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Isoflavonoids. III*
Constituents of *Cotoneaster* Species

R. G. Cooke and R. A. H. Fletcher

Department of Organic Chemistry, University of Melbourne,
Parkville, Vic. 3052.

Abstract

Extracts of *Cotoneaster serotina* yielded biochanin-7-*O*-glucoside, biochanin, the triterpenoid ursolic acid, benzoic acid and mandelic acid. The first three of these compounds and the triterpenoid uvaol were isolated from *Cotoneaster pannosa.*

In contrast to the extremely wide distribution of flavonoids in higher plants the isoflavonoids are largely confined to the family Fabaceae (Papilionaceae) and there are only isolated occurrences reported for other families, e.g. Podocarpaceae, Amarantaceae, Moraceae, Iridaceae and Rosaceae.

Some isoflavones have a mild estrogenic activity in some animals,[1] a well known example of their effect being the infertility of sheep grazing on pastures of clover (*Trifolium* species).[2] It seemed of interest to explore the possible occurrence of isoflavones in other plants which might be commonly included in the diet of animals, particularly birds, and which have botanical relationship with known sources of isoflavonoids. The family Rosaceae seemed an obvious choice because many of its members are commonly cultivated and have brightly coloured fruits known to be eaten by birds and other animals. Examples of the occurrence of isoflavonoids in this family are provided by the detection of prunitrin, prunetin and genistein in *Prunus* species.[3,4]

We have now examined several other plants from this family. The flowers and fruits of *Cotoneaster serotina* and *C. pannosa* contain biochanin-7-*O*-glucoside and ursolic acid. In some experiments biochanin (5,7-dihydroxy-4-methoxyisoflavone) was also isolated but this may have been formed by hydrolysis of the glucoside during the extraction procedure. Benzoic and mandelic acids were also obtained from the fruits of *C. serotina* whilst uvaol was isolated from the fruits of *C. pannosa.* The leaves of both species contained ursolic acid but no isoflavone. In preliminary examinations no isoflavonoid was detected in three *Sorbus* species and two *Crataegus*

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[1] Biggers, J. D., in 'The Pharmacology of Plant Phenolics' (Ed. J. W. Fairbairn) p. 51 (Academic Press: New York 1959).
[2] Bennetts, H. W., Underwood, E. J., and Shier, F. L., *Aust. Vet. J.*, 1946, **22**, 2.
[3] Finnemore, H., *Pharm. J.*, 1910, **31**, 604.
[4] Harborne, J. B., 'The Comparative Biochemistry of the Flavonoids' p. 160 (Academic Press: New York 1967).

species. Both ursolic acid and uvaol have been isolated previously from plants of the family Rosaceae.[5]

Marshall has discussed the effects of estrogens on the development and reproductive cycle of birds,[6] and there has been considerable interest in the metabolism of isoflavones in the domestic fowl.[7–10] Although food supply is obviously of importance in the behaviour of birds there is not, to our knowledge, any evidence that specific seasonal variation in diet can affect mating, migration etc. More general investigation of the effects of ingested isoflavonoids might be of some interest.

Experimental

The plant materials were extracted first with cold ethanol or methanol and then with the hot solvent. After concentration of the extracts the products were obtained either by direct crystallization or by chromatography on columns of silica gel (B.D.H. 200–335 mesh) or on 20 cm by 20 cm plates coated with Merck Kieselgel G. Fruits were usually first mashed in a blendor and allowed to stand at least 24 h in water before filtration and drying of the residue which was then extracted with ethanol. The biochanin isolated from the fruits may have been formed by hydrolysis during this treatment, but the consequent removal of water-soluble materials simplified the subsequent extractions. Dried flowers of *Cotoneaster serotina* (100 g) were extracted by heating for 24 h in methanol (1 l.). The extract was concentrated and the constituents separated by chromatography on silica gel using benzene, chloroform and chloroform–methanol consecutively for elution. Biochanin-7-glucoside was eluted by chloroform–methanol (5 : 1). The only other constituent identified was ursolic acid.

Fresh fruits (640 g) were mashed with cold water (1·5 l.), allowed to stand one week and then centrifuged. The solid was soaked in cold ethanol for two weeks and then, after filtration, was further extracted with boiling ethanol for 24 h. Chromatography of the cold ethanol extract on silica gel gave biochanin, benzoic acid and mandelic acid. The hot ethanol extract yielded ursolic acid, biochanin, benzoic acid and a small quantity of biochanin-7-glucoside.

Fresh flowers of *Cotoneaster pannosa* (25 g) were extracted with hot methanol (1 l.), the extract was concentrated and then chromatography on silica gel as above gave biochanin-7-glucoside.

Fresh fruits of this species (520 g) were extracted with cold and then hot ethanol. Concentration of the cold ethanol extract caused deposition of a solid which, by chromatography on silica gel, gave uvaol. A small amount of the viscous residue from the cold ethanol extract was hydrolysed by heating with hydrochloric acid (1M). The precipitate was shown to contain biochanin by thin-layer chromatography on silica gel.

The various fractions on plates were detected by examination under daylight and u.v. light, and by treatment with ammonia vapour, iodine vapour, ethanolic ferric chloride or diazotized sulphanilic acid. Ultraviolet and infrared spectrometry were used to search for aromatic compounds such as isoflavonoids.

Biochanin-7-O-glucoside, m.p. 217–218°, was identified by comparison with a synthetic specimen[11] (mixed m.p. and i.r. spectrum). The yield from dried *Cotoneaster serotina* flowers was 0·73%.

Biochanin, m.p. 215–216°, was also identical with a synthetic specimen. The yield from fresh fruits of *Cotoneaster serotina* was 0·03%.

Ursolic acid, m.p. 277–280°, was identified with an authentic specimen (i.r. spectrum).

Uvaol, m.p. 229–230°, was also identified by its i.r. spectrum in comparison with an authentic specimen.

Benzoic acid, m.p. and mixed m.p. 120–121°.

[5] Karrer, W., 'Konstitution und Vorkommen der organischen Pflanzenstoffe' pp. 823, 824 (Birkhäuser: Basel 1958).
[6] Marshall, A. J., in 'Biology and Comparative Physiology of Birds' (Ed. A. J. Marshall) Vol. 2, p. 195 (Academic Press: New York 1961).
[7] Common, R. H., and Ainsworth, L., *Biochim. Biophys. Acta*, 1961, **53**, 403.
[8] Cayen, M. N., Carter, A. L., and Common, R. H., *Biochim. Biophys. Acta*, 1964, **86**, 56.
[9] Cayen, M. N., Tang, G., and Common, R. H., *Biochim. Biophys. Acta*, 1965, **111**, 349.
[10] Tang, G., and Common, R. H., *Biochim. Biophys. Acta*, 1968, **158**, 402.
[11] Wong, E., Mortimer, P. I., and Geissman, T. A., *Phytochemistry*, 1965, **4**, 89.

Mandelic acid, m.p. 132–134°, was presumably one of the active forms but the quantity was too small for determination of the optical rotation. The m.p. and i.r. spectrum differed from those of racemic mandelic acid but the mass spectrum was identical. The most common naturally occurring form is the (−)-isomer (lit.[12] 133°).

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[12] 'Dictionary of Organic Compounds' 4th Edn, Vol. 4, p. 2052 (Eyre & Spottiswoode: London 1965).