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The separation of phenolic glucosides by gel filtration

The naturally-occurring phenolic glucosides are heterogenous according to their structural components and to their molecular size. In different natural sources the main phenolic glucoside is always accompanied by a few other phenolic glucosides and carbohydrates. The methods of separation and purification of phenolic glucosides therefore attract great interest.

In order to separate the phenolic glucosides and carbohydrates which occur in the bark and leaves of *Populus tremula*, we applied the method of gel filtration on Sephadex G-25 with water as eluent. In our previous paper[1], we established that it is possible to separate the glucosides salicin and tremuloidin from carbohydrates. Salicin and tremuloidin adsorb in a manner which does not conform with the theory of gel filtration. The phenolic glucoside salicin (mol. wt. 286) has an adsorbing aromatic ring in its molecule and the distribution coefficient is higher than expected. Tremuloidin (2-benzoylsalicin, mol. wt. 390) is adsorbed more strongly than salicin because of two aromatic rings in the molecule.

In continuing our studies on the separation of phenolic glucosides, we applied the method of gel filtration to more complex mixtures of the naturally-occurring phenolic glucosides.

The phenolic glucosides tested and their structures, are listed in Table I.

TABLE I

PHENOLIC GLUCOSIDES AND THEIR STRUCTURES

Glucoside	*Structure*	*Mol. wt.*
Salicin	2-oxy-benzylalcohol-β-D-glucopyranoside	286
Populin	6-benzoylsalicin	390
Tremuloidin	2-benzoylsalicin	390
Fragilin	6-acetylsalicin	328
Salireposide	2,5-dioxy-benzylalcohol-β-D-(6-O-benzoyl)-glucopyranoside	406
Grandidentatin	*cis*-cyclohexandiol(1,2)-1-β-D-(2-*p*-cumaroyl)-glucopyranoside	424
Triandrin	3-(4-hydroxyphenyl)-2-propen-1-ol-1-β-D-glucopyranoside	312

We isolated from different natural sources three additional phenolic glucosides besides salicin: tremuloidin, populin and salireposide.

The freshly-obtained bark of various Salicaceae species (*Populus tremula, Salix alba, Salix repens*) was extracted with 96% ethanol and the extract treated with an excess of basic lead acetate. The mixture was filtered and the lead removed from the clear filtrate by treatment with hydrogen sulphide. The resulting clear solution was concentrated under reduced pressure. After evaporation, crystals were collected and examined by means of paper or thin-layer chromatography.

In our experiments we also tested samples of the phenolic glucosides, fragilin, triandrin and grandidentatin.

Filtration experiments were carried out using Sephadex G-25 and Sephadex .H-20 (Pharmacia, Uppsala, Sweden). Most of the experiments were performed on ephadex columns with a dimension of 1.104 cm. The dry Sephadex was allowed to well in water or in a mixture of water and organic solvents used as an eluent. The ubstances to be tested were dissolved in an appropriate eluent and put on the olumn in a volume of 2–3 ml. The concentration of the tested substances varied etween 10 and 15 mg. All experiments were carried out in 12–18 h at room temper- .ture. The flow rate was approximately 2 ml/10 min.

The fractions were analysed using Millon's reagent (H. THIEME[2]; B. DOBROVOLSKA AND K. TWARDOWSKA[3]). Our investigation showed that, instead of Millon's eagent, concentrated sulphuric acid can be used as a colorimetric reagent for the letermination of phenolic glucosides which give a red-coloured complex with sulphuric cid (only salireposide gives a yellow complex[4]). The absorption maximum of the oloured complex salicin–sulphuric acid is at 510 mμ. Concentrated sulphuric acid 3 ml) was added to the fraction to be tested (1 ml). After 20 min at room temperature he optical density was measured at 510 mμ.

Fractionated glucosides were identified by means of thin-layer chromatography n silica gel in a system of chloroform–methanol (4:1)[5].

Besides the distilled water for gel filtration, mixtures of water–ethanol (9:1), water–methanol (9:1) and 96% ethanol were used as eluents. Consequently, our experiments are divided into three groups.

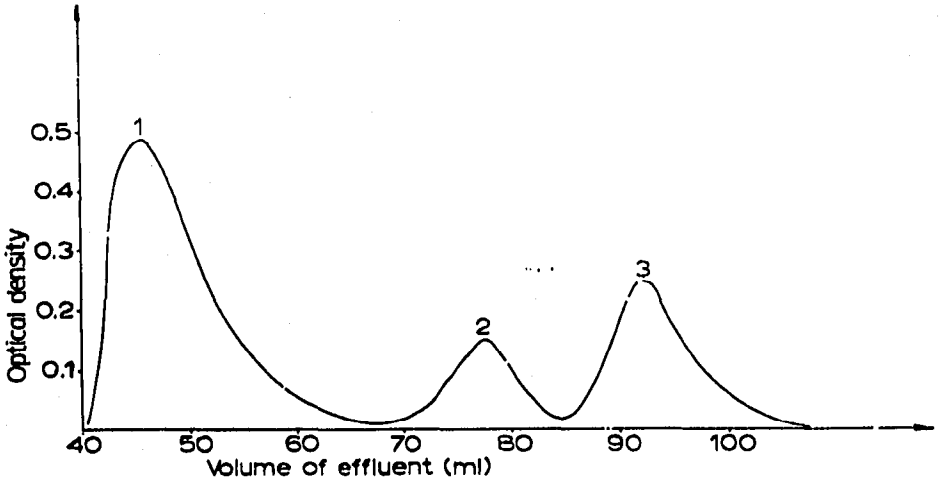

Fig. 1. Separation of phenolic glucosides on Sephadex G-25 in distilled water. Bed dimensions: 1.104 cm. Sample: 2 ml containing 10 mg of glucosides. Flow rate 2 ml/10 min. 1 = salicin; 2 = populin; 3 = salireposide.

Separation of phenolic glucosides on Sephadex G-25 with distilled water as an eluent

With distilled water as an eluent we separated salicin, populin (or tremuloidin) and salireposide (Fig. 1). Fragilin (mol. wt. 328) is collected in the same fractions as salicin (mol. wt. 286), which indicates the possibility of separating fragilin from populin (or tremuloidin) and salireposide, but not from salicin. With water as an eluent it is impossible to separate populin (6-benzoylsalicin, mol. wt. 390) from tremuloidin (2-benzoylsalicin, mol. wt. 390).

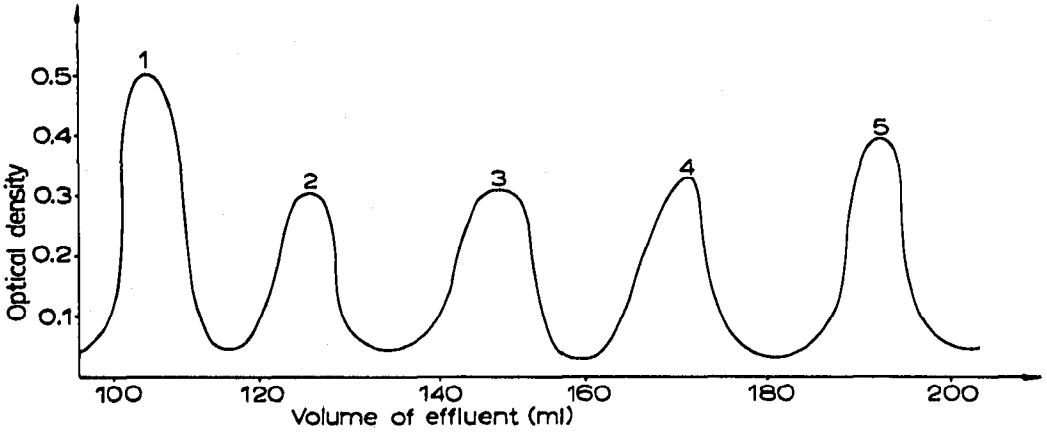

Fig. 2. Separation of phenolic glucosides on Sephadex G-25 in water–methanol (9:1). Bed dimensions: 1.104 cm. Sample: 3 ml containing 3 mg of each glucoside. Flow rate 2 ml/10 min. 1 = salicin; 2 = populin; 3 = tremuloidin; 4 = grandidentatin; 5 = salireposide.

Separation of phenolic glucosides on Sephadex G-25 with water–methanol (9:1) as an eluent

In order to increase the low solubility of phenolic glucosides in water, we performed some experiments with a mixture of water and methanol (Fig. 2). The results obtained show the possibility of separating populin (6-benzoylsalicin, mol. wt. 390) from tremuloidin (2-benzoylsalicin, mol. wt. 390); tremuloidin is adsorbed more strongly than populin. With a mixture of water and methanol, we succeeded in separating salicin, populin, tremuloidin, salireposide and grandidentatin. Similar results were obtained when elution was carried out with water–ethanol (9:1).

Separation of phenolic glucosides on Sephadex LH-20 with 90% ethanol as an eluent

With ethanol as an eluent on Sephadex LH-20, we separated salicin, populin, tremuloidin and salireposide. The results obtained are basically similar to the results obtained on Sephadex G-25, but the separation volumes are smaller (Fig. 3).

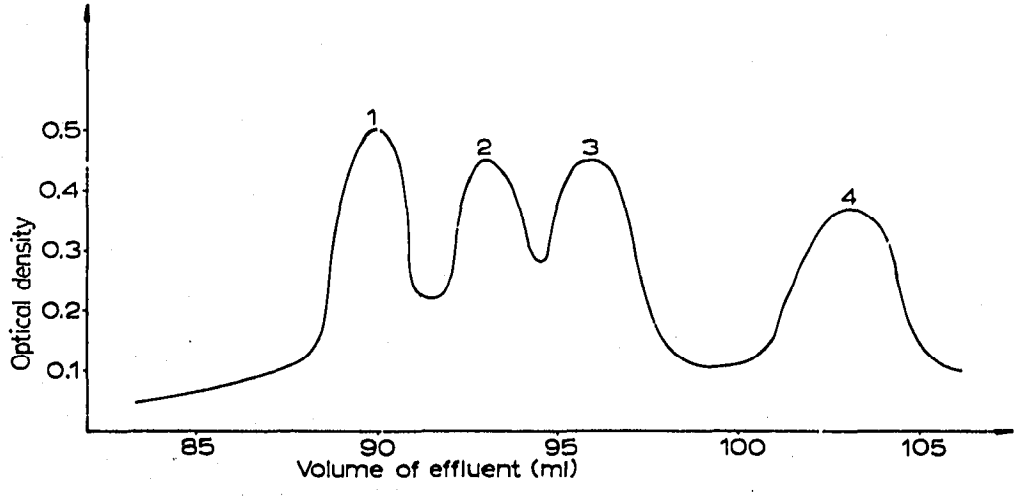

Fig. 3. Separation of phenolic glucosides on Sephadex LH-20 in ethanol. Bed dimensions: 1.106 cm. Sample: 3 ml containing 4 mg of each glucoside. Flow rate 2 ml/15 min. 1 = salicin; 2 = populin; 3 = tremuloidin; 4 = salireposide.

Gel filtration on Sephadex G-25 and Sephadex LH-20 may give good separation f phenolic glucosides. The phenolic glucosides adsorb in a manner which does not onform with the theory of gel filtration and leads to higher elution volumes than an be expected from the molecular size. The adsorption effect is related to the ructure of the phenolic glucosides and increases with the number of aromatic rings cluded in the structure of glucosides. Therefore, populin or tremuloidin (mol. wt. 90) which have two adsorbing aromatic rings in their molecule, are adsorbed more trongly than salicin (mol. wt. 286). The adsorption effect also depends on hydroxyl roups included in the agluconic part of the glucosides: hydroxyl groups in the olecule seem to increase adsorption. None of the glucosides tested are irreversibly dsorbed onto the column.

When elution was carried out with distilled water, we separated salicin, populin or tremuloidin) and salireposide. Better results in separation were obtained on ephadex G-25 with water–ethanol (9:1) or water–methanol (9:1). With these luents, we separated salicin, populin, tremuloidin, salireposide and grandidentatin. The separation of populin from tremuloidin is completely eliminated when elution is arried out with distilled water. In all experiments fragilin and triandrin were eluted imultaneously with salicin.

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Chemistry Department,
Faculty of Science,
University of Sarajevo,
Sarajevo (Yugoslavia)

A. REPAŠ
B. NIKOLIN
K. DURSUN

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