Isoflavonoid Phytoalexins of *Parochetus communis* and *Factorovskya aschersoniana*

John L. Ingham

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

Z. Naturforsch. **34 c**, 290–292 (1979);
received December 4, 1978

Leguminosae, *Parochetus*, *Factorovskya*, Isoflavonoids, Phytoalexins

Medicarpin has been isolated as a phytoalexin from the fungus-inoculated leaflets of *Parochetus communis* and *Factorovskya aschersoniana;* a second isoflavonoid derivative, vestitol, is also produced by *F. aschersoniana*. Some taxonomic aspects of phytoalexin formation by the title species are briefly discussed.

It has recently been shown that isoflavonoid and/or non-flavonoid phytoalexins are frequently produced by the excised, fungus *(Helminthosporium carbonum)*-inoculated leaflets of species belonging to two major tribes – the Trifolieae [1–4] and Vicieae [5–9] – of the Leguminosae (subfamily Papilionoideae). Surveys within the four principal genera *(Melilotus* [1]; *Medicago* [2]; *Trifolium* [3]; and *Trigonella* [4]) of the Trifolieae have revealed a rich assortment of induced pterocarpan (*e. g.* medicarpin, **1**) and isoflavan (*e. g.* vestitol, **2**) derivatives several of which are apparently absent from, or of extremely rare occurrence in, other tribes of the Papilionoideae including the Vicieae *sensu lato* [10]; the latter tribe is similarly characterised by the production of many phytoalexins – pisatin from *Pisum* [6] and *Lathyrus* [8, 9] is a notable example – not yet associated with species of the Trifolieae. This paper describes the isolation of phytoalexins from two monospecific genera *(Parochetus* and *Factorovskya)* of the Trifolieae and additionally provides some evidence to suggest that *P. communis* represents a chemical link between this tribe and the Vicieae.

a) Parochetus communis

Plant: P. communis Buch.-Ham. (Ceylon clover; shamrock pea) is a creeping, moisture-loving herb indigenous to tropical East Africa and Asia; it is particularly abundant in the Himalayan foothills up to 2500 m. *P. communis* is unique within the Trifolieae in that it produces solitary, purple-blue flowers which resemble those of a miniature sweet pea *(Lathyrus odoratus;* tribe Vicieae). The stems and leaves are also marked with bands of anthocyanin pigment.

Uses: As a rock garden or greenhouse ornamental and in its native habitat as an occasional browse plant for livestock.

Previous Phytochemical Work: None.

Present Work: Leaves of *P. communis* were obtained from a mature plant growing at the University of Oxford (Magdalen College) Botanic Garden. Extracts (EtOAc) of diffusates [11] from *H. carbonum*-inoculated leaflets were chromatographed (Si gel TLC [12]; $CHCl_3$: MeOH, 50 : 1) to afford a single phenolic compound indistinguishable (UV, MS, Co-TLC) from authentic medicarpin (**1**) (3-hydroxy-9-methoxypterocarpan). There was no evidence to suggest that leaf diffusates contained pisatin – a common *Lathyrus* phytoalexin [8, 9] – or, as judged by TLC bioassay against *Cladosporium herbarum*, any other fungitoxic material of flavonoid or non-flavonoid origin. (**1**) was absent from the control (H_2O) diffusates. In 4 separate experiments, the medicarpin concentration (based on log $\varepsilon = 3.89$ at 287 nm[13]) in 48 h fungus-induced diffusates was found to be 202, 148, 173 and 134 μg/ml. Large quantities (488, 394, 627 and 432 μg/g fr. wt respectively) of **1** were also isolated from tissue extracts (EtOH) [1] of the *H. carbonum*-inoculated leaflets.

Taxonomic Considerations: In terms of its phytoalexin response, *Parochetus* clearly resembles other genera of the Trifolieae – particularly *Melilotus* [1] – although chemically it would appear to be less advanced than *Factorovskya* (see below), *Medicago* [2], *Trifolium* [3] and *Trigonella* [4] where medicarpin frequently co-occurs with isoflavonoid derivatives of equivalent, or considerably greater, fungitoxicity. In the Vicieae, **1** accumulates rarely and then only in trace amounts [5, 8]; as mentioned earlier, no phytoalexins characteristic of the Vicieae (*e. g.* pisatin and variabilin of *Pisum*, *Lathyrus* and *Lens* or the biogenetically distinct furanoacetylenes of *Vicia)* were isolated from *P. communis* despite its floristic similarity to *Lathyrus*. It is worth noting, however, that the major flower pigment of *P. communis* has recently been identified as malvidin-3-rhamnoside [14], a very uncommon

Reprint requests to Dr. J. L. Ingham.

0341-0382 / 79 / 0300-0290 $ 01.00/0

anthocyanin first obtained from *Lathyrus sativus* [15]; moreover, the stems and leaves of shamrock pea contain small quantities of delphinidin-3-lathyroside (lathyrose = xylosylgalactose) [14, 15] which occurs in flowers of crimson and scarlet sweet pea *(L. odoratus)* mutants [15]. In fact, anthocyanins with 3-rhamnoside or 3-rhamnoside-5-glucoside residues are common in the Vicieae [15] whereas glucosylated pigments (3-glucosides and 3,5-diglucosides) are found in petals of blue- and red-flowered *Medicago* and *Trifolium* species. The limited phytoalexin/anthocyanin data described above suggest, therefore, that *P. communis* may represent a link between the Trifolieae and Vicieae. *Parochetus* almost certainly lies at or near the periphery of the Trifolieae and its relationship to other genera within the tribe remains obscure; however, a connection through *Trigonella* is a distinct possibility since malvidin-3-rhamnoside has been provisionally identified in the pale-blue flowers of *T. coerulea* [14] (azure fenugreek), a species which also produces large quantities of **1**.

b) Factorovskya aschersoniana

Plant: F. aschersoniana (Urb.) Eig is a prostrate, yellow-flowered annual found on dry, sandy soils in an area of the Middle East extending from Turkey through southern Israel (Negev) and the Sinai Peninsula to Egypt. Originally named *Trigonella aschersoniana*, this species was later transferred to the separate genus *Factorovskya* [16] on the basis of several characters but in particular its geocarpic habit, a very rare feature in the Leguminosae. In general appearance, *F. aschersoniana* is strikingly similar to those *Trigonella* species which Ingham [4, 17] describes as being "*Medicago*-like".

Uses: None.

Previous Phytochemical Work: None.

Present Work: Seeds of *F. aschersoniana* (collected near Be'er Sheva, Israel) were germinated and the resulting plants grown for 10 – 12 weeks [18] prior to fungus-inoculation of the excised leaflets. Diffusates were collected after 36 h incubation [18]. Medicarpin (**1**) (33 μg/ml) and its isoflavan derivative, vestitol (**2**) (7,2′-dihydroxy-4′-methoxyisoflavan; 36 μg/ml) were readily isolated (Si gel TLC, $CHCl_3$: MeOH, 50 : 1) from EtOAc extracts of the *H. carbonum*-induced diffusates. No other compounds were detected. **1** and **2** were not produced by leaflets treated with droplets of de-ionised H_2O.

Taxonomic Considerations: Despite its geocarpic nature, *F. aschersoniana* is undoubtedly allied to *Trigonella*, a genus characterised by the accumulation of both pterocarpan (**1** and maackiain) and isoflavan (**2** and sativan) phytoalexins [4]. In fact, from an examination of over 40 species [4, 19] it has been possible to divide *Trigonella* into three chemical groups depending on whether the leaf phytoalexin response is typical of *Medicago, Melilotus* or *Trifolium;* further subdivision is also feasible when quantitative features are taken into account. Phytoalexin data for *F. aschersoniana* suggest that this species is most closely related to the "*Medicago*-like" *Trigonella* subgroup exemplified by *T. brachycarpa, T. noëana* [4] and, after recent re-examination, *T. monspeliaca* [19]. All three species produce **1** and **2** but, like *F. aschersoniana*, do not accumulate sativan (vestitol-2′-O-methyl ether). The latter phytoalexin is commonly encountered in *Medicago* [2] and in species such as *T. monantha* and *T. geminiflora* [4] which belong to the second, and considerably larger, "*Medicago*-like" subgroup of *Trigonella. F. aschersoniana* is chemically distinct from members of the two other *Trigonella* groups *("Melilotus*-like" and "*Trifolium*-like") mentioned above; all the "*Trifolium*-like" species produce **1** and maackiain (a compound absent from *Factorovskya)* whilst the "*Melilotus*-like" group is typified by, (a) absence of isoflavan phytoalexins and, (b) rapid formation of coumarin upon tissue maceration [1]. Both (a) and (b) are characteristic features of the genus *Melilotus* [1]. *Factorovskya, Medicago* and all the "*Medicago*-like" members of *Trigonella* have been found to be coumarin-negative.

The author thanks M. Awishai (Department of Botany, The Hebrew University, Jerusalem, Israel) for providing seeds of *F. aschersoniana.*

[1] J. L. Ingham, Z. Naturforsch. **32 c**, 449 (1977).
[2] J. L. Ingham, Biochem. Syst. Ecol. in press.
[3] J. L. Ingham, Biochem. Syst. Ecol. **6**, 217 (1978).
[4] J. L. Ingham and J. B. Harbone, Nature **260**, 241 (1976).
[5] J. A. Hargreaves, J. W. Mansfield, and D. T. Coxon, Nature **262**, 318 (1976).
[6] I. A. M. Cruickshank and D. R. Perrin, Aust. J. Biol. Sci. **18**, 829 (1965).
[7] D. J. Robeson, Phytochemistry **17**, 807 (1978).
[8] D. J. Robeson, Ph. D. thesis, University of Reading, U. K. 1978.
[9] D. J. Robeson and J. B. Harborne, Z. Naturforsch. **32 c**, 289 (1977).

[10] F. K. Kupicha, Botan. J. Linn. Soc. **74,** 131 (1977).
[11] J. L. Ingham and R. L. Millar, Nature **242,** 125 (1973).
[12] J. L. Ingham, Z. Naturforsch. **31 c,** 504 (1976).
[13] S. H. Harper, A. D. Kemp, and W. G. E. Underwood, Chem. Ind. **1965,** 562.
[14] J. B. Harborne, personal communication.
[15] J. B. Harborne, Comparative Biochemistry of the Flavonoids, Academic Press, London 1967.
[16] A. Eig, Zionist Org. Inst. Agric. Nat. Hist. Bull. **6,** 11 (1927).
[17] J. L. Ingham, Ph. D. thesis, University of Reading, U. K. 1976.
[18] J. L. Ingham, Phytochemistry **15,** 1489 (1976).
[19] J. L. Ingham, unpublished data.