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METABOLISM OF PHASEOLLIN BY *COLLETOTRICHUM LINDEMUTHIANUM*

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Key Word Index—*Phaseolus vulgaris*; Leguminosae; *Colletotrichum lindemuthianum*; phaseollin; phytoalexin; metabolism.

Abstract—Two metabolites of the phytoalexin phaseollin (**1**) produced by cultures of the fungus *Colletotrichum lindemuthianum* have been assigned structures (**3**) and (**4**).

Phaseolus vulgaris inoculated with various fungal pathogens produces the pterocarpan phaseollin (**1**), together with several other phytoalexins.[1–3] In evaluating the role of these compounds in disease resistance, it is desirable to know both the rate at which they are induced in the host plant and the capacity of the pathogen to metabolize and detoxify them. Two studies of the metabolism of phaseollin by fungi have already appeared. The alfalfa pathogen *Stemphylium botryosum* was found to produce a reduction product, phaseollinisoflavan,[4,5] while *Fusarium solani* f. sp. *Phaseoli* gave rise to an unidentified compound $C_{20}H_{18}O_5$ containing a carbonyl group.[6]

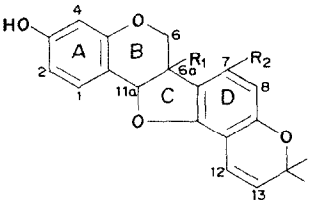
The *in vitro* metabolism of phaseollin by *Colletotrichum lindemuthianum*, the cause of anthracnose disease, is being investigated in our laboratories. In preliminary studies[7] the fungus was found to convert phaseollin into two phenolic metabolites, designated 1 and 2. In the present communication this finding has been confirmed using [^{14}C]-phaseollin and the structures of the two metabolites have been elucidated.

[^{14}C]-Phaseollin was extracted from etiolated bean hypocotyls which had been inoculated with tobacco necrosis virus and sodium acetate U-[^{14}C], specific activity 57 μCi/μmol.[8] After addition of a sample of phaseollin, specific activity 8600 dpm/mg, to a mycelial culture of *C. lindemuthianum*, race γ, subsequent ether extraction after 6 hr and TLC afforded unchanged phaseollin and also metabolites 1 (18% yield) and 2 (17% yield). The specific activities were calculated to be 8529, 9537 and 8503 dpm/mg respectively and no other labelled products were detected in the ether soluble extract.

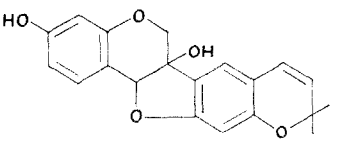
Metabolite 1, $C_{20}H_{18}O_5$ (M^+ 338), had a lower R_f than phaseollin on silica gel chromatograms[7] and gave a yellow colour with diazotized *p*-nitroaniline. Its UV spectrum (EtOH) λ_{max} 280, 286 and 314 nm was virtually superimposable with that of phaseollin, while the

[1] PERRIN, D. R. (1964) *Tetrahedron Letters* 29.
[2] PERRIN, D. R. and BATTERHAM, T. J. (1972) *Tetrahedron Letters* 1673.
[3] BURDEN, R. S., BAILEY, J. A. and DAWSON, G. W. (1972) *Tetrahedron Letters* 4175.
[4] HEATH, M. C. and HIGGINS, V. J. (1973) *Physiol. Plant Pathol.* **3**, 107.
[5] HIGGINS, V. J., STOESSL, A. and HEATH, M. C. (1974) *Phytopathology* **64**, 105.
[6] VAN DEN HEUVEL, J. and VANETTEN, H. D. (1973) *Physiol. Plant Pathol.* **3**, 327.
[7] BAILEY, J. A. *Physiol. Plant Pathol.* In press.
[8] VANETTEN, H. D. and BATEMAN, D. F. (1971) *Phytopathology* **61**, 1363.

IR spectrum ($CHCl_3$) showed aromatic absorption at 1595 and 1626 cm^{-1} but no bands in the carbonyl region. In the mass spectrum, peaks were present at *m/e* 338 (30%, M^+), 323 (100, M^+–Me), 320 (21, M^+–H_2O), 305 (45, M^+–Me–H_2O) and 295 (34, M^+–Me–CO).



(1) $R_1 = R_2 = H$
(3) $R_1 = OH$, $R_2 = H$
(4) $R_1 = R_2 = OH$



(2)

The NMR spectrum (CD_3CN) indicated that the 2,2-dimethylchromen ring had been retained (2 three-proton singlets at 1·33 and 1·37 and an AB quartet, *J* 10 Hz, at δ5·64 and 6·41) while two low-field aromatic doublets at 7·11 and 7·32 (both *J* 8 Hz) were also in similar positions to those observed for C_1 and C_7 in phaseollin.[1] Three remaining aromatic protons appeared in a multiplet δ6·3–6·7 which was not fully resolved although a doublet at 6·62 ($\frac{1}{2}$H, *J* 2 Hz) was clearly one arm of an aromatic doublet (*J* 8 Hz) further split by a *meta*-proton. These data leave little doubt that the aromatic ring substitution of phaseollin had remained unchanged. However, the characteristic spectral pattern of the protons on the central oxygen heterocyclic rings of phaseollin[1,2] was no longer apparent. Instead, there were two sharp singlets at δ4·05 (2H) and 5·30 (1H) which suggested that hydroxylation had occurred at C_{6a}, the protons at C_6 showing accidental chemical equivalence. In the pterocarpan tuberosin (**2**) similar resonances occur at δ4·0 and 5·25.[9]

Structure **3** for the metabolite was confirmed by treatment of 1 with formic acid at room temperature. This yielded a phenolic product of higher R_f, λ_{max} 284, 293, 331 and 351 nm, M^+ 320, consistent with the dehydration of the tertiary alcohol to yield a pterocarpen. Metabolite 1 is thus identical with the structure **3** which has previously been formulated for an induced antifungal compound from soybean.[10] Both the NMR and mass spectral data are in good agreement with the soybean phytoalexin but the UV data differ slightly. Nevertheless, the evidence given in this paper fully defines metabolite 1 as 6a-hydroxyphaseollin (**3**).

Metabolite 2, $C_{20}H_{18}O_6$ (M^+ 354), λ_{max} 282, 286 and 308 (*sh*) nm had a lower R_f than metabolite 1 on TLC and gave an ochre colour with diazotized *p*-nitroaniline. Only two prominent fragment ions appeared in the MS, at 336 (63%, M^+–H_2O) and 321 (100%, M^+–H_2O–Me). The possibility that metabolite 2 was formed from metabolite 1 by further hydroxylation was supported by the NMR spectrum (CD_3CN) which defined the structure. Thus, resonances at δ1·30 (*s*, 3H), 1·33 (*s*, 3H), 5·40 (*d*, 1H, *J* 10 Hz) and 6·34 (*d*, 1H, *J* 10) confirmed the presence of the 2,2-dimethylchromen ring and an hydroxyl substituent at 6a was indicated by peaks at 5·26 (*s*, 1H) and 4·26 (*q*, 2H, *J* 12). The aromatic region of the spectrum exhibited substantial differences from phaseollin and metabolite 1. In particular, only one low-field doublet was present (δ7·30, 1H, *d*, *J* 8) while a high-field aromatic proton appeared as a sharp singlet at 5·77. Two other aromatic protons were present

[9] JOSHI, B. S. and KAMAT, V. N. (1973) *J. Chem. Soc.* (*C*) 907.
[10] SIMS, J. J., KLEN, N. T. and HONWAD, V. K. (1972) *Phytochemistry* **11**, 826.

at 6·30 (*d*, *J* 2) and 6·51 (*dd*, *J* 8, *J* 2). This indicates that the 3 protons of ring A have been retained but the loss of an *ortho*-aromatic quartet and the appearance of a one proton singlet demonstrates that hydroxylation has occurred in ring D. The location of the single D ring proton is obtained from a consideration of the value of the chemical shift. Thus if the hydroxyl occupies the C-7 position, the substituent shielding effects, based on the data compiled by Ballantine and Phillinger,[11] would lead to an expected chemical shift of 6·00. If, however, C-8 is substituted, then the calculated shift for the proton at C-7 is now 6·75. The observed value of 5·77 is therefore in good agreement with the location of the hydroxyl at C-7 and the difference between the calculated values (0·75 ppm) is sufficiently large to minimize possible error arising from the multiple substitution. Metabolite 2 is hence represented as **4**.

The metabolism of phaseollin by *Colletotrichum lindemuthianum* would thus appear to follow a different pathway to that reported for two other fungi.[4–6] Preliminary indications are that both metabolites 1 and 2 are fungitoxic and hence their formation cannot be considered a detoxification process *per se*. However, with incubation periods of over 12 hr both metabolites disappear from the liquid medium and hence they may be intermediates in an overall detoxification process. Further investigations are now in progress.

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[11] BALLANTINE, J. A. and PILLINGER, C. T. (1967) *Tetrahedron* **23**, 1691.