Phytochemistry, 1967, Vol. 6, pp. 1585 to 1586. Pergamon Press Ltd. Printed in England

SHORT COMMUNICATION

THE FLAVONOID GLYCOSIDES OF *SALIX PURPUREA*

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(*Received* 10 *May* 1967)

Abstract—Isosalipurposide, the 2′-glucoside of chalconaringenin, has been shown to be the principal flavonoid of young bark of *Salix purpurea*; in the old bark it is accompanied by isomeric forms of naringenin 5-glucoside, salipurposide. The leaf of *S. purpurea* shows a very different flavonoid pattern; luteolin 7-glucoside predominates, together with smaller amounts of the 7-glucosides of eriodictyol and naringenin. The distribution of these compounds among *Salix* species has been surveyed, and their possible significance in flavonoid biosynthesis is discussed.

INTRODUCTION

IN 1931, Charaux and Rabaté[1] isolated salipurposide from the bark of *Salix purpurea* L. and later[2] the isomeric isosalipurposide was obtained from the same source; both compounds gave on hydrolysis glucose and naringenin.[3] The u.v. spectra were shown to be quite different and chalcone-flavonone isomerism deduced. At a later date Hänsel, Heise and Pinkewitz[4] isolated diastereoisomeric forms of naringenin 5-glucoside from the same material, and confirmed the structure by hydrogenation to phloridzin. Rabaté[5] obtained a flavonoid from the leaf of *S. purpurea*, which gave glucose and an unidentified aglycone on hydrolysis.

The present paper described the separation and identification of these and other flavonoids of *S. purpurea*, and their distribution in different tissues and different varieties.

RESULTS

A paper chromatographic survey of the phenolic compounds of willow species and varieties, showed that the *Salix purpurea* group was distinguished by a much higher content of flavonoids than all the other species, except *S. daphnoides*. Growth of the 1966 season was sampled, and leaf and bark extracted separately with alcohol. In the bark of the 1966 growth of *S. purpurea* "Helix", only isosalipurposide, the 2′-glucoside of chalconaringenin was found in any quantity. This contrasted with the previous reports,[1,4] so the bark of several years' age was examined. In this extract salipurposide (naringenin 5-glucoside) was found in quantity, separating into two stereoisomeric forms on paper in aqueous solvents.[4] The leaf of this variety showed a pattern of flavonoid compounds differing completely from those found in the bark. Luteolin 7-glucoside and eriodictyol 7-glucoside were the main spots on the chromatograms, with a weaker spot due to naringenin 7-glucoside.

1 C. CHARAUX and J. RABATÉ, *Bull. Soc. Chim. Biol.* **13**, 590 (1931).
2 C. CHARAUX and J. RABATÉ, *Compt. Rend.* **196**, 816 (1933).
3 J. RABATÉ, *Bull. Soc. Chim. Biol.* **17**, 314 (1935).
4 R. HÄNSEL, D. HEISE and G. PINKEWITZ, *Pharm. Acta Helv.* **35**, 27 (1960).
5 J. RABATÉ, *Bull. Soc. Chim. Biol.* **17**, 439 (1935).

A large number of other named varieties of *S. purpurea* were examined and all resembled "Helix" in containing isosalipurposide as the main flavonoid of the current year's bark. In those varieties whose older bark was examined, substantial amounts of the stereoisomeric forms of salipurposide as well were found in all cases. *S. daphnoides* bark showed a similar pattern. In the leaf of all varieties of *S. purpurea*, luteolin 7-glucoside was found, but the amounts of eriodictyol 7-glucoside varied widely. *S. daphnoides* leaf contained only luteolin 7-glucoside in appreciable amount.

DISCUSSION

The apparently universal occurrence of salicin in the bark of *Salix* species has been long recognized. Other glycosides with structures related to salicin are of more limited distribution.[6] Turning to the flavonoids, the *S. purpurea* and *S. daphnoides* groups are clearly distinguished from nearly all the rest by the presence of the chalcone isosalipurposide (chalconaringenin 2′-glucoside) in the bark. In the extensive collection of willows at Long Ashton, comprising more than thirty species and 250 varieties, only three, apart from those belonging to the *purpurea* and *daphnoides* groups or their hybrids, were found to contain easily detectable amounts of chalcones.

The clear differentiation between the phenolic patterns of the leaf and young bark is interesting and must reflect different patterns of activity of the synthetic enzymes involved. There is surprisingly little overlap; some luteolin 7-glucoside can be detected in very young bark, and in a few *purpurea* varieties a little chalcone is found in the leaf, but generally the division is very clear. The occurrence of substantial amounts of naringenin 5-glucoside only in old bark (over 1 yr old) suggests that the chalcone-flavanone change is chemical rather than enzymic in nature in *Salix*; the formation of both optical isomers rather than a single form supports this.

The minor components of *purpurea* leaf are of interest since they could be linked biosynthetically by successive hydroxylation and oxidation.[7] The quantitative variability of naringenin and eriodictyol 7-glucosides in *S. purpurea* varieties suggests that these flavanones are active intermediates in the synthesis of luteolin 7-glucoside. The build-up of the chalcone 2′-glucoside in bark suggests that it, too, is an end-product of synthesis. Further transformation of intact chalcone 2′-glucosides, apart from isomerization to the flavanone 5-glucoside, would appear to be very infrequent compared with that of the chalcone 4′-glucosides (or the corresponding flavanone 7-glucosides). The only clearly demonstrated case seems to be the reduction of isosalipurposide to phloridzin in apple leaf.[7]

EXPERIMENTAL

Isolation of compounds. Flavonoids were extracted from fresh leaf and bark of *Salix* with boiling ethanol, and separated by chromatography on Whatman 3MM paper, using 2% and 50% aqueous acetic acid, and *sec.*-butanol:acetic acid:water (70:2:28).

Identification. The structure of isosalipurposide was confirmed by catalytic hydrogenation to phloridzin, the product being indistinguishable from authentic phloridzin by co-chromatography in three solvents and comparison of u.v. spectra and shifts. Luteolin 7-glucoside was identified by comparison of its chromatographic and spectral properties with authentic material, and acid hydrolysis to luteolin and glucose. The flavanones were distinguished on paper chromatograms by their colour and fluorescence. All gave colours with the KBH_4/HCl reagent;[8] naringenin and its 5- and 7-glucosides red, eriodictyol and its 7-glucoside violet. On the untreated paper, none showed fluorescence; in the presence of ammonia, the 7-glucosides showed a green fluorescence; after dipping in alcoholic $AlCl_3$, all except naringenin 5-glucoside gave a green fluorescence. Their identities were confirmed by spectral and chromatographic comparison with authentic specimens.

[6] H. THIEME, *Pharmazie* **20**, 570 (1965).
[7] H. GRISEBACH, *Chemistry and Biochemistry of Plant Pigments*, p. 279. Academic Press, New York (1965).
[8] E. EIGEN, M. BLITZ and E. GUNSBERG, *Arch. Biochem. Biophys.* **68**, 501 (1957).