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SHORT COMMUNICATION

PHENOLS IN *SALIX* SPECIES

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Abstract—The phenolic compounds present in the leaves of 20 species of *Salix* have been surveyed. A comment is made upon their use as taxonomic tracers.

INTRODUCTION

THE GENUS *Salix* is noteworthy for the metabolism of the $C_6.C_1$ phenol saligenin and its derivatives.[1] In earlier work Thieme[2] noted distinct differences between species based on the occurrence of the glucoside salicin. As an adjunct to biosynthetic studies of saligenin formation, a survey of phenolic compounds in *Salix* species was undertaken. A recent publication[3] prompts the communication of some of our preliminary observations.

RESULTS AND DISCUSSION

Phenols were extracted from fresh leaves by the usual methods and analysed by paper chromatography. Semi-quantitative analysis of salicin derivatives was carried out by the procedures of Thieme[2] and of leucoanthocyanins by conversion to the anthocyanidin and spectroscopic assay at 550 nm. All species showed the presence of flavonol glycosides (most commonly quercetin derivatives[4]) and esters of the various hydroxycinnamic acids.[5] In agreement with the observations of Thieme,[2] salicin derivatives (principally salicin, fragilin and salicortin) were found in *S. nigricans, pentandra, fragilis, fragilis* (var. *latifolia*), *pentandra* × *fragilis, repens, purpurea* and also in *S. daphnoides, calodendron* and *baysfordiana*. Conversely, except for *S. daphnoides*, leucoanthocyanin formation was not detected in this group of species. Salicin derivatives were not found in the remaining species, (*S. caprea, cinerea, aurita, alba, viminalis, viminalis* × *cinerea, caerulia, phylicifolia* and *caprea* × *lanata*) but all contained high concentrations of leucoanthocyanins (yielding principally cyanidin, accompanied in some cases by delphinidin, on acid treatment) and substances provisionally identified as (+)-catechin and (+)-gallocatechin.[6] However *S. triandra* appeared to be unique in the survey in the absence from the leaf tissue of both salicin derivatives and leucoanthocyanins. These observations differ in some respects from those of Binns, Blunden and

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[4] J. B. HARBORNE, *Comparative Biochemistry of the Flavonoids*, Academic Press, New York (1967).
[5] J. B. HARBORNE and J. J. CORNER, *Biochem. J.* **81**, 242 (1961).
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Woods[3] who found phenolic glucosides in all species of *Salix* which they examined including *S. alba, caprea* var. *lanata, cinerea, phylicifolia, triandra* and *viminalis*, but agree with those of Thieme[2] who could not detect these derivatives in these species. In addition Binns, Blunden and Woods detected leucoanthocyanin formation in all but one of the species which they examined including *S. pentandra, fragilis, purpurea* and *calodendron*. Whilst there is no evidence to suggest that the ability to produce leucoanthocyanins and catechins on the one hand and saligenin derivatives on the other are related metabolic functions, measurement of the concentration of these compounds in the leaf points to a useful chemical classification of the genus.

EXPERIMENTAL

Extraction

Frozen leaves (25 g) were crushed in a mortar with powdered glass and water (40 ml) to a fine paste. After 5 min the solution was filtered through iron-free cellulose (15g) in a Hirsch funnel. The residue was re-extracted with water (30 ml) and the combined filtrates acidified (N-H_2SO_4, 2 ml) and extracted with ethyl acetate (10 × 100 ml). Removal of the solvent at 30° and freeze-drying from *t*-butanol gave the extract as a light brown or buff coloured powder (approx. 0·5 g).

Analysis

Two dimensional paper chromatographic analysis was carried out with (1) 6% acetic acid and (2) butan-2-ol: acetic acid: water (14:1:5). Phenols were revealed by their absorption under u.v. light (with and without ammonia vapour) and by spraying with Gibbs reagent,[7] potassium ferricyanide—ferric chloride[8] and Millons reagent.[9] Salicin, fragilin and salicortin were also confirmed by co-chromatography with authentic samples.

Aliquots of the extract (100 mg) were heated at 90° under N_2 with $NaHCO_3$ solution (5 ml) for 2 hr, neutralized (N HCl) and treated at 20° with β-glucosidase for 24 hr. The solution was extracted with ether (10 × 10 ml) and the extract chromatographed as above. Saligenin was detected with Gibbs reagent.

Quantitiative analysis of salicin and its derivatives was carried out by the method of Thieme.[2] Anthocyanidin derivatives were determined by treating an aliquot of the extract (50 mg) in methanol (4 ml) containing conc. HCl (0·5 ml) at 60° for 20 min. The solution was analysed by TLC on cellulose in Forestal solvent[4] and, after dilution with methanol (45 ml), the light absorption measured from 500 to 600 nm. Extracts showing a distinct λ_{max} *ca.* 550 nm were calibrated against standard cyanidin solutions to give a measure of the anthocyanidin concentration.

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