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JOHN L. INGHAM

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

INDUCED ISOFLAVANS FROM THE LOTEAE

In recent years it has been demonstrated that low-molecular weight antibiotics, or phytoalexins, are produced following the interaction between plants and various biotic or abiotic agents (Ingham, 1972). These substances are considered to play an important role in the disease resistance of higher plants including numerous species from the Lotoideae subfamily of the Leguminosae. Phytoalexins are not detectable in the tissues of healthy plants but are produced following inoculation or infection with a wide range of micro-organisms.

During an investigation of the family Leguminosae, it was found that phytoalexins were produced by species from several genera (Lotus, Tetragonolobus and Anthyllis) of the tribe Loteae. Phytoalexin formation was induced by inoculation of detached leaves with the non-pathogenic fungus, Helminthosporium carbonum (Higgins and Millar, 1968). After 48 hours incubation, the inoculated tissues were excised, extracted with ethanol and the active compounds separated by thin-layer chromatography. Phytoalexins were not produced when leaves were inoculated with de-ionised water.

Two species from the genus Lotus namely, L. corniculatus L. (birdsfoot-trefoil) and L. uliginosus Schkuhr (marsh birdsfoot-trefoil), were examined for phytoalexin production. Both species are closely related and are difficult or impossible to distinguish on the basis of their morphology or leaf flavonoid patterns. Leaves of L. corniculatus 'Viking' have previously been reported to produce the isoflavan phytoalexins, vestitol (7,2'-dihydroxy-4'-methoxyisoflavan) and sativan (7-hydroxy-2',4'-dimethoxyisoflavan) (Bonde, Millar and Ingham, 1973).

In addition to the aforementioned phytoalexins, it has been found that leaf tissues of L. corniculatus also produce 7,2',4'-trihydroxyisoflavan, a compound not previously reported as a plant constituent; the trivial name, demethylvestitol, is proposed for this substance. In contrast to L. corniculatus, only demethylvestitol and vestitol are produced by L. uliginosus. This latter species apparently does not produce sativan. Thus, whilst L. corniculatus and L. uliginosus are morphologically similar, they can be chemically distinguished by reference to their respective phytoalexins. The above compounds could not be isolated from the control tissues of either L. corniculatus or L. uliginosus. For these two species the terminal stages of phytoalexin biosynthesis would appear to involve the methylation of demethylvestitol to afford vestitol; in L. corniculatus, vestitol can be further methylated to sativan. It is noteworthy that bioassays against the mycelial growth of H. carbonum indicate that increasing isoflavan methylation greatly enhances antifungal activity. Quantitative analysis of inoculated leaf tissues indicates that vestitol and sativan are present in concentrations sufficient to account for cessation of fungal development.

In addition to Lotus, several species from the genus Tetragonolobus have also been examined for phytoalexin formation. Tetragonolobus is closely related to Lotus and is regarded by some authorities (Hutchinson, 1964) as synonymous with it. None of the four species examined (T. biflorus (Desr.) Ser., T. maritimus (L.) Roth, T. purpureus Moench and T. requienii (Mauri ex Sang.) Sang.) produced sativan. However, all the species accumulated a hitherto undescribed phytoalexin isomeric with vestitol. This compound (7,4'-dihydroxy-2'methoxyisoflavan) has been assigned the trivial name, isovestitol. With the exception of T. maritimus, isovestitol was also accompanied by demethylvestitol. From the above data delimitation of Tetragonolobus from Lotus would appear to have some tentative chemical support although clearly a much greater number of species must eventually be examined. Unfortunately, the small leaflets characteristic or many Lotus species renders inoculation and subsequent sample collection extremely difficult.

In the tissues of other leguminous species isoflavans occur together with pterocarpans, a group of isoflavonoid compounds from which the isoflavans are generally considered to be derived. However, no evidence has yet been obtained for the production, by species from the Loteae, of 3,9-dihydroxypterocarpan, the most logical biosynthetic precursor of demethylvestitol.

References

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EDITOR'S NOTE: Marked differences in phenolics between Lotus siliquosus (Tetragonolobus siliquosus) and other species of Lotus clearly favored the former species as a member of the genus Tetragonolobus rather than Lotus (Harney and Grant, 1965).

Reference

Harney, P. M. and Grant, W. F. 1965. A polygonal presentation of chromatographic investigations on the phenolic content of certain species of Lotus. Can. J. Genet. Cytol. 7: 40-51.