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ISOLATION AND STRUCTURE ELUCIDATION OF GENUINE OAT PHYTOALEXIN, AVENALUMIN I

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Abstract: The major phytoalexin from oat leaves has been identified as 2-[2-(4-hydroxyphenyl)ethenyl]-6-hydroxy-4H-3,1-benzoxazin-4-one (A).

Phytoalexins are antimicrobial substances produced by plants following infection with microorganisms. On inoculation with incompatible races of oat crown rust, oat leaves accumulate luminescent substances, designated as avenalumin I, II and III, which are inhibitory to the growth of rust fungi.[1] Possible significance of avenalumins in induced resistance of oat cultivars to rust races has been demonstrated for the combination of twenty oat Pc lines and two crown rust races. We report herein the isolation and structure elucidation of avenalumin I which is a major compound found in any combination of the oat cultivars and rust races.

Hot methanol extract of primary leaves of oat, infected with an incompatible race of *Puccinia coronata* f. sp. *avenae*, was fractionated by passing twice through Sephadex LH-20 column (eluted with methanol). The fraction containing avenalumins was subjected to preparative thin layer chromatography [Kieselgel 60 G, E. Merck, 0.35mm, 20 x 20cm, developed with $CHCl_3$-MeOH-H_2O(65:35:10 by volume, lower layer)] to give pure avenalumin I (Rf 0.30, by UV-detection; 100mg from 10kg of infected fresh leaves).

Avenalumin I, $C_{16}H_{11}NO_4$, which gives no distinct melting point, is an optically inactive phenolic compound. Its ^{1}H-NMR and ^{13}C-NMR spectra (in DMSO-d_6) indicate the presence of two aromatic rings and a conjugated double bond in its molecule: ^{1}H-NMR, δ, 8.40, 1H, d, J=9.0Hz; 7.2-7.8, 4H, m; 6.2-6.9, 3H, m; 6.38, 1H, d, J=16.0Hz; and ^{13}C-NMR spectrum was shown in Fig. 1 and UV $\lambda_{max.}$ nm(ε); 317(15200), 336(15900) in methanol and 320(15400), 344(13400) in methanol containing hydrochloric acid, and 366(16600) in methanol containing sodium hydroxide.

Acetylation of avenalumin I with acetic anhydride in pyridine at room temperature overnight gave crystalline avenalumin I diacetate, $C_{20}H_{15}NO_6$, mp 226-228°C, UV $\lambda_{max.}$ nm(ε) 226(17500), 306(18900), 318(21300), 341(20200) in methanol. The ^{1}H-NMR (220MHz in $CDCl_3$) and IR spectra of the diacetate suggestid that it contains following structure moieties: two acetoxyl groups [δ, 2.31 and 2.32, each 3H, s and 1760($\nu_{C=O}$) and 1190cm^{-1}], a *trans* disubstituted ethylenic double bond (δ, 6.71 and 7.81, each 1H, d, J=16.0Hz), a 1,4-disubstituted benzene

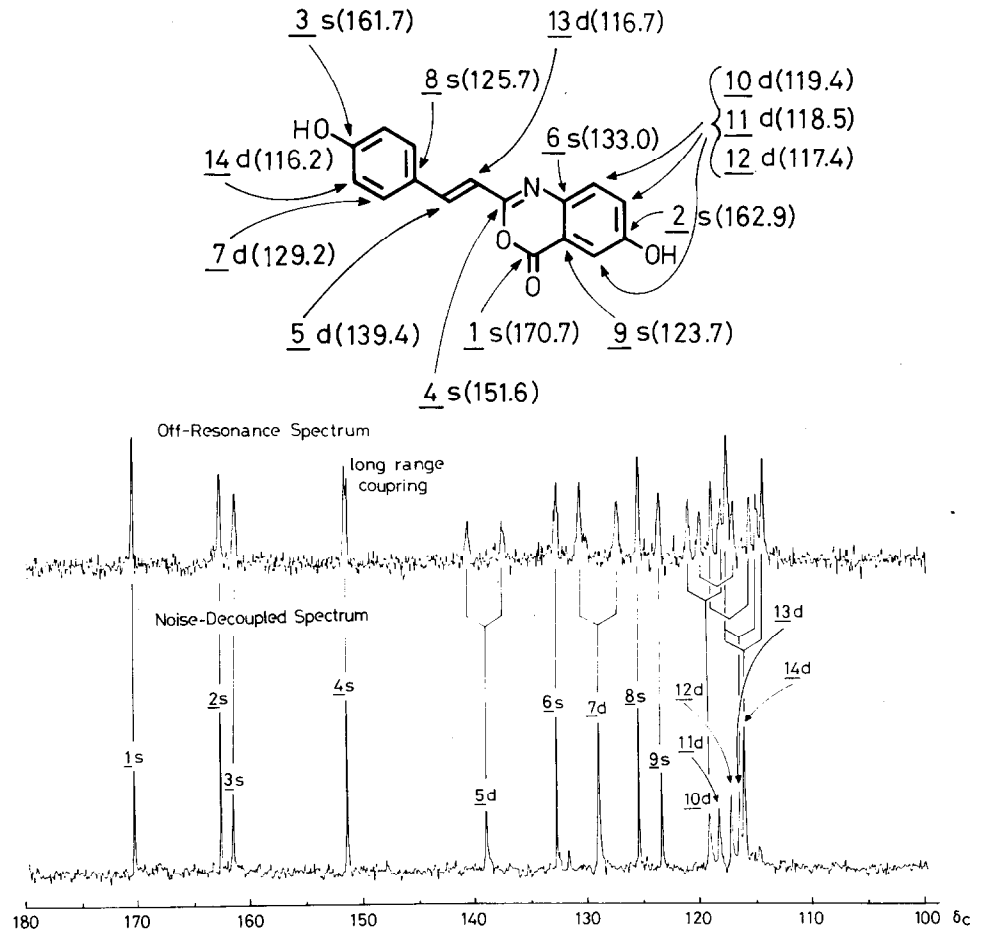

Fig. 1 ^{13}C-NMR spectrum and its signal assignment of avenalumin I

ring (δ, 7.13 and 7.56, each 2H as a AB-type quartet, J=8.0Hz) and a 1,2,4-trisubstituted benzene ring (δ, 7.52, 1H, dd, J=8.0 and 2.5Hz; 7.62, 1H d, J=8.0Hz and 7.92, 1H, d, J=2.5Hz).

Methanolysis of avenalumin I (with methanol containing hydrogen chloride in a sealed tube at 110°C for 24h) gave almost quantitatively methyl *p*-coumarate [m/z, 178(80%)($M^{+\cdot}$), 147(base peak), 119(31%) and 91(23%)] and methyl 4(or 5)-hydroxyanthranilate [m/z, 167(base peak)($M^{+\cdot}$), 135(92%) and 107(68%)], which were identified by a GC-MS system. From these methanolysis products, avenalumin I is concluded to be 2-[2-(4-hydroxyphenyl)ethenyl]-6(or 7)-hydroxy-4H-3,1-benzoxazin-4-one (A) or (A'). The conclusion is also supported by mass spectroscopic analysis of avenalumin I diacetate as shown in Fig. 2. Position of the hydroxyl group on the benzoxazinon ring was determined by synthesis of diacetates of both compounds (A) and (A').

Treatment of each 5- and 4-hydroxyanthranilic acid with acetic anhydride under reflux for 4h gave the acetoxybenzoxazinones (B) and (C), respectively.[2,3] The former (B) was subjected to a condensation reaction with *p*-acetoxybenzaldehyde in the presence of *p*-toluenesulfonic acid in benzene under reflux for 2 days to give a crystalline product, mp 226-228°C (in 75% yield), which was identical with avenalumin I diacetate in all respects. The same treatment of the

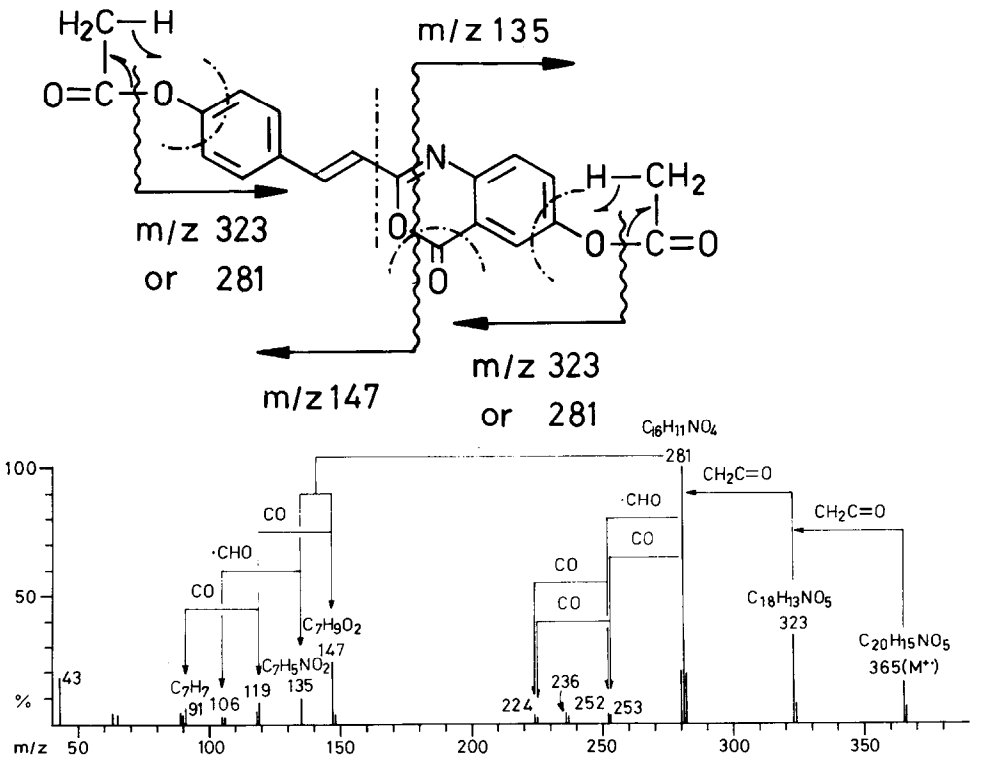

Fig. 2 EI mass spectrum (70eV) of avenalumin I diacetate

latter (C) gave an isomeric benzoxazinon [diacetate of (A')], mp 194-195°C (in 45% yield). Removal of acetyl groups from the synthesized diacetate of (A) was performed by treatment with sodium borohydride in dimethoxyethane to give (A)(in 45% yield), which indicated the same antifungal activity with avenalumin I (50% inhibition of germination of *P. coronata* f. sp. *avenae*, 200μg/ml).

This is the first report to demonstrate the occurence of a nitrogen-containing phytoalexin with benzoxazin-4-one structure. Significance of avenalumins in disease resistance of oat will be published in other reports.[1,4)]

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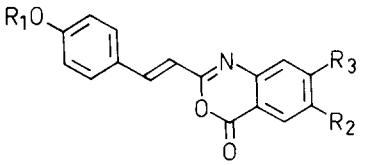

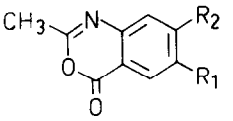

(A) R_1=H, R_2=OH, R_3=H (B) R_1=OAc, R_2=H

(A') R_1=H, R_2=H, R_3=OH (C) R_1=H, R_2=OAc

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