

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

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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 pterocarpans (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<sub>3</sub> : MeOH, 50 : 1) to afford a single phenolic compound indistinguishable (UV, MS, Co-TLC) from authentic medicarpin (**1**) (3-hydroxy-9-methoxypterocarpans). 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<sub>2</sub>O) diffusates. In 4 separate experiments, the medicarpin concentration (based on log  $\epsilon$  = 3.89 at 287 nm<sup>13</sup>) in 48 h fungus-induced diffusates was found to be 202, 148, 173 and 134  $\mu$ g/ml. Large quantities (488, 394, 627 and 432  $\mu$ g/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

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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 Viciae [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 Viciae. *Paroetus* 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<sub>3</sub> : 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<sub>2</sub>O.

**Taxonomic Considerations:** Despite its geocarpic nature, *F. aschersoniana* is undoubtedly allied to *Trigonella*, a genus characterised by the accumulation of both pterocarpin (**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.

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