**D-40: High performance liquid chromatographic study of the genus *Lathyrus* for isoflavones with emphasis on orobol and biochanin A**

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The isoflavones represent an important and very distinctive subclass of the flavonoids. These compounds are based on a 3- phenyl chroman skeleton that is biogenetically derived by an aryl migration mechanism from the 2-phenyl chroman skeleton of the flavonoids. In contrast to the flavonoids, the isoflavonoids have a very limited distribution in the plant kingdom, and are almost restricted to the subfamily Papilionoideae of the Leguminosae. The 4 simple isoflavones diadzein, formononetin, genistein and biochanin A are extremely common with wide range of different oxygenation and substitution patterns.

*Lathyrus*, a herbaceous genus with 144 species, belongs to the subfamily Papilionoideae in the family Leguminosae. The main objectives of this study are, to isolate and identify the isoflavones and a chemosystematical study of the distribution pattern of isoflavones in genus *Lathyrus*. Due to the unavailability of the standard markers, this study was mainly based on the compounds, orobol and biochanin A.

To study the distribution of isoflavones, 22 species of the genus *Lathyrus* were studied for the presence of isoflavones in the leaves using reverse phase high performance liquid chromatography (HPLC) with diode array detection. 100 mg of dried leaves from herbarium material were used for acid hydrolysis. The leaves were hydrolysed with 2N HCl for 40 min and extracted into ethyl acetate. The extractions were evaporated to dryness and dissolved in 0.5 ml 80% methanol. From that 0.25 ml were taken for HPLC studies, diluted with another 0.25 ml of 80% methanol and filtered for HPLC injection. The HPLC studies

were carried out using a 0.4 mm ID, 4.0 cm column with C18 phenyl packing material. The following solvent gradients were used: 65% A and 35% B, changing linearly to 0% A and 100% B in 20 min (Composition of solvent A: 5% acetic acid in $H_2O$, solvent B: methanol: $H_2O$: acetic acid = 18: 1: 1). Flow rate was 1.00 ml/min and the pressure 3000.0 PSI. Orobol and biochanin A were run as the standard markers in order to compare the retention times and UV spectra. For each extract 50 microliters were injected.

A number of peaks were observed on the HPLC chromatograms of the hydrolysed extracts which gave isoflavonoid-like UV absorption spectra (maximum absorbency at around 260 nm and a shoulder at 300 nm or higher wavelength).

*L.latifolius,* and *L.montanus* gave peaks of the same retention time and UV spectra as the marker orobol while in *L.incurvus* had peak as biochanin A. Whereas other species gave similar UV spectra but different retention times. They could not be identified because of lack of the standard compounds.

The amounts of the isoflavones found per weight of leaf were calculated by comparing the height of the peaks on the HPLC chromatograms with those of known concentrations of orobol and biochanin A standard. Always a known amount of plant material and the volume of extracts were used in order to calculate the quantity of the isolated compounds. The percentage amounts of isoflavonoids found per dry weight of leaf were grouped into 4 ranges for ease of comparison.

The amount of orobol found was 1% of dry weight of leaf of *L.montanus* and 0.001-0.01% in *L.latifolius*. In *L.incurvus*, biochanin A was found in 0.001-0.01% of dry weight of leaf. The compound A was found in *L. tingitanus*, *L. hierosolymitanus* and *L. clymenum*. The compound B,C,D,E and F were found in *L.hirsutus*, *L. montanus* and *L. vernus* respectively but their quantities were very low.

Although isoflavones do not appear to be common in the genus *Lathyrus* they were still found in about a third of the species investigated, but the quantities were generally low. Only *L.montanus* accumulates them in large concentrations. Sections *Lathyrus* and *Orobus* were seem to be comparatively rich in isoflavones. Isoflavones were not detected in sections *Pratensis*, *Orobastrum*, *Nissolia* and *Aphaca*.