Structure of a Fungal Metabolite of the Phytoalexin Wyerone Acid from *Vicia faba L.*

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The isolation and identification of the major detoxification product of wyerone acid (a phytoalexin from *Vicia faba L.*), produced by *Botrytis fabae*, are described. The compound has been shown to be 3-[5-(1-hydroxy-*cis*-hept-4-enyl)-2-furyl]-*trans*-prop-2-enoic acid, by total synthesis of the methyl ester.

THE concept that plants are able to resist fungal attack by the production of antifungal compounds which inhibit invading hyphae was formalised by Müller and Borger,[1] who proposed that such substances be called phytoalexins. The broad bean (*Vicia faba* L.) has been shown to produce the phytoalexin wyerone acid[2] (I) after infection with *Botrytis fabae* and *B. cinerea*.[3,4]

The differential pathogenicity of *B. fabae* (virulent) and *B. cinerea* (avirulent) in *V. faba* has been attributed to their interaction with wyerone acid.[3-5] *B. fabae*, unlike *B. cinerea*, is able to detoxify wyerone acid rapidly and thus prevent its accumulation to antifungal concentrations in the infected plant.[5,6] A metabolite of wyerone acid produced both *in vitro* and *in vivo* by *B. fabae* has been tentatively identified as reduced wyerone acid (II).[6] We report the isolation, structure determination, and synthesis of the methyl ester of the detoxification product.

The addition of wyerone acid to germinating conidia of *B. fabae* (but not *B. cinerea*) resulted in the rapid loss (assayed at $\lambda_{max.}$ 350 nm) of wyerone acid and the accumulation of another acid with $\lambda_{max.}$ 310 nm. This latter acid was found in high concentrations in leaf tissue blackened and colonised by hyphae of *B. fabae*. The suspected metabolite could not be found in tissue infected by *B. cinerea*.

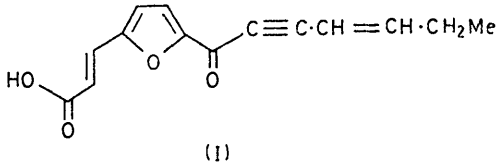
Larger quantities of the metabolite were isolated from droplets of *B. fabae* spore suspension, incubated in pod seed cavities as previously reported.[6] The i.r. spectrum of the crude isolate suggested the presence of an αβ-unsaturated carboxylic acid. The sample was esterified with diazomethane and the ester isolated and purified by t.l.c.

The i.r. spectrum of this product showed an αβ-unsaturated ester carbonyl absorption (1722), a hydroxy-band (3450), C=C stretching absorption (1640), and absorptions characteristic of the furan system at (1200, 1018, and 855 cm^{-1}). The n.m.r. spectrum showed signals at τ 3·80 and 2·68 (each 1H, d, H_a and H_b), 3·53 and 3·76 (each 1H, d, furan ring protons), 4·68 (2H, m, side-chain CH=CH), 5·36 (1H, t, C*H*·OH), 6·29 (3H, s, CO_2Me), 7·7—8·3 (6H, m, 3 × CH_2), and 9·08 (3H, t, Me). The mass spectrum showed a molecular ion at *m/e* 264·136 ($C_{15}H_{20}O_4$) and a fragmentation pattern

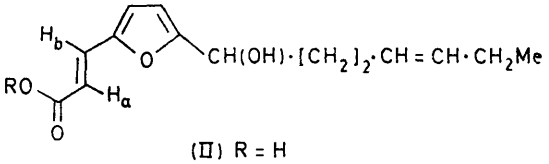
[1] K. O. Müller and H. Borger, *Arb. Biol. Anst.* (*Reichsaust*) *Berlin*, 1941, **23**, 189.
[2] C. H. Fawcett, D. M. Spencer, R. L. Wain, A. G. Fallis, Sir Ewart R. H. Jones, M. Le Chan, C. B. Page, V. Thaller, D. C. Shubrook, and P. M. Whitmuns, *J. Chem. Soc.* (*C*), 1968, 2455.
[3] R. M. Letcher, D. A. Widdowson, B. J. Deverall, and J. W. Mansfield, *Phytochemistry*, 1970, **9**, 249.
[4] B. J. Deverall and J. Vessey, *Ann. Appl. Biol.*, 1969, **65**, 449.
[5] B. J. Deverall and J. W. Mansfield, in preparation.
[6] J. W. Mansfield and D. A. Widdowson, *Phys. Plant Path.*, 1973, **3**, 393.

consistent with the metabolite being a hexahydro-derivative (II) of wyerone acid.

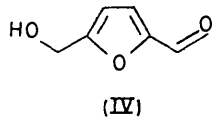
Wyerone acid (I) has been successfully synthesised [2] by the conversion of sucrose into 5-hydroxymethyl-2-furaldehyde (IV) and subsequent reactions of this



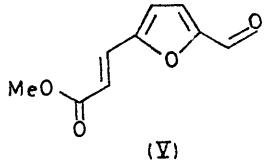
(I)



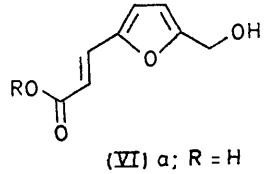
(II) R = H
(III) R = Me



(IV)



(V)



(VI) a; R = H
b; R = Me

molecule to introduce side chains into the 2- and 5-positions of the furan ring. In our hands condensation of malonic acid with the aldehyde (IV) gave the αβ-unsaturated acid (VIa), but in considerably lower yield than reported,[2] and the work-up procedure was altered to improve this yield. The crude acid was not isolated but esterified with diazomethane and the product was oxidised with active manganese dioxide. Direct sublimation then gave the pure aldehyde (V), whose physical properties were identical with those previously reported.

Initial attempts to effect a Grignard reaction of the aldehyde (V) with *cis*-hex-3-enylmagnesium bromide in tetrahydrofuran gave the alcohol (VIb) as the only product. In ether and by use of the inverse addition procedure the desired product (III) was obtained contaminated with minor amounts of the alcohol (VIb), from which (III) was conveniently isolated by p.l.c.

The i.r. and n.m.r. spectra of the purified product were virtually identical with those of the esterified natural sample (pale yellow oil). The coupling constants of the side-chain olefinic protons in the natural sample suggested the *cis*-orientation about the double bond, as present in wyerone acid. The olefinic proton signals from both synthetic and natural samples showed $W_{\frac{1}{2}}$ 8 Hz.

The toxicity of the metabolite to *B. fabae* and *B. Cinerea* is considerably less than that of wyerone acid.[6] This suggests that the biological activity is associated with the acetylenic ketone function, a factor noted with many other naturally occurring acetylenes and polyacetylenes.[7]

EXPERIMENTAL

I.r. spectra were recorded on a Unicam SP 257, u.v. spectra on a Unicam SP 800, n.m.r. spectra on a Varian HA100, and mass spectra on an A.E.I. MS9 instrument. M.p.s were determined on a Kofler hot-stage apparatus. Petroleum refers to the fraction of b.p. 60—80°.

Isolation and Identification of the Metabolite.—*B. fabae* infection droplets (51 ml) incubated in seed pod cavities were collected 70 h after inoculation. The aqueous diffusate was extracted with ether (3 × 50 ml), the volume of the extract was reduced, and the mixture was separated by t.l.c. (silica gel; eluant ether–methanol, 2 : 1). The band of R_F 0·8 was extracted with 50% aqueous ethanol. The eluate was further subjected to t.l.c. (silica gel; eluant chloroform–methanol, 1 : 1) and the product extracted from a band at R_F 0·75 with 50% aqueous ethanol. The crude product (6 mg) showed $\nu_{max.}$ 3500—2800br and 1690—1720s cm^{-1}. Treatment with ethereal diazomethane, followed by t.l.c. (silica gel; eluant ethyl acetate–petroleum, 3 : 1) and extraction of the band at R_F 0·8 with chloroform gave *methyl* 3-[5-(1-*hydroxy*-cis-*hept*-4-*enyl*)-2-*furyl*]-trans-*prop*-2-*enoate* (III) (5 mg), $\nu_{max.}$ ($CHCl_3$) 3450, 1722, 1640, 1200, 1018, and 855 cm^{-1}, $\lambda_{max.}$ (EtOH) 312 nm, τ ($CDCl_3$) 2·68 (1H, d, *J* 16 Hz), 3·80 (1H, d, *J* 16 Hz), 3·53 (1H, d, *J* 3·5 Hz), 3·76 (1H, d, *J* 3·5 Hz), 4·68 (2H, m), 5·35 (1H, t, *J* 6 Hz), 6·29 (3H, s), 7·7—8·3 (6H, m), and 9·08 (3H, t, *J* 7 Hz), M^+ 264·136 ($C_{15}H_{20}O_4$ requires *M*, 264·136), *m/e* 246, 233, 231, 207, 194, 181, 149, and 121.

Methyl 3-(5-*Formyl*-2-*furyl*)*prop*-*trans*-2-*enoate* (V).—A solution of 5-hydroxymethyl-2-furaldehyde (6·3 g) and powdered malonic acid (5·5 g) in ethanol (15 ml) and pyridine (5 ml) was heated under reflux for 4 h. The solvent was removed under reduced pressure and the residual oil was treated with aqueous sodium hydroxide until strongly alkaline and extracted with ether (2 × 20 ml). The aqueous phase was acidified (2N-H_2SO_4) and extracted with chloroform (3 × 25 ml); the combined extracts were dried (Na_2SO_4), filtered, and evaporated to yield the crude acid (2·5 g). This was suspended in ethereal diazomethane (25 ml) and left in a refrigerator overnight. The excess of diazomethane and ether were removed under reduced pressure and the residual solid was degassed (oil pump).

The crude ester was dissolved in dry chloroform (100 ml) and active manganese dioxide (10 g) was added. The mixture was stirred at room temperature overnight and filtered, and the manganese dioxide was washed with chloroform. Evaporation of the solution yielded a white solid which was sublimed at 100—110° and 10 Torr to yield the formyl ester (V) (2·2 g), m.p. 106° (lit.,[2] 106—106·5°).

Methyl 3-[5-(1-*Hydroxyhept*-cis-4-*enyl*)-2-*furyl*]*prop*-trans-2-*enoate* (III).—*cis*-Hex-3-enyl bromide (0·5 g) in ether (3 ml) was added dropwise to magnesium turnings (0·15 g) in ether (3 ml) at such a rate as to maintain refluxing. The mixture was then refluxed for a further 30 min and

[7] J. D. Bu'Lock. *Progr. Org. Chem.*, 1964, **6**, 86.

cooled to room temperature; the supernatant liquid was removed with a syringe and added dropwise during *ca.* 5 min to a suspension of the furan (V) in ether (10 ml). The mixture was stirred at room temperature for 15 min and then refluxed for 30 min, cooled, and partitioned between ether and saturated aqueous ammonium chloride. The organic layer was separated, washed with water, dried, and evaporated to yield a pale yellow solid. T.l.c. (silica gel; ethyl acetate–petroleum, 1 : 1) showed that the product consisted of starting material, the ester (III), and the alcohol (VIb). P.l.c. gave starting material (0·28 g), and the *hydroxy-ester* (III) (0·24 g) as a clear pale yellow oil, $\nu_{max.}$ ($CHCl_3$) 3450, 3050, 3000—2850, 1722, 1640, 1200, 1018, and 855 cm^{-1}, τ ($CDCl_3$) 2·67 (1H, d, J 16 Hz), 3·79 (1H, d, J 16 Hz), 3·53 (1H, d, J 3·5 Hz), 3·75 (1H, d, J 3·5 Hz), 4·68 (2H, m), 5·35 (1H, t, J 6 Hz), 6·29 (3H, s), 7·7—8·3 (6H, m), and 9·07 (3H, t, J 7 Hz), $\lambda_{max.}$ (EtOH) 312 nm (ε 25,400), M^+ 264 and same fragmentation as the esterified natural sample (Found: C, 68·0; H, 7·5. $C_{15}H_{20}O_4$ requires C, 68·2; H, 7·6%).

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