues of Avenacin A-1.—Avenacin A-1 (110 mg) or A-2 (85 mg) in dry dimethylformamide (0.7 ml) was stirred (24 h) at 20 °C with freshly prepared and dried silver oxide (500 mg) and methyl iodide (0.3 ml). Further additions of silver oxide and methyl iodide were then made after 24, 48, and 120 h. After 6 days the mixture was filtered and the filtrate extracted with chloroform. The chloroform extracts were washed, dried ($MgSO_4$), and evaporated and dispersed onto silica which was packed on a dry silica column (1 × 7 cm). Elution was with light petroleum (b.p. 60—80 °C)→ethyl acetate→ethyl acetate–methanol (9:1). Four main bands, each showing a strong blue fluorescence in u.v. light, were collected and each was treated as follows.

The methylated avenacin was heated in a sealed tube at 100 °C for 2.5 h with 2M-hydrochloric acid (0.5 ml). Solid silver carbonate was added and a portion of the solution was used for g.l.c. (5 ft glass column, 5% neopentyl glycol succinate at 155 °C) or t.l.c. (silica plates, eluting with ethyl acetate: spray 10% sulphuric acid heated to 100 °C) with results as below.

	G.l.c. [R_t (min)]			R_F
2,3,4,6-Tetramethoxyglucose	5.5	8.0		0.61, 0.73
3-*O*-Methylarabinose			18.5*	0.11
Avenacin A-1 products	5.5	8.0	18.5	0.61, 0.73, 0.11
Avenacin A-2 products	5.5	8.0	18.5	

* Major peak

The remainder of the solution of methylated methyl glycosides was evaporated under reduced pressure, dissolved in 1M-trifluoroacetic acid (1 ml) and heated at 100 °C for 2 h. The acid was evaporated and the residue dissolved in water and deionised. Solid sodium borohydride was added slowly at room temperature with stirring and the latter continued for 2 h. Excess of 0.2M-acetic acid was added and stirring continued (2 h). After evaporation under reduced pressure, and re-evaporation with successive 10 ml amounts of methanol, the residue was acetylated by heating overnight at 100 °C with dry

pyridine (0.4 ml) and acetic anhydride (0.4 ml). Work-up gave a chloroform solution of acetylated alditols which was examined by g.l.c.-m.s. (5 ft glass column using 3% ECNS nitrile silicone polyester,[6] at 185 °C) as follows:

	R_t (min)	Major ions
2,3,4,6-Tetra-*O*-methyl-glucitol diacetate	10.0	43, 45, 71, 87, *101*, 117, 129, 145, 161, 205.
3-*O*-Methylarabinitol tetra-acetate	24.5	43, 87, *129*, 189.
Avenacin A-1 products	10.0	43, 45, 71, 87, *101*, 117, 129, 145, 161, 205.
	24.5	43, 87, *129*, 189.
Avenacin A-2 products	10.0	43, 45, 71, 87, *101*, 117, 129, 145, 161, 205.
	24.5	43, 87, *129*, 189.

T.l.c. also confirmed these conclusions.

Isolation of Deglucoavenacin A-1.—Large-scale C_{18}-reversed phase chromatography of fractions, from oat root extraction enriched in avenacin B-1 gave fractions 9—11 (mainly A-1), 12 and 13 (mainly B-1), and finally fractions 14—16. Examination of these final fractions by t.l.c. [silica, eluting with chloroform–methanol–water (65:30:3)] revealed a new blue fluorescent spot of R_F 0.63 (avenacin B-1 had R_F 0.33). It was purified by h.p.l.c. on a C_{18}-reversed phase column (elution 72.5% methanol in water at 2 ml/min) to give *deglucoavenacin* A-1, R_t 17 min (avenacin A-1 has R_t 10 min). Mass spectrometry using a f.a.b. source showed *m/z* 933 (*M* + 2), 932 (*M* + 1), 770 (−162), 755 (−162, −15), and *638* (aglycone + 1). The molecular ion corresponds to loss of 162 from avenacin A-1 (M^+, 1 093.5). The degluco compound had ^{1}H n.m.r. ([$^2H_5$]pyridine + D_2O) δ 10.20 (1 H, s, CHO), 8.16 (1 H, dd, *J* 8 and 1.5 Hz), 7.46 (1 H, t), *ca.* 6.72 (2 H, m, ArH), *ca.* 7.9 (1 H, m, exchanges with D_2O, NH), 5.73 (1 H, dd, *J* 4, 12 Hz, C*H*-O-ester), 5.20 (2 H, d, *J* 6.8 Hz, two anomeric protons), *ca.* 4.6 (1 H, br, C-16 C*H*OH) under 4.63 (1 H, t, *J* 7 Hz) 4.58—4.38 (2 H, m), 4.37—4.25 (6 H, m), 4.24—4.07 (6 H, m) (sugar protons and 3-C*H*O), 3.74 (1 H, d, *J* 12 Hz), 3.70 (1 H, d, *J* 12.1 CH_2OH), 3.01 (2 H, m) [without D_2O 3.05 (1 H, dd, *J* 4, 13 Hz, 22-H)], 2.96 (1 H, d, *J* 3 Hz, epoxide H), 2.80 (3 H, s, NH*Me*) 2.3—2.1 (4 H, m), 2.0—1.6 (*ca.* 3 H, m), 1.46 (3 H, s), 1.29 (3 H, s), 1.25 (3 H, s), 1.19 (3 H, s), and 1.00 (3 H, s) and 0.88 (3 H, s) (6 methyls).

The compound corresponds in chromatographic and mass-spectral characteristics to that produced by the incubation of *Gaeumannomyces graminis* var. *avenae* (Gga) with avenacin A-1.[13]

Isolation of Glucoavenacin A-1.—Large scale C_{18}-reversed phase h.p.l.c. of partially purified avenacin mixture enriched in avenacin A-2, eluting with 72.5% methanol in water, gave fractions 5—8 containing avenacin A-2 (0.65 g) whilst 11—13 gave avenacin A-1 (0.48 g). The intermediate fraction 9—10 (0.28 g) contained avenacin B-2 and in addition a minor component identified by having a lower R_F on t.l.c. [silica, chloroform–methanol–water (65:30:4)]. Purification by p.l.c. using the above solvent gave < 1 mg (from 600 g dry roots) of a new compound R_F 0.30 (avenacin A-2 R_F 0.43). The R_t on h.p.l.c. using the C_{18}-reversed phase method and eluting with 72.5% methanol in water at 1.5 ml/min was 9 min (avenacin B-2 was 8.4 min). The compound had $\lambda_{max.}$(ethanol) 355 and 254 nm with strong end absorption: this, and its bright blue fluorescence, are in agreement with an *N*-methylanthranilate component. In the mass spectrum (f.a.b.) there were ions at *m/z* 1 278.3 (*M* + 23), 1 256.5 (*M* + 1), 1 238.3 (*M* − 18), and ions for loss of one hexose 1 094.8 (*M* − 162), two hexoses 932.1 (*M* − 324), three hexoses 770 (*M* − 486), and three hexoses plus one pentose 638 (*M* − 619), followed by loss of an H_2O from the aglycone (620) ($C_{60}H_{93}NO_{26}$ requires 1 255.6).

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