

LETTERS TO THE EDITORS

The Editors do not hold themselves responsible for opinions expressed by their correspondents. No notice is taken of anonymous communications

Anthocyanins of the Leaf of the Copper Beech (*Fagus sylvatica*) and the Fruit of the Cultivated Strawberry (*Fragaria virginiana*)

THE pigment of the copper beech was identified in 1932¹ as cyanidin 3-galactoside; two years later, the colouring matter of wild strawberries was stated to be pelargonidin 3-galactoside².

In the former case a small specimen of the anthocyanin was isolated in substance; but in the latter case, the conclusion was reached from the results of reactions in solution. In these tests the distinction between isomeric glycosides was made only by observation of distribution between partly miscible solvents, and this is a property known to be affected by the presence of other substances. It therefore seemed desirable to examine the chromatographic behaviour of these anthocyanins. Using synthetic specimens³ and the method of Bate-Smith and Westall⁴ (paper chromatograms; *n*-butanol-acetic acid-water, 4:1:5, as solvent; original solution in 1 per cent hydrochloric acid, 0.1 per cent with respect to anthocyanin), it was found that chrysanthemine (cyanidin 3-glucoside) and idaein (cyanidin 3-galactoside), though very similar in behaviour, could be definitely distinguished, or even separated, in a run of 24-30 hr. The *R_F*'s are 0.395 and 0.38 for the glucoside and galactoside respectively, but careful standardization of the conditions ensured unambiguous results. A mixture gave an elongated band, and there was no evidence of any holding back of the faster-moving glucoside by the galactoside.

The copper beech pigment (25 mgm.) was isolated as already described from leaves (1 kgm.) collected in June 1954 from a tree near the laboratory, except that the purification through the picrate was omitted. On chromatography the pigment ran like idaein and similarly, as a compact spot, when mixed with idaein. When mixed with chrysanthemine, the elongation of the coloured region was the same as that obtained with a mixture of synthetic idaein and chrysanthemine. The identification of this anthocyanin as idaein was therefore confirmed.

Sondheimer and Kertesz⁵ purified the anthocyanin of a variety of strawberry grown in the United States and found it to be callistephin, since cyanidin and glucose (as D-glucosazone) were obtained on hydrolysis. Although the wild strawberry (*Fragaria vesca*) was not available in sufficient quantity, we thought it of interest to examine garden strawberries (from Norfolk) with the aid of the chromatographic procedure.

Preliminary experiments showed that the divergence between pelargonidin glucoside (callistephin) and pelargonidin galactoside (called fragarin, possibly erroneously) was greater than that between the corresponding cyanidin derivatives. The *R_F* of the glucoside was about 0.45 and that of the galactoside, 0.38. The pigment (c. 35 mgm.) from the cultivated strawberries (1.5 kgm.) was isolated by means of a method closely similar to that used for the beech leaves. Its chromatographic behaviour was the same as that of synthetic callistephin and differed from that of synthetic pelargonidin 3-galactoside.

The possibility remains that the anthocyanin of wild strawberries may differ from that of the cultivated kinds; a precedent is the occurrence of cyanidin galactoside in European cranberries⁶, whereas those obtained from Newfoundland were found to be coloured by peonidin glucoside⁷.

R. ROBINSON
HERCHEL SMITH

Dyson Perrins Laboratory,
University, Oxford.
Feb. 21.

¹ Robinson and Robinson, *Biochem. J.*, **26**, 1650 (1932).

² Robinson, *Ber. dtsh. chem. Ges.*, **67**, 98 (1934).

³ Robertson and Robinson, *J. Chem. Soc.*, 1460 (1928). Murakami, Robertson and Robinson, *ibid.*, 2685 (1931). Grove and Robinson, *ibid.*, 2722 (1931). Nair and Robinson, *ibid.*, 1611 (1934).

⁴ Bate-Smith and Westall, *Biochim. Biophys. Acta*, **4**, 427 (1950).

⁵ Sondheimer and Kertesz, *J. Amer. Chem. Soc.*, **70**, 3476 (1948).

⁶ Willstätter and Mallison, *Liebig's Ann.*, **408**, 15 (1915).

⁷ Grove and Robinson, *Biochem. J.*, **25**, 1706 (1931). Levy and Robinson, *J. Chem. Soc.*, 2715 (1931). Grove and Robinson, *ibid.*, 2722 (1931).

Release of Aromatic Compounds from Birch and Spruce Sawdusts during Decomposition by White-rot Fungi

DURING investigations into the decomposition of lignin by soil micro-fungi, it was found that a large number of these were able to decompose several aromatic compounds which are believed to be related to lignin¹. In an attempt to discover whether such compounds are released on biological decomposition of lignin, two wood-rotting fungi, *Polystictus versicolor* and *Trametes pini*, both white-rots, were grown on birch and spruce sawdusts. 25 gm. of sawdust (previously extracted with alcohol-benzene) was added to each flask and moistened with 40 ml. of a mineral salts medium (0.1 gm. potassium dihydrogen phosphate, 0.05 gm. potassium chloride, 0.05 gm. magnesium sulphate, 0.5 gm. peptone, 1 gm. glucose, 100 ml. water). The flasks were sterilized by steaming for one hour on three successive days. The fungi produced much growth which penetrated the sawdust, causing it to become lighter in colour. After six months incubation at 22° C.,

9 gm. of this material was extracted with 2 per cent sodium hydroxide. On acidification of the extract a considerable precipitate was formed which was then centrifuged off; the supernatant was then extracted with ether. The ether was evaporated off and the residue dissolved in a small volume of absolute alcohol for application to chromatography papers. These were developed with a single-phase mixture consisting of *n*-propanol-ammonia (sp. gr. 0.880) - water (80:5:15), run for two periods of 24 hr. at 21° C. and dried between successive runs. Two sprays were used: (a) diazotized sulphanilic acid² for phenolic groups, and (b) 2:4-dinitrophenylhydrazine³ for aldehydes.



Fig. 1. A, vanillic acid; B, syringic acid