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2-HYDROXYPUTRESCINE AMIDES AS ABNORMAL METABOLITES OF WHEAT

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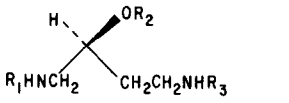
On inoculation with stem rust or leaf rust, wheat leaves resistant to these pathogens produce small amounts of two substances which are not detectable in susceptible or in uninoculated resistant leaves (1,2). Ion-exchange and chromatographic techniques have given the compounds as syrups which were sufficiently pure for structural studies.

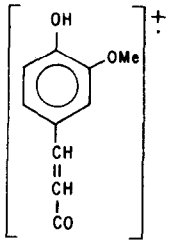


(I) $R_2 = R_3 = H$, R_1 = 4-OH-3-OMe-C_6H_3-CH=CH-CO· (γ β α)

(II) $R_2 = R_3$ = Ac, R_1 = 4-OH-3-OMe-C_6H_3-CH=CH-CO· (γ β α)

(V) $R_1 = R_2 = R_3 = H$

(VI) $R_2 = R_3 = H$, R_3 = 4-OH-C_6H_4-CH=CH-CO·



(III)

Structure (I) is proposed for one of the metabolites on the following evidence. The substance (8 mg from 10,000 leaves of the resistant line of Chinese spring wheat (1) after inoculation with Puccinia graminis f. sp. tritici; 15 mg from 30,000 leaves of the variety "Selkirk" after inoculation with P. recondita (2)) behaves as a moderately strong base which forms a stable salt with 1 mol-equiv. of acetic acid (n.m.r.). It is reversibly absorbed on strongly basic ion-exchangers but does not possess a free carboxyl group (electrophoretic behaviour). The u.v. spectrum, with λ_{max} 316 and 293 nm (log ϵ 3.92 and 3.85 (neutral or acid) and λ_{max} 363 and 309 nm (5×10^{-4}N OH^-) reveals the presence of a phenolic group and corresponds to a feruloyl chromophore (predominantly but not exclusively in the trans form, see inf.). Ferulic acid, identified by chromatographic methods, is liberated by alkaline hydrolysis.

On acetylation, the metabolite furnishes the neutral triacetyl derivative (II), $[\alpha]_D^{24^\circ}$ -20° (c, 0.7% in $CHCl_3$), with the u.v. spectrum of an O-acetylated ferulamide, λ_{sh} 298 and λ_{max} 273 nm (log ϵ 3.89 and 4.12). The i.r. spectrum (in $CHCl_3$) shows bands corresponding to NH (3460 cm^{-1}), amide (1666 cm^{-1}), aryl acetate (1758 cm^{-1}) and, most importantly, aliphatic acetate (1730 cm^{-1}). Prominent peaks in the mass spectrum (Morgan-Schaffer Corp., Montreal), with the molecular ion (6.5%) at 406 m.u., are tentatively assigned to ions resulting from the consecutive loss of 42 (CH_2CO)(29%), 60 (CH_3CO_2H)(10%), 59 (CH_3CONH_2)(8.5%), and 52 (2 C_2H_2?)(18%). The base peak at 177 m.u. corresponds to the ion (III).

The n.m.r. spectrum confirms that (II) is a ferulic acid derivative. However, it is a misture of the geometrical isomers (double bond trans and cis respectively) because the resonances from the corresponding vinyl protons (2 AB systems of unequal areas, J_{AB} 15.5 and 12.5 Hz respectively) can be clearly identified. Most of the other bands are similarly paired and can be assigned (Fig. 1) by a comparison with the spectra of the model compound (IV). The pure

4-AcO-3-OMe-C_6H_3-CH=CH-CO-NH-$(CH_2)_3$-NHAc
(IV)

trans-isomer of (IV), m.p. 155-157.5°, λ_{sh} 299 and λ_{max} 276 nm (log ϵ 4.04 and 4.31), prepared by the reaction of 1,3-diaminopropane with 1 mol-equiv. of O-acetyferulic acid in the presence of dicyclohexylcarbodiimide and subsequent acetylation, has the spectrum reproduced in Fig. 2. On brief irradiation with light, it gives a mixture of the trans- and cis-isomers as a syrup, λ_{sh} 299 and λ_{max} 273 nm (log ϵ 3.77 and 4.01), whose spectrum (Fig. 3) shows chemical shift differences analogous to those of (II). The metabolite (I) can be shown similarly to be a mixture of the trans- and cis-isomers. The only relevant differences in its n.m.r. spectrum (free base in D_2O) from that of (II) are the absence of bands assignable to acetate and the substitution of a multiplet (1H) near δ 4.3 ($>C\underline{H}(OH)$) for that near 5.0 ($>C\underline{H}OAc$).

Vigorous acid hydrolysis of (II) gave, as essentially the sole water-soluble product, a crystalline salt which is identical with S-(+)-2-hydroxyputrescine dihydrochloride (V) by co-chromatography, co-electrophoresis, n.m.r. and i.r. spectrum. Too little of the hydrolysis product, m.p. 246-252°, was available for a determination of its optical rotation. The configurational assignment rests on the fact that its 1:1 mixture with an authentic specimen ($[\alpha]_D^{22^\circ}$ + 7°) melted at 249-254° whereas a similar mixture with R-(-)-2-hydroxyputrescine hydrochloride (V, opposite configuration, $[\alpha]_D^{22^\circ}$ - 7°) melted at 232-236°. The authentic compounds were prepared in about 15% yield by the $LiAlH_4$ reduction of the diamides of S-(-)- and

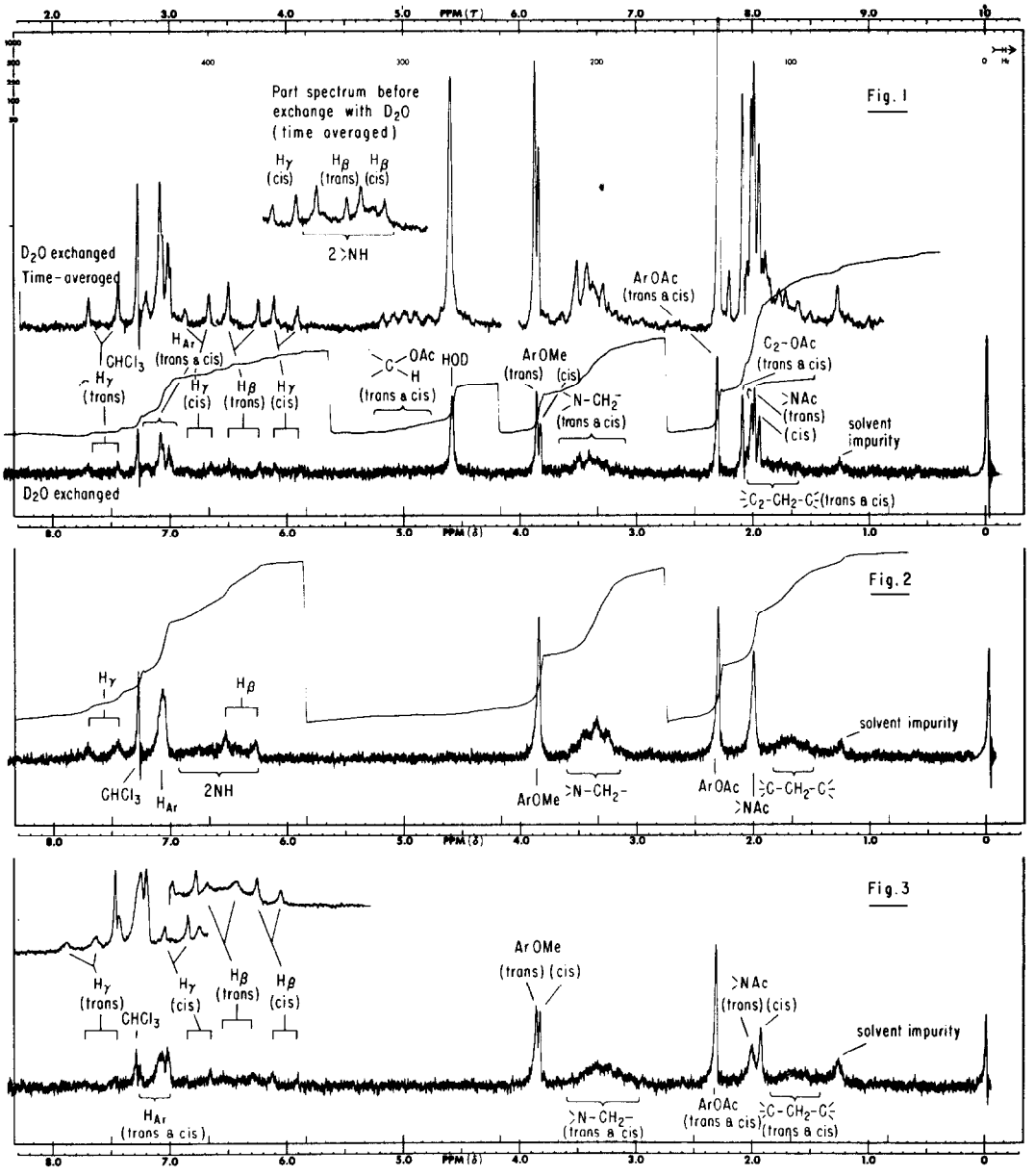


Fig. 1. 60 MHz spectrum of (II). Time-averaged sections, 16 scans through 100 Hz. The last section (δ 2.5-.85) shows peaks, immediately to the right of the bands assigned to ArOAc and cis-NAc, which arose from a field shift during the run and should be disregarded.

Fig. 2. 60 MHz spectrum of (IV).

Fig. 3. 60 MHz spectrum of irradiated (IV).

R-(+)-malic acid respectively; the melting points were 250-253° and 249-252° respectively, and 235-239° in 1:1 admixture.

After methylation of the phenolic hydroxyl (CH_2N_2), (I) fails to react with the periodate-benzidine reagent diagnostic for vic-aminoalcohols. Formulation of the metabolite as the 2-hydroxyamide, as in (I), is therefore favoured but awaits confirmation by synthesis.

On analogous spectroscopic and hydrolytic evidence, the second metabolite, isolated from inoculated wheat in similar yields, must be formulated as (VI), the coumaric acid amide of 2-hydroxyputrescine.

The relatively few amides of cinnamic acids which have been isolated from nature include coumaroylagmatine and the hordatines from Hordeum vulgare (Gramineae)(3), and subaphylline (feruloylputrescine) from Salsola subaphylla (Chenopodiaceae)(4) and Citrus spp. (Rutaceae)(5).

This report is the first record of an hydroxylated putrescine from natural sources. It is of interest that racemic (V) has recently been synthesized, by a route different from the one adopted here, in the expectation that it would be of biological significance (6). Oxidative processes have been implicated in the resistant reactions of many plants (7) but are little understood. Further work on the significance of (V) in this context is in progress.

Acknowledgements

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