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THE ANTIFUNGAL FACTORS IN BARLEY - THE CONSTITUTIONS OF HORDATINES A AND B.

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One of the antifungal factors** isolated (1,2) from barley seedlings consists, essentially, of the glucosides of two closely related compounds, hordatine A and hordatine B. Attempts to achieve a preparative resolution of the glucosides were of no avail but, fortunately, it was possible to treat the mixture, for many purposes, as a single chemical entity. It could thus be shown (2) that it comprises, per molecule, two agmatine residues bound in amide bonds through their aminonitrogens, a D-glucopyranose unit, and a substituted coumaric acid residue attached to a further, undefined moiety. Mild methanolysis liberated the hordatines which were also investigated mainly in admixture.

The hordatines have λ_{max}^{ca} 300 mμ (log ε 4.31) which is essentially stable to base. The phenolic oxygen in the coumaroyl moiety of the compounds is therefore still bound in an ether link. On the other hand, a second maximum at 229 mμ (log ε 4.35) shifts to 238 mμ on basification. Hydrogenation of the hordatines furnishes the dihydro derivatives, with $\lambda\lambda_{max}$ 281, 220 mμ (log ε 3.49, 4.15) in neutral, and λ_{max} 287, λ_{sh} 281, λ_{max} 246 mμ in alkaline solution. Clearly, the hordatines contain a free phenol which is not conjugated to the coumaroyl chromophore.

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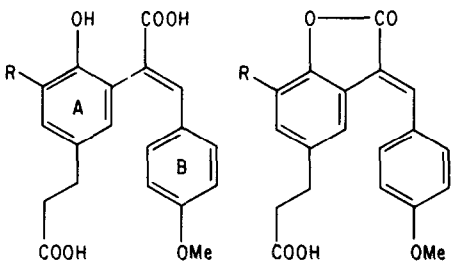
* Contribution No. 323
** the fraction previously (2) designated as M.

The dihydrohordatines, also accessible by methanolysis of the dihydroglucosides, have been partially resolved by countercurrent distribution into fractions consisting of dihydrohordatine A ($[\alpha]_D^{23^\circ}$ + 49° when almost pure), admixed with increasing amounts of dihydrohordatine B. Methylation (Me_2SO_4/K_2CO_3) followed by vigorous alkaline hydrolysis, furnished the crystalline acids I (40mg) and III (3mg) from material rich in the A component. Poorer fractions gave the same acids in the ratio 4:3 but in both cases, I and III together were obtained in about 80% yield.

I is a colourless dicarboxylic acid $C_{19}H_{18}O_6$*, m.p. 164-8°, $\lambda\lambda_{max}$ 298, 218 mμ (log ε 4.23, 4.27, 96% EtOH); λ_{max} 288 mμ (log ε 4.37, .06N NaOH, 50% EtOH); $\lambda\lambda_{max}$ 310, 220 mμ (log ε 4.29, 4.29; acidified EtOH) and ν_{max}^{KBr} 1696, 1652 cm^{-1}. At the melting point, it is transformed into the yellow, crystalline lactone II, $C_{19}H_{16}O_5$, m.p. 155-8°, $\lambda\lambda_{max}$ 381, 250 mμ (log ε 4.28, 4.23), ν_{max}^{KBr} 1762, 1710 cm^{-1}, reopened to I by dilute base. Ozonolysis of I affords a salicylic acid. The nmr spectrum of I (in d_6-acetone) shows a singlet at τ 2.15 (1H, deshielded olefinic proton); a typical A_2B_2 system (4H, multiplet approximating to a pair of doublets centred at 2.81 and 3.22, J_{AB} 9 c.p.s.) superimposed on the only partly resolved signals due to three other aromatic protons; a singlet at 6.23 (3H, aromatic OMe) and a multiplet approximating to a quartet centred at 7.32 (4H, β-arylpropionic acid).



(I, R=H) (II, R=H)
(III, R=OMe) (IV, R=OMe)

*Satisfactory analyses were obtained for all compounds characterized by constitutional formula.

The structures* represented by (I) and (II) respectively, followed from the sum of this evidence and were confirmed by synthesis. Addition of allyl bromide to phloretic acid methyl ester gave methyl-β-4-allyloxyphenyl-propionate, characterized as the corresponding acid $C_{12}H_{14}O_3$, m.p. 89-90°. Claisen rearrangement of the ester at 210°, followed by acetylation of the crude product, ozonolysis, alkaline hydrolysis and chromatography over silica gel, afforded 50% of β-(3-carboxymethyl-4-hydroxyphenyl)propionic acid, $C_{11}H_{12}O_5$, m.p. 155-7°. This was condensed with anisaldehyde in the presence of pyridine to give 70% of the expected product, identical with lactone II by m.p., mixed m.p., uv and ir spectra and by hydrolysis to acid I (criteria as above).

Acid III, $C_{20}H_{20}O_7$, m.p. 180-4°, is very similar to I in uv spectrum. At the melting point, it forms a lactone IV, $\lambda\lambda_{max}$ 380, 250 mμ, ν_{max}^{KBr} 1757, 1705 cm^{-1}, while the nmr spectrum indicates that III is a ring A methoxy derivative of I. Of the three possible structures, (III), derived from ferulic acid, was the most probable on biogenetic grounds. This surmise was substantiated by a synthesis of III from dihydroferulate along the route described above.

I and III can be derived directly from dihydrohordatine A and B respectively, if these are formulated as in (V) and VI). The hordatine glucosides can then be represented as a mixture consisting predominantly of (VII), smaller amounts of (VIII), and their double bond (cis) isomers (proof for the presence of the latter is available). The assigned structures are consistent with all the available evidence, except for two earlier analyses (2) for salts of the glucosides, which, when considered together**, supported a

* Stereochemistry about the olefinic bond not implied.

**The dipicrate, in contrast to the dihydrochloride of the mixture, could be purified by repeated precipitation. The analytical data obtained for it are also compatible with the present formulations. Found, C, 47.29, H, 5.16, N, 16.23, O, 31.64%. $C_{34}H_{48}N_8O_9$. $2C_6H_3N_3O_7$ requires C, 47.19, H, 4.65, N, 16.75, O, 31.43%. $C_{35}H_{50}N_8O_{10}$. $2C_6H_3N_3O_7$ requires C, 47.00, H, 4.70, N, 16.33, O, 31.97%.

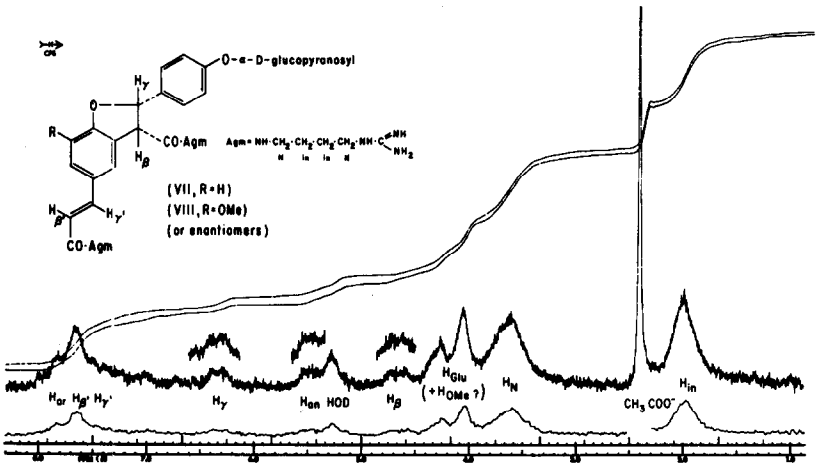


Fig. 1. Nmr spectrum of hordatine (A and B) glucoside diacetate in D_2O.

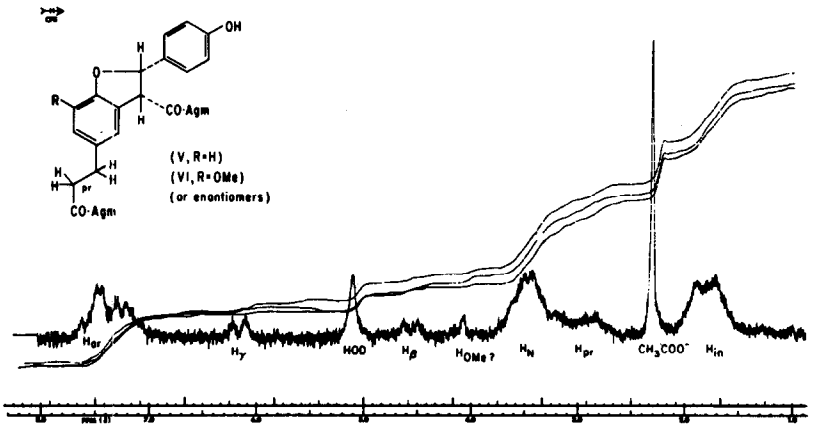


Fig. 2. Nmr spectrum of dihydrohordatine (A and B) diacetate in D_2O.

$C_{37}H_{58}N_8O_{11}$ formulation. The proposal was only tentative (2) and can be rejected when the nmr spectra of hordatine derivatives are considered. These, when allowance is made for the known number of proton-bare carbon atoms and NH and OH groups, lead, within the limits of integration, to constitutions $C_{34}H_{48}N_8O_9$ or $C_{35}H_{50}N_8O_{10}$ as required by (VII) or (VIII) respectively.

All bands can be assigned in conformity with the proposed structures, as shown by typical spectra for the glucosides (Fig. 1) and the dihydrohordatines (Fig. 2) and data for some other derivatives (Table 1).

TABLE 1.

Nmr Spectra in D_2O.

τ [a], (multiplicity)
J c.p.s., (relative area)

Compound	1	2[b]	3[b]
$H_{ar}, H_{\beta}, H_{\gamma}$	2.0–2.9, (m) –, (7)	2.0–3.5, (m) –, (9)	2.0–3.5, (m) –, (8)
H_{γ}	3.67, (d) 7.5, (1)	3.85, (d) 7.5, (1)	3.75, (d) 7.5, (1)
H_{an}	4.46, (d) 5.0, (1)	–	–
H_{β}	5.32, (d) 7.5, (1)	5.50, (d) 7.5, (1)	5.50, (d) 7.5, (1)
H_{Glu}, H_{OMe}	5.5–6.1, (m) –, (6)	5.95, (s) –, (ca 0.5)	5.98, (s?) –, (ca 0.3)
H_N	6.1–6.7[c], (m) –, (8)	6.1–6.9, (m) –, (8)	6.1–6.9, (m) –, (8)
H_{pr}	6.6[c]–7.3, (m) –, (4)	–	–
$CH_3.CO$	–	–	7.38(s),7.45(s) –, (3),(–,weak)
$CH_3.COO^-$	7.60, (s) –, (6)	–	–
H_{in}	7.8–8.5, (m) –, (8)	7.8–8.5, (m) –, (8)	7.8–8.6, (m) –, (8)

1) dihydrohordatine glucoside diacetate 2) hordatine di-d_3-acetate 3) obtained by acetylation (Ac_2O, room temperature) of hordatine at the phenolic hydroxyl, as di-d_3-acetate. Some products of N-acylation are probably present but no shifts characteristic for acylation of primary or secondary alcohols can be detected. a) determined on a Varian A60 instrument to tetramethylsilane as external reference. b) note the signals assignable to methoxyl. c) bands overlap in this region.

The stereochemistry of the hordatine glucosides at the anomeric centre follows from the position (3) of the signal due to H_{an}. The cis configuration has been assigned to H_β, H_γ by analogy with dehydrodiconiferyl alcohol (4) (H_β 5.81, H_γ 4.46, $J_{\beta\gamma}$ 7.2 c.p.s., in $CDCl_3$) but must be regarded as tentative (4).

Trace amounts of dehydrodiconiferyl alcohol and related compounds have been detected in spruce (5), but only by chromatographic procedures and, further, the compounds may have been artifacts (6). The hordatine glucosides are isolated in relatively substantial amounts and they cannot be artifacts, as evidenced by the optical activity of the aglucones. They thus appear to be the first authentic examples of the occurrence, in nature, of the phenylpropanoid dimers which have been discussed as lignin precursors (5,6). Further, evidence is available that hordatines A and B occur in barley also in the free form. The structure of hordatine A has been confirmed by a synthesis of the racemate, which will be reported in the near future.

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