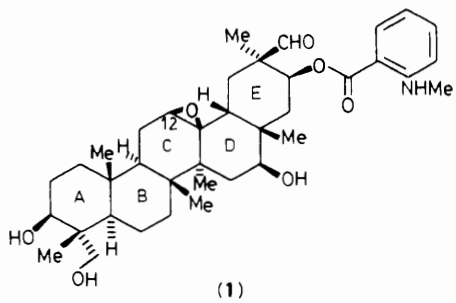
Structures of the Four Avenacins, Oat Root Resistance Factors to 'Take-All' Disease

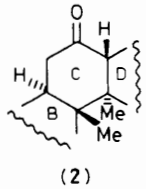
Leslie Crombie,* W. Mary L. Crombie, and Donald A. Whiting*
Department of Chemistry, The University, Nottingham, NG7 2RD, U.K.

The true aglycones of the avenacins are acid sensitive triterpene 12,13-epoxides, not 12-ketones, and one such avenestergenin epoxide is isolated direct from oat roots; this, together with study of the attached trisaccharide, permits complete structures to be proposed for the four avenacins (**3a—d**); avenestergenins readily undergo an acid-catalysed anhydrodimerisation *via* acetal formation.

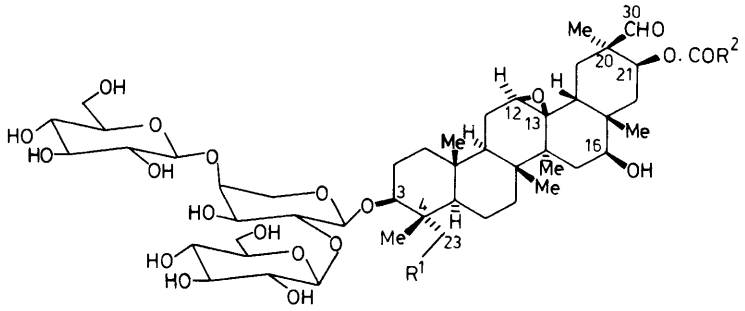
Although the structures of the four avenestergenins A–1, A–2, B–1, and B–2 formed by acid hydrolysis of the four avenacins from oat roots are deduced in the preceding communication,[1] closer examination shows that these are apparent rather than true aglycones. Thus, whilst i.r. absorption attributable to the 30-aldehyde [ν_{max} (KBr) 1721 cm^{-1}] and C–21 ester (1677 cm^{-1}) is present in the avenacins, a keto-carbonyl found near 1695 cm^{-1} in the avenestergenins is missing. Further, the ^{13}C n.m.r. (C_5D_5N) carbonyl resonance at δ 211.4 p.p.m. in avenestergenin A–1 is lacking in avenacin A–1; on the other hand, a new carbon resonance is obvious at



(1)



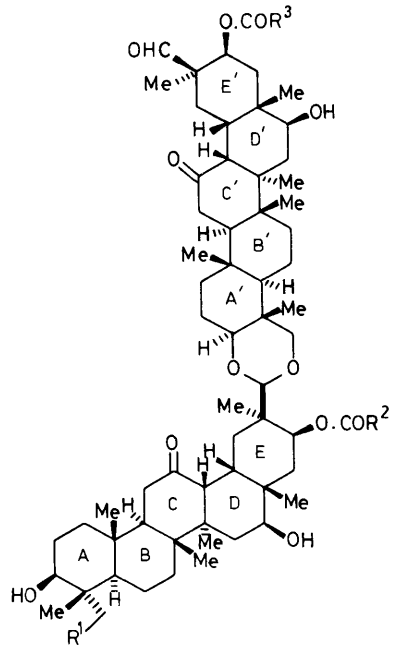
(2)



(3) **a**; R^1 = OH, R^2 = *o*-$MeHNC_6H_4$ Avenacin A–1
b; R^1 = OH, R^2 = Ph Avenacin A–2
c; R^1 = H, R^2 = *o*-$MeHNC_6H_4$ Avenacin B–1
d; R^1 = H, R^2 = Ph Avenacin B–2

δ 54.2 p.p.m. (d). Since the avenestergenins have the molecular formulae one would expect for deglycosylated avenacins, we were led to the view that the real aglycones were 12,13-epoxides which rearranged to 12-carbonyl compounds on treatment with acid. Verification came from further direct isolation work on oat roots which yielded a 12,13-epoxy-avenestergenin A–1 (**1**), $C_{38}H_{55}O_7N$, m.p. 218–221 °C, isomeric with the customary 12-keto-avenestergenin A–1 (**2**). It contained *N*-methylanthranilic acid and its ^1H n.m.r. spectrum ($CDCl_3$ or C_5D_5N) showed close similarity to avenestergenin A–1 except in the C ring region. The compound had ν_{max} ($CHCl_3$) 1725 and 1675 cm^{-1} with no trace of a 12-keto-carbonyl absorption near 1695 cm^{-1} when scrutinised by high-resolution Fourier transform (F.t.) i.r. spectroscopy. Acid treatment decisively showed its nature, converting the compound into the familiar 12-keto-avenestergenin A–1 (h.p.l.c. and t.l.c. comparisons). In avenacin A–1 the ^{13}C n.m.r. resonances for the epoxide carbons [C–12, δ 54.2 (d); C–13, 66.0 p.p.m. (s)] signify a β-orientation since the 12β,13β carbons in 3β-acetoxy-12β-epoxyoleanane resonate at δ 53.8 and 66.3 p.p.m. whereas in the 12α,13α-form they resonate at δ 65.5 and 63.1 p.p.m.[2]

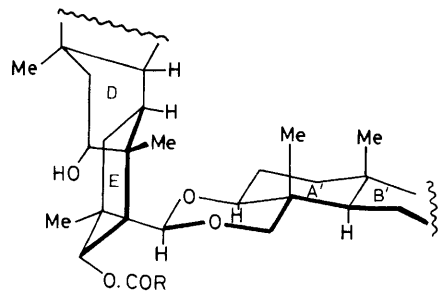
The trisaccharide component, common to all four avenacins, and built from two glucose and one arabinose molecules, was located at C–3 by the deglycosylation shift[3] at C–3 and C–2 in the ^{13}C n.m.r. spectrum (in C_5D_5N, the C–3 and C–2 resonances at δ 82.2 and 25.8 p.p.m. in avenacin A–1 move to δ 72.9 and 27.6 p.p.m., respectively in avenestergenin A–1). In the positive-ion fast atom bombardment (f.a.b.) mass spectrum (glycerol), the $M + 1$ parent ion of avenacin A–1 at *m/z* 1094 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 that arabinose is attached directly to the triterpene C–3 oxygen. Similar data were obtained for the other avenacins using f.a.b. mass spectroscopy (m.s.) in either the positive- or negative-ion modes. The location of the two glucose units on the arabinose, and the pyranose form of the glucose, was shown by exhaustive methylation (Ag_2O–MeI–dimethylformamide) of avenacin A–1, followed by methanolysis to give the methyl glycosides of 2,3,4,6-tetra-*O*-methyl-D-glucose and 3-*O*-methylarabinose (t.l.c. and g.l.c. on 5% neopentyl glycol succinate at 155 °C and comparison with authentic specimens). These results were confirmed by treatment of the methyl glycosides with trifluoroacetic acid, followed by reduction with sodium borohydride and acetylation. The products were identified as 2,3,4,6-tetra-*O*-methylglucitol diacetate and 3-*O*-methylarabinitol tetra-acetate by g.c.–m.s. [3% ECNSS at 185 °C, ECNSS is a nitrile silicone polyester copolymer (U.S. Pat., 3 263 401)] comparing with authentic specimens.



(4) **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

Similar results were obtained with avenacin A–2. These findings are in agreement with work by Tschesche[4] on his 'avenacin A' specimen: he identified 2,3,4,6-tetra-*O*-methyl-D-glucose and 3-*O*-methyl-L-arabinose.

With the fact that two glucopyranose residues are attached at the 2- and 4-positions of arabinose clearly established, further progress could be made by n.m.r. methods. ^{13}C N.m.r. assignments (C_5D_5N) could be readily made for the two glucose residues and the C–1 resonances (δ 105.6 and 105.5 p.p.m.) showed that both were β-pyranoses.[5,6] This is in agreement with ^1H n.m.r. data where 1–H resonates at δ 5.31 (d) and 5.08 (d) in the two glucoses, both with $J_{1,2}$ 7.7 Hz indicating a β-linkage.[7] The remaining ^{13}C n.m.r. carbohydrate resonances (δ 103.5, 81.1, 72.6, 77.1, and 63.6 p.p.m.) are in good agreement with those calculated for an α-arabinopyranoside having 2- and 4-β-glucopyranoside attachments.[8] Furanose forms are ruled out, and fits are distinctly



(5)

less satisfactory for a β-linked arabinopyranoside. The anomeric arabinose proton resonates at δ 5.18 (d, *J* 5.5 Hz), a coupling accommodated by the α-arabinopyranose form illustrated in (**3**), which summarises completed structure proposals for the four avenacins.

During chromatographic isolation of the avenestergenins, produced by hydrolysis of the avenacins, higher molecular weight material was encountered which frequently caused interference in h.p.l.c. work. As a result, it was discovered that avenestergenins readily form anhydrodimers (and probably higher 'oligomers') on acid treatment. Thus, avenestergenin A–1 anhydrodimerises to form A–1/A–1 (**4a**) as a high melting powder for which no M^+ could be obtained by electron impact or chemical ionisation m.s. techniques. Using f.a.b.m.s. methods [*ex* Carbowax 200 or diamylphenol (DAP) benzonitrile] the compound gave a strong, clear, M^+ at *m/z* 1257 $\{[637 + 637 - H_2O + 1]^+\}$. Only one ·CHO proton per molecule (δ 9.91) was present and the new acetal ring could be recognised by, among other features, the single acetal proton located at δ 4.98 (s, 1H). The conformational situation around the dioxane ring is shown in (**5**). The A–2/A–2 anhydrodimer (**4b**) had similar characteristics and formed the expected tetra-acetyl derivative. Anhydrodimerisation of avenestergenin A–1 with A–2 gave a pair of products A–1/A–2 (**4c**) and A–2/A–1 (**4d**), readily recognisable from their ^{1}H n.m.r. spectra. In the case of avenestergenins of the B series, anhydrodimers can only form with the B component providing the aldehyde part of the dioxane, *e.g.* B–1/A–1 (**4f**) and B–1/A–2 (**4g**). The B–2/A–2 compound (**4e**) gave a f.a.b. M^+ (*ex* DAP–benzonitrile) cluster *m/z* 1183/1184 $\{[608 + 592 - H_2O + 1]^+\}$ and the expected ^{1}H n.m.r. data.

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8 From data of A. Lipták, Z. Szurmai, P. Nánási, and A. Neszmélyi, *Tetrahedron*, 1982, **38**, 3489; P. A. J. Gorin and M. Mazurek, *Can. J. Chem.*, 1975, **53**, 1212, the following ^{13}C n.m.r. resonances for C–1—C–5 of an α-arabinopyranoside having β-glucopyranosides at C–2 and C–4 were calculated as: δ 102.9, 79.8, 72.3, 77.8, and 64.6 p.p.m.