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CHEMISTRY AND TAXONOMY OF *FOUQUIERIA SPLENDENS* ENGELM: A NEW MEMBER OF THE ASPERULOSIDE GROUP

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In his book *The Taxonomy of Vascular Plants*,[1] Lawrence writes: "The phyletic position of the Fouquieriaceae is unsettled. In earlier Englerian works, and by Wettstein, it was included in the Parietales, and was advanced to its present position [following the Polemoniaceae in the Tubiflorae] by Engler and Gilg (1924). Bessey included it in his Ebenales and Hutchinson in his Tamaricales." It was suggested that an examination of the phenolic constituents of *Fouquieria* species might assist in current studies.* The leaves, outer bark and inner bark from *F. splendens* Engelm. ("Ocotillo") were therefore examined by the methods routinely employed in this laboratory in surveys of the phenolic constituents of leaves.[2] From the systematic point of view the most significant constituents which were found were leucocyanidin (leucodelphinidin being absent) and ellagic acid. In addition, the flavonols kaempferol and quercetin (but not myricetin), the cinnamic acids, *p*-coumaric, caffeic and (in low concentration) ferulic acids; and the coumarin, scopoletin, were all present.

The occurrence together in one plant of leuco-anthocyanins and ellagic acid is of quite common occurrence in the dicotyledons, especially in woody families, but this combination associated with the absence of leucodelphinidin and myricetin is rare. Families in which this pattern has been found include Rosaceae-Rosoideae, the Tamaricaceae and the Cornaceae. Ellagic acid has not been found in any of the families in the Englerian system beyond the Ebenaceae, and leuco-anthocyanins relatively rarely in these families. It seems unlikely, therefore, that the affinities of the Fouquieriaceae lie in this systematic area. This leaves a wide choice, from the chemical evidence so far considered, as to where these affinities might be, but relationship with the Tamaricaceae is certainly included among the possibilities. However, there is further evidence which may ultimately help to narrow down the area of possibility.

In the course of acid hydrolysis of the leaves and bark it was observed that a blue colour developed in the aqueous extract, soon replaced by a dark precipitate. This behaviour is characteristic of asperuloside (I). The precursor substance, which was not present in any great quantity in any of the tissues examined, was extracted by boiling with water, diluting the extract with an equal volume of *n*-propanol, salting out the propanol with NaCl, and evaporating the alcoholic solution to dryness in a stream of warm air. The ethanolic extract of the residue was separated on Whatman No. 3 paper in either 6% aqueous acid or in butanol: acetic acid: water (6:1:2), the position of the chromogen being ascertained by boiling test sections in 2 N HCl. The corresponding strips were eluted with 70% ethanol, and the eluates

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* I am grateful to Professor B. L. Turner, Austin, Texas, for this suggestion.

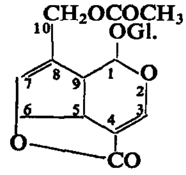
[1] G. H. M. Lawrence, *The Taxonomy of Vascular Plants*, The MacMillan Company, New York (1951).

[2] E. C. Bate-Smith, *J. Linnean. Soc.* (*Botany*.) **58**, 95 (1962).

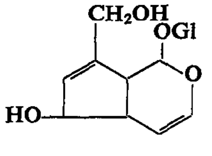
rechromatographed on Whatman No. 1 paper in the alternate solvent. Owing to the low concentration of the compound in the starting material, the eluates were still contaminated with phenolic and other glycosides but were sufficiently pure for its properties to be compared with those of asperuloside.

The *Fouquieria* substance gives a similar blue colour to asperuloside when heated with the glacial acetic acid–copper sulphate reagent described by Trim and Hill;[3] the reaction product in each case had λ_{max} 636 mμ with a shoulder at ~615 mμ. Heated with methylamine at neutral or slightly acid pH, asperuloside gives a violet product λ_{max} 583 and 563 mμ[3]; the reaction product from *Fouquieria* also had an absorption maximum in this region of the spectrum, but the actual position of the peak could not be accurately determined owing to the presence of impurities which gave an orange-yellow coloration with the reagent.

The *Fouquieria* substance differed however from asperuloside in its R_f values. The position of the two substances on the paper was determined by means of a modified Trim and Hill reagent. The R_f values of asperuloside and *Fouquieria* substance were 0·51 and 0·45, respectively, in butanol–acetic acid–water; 0·58 and 0·46 in ethyl acetate–formic acid–water (10:2:3); both 0·92–0·93 in 6% aqueous acetic acid; and 0·25 and 0·21 in butanol–ethanol–water (4:1:2·2). In view of these differences, it is suggested that this compound should be named asperocotillin.



(I)



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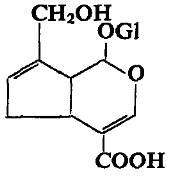

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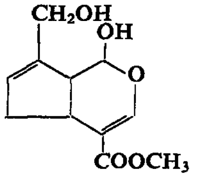

(IV)

Asperuloside belongs to a class of substances, of which aucubin (II) has been regarded as the type compound.[3] Asperuloside itself is especially associated with the Rubiaceae, but is present also in numerous species of *Escallonia*[4] and in *Daphniphyllum macropodum*.[3] Aucubin and other nearly related members of the class (e.g. catalpol) are found especially in the families in the Tubiflorae, but are also present in the Loganiaceae, in *Aucuba japonica* (Cornaceae) and *Garrya elliptica* (Garryaceae). The two sections differ sufficiently in their properties to merit separation as "asperulosides" and "aucubins" respectively. Another representative of the former, monotropein, occurs in *Monotropa hypopitys* and other members of the Pyrolaceae[5] and a third in *Genipa americana* (Rubiaceae). The structure of the former (III) has recently been ascertained by Inouye *et al.*[5] and that of the aglycone of the latter, genipin (IV) by Djerassi *et al.*[6] Its glucoside, which occurs in unripe fruit, has the same R_f as monotropein in butanol–acetic acid–water.[3]

The main structural difference between asperulosides and aucubins lies in the presence of a carbonyl function in position 4 of the former. They differ in behaviour to the reactions described above. With the Trim and Hill reagent, aucubin gives a purple colour with an absorption maximum at 600 mμ, and no reaction with methylamine. On the other hand, aucubin and catalpol react with the benzidine–trichloroacetic acid spraying reagent for

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[3] A. R. TRIM and R. HILL, *Biochem. J.* **50**, 310 (1951).

[4] V. PLOUVIER, *Compt. rend.* **242**, 1643 (1956).

[5] H. INOUYE, T. ARAI, Y. MIYOSHI and Y. YAOI, *Tetrahedron Letters* 1031 (1963).

[6] C. DJERASSI, J. D. GRAY and F. KIND, *J. Org. Chem.* **25**, 2174 (1960).

sugars (Bacon and Edelman[7]), aucubin giving a dark spot with pink fluorescence and catalpol an orange spot with very strong yellow fluorescence, whilst asperuloside gives no reaction.[8] The preparation from *Fouquieria* behaved like asperuloside.

From the taxonomic point of view, it is clearly legitimate to expect the Fouquieriaceae to possess some affinity with other families in which the asperulosides occur. The difficulty is that, with the exception of the Rubiaceae, the systematic position of all those families is at present in question. *Escallonia* is now separated from the Saxifragaceae and *Daphniphyllum* from the Euphorbiaceae, in which they have until recently been included, and the Pyrolaceae, in which *Monotropa* is usually placed, is by some authorities included with, by others excluded from the Ericaceae. Although these substances can so easily be demonstrated, it is unlikely that they have been detected wherever they occur (indeed the present observation is itself evidence of this), so that it is premature to venture taxonomic conclusions from the present chemical evidence. It can only be remarked that none of the families recorded by Lawrence is indicated as closely related to the Fouquieriaceae by that evidence.

EXPERIMENTAL

Examination of Phenolic Constituents

The tissue was heated in 2 N HCl for 30 min and the phenolic compounds extracted with a small quantity of iso-amyl alcohol. Chromatograms were run in acetic acid: HCl: water (30:3:10) and toluene: acetic acid: water (4:1:5 top layer).[2]

Detection of Asperuloside on Paper

The Trim and Hill reagent[3] is too volatile to react with asperuloside at 100° when sprayed on paper. Of the non-volatile acids of the necessary strength to replace HCl, toluene-*p*-sulphonic acid (T.P.S.) has proved satisfactory, provided the paper does not dry before the reaction has time to take place. This is prevented by heating the papers sprayed with acetic acid: 0·2% $CUSO_4$ soln.: T.P.S. (10:1:1) in the vapour of boiling acetic acid (118°). The asperuloside is quickly revealed as a blue spot on the paper which spreads very little and does not fade. In u.v. light the spot has a pink fluorescence.

Acknowledgements—I am indebted to Dr. T. Swain for suggesting the modification of the Trim and Hill reaction. I am also grateful to Professor B. L. Turner and Mr. J. W. Walker for supplying the plant material, Dr. A. H. Trim for the specimen of asperuloside and to Dr. J. D. S. Bacon for allowing me to see unpublished results of the late Dr. R. B. Duff.

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[7] J. S. D. Bacon and J. Edelman, *Biochem. J.* **48**, 114 (1951).

[8] R. B. Duff, (unpublished).