Pisatin as a Major Phytoalexin in *Lathyrus*

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(Z. Naturforsch. **32 c**, 289 [1977]; received December 13, 1976)

Leguminosae, *Lathyrus*, Phytoalexins, Pterocarpans, Pisatin

The pterocarpan pisatin has been identified as a major phytoalexin in fifteen species of *Lathyrus*. Thus, the genus is closer in its phytoalexin response to *Pisum* than to *Vicia*, which is known to produce furanoacetylenes on fungal invasion.

Recent studies [1] have indicated that the induction of phytoalexins in higher plants by fungi is interesting not only because it increases our understanding of disease resistance mechanisms but also because it provides new insights into systematic and evolutionary relationships among plants. Most attention has been given to the family Leguminosae, which in general produce isoflavonoid phytoalexins [2]. Although an increasing number of legume genera have been studied, little attention has yet been paid to *Lathyrus*, which is one of the largest genera of the tribe Vicieae, with some 130 species, several of which are used for food or as animal fodder and one, *L. odoratus*, the sweet pea, is an important ornamental crop. Taxonomically, the genus is closest to *Vicia* and indeed taxa of the two genera are so closely similar morphologically that they are often difficult to separate [3]. *Lathyrus* and *Vicia* are distinguished chemically from other Vicieae in having a rich assembly of non-protein amino acids in the seeds, some of which are toxic [4]. *Vicia* is of particular interest from the pathological viewpoint, since at least three species including *V. faba* are completely anomalous among legumes in producing acetylenic phytoalexins, based on the furanoacetylenic acid methyl ester, wyerone [5]; the only family where acetylenes occur otherwise as phytoalexins is the Compositae [6]. It was, therefore, of interest to see if *Lathyrus* showed a similar response to *Vicia* in this way.

Using the now standard drop diffusate technique [2], leaves of fifteen *Lathyrus* species were variously induced to produce phytoalexins by inoculation with spore suspensions of *Helminthosporium carbonum* Ullstrup, water controls being set up at the same time. The substances so produced *de novo* were isolated, bioassayed for antifungal activity [7] and then examined by standard spectral and chromatographic procedures. In every case, pisatin (6α-hydroxy-3-methoxy-8,9-methylenedioxypterocarpan), the characteristic phytoalexin of *Pisum sativum* [8], was present. It was identified by direct comparison (UV, TLC and MS) with an authentic sample.

The fifteen species tested variously represent six sections of the genus, so that pisatin formation would appear to be a representative feature of *Lathyrus* as a whole. In *L. tingitanus* L., pisatin synthesis was induced in both pods and leaves. Certain species (*e.g. L. odoratus* L.) yielded other isoflavonoid phytoalexins, the identities of which are under active investigation. In no case yet, however, has any evidence been obtained of acetylenic derivatives being induced in the genus; wyerone, the major acetylene of *Vicia faba*, was available and run as a control during these investigations.

The results of a limited survey of the genus *Lathyrus* thus indicate that the genus is closer to *Pisum* than to *Vicia* in phytoalexin synthesis. This is because a major product is pisatin, which is characteristically produced not only by *Pisum sativum* but all other *Pisum* species investigated [9], a result confirmed in this laboratory. The response in *Lathyrus* is clearly different from that in *Vicia faba* and two allied species *V. galilea* and *V. narbonensis*, where the phytoalexins induced are acetylenic. Recent investigations of *Vicia faba* [10] have shown that this taxon is not completely anomalous in phytoalexin response, since trace amounts of medicarpin (3-hydroxy-9-methoxypterocarpan) were formed in addition to the three major furanoacetylenes, following infection with *Botrytis cinerea* Fr. Pisatin, at least, is not present and we have tested a range of *Vicia* species for phytoalexin production and none has so far yielded any evidence of pisatin formation. The surprising conclusion that phytoalexin induction links *Lathyrus* to *Pisum* rather than to *Vicia* is based on a relatively small sampling and further work is in progress to clarify these biochemical inter-relationships among the Vicieae.

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The authors are grateful to J. L. Ingham for advice and one of us (D. J. R.) is in receipt of an S.R.C. CASE award, supported in part by I.C.I. (Plant Protection) Ltd.

[1] J. L. Ingham and J. B. Harborne, Nature **260**, 241 [1976].
[2] J. L. Ingham, Ph. D. thesis, University of Reading 1976.
[3] P. W. Ball, Flora Europea **2**, 136 [1968].
[4] E. A. Bell, Chemotaxonomy of the Leguminosae (ed. J. B. Harborne, D. Boulter, and B. L. Turner), p. 179, Academic Press, London 1971.
[5] J. A. Hargreaves, J. W. Mansfield, and P. T. Coxon, Phytochemistry **15**, 651 [1976].
[6] J. L. Ingham, Botan Rev. **38**, 434 [1972].
[7] A. L. Homans and A. Fuchs, J. Chromatogr. **51**, 327 [1970].
[8] I. A. M. Cruickshank and D. R. Perrin, Biochemistry of Phenolic Compounds (ed. J. B. Harborne), p. 511, Academic Press, London 1964.
[9] I. A. M. Cruickshank and D. R. Perrin, Austral. J. Biol. Sci. **18**, 829 [1965].
[10] J. A. Hargreaves, J. W. Mansfield, and D. T. Coxon, Nature **262**, 318 [1976].