Isolation of Avenacins A–1, A–2, B–1, and B–2 from Oat Roots: Structures of their 'Aglycones', the Avenestergenins

Leslie Crombie,* W. Mary L. Crombie, and Donald A. Whiting*

Department of Chemistry, The University, Nottingham, NG7 2RD, U.K.

Four antifungal avenacins are isolated from oat roots: the structures of their aglycones', avenestergenins A–1, A–2, B–1, and B–2, are shown to be *N*-methylanthranilic or benzoic esters of an unusually oxygenated oleanane triterpene.

'Take-all' is a complex fungal disease of cereals, serious and widespread in Britain and the rest of the world.[1] The roots and tiller-bases of wheat are attacked by the fungus *Gaeumannomyces graminis* (Sacc.) Arx and Olivier var. *tritici* Walker, (Ggt), but oats are resistant. It is generally agreed that pre-formed inhibitors are implicated in this resistance,[2,3] and a substance 'avenacin' which proved to be a potent growth inhibitor for this and other fungi was isolated by Maizel *et al.*[4] It was recognised as a triterpene glycoside in type, esterified with *N*-methylanthranilic acid.[4] Further work by Tschesche and his colleagues[5] did not permit definition of the triterpene class, but allowed revision of the functional groups and a closer definition of the trisaccharide attachment. A second compound having one Me replaced by $\cdot CH_2OH$, reported to be a disaccharide, was also found.[5]

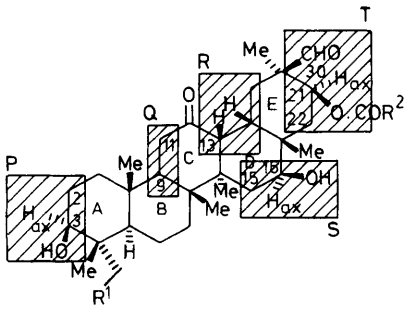
In our experiments, oats were grown hydroponically, the roots freeze-dried, and worked up to obtain the 'avenacin' fraction which, after extensive h.p.l.c. separations (C_{18}-reversed phase eluting with methanol–water, 3 : 1) gave the four avenacins shown in Table 1. Avenacin A–1 and B–1 had an intense blue fluorescence in methanol, and on methanolysis each gave 1 mol of methyl *N*-methylanthranilate (g.c.–mass spectroscopy and n.m.r.); similarly A–2 and B–2 each gave 1 mol of methyl benzoate. The A–1 and A–2 compounds each contain one extra oxygen atom relative to their B–1 and B–2 counterparts, but all four are trisaccharides of the same type giving on hydrolysis (1 M HCl, reflux) 1 mol of arabinose and 2 mol of glucose [g.l.c. as trimethylsilyl (TMS) derivatives]. Short term hydrolysis gave the two sugars in a ratio 1 : 13 indicating that arabinose is the residue adjacent to the terpene. No disaccharide was encountered.

Four crystalline avenestergenins A–1, A–2, B–1, and B–2

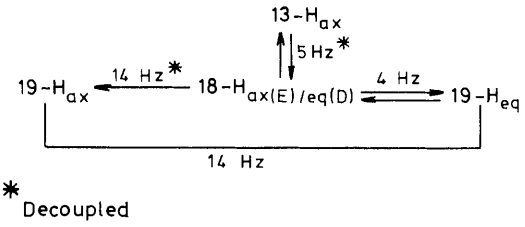
Table 1. Avenacins isolated from oat roots.

	M.p.	Molecular formula (*M*)[a]	λ_{max} (EtOH), nm
Avenacin A–1	228—233 °C	$C_{55}H_{83}O_{21}N$ (1093)	223 (ε 25 250), 255(7 900), 357(5 500)
Avenacin A–2	237—239 °C	$C_{54}H_{80}O_{21}$ (1064)	230 (ε 14 700), 274(1 200), 281(1 100)
Avenacin B–1	Glass	$C_{55}H_{83}O_{20}N$ (1077)	223 (ε 23 300), 255(7 900), 356(5 300)
Avenacin B–2	Glass	$C_{54}H_{80}O_{20}$ (1048)	228 (ε 14 600), 274(1 300), 281(1 250)

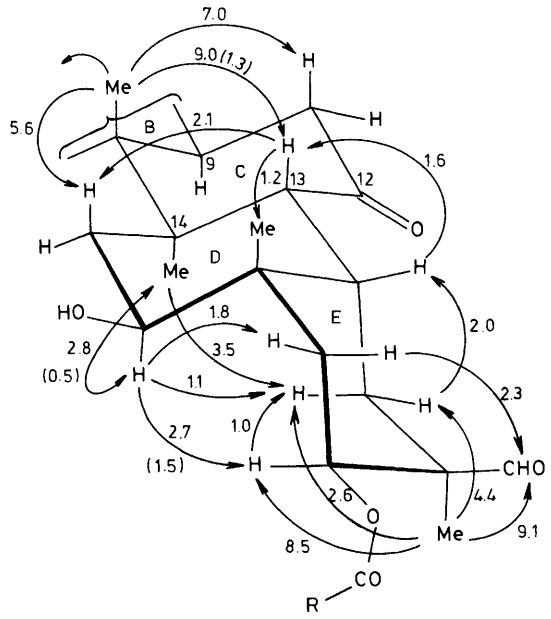
[a] Positive ion fast atom bombardment (f.a.b.) from $M + 23$ and $M + 1$; negative ion f.a.b. from $M - 1$.



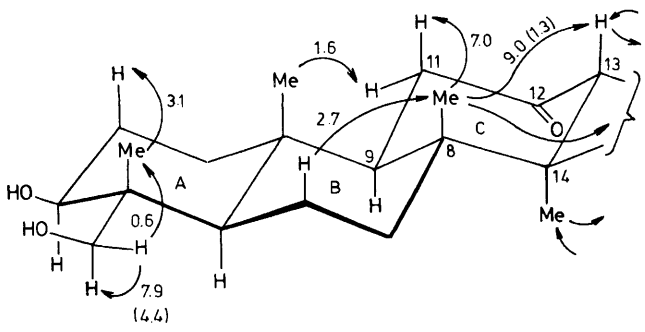
(1) **a**; R^1 = OH, R^2 = *o*-MeHNC$_6$H$_4$
b; R^1 = OH, R^2 = Ph
c; R^1 = H, R^2 = *o*-MeHNC$_6$H$_4$
d; R^1 = H, R^2 = Ph



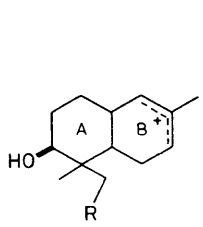
(A)



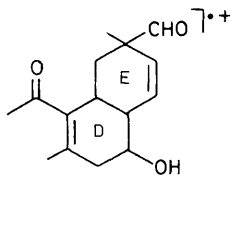
(2a)



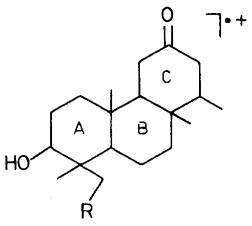
(2b)



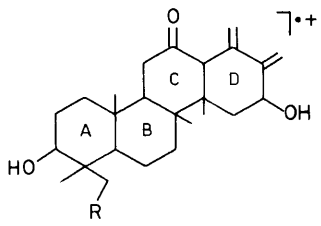
(3)



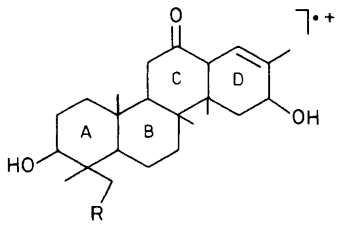
(4)



(5)



(6)



(7)

R = H or OH

were derived from the four oat-root avenacins after acid hydrolysis of the carbohydrate section: they thus constitute the apparent aglycones. Avenestergenin A–1 ($C_{38}H_{55}O_7N$, m.p. 210—211 °C)† and B–1 ($C_{38}H_{55}O_6N$, m.p. 186—187 °C) release 1 mol of methyl *N*-methylanthranilate on methanolysis, whilst A–2 ($C_{37}H_{52}O_7$, m.p. 189—190 °C) and B–2 ($C_{37}H_{52}O_6$, m.p. 177—178 °C) release 1 mol of methyl benzoate. All four 'aglycones' have u.v. data closely similar to their parent avenacins, the chromophores being the esterifying acids. Though many similar data are available for all four avenestergenins, for reasons of economy the structural discussion is focussed mainly on A–1 with mention of the others when necessary.‡

Avenestergenin A–1 (M^+ *m/z* 637.396) contains three free hydroxyl groups (triacetate, m.p. 168—170 °C; tri-TMS-derivative, M^+ *m/z* 853), two secondary and one primary (^{1}H n.m.r. and acetylation shifts), and a fourth hydroxy group esterified by *N*-methylanthranilic acid. An aldehyde group is present [ν_{max} ($CHCl_3$) 1722 cm^{-1}; ^{1}H n.m.r. δ 9.91] and a carbonyl apparently in a 6-membered ring [ν_{max} 1695 cm^{-1}, ^{13}C n.m.r. δ 211.4 p.p.m. (s)]. Bearing in mind these functionalities and their effect on the n.m.r. data, which show 6 tertiary methyls along with the two oxidised methyls (·CHO,

† This probably corresponds to the avenamine of Maizel *et al.* (ref. 4) and the avenamine-A of Tschesche *et al.* (ref. 5) but we have altered the 'amine' naming of the 'aglycones' since two of ours are nitrogen-free.

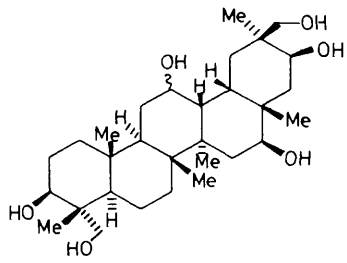
‡ A number of attempts to obtain a crystal suitable for *X*-ray analysis were made using the avenacins and avenestergenins, together with heavy-atom derivatives (bromofuroates, *p*-iodobenzoates) of the latter. None proved satisfactory, so structure work was continued as a chemical/spectroscopic problem.

CH_2OH), together with molecular formulae data, an oleanane framework seemed highly probable. Our structural conclusions are indicated in (**1a—d**) and the evidence is developed in summary below. ^1H N.m.r. data (250 MHz, $CDCl_3$) for all four avenestergenins and their acetates, and the spin-decoupling results for avenestergenin A–1, permit construction of a 'patch diagram' (**1**) showing local shaded areas (P to T) in which connectivity is established. The type of information used is indicated in (A). It is noted that there is a small 'W'-coupling (1.0 Hz) between the 30–*CH*O and the C–21 H_{ax} proton, the latter carbon carrying the aryl ester, thus relating functionalities within block T.

Results from nuclear Overhauser difference (n.O.e.) spectroscopy are summarised in (**2a**) and (**2b**) showing two segments of the molecule in different views. These have given valuable information. First, although P is an isolated block on (**1**), connectivities can be established *via* the n.O.e. enhancements for Q–S, Q–R, R–S, S–T, R–T, and Q–T. This may be direct as in the case of 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–$(CH_3)_{ax}$→15–H_{ax}]. Apart from interconnecting the ^1H-coupled blocks, a number of other valuable stereochemical points arise. Thus, in ring A the 24–Me is axial with the ·CH_2OH (α–). The *cis*–D/E fusion is verified (*e.g.* n.O.e. of 27–Me_{ax} with 19–H_{ax}, or 16–H_{ax} with 21–H_{ax}). The latter n.O.e. also shows that the C–16 hydroxy group is equatorial (β–), whilst the ester at C–21 is also equatorial (β–), supplementing the evidence derived from ^1H n.m.r. coupling constant data. From its n.O.e. with 22–H_{ax}, the 30–CHO must be axial, whilst the 29–Me_{eq} shows n.O.e. effects with 19– and 21–H_{ax}.

^{13}C N.m.r. data are consistent with structure (**1**), the majority of the signals being specifically assignable. Excellent analogies for the A/B systems of both the A and B series are available,[6] but because of the unusual functionalities of the C/D/E system (12-carbonyl, 30-aldehyde, and 21-ester) existing triterpene data are of less direct value and need to be combined with general ^{13}C n.m.r. considerations. Full ^{13}C n.m.r. data have been measured for all four avenestergenins and, together with some specific C–H decoupling, this strengthens the assignments.

A study of cleavage fragments in the electron impact mass spectrum[7] adds useful confirmatory evidence for the distribution of functional groups within the molecules. Five fragments which have been accurately mass-measured, and for which logical cleavage mechanisms can be proposed, are illustrated as (**3**)—(**7**). Both avenestergenins of the A and B series give the appropriate fragments: *N*-methyl-anthranilic acid is a readily recognised elimination product of A–1 and B–1.



(**8**)

Reduction of avenestergenin with lithium aluminium hydride gives avenagenol.[4] The compound forms a hexa-acetate (m.p. 220—223 °C) and can be formulated as (**8**).

We thank the Agricultural Research Council for support, and the S.E.R.C. for the provision of instrumentation. Dr. B. Mann and Dr. I. Sadler are thanked for their help with n.O.e. difference spectra.

Received, 7th November 1983; Com. 1445

References

1 'Biology and Control of Take-All,' eds. M. J. C. Asher and P. J. Shipton, Academic Press, London, 1981.
2 E. M. Turner, *J. Exp. Bot.*, 1961, **12**, 169; R. H. Goodwin and B. M. Pollock, *Am. J. Bot.*, 1954, **41**, 516.
3 J. Holden, *Trans. Br. Mycol. Soc.*, 1980, **75**, 97.
4 J. V. Maizel, H. J. Burkhardt, and H. K. Mitchell, *Biochemistry*, 1964, **3**, 424; 426.
5 R. Tschesche, H. Chandra Jha, and G. Wulff, *Tetrahedron*, 1973, **29**, 629.
6 *E.g.*, K. Tori, Y. Yoshimura, S. Seo, K. Sakurawi, Y. Tomita, and H. Ishii, *Tetrahedron Lett.*, 1976, 4163; K. Tori, S. Seo, A. Shimaoka, and Y. Tomita, *Tetrahedron Lett.*, 1974, 4227.
7 H. Budzikiewicz, J. M. Wilson, and C. Djerassi, *J. Am. Chem. Soc.*, 1963, **85**, 3688.