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IDENTIFICATION OF A SECOND ANTIFUNGAL ISOFLAVAN FROM DISEASED *PHASEOLUS VULGARIS* TISSUE

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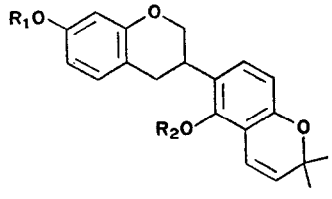
Key Word Index—*Phaseolus vulgaris*; leguminosae; bean; 2′-methoxyphaseollinisoflavan; *Fusarium solani* f. sp. *phaseoli*; antifungal compounds; phytoalexins.

Abstract—A second antifungal isoflavan has been isolated from diseased bean hypocotyls and identified as 2′-methoxyphaseollinisoflavan.

INTRODUCTION

FRENCH bean, *Phaseolus vulgaris* L., synthesizes at least four antifungal isoflavanoids in response to virus or fungal infections.[1,2] These isoflavanoids are the pterocarpans, phaseollin[3] and phaseollidin,[4] the isoflavanone, kievitone[1] and the isoflavan, phaseollinisoflavan[1] (I). While investigating the occurrence of these compounds in bean hypocotyl tissue, infected with *Fusarium solani* (Mart.) Sacc. f. sp. *phaseoli* (Burk.) Snyd. and Hans., a fifth antifungal isoflavanoid was discovered. This paper presents evidence that the structure of this compound is 2′-methoxyphaseollinisoflavan (II).



(I) $R_1 = H$; $R_2 = H$
(II) $R_1 = H$; $R_2 = Me$
(III) $R_1 = Me$; $R_2 = Me$
(IV) $R_1 = Me$; $R_2 = H$

RESULTS

This previously undescribed isoflavanoid has essentially the same mobility in all TLC systems tested as phaseollin, but can be separated from the latter by using either the GLC system described by Keen *et al.*[5] (R_t relative to catechin 0·8) or by column chromatography on Sephadex LH20 with 95% ethanol as the eluant ($V_e/V_o = 3{\cdot}0$). The compound is a colorless solid exhibiting UV absorption at [λ_{max}^{EtOH} (log ϵ)] 228 (4·60), 271 (3·92), 280 (3·97), 290 sh (3·67) and 313 (3·31) nm. It exhibits an optical rotation of $[\alpha]_D^{24} + 19{\cdot}5°$ (EtOH)

[1] BURDEN, R. S., BAILEY, J. A. and DAWSON, G. W. (1972) *Tetrahedron Letters* 4175.
[2] SMITH, D. A., VANETTEN, H. D. and BATEMAN, D. F. (1973) *Physiol. Plant Pathol.* **3**, in press.
[3] PERRIN, D. R. (1964) *Tetrahedron Letters* 29.
[4] PERRIN, D. R., WHITTLE, C. P. and BATTERHAM, T. J. (1972) *Tetrahedron Letters* 1673.
[5] KEEN, N. T., SIMS, J. J., ERWIN, D. C., RICE, E. and PARTRIDGE, J. E. (1971) *Phytopathology* **61**, 1084.

which is similar to that reported for other isoflavans.[6,7] The compound reacts with diazotised *p*-nitroaniline to form an orange-colored product, but does not appear to react with Gibbs reagent[8] or with 2,4-dinitrophenylhydrazine. The low resolution mass spectrum reveals a parent ion at *m/e* 338. High resolution mass spectrometry (obtained with an AEI M.S. 902 instrument using a heated direct insertion probe) indicates an elemental composition of $C_{21}H_{22}O_4$. Other major ions in the spectrum were at *m/e* 323 (100%, $C_{20}H_{19}O_4$), 216 (8%, $C_{14}H_{16}O_2$), 203 (7%, $C_{13}H_{15}O_2$), 201 (63%, $C_{13}H_{13}O_2$) and 185 (14%, $C_{12}H_9O_2$). An intense M-15 peak (323) is consistent with the presence of a 2,2-dimethylchromene ring.[4] The presence of ions at *m/e* 216 and 201 would be expected from a retro-Diels–Alder fragmentation of the 338 and 323 ions, as reported for other isoflavans.[9,10] The MS of the acetylated product, formed on acetylation with pyridine–acetic anhydride, reveals a parent ion at *m/e* 380, a base peak at *m/e* 365 (M-15) and a prominent peak at *m/e* 323 (M-15-42) indicating the presence of an aromatic hydroxyl group.

Verification of the unknown as 2′-methoxyphaseollinisoflavan (II) was accomplished in the following manner. A sample of phaseollinisoflavan (I) was obtained from Burden and Bailey and upon incomplete methylation with diazomethane, three products were obtained. One was identical with the unknown, as determined by UV spectrophotometry, TLC, GLC, MS and degree of antifungal activity. The unknown was methylated with diazomethane, and the product proved identical with a second product obtained from methylation of phaseollinisoflavan. These compounds had identical UV spectra, TLC mobility, and MS (parent ion at *m/e* 352, a base peak at *m/e* 337 and prominent peaks at *m/e* 216, 203, 201 and 185). These compounds were thus identified as the dimethylated homologue of phaseollinisoflavan (III). The MS of the third product obtained from methylation of phaseollinisoflavan revealed major ions at *m/e* 338 (44%), 323 (100%), 187 (56%), 137 (19%), consistent with 7-methoxyphaseollinisoflavan (IV).

When bioassayed against *Fusarium solani* (Mart.) Appel. and Wr. f. sp. *cucurbitae* Snyd. and Hans., a non-pathogen of bean, 2′-methoxyphaseollinisoflavan causes a 50% inhibition of radial mycelial growth at *ca.* 12 μg/ml. There was no inhibition of the bean pathogen *F. solani* f. sp. *phaseoli* at twice this concentration. Preliminary attempts to induce the synthesis of this compound in bean tissue abiotically and attempts to find it in *Rhizoctonia solani*-infected bean tissue have failed. Thus, this isoflavan could arise by a specific induction of its synthesis in bean tissue by *F. solani* f. sp. *phaseoli*, or it could be a fungal metabolite of one of the other isoflavanoids present in bean.

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