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The separation of phenolic glucosides and carbohydrates from the bark of *Populus tremula* by gel filtration

The phenolic glucoside salicin is present in the bark and leaves of various *Populus* species and has been used in medicine as an analgesic and anti-rheumatic. During the past few years other phenolic glucosides have been isolated from *Populus* species. We have established that the bark of two-year-old seedlings of *Populus tremula* contains tremuloidin (2-benzoyl salicin) besides salicin, which is in agreement with the work of PEARL AND DARLING¹. From the bark of two-year-old seedlings we isolated salicin (0.75%), tremuloidin (0.45%) and saccharose (1.20%). The chromatographic investigations indicate the presence of glucose, fructose and some unknown trisaccharide².

In order to separate carbohydrates which occur in the bark of *Populus tremula* from glucosides, and the glucoside salicin from tremuloidin, we applied the method of gel filtration on Sephadex G-25.

The bark of two-year-old seedlings of *Populus tremula*, cut in May 1967 at the nursery garden at Nedjarići near Sarajevo, was extracted with 96% ethanol. The ethanol was recovered and the residue extracted with water. The aqueous solution was treated with lead subacetate and filtered. The filtrate was saturated with hydrogen sulphide and the resulting precipitate filtered off. The colourless filtrate was concentrated and some crystalline material was collected.

Isolated material (10 mg) was dissolved in water (2 ml) and applied to the column. The Sephadex G-25 column had a volume of 145 ml. The column was washed with distilled water and the fractionation was carried out in 12–15 h. The concentration of carbohydrates was measured by the orcinol-sulphuric acid method³. The glucoside content was determined using the nitric acid-barbituric acid method⁴ or by the method of DOBROWOLSKA⁵. The experiment with isolated glucosides, performed

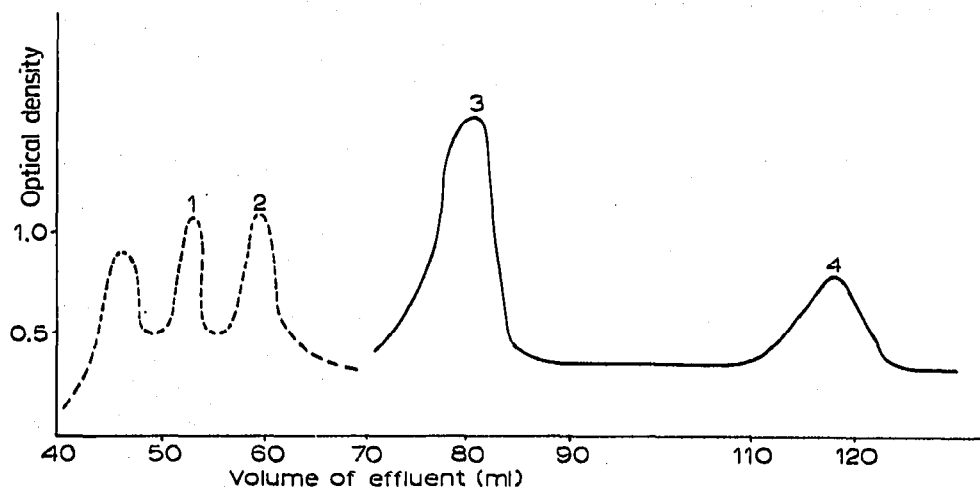


Fig. 1. Gel filtration on Sephadex G-25. The separation of salicin, tremuloidin and carbohydrates. (— — — —) carbohydrates; orcinol-sulphuric acid method (505 m μ). (—) Phenolic glucosides; barbituric acid method (410 m μ). 1 = Sucrose (mol. wt. 342); 2 = glucose (mol. wt. 180); 3 = salicin (mol. wt. 286); 4 = tremuloidin (mol. wt. 390).

in order to demonstrate the possibility of separating phenolic glucosides from carbohydrates and salicin from tremuloidin, is presented in Fig. 1.

We have observed that the glucosides salicin and tremuloidin produce adsorption effects which do not conform with the theory of gel filtration. The phenolic glucoside salicin (2-oxy-benzylalcohol- β -D-glucopyranoside) has an adsorbing aromatic ring in its molecule and the distribution coefficient is higher than expected. Because of the adsorption effect, salicin (mol. wt. 286) was collected in the fraction after glucose. Tremuloidin (mol. wt. 390) was collected in the fractions after salicin. From our results is evident that tremuloidin is adsorbed more strongly than salicin because of the two aromatic rings in the tremuloidin molecule. Our results show that gel filtration on Sephadex G-25 may give a separation of carbohydrates from glucosides and, furthermore, separate the glucoside salicin from tremuloidin. Experiments on a preparative scale are in progress.

The glucosides salicin and tremuloidin were examined by means of thin-layer chromatography on silica gel. The plates were developed with chloroform-methanol (4:1) and sprayed with 25% nitric acid. After spraying, the plates were left for 15 min at 80°. The plates were then sprayed with 1% 1,3-diphenylthiobarbituric acid in 1% sodium hydroxide and heated for 20 min at 100° to produce colour spots (salicin, yellow spots, and tremuloidin, orange spots).

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