Phytochemistry, 1978, Vol. 17, p 165 Pergamon Press Printed in England

PHASEOLLIDIN, A PHYTOALEXIN OF *PSOPHOCARPUS TETRAGONOLOBUS*

JOHN L. INGHAM

Phytochemical Unit, Department of Botany, University of Reading, Whiteknights, Reading RG6 2AS, England

(*Received* 31 *May* 1977)

Key Word Index—*Psophocarpus tetragonolobus*; Leguminosae; winged bean; isoflavonoid; pterocarpan; phytoalexin; antifungal activity.

Winged bean or Goa bean (*Psophocarpus tetragonolobus* [L.]DC.) is a herbaceous vine traditionally grown for food in the highlands of Papua–New Guinea. It is also cultivated in other tropical areas (Burma, India) and has been introduced into the West Indies. *P. tetragonolobus* is a potentially valuable crop species because of the high protein content of its seeds and tubers.

Excised, etiolated stems [1] of *P. tetragonolobus* (variety UPS 122) were inoculated with droplets of a conidial suspension of the fungus *Helminthosporium carbonum* Ullstrup and incubated for 48 hr [1]. Control stems received drops of de-ionised H_2O. Tissues underlying the droplets were then removed and extracted with EtOH [1]. TLC bioassay [1, 2] (using *Cladosporium herbarum* Fr. as the test organism) revealed that a single, highly antifungal fraction (R_f *ca* 0.55 in $CHCl_3$–MeOH, 25:1) was associated with the inoculated (but not the control) tissue extract. Elution (EtOH) and additional TLC purification ($CHCl_3$, × 3) of this fraction afforded a phenolic compound (diazotised *p*-nitroaniline, orange) indistinguishable (MS, UV, TLC) from an authentic sample of the pterocarpan, phaseollidin (**1**) (3,9-dihydroxy-10-*iso*pentenylpterocarpan). Methylation (CH_2N_2) and acetylation (Ac_2O–Py) gave respectively a diMe ether (M^+ 352; λ_{max}^{EtOH} 211, 233 *sh*, 281, 286 nm) and a diacetate (M^+ 408; λ_{max}^{EtOH}: 211, 226 *sh*, 278 *sh*, 285 nm) neither of which appears to have previously been prepared. *Psophocarpus* phaseollidin was chromatographically homogeneous in 6 TLC systems; the MS gave no evidence for the presence of 2′-*O*-methylphaseollidinisoflavan (M^+ 340) [3], a compound difficult to separate from **1** (M^+ 324).

Phaseollidin is a rare isoflavonoid phytoalexin having previously been obtained only from *Phaseolus vulgaris* [4] and *Vigna unguiculata* [3, 5] (tribe Phaseoleae). Nevertheless, its production by *Psophocarpus* is not entirely unexpected since this genus also belongs to the Phaseoleae, a tribe in which complex induced or constitutive (e.g. *Neorautanenia* and *Pachyrrhizus* sp. [6]) isoflavonoids are relatively common. However, no evidence was obtained to suggest that *P. tetragonolobus* produced any of the other phytoalexins (e.g. phaseollin, phaseollinisoflavan, kievitone and vignafuran [3, 7–9]) characteristic of *Phaseolus* and *Vigna*. It is noteworthy that hypocotyls of the taxonomically related grain legume, *Lablab niger* (hyacinth bean) also accumulate **1** following inoculation with *H. carbonum* [10]. However, like *Phaseolus* and *Vigna* (but in contrast to *Psophocarpus*), hyacinth bean hypocotyls produce many other isoflavonoids including the prenylated isoflavanone, kievitone [10].

Large quantities of phaseollidin were obtained from the fungus-inoculated stems of winged pea; on 3 occasions recorded concentrations of **1** (based on $\log \varepsilon = 3.78$ at 286.5 nm [11]) were 723, 809 and 751 μg/g fr. tissue. In contrast, **1** was never detected in control tissue extracts. The concentration of phaseollidin in stem samples collected 12, 24, 36, 48 and 72 hr after inoculation with *H. carbonum* was 108, 317, 688, 744 and 829 μg/g respectively. In a TLC bioassay against *C. herbarum* [1, 2], **1** exhibited marked antifungal activity at a level of 15 μg; when incorporated into agar (5–50 μg/ml) [1] and tested against mycelial growth of *H. carbonum*, **1** gave an ED_{50} value of between 30 and 35 μg/ml. Phaseollidin is also inhibitory to other plant pathogenic fungi [11]. In view of its antifungal properties and high tissue concentration there would appear little doubt that phaseollidin contributes substantially to the disease resistance of winged pea.

Acknowledgements—The author thanks W. Erskine for seeds of winged pea and J. A. Bailey for a specimen of **1**. Financial assistance from the SRC is also gratefully acknowledged.

REFERENCES

1. Ingham, J. L. (1976) *Phytopathol. Z.* **87**, 353.
2. Homans, A. L. and Fuchs, A. (1970) *J. Chromatog.* **51**, 327.
3. Preston, N. W. (1975) *Phytochemistry* **14**, 1131.
4. Perrin, D. R., Whittle, C. P. and Batterham, T. J. (1972) *Tetrahedron Letters* 1673.
5. Bailey, J. A. (1973) *J. Gen. Microbiol.* **75**, 119.
6. Wong, E. (1975) in *The Flavonoids* (Harborne, J. B., Mabry, T. J. and Mabry, H. eds) p. 743. Chapman & Hall, London.
7. Perrin, D. R. (1964) *Tetrahedron Letters* 29.
8. Burden, R. S., Bailey, J. A. and Dawson, G. W. (1972) *Tetrahedron Letters* 4175.
9. Preston, N. W., Chamberlain, K. and Skipp, R. A. (1975) *Phytochemistry* **14**, 1843.
10. Ingham, J. L. unpublished observations.
11. Perrin, D. R., Biggs, D. R. and Cruickshank, I. A. M. (1974) *Australian J. Chem.* **27**, 1607.