USE OF SEPHADEX IN THE ANALYSIS OF SOME FLAVONOIDS

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In 1960, Gelotte [1] reported that Sephadex possesses a capacity for adsorbing aromatic compounds, especially phenols. It was established later that flavonoids and tannin can also be adsorbed by Sephadex, and this so strongly that some of these compounds autoxidize under the influence of alkali [2]. At the same time, Nilson [3] by means of Sephadex successfully separated a mixture of isoflavones without disturbing their structures. In 1966, Somers [4], using type G-25 Sephadex and an ethanolic aqueous medium, eliminated the adsorption phenomenon and isolated fractions containing tannin. Vancraenenbroeck [5] used Sephadex to separate anthocyanins. At present, Sephadexes are being used successfully for separating various polyphenolic compounds [5–8].

We have studied the conditions for the separation and purification of 14 flavones, flavonols, and flavanones on a column filled with Sephadex gel and have also investigated the possibility of using Sephadex in thin-layer chromatography (Table).

Structure and Molecular Weights of Flavonoids Subjected to Gel Filtration

Flavonoids	Structure	Empirical formula	Mol wt
Aglycones			
Apigenin	5,7,4'-trihydroxyflavone	$C_{15}H_{10}O_5$	270
Luteolin	5,7,3',4'-tetrahydroxyflavone	$C_{15}H_{10}O_6$	286
Kaempferol	5,7,4'-trihydroxyflavonol	$C_{15}H_{10}O_6$	286
Quercetin	5,7,3',4'-tetrahydroxyflavonol	$C_{15}H_{10}O_7$	302
Hesperetin	5,7,3'-trihydroxy-4'-methoxyflavanone	$C_{16}H_{14}O_6$	302
Monoglycosides			
Cosmosiin	7-O-β-D-glucopyranosyl-5,4'-dihydroxyflavone	$C_{21}H_{20}O_{10}$	432
Luteolin 7-glucoside	7-O-β-D-glucopyranosyl-5,3',4'-trihydroxyflavone	$C_{21}H_{20}O_{11}$	448
Astragalin	3-O-β-D-glucosyl-5,7,4'-trihydroxyflavonol	$C_{21}H_{20}O_{11}$	448
Isoquercitrin	3-O-glucopyranosyl-5,7,3',4'-tetrahydroxyflavonol	$C_{21}H_{20}O_{12}$	464
Quercimeritrin	3-O-β-D-glucopyranosyl-5,3',4'-trihydroxyflavonol	$C_{21}H_{20}O_{12}$	464
Diglycosides			
Luteolin 7-β-diglycoside	7-O-β-D-glucopyranosyl-(1 → 3)-β-D-glucopyranosyloxy-5,3',4'-trihydroxyflavone	$C_{27}H_{30}O_{16}$	610
Apigenin 7-β-D-glucosylxyloside	7-O-β-D-glucopyranosyl-(1 → 2)-β-xylosyloxy-5',4'-dihydroxyflavone	$C_{26}H_{29}O_{14}$	567
Rutin	5,7,3', 4'-tetrahydroxy-3-O-rutinosyloxyflavonol	$C_{27}H_{30}O_{16}$	610
Hesperedin	5,3'-dihydroxy-4'-methoxy-7-O-β-rutinosyloxyflavanone	$C_{28}H_{34}O_{15}$	590

In gel filtration through Sephadex, the molecules of a solute with dimensions greater than the dimensions of the pores of the Sephadex granules are not retained by the gel and issue from the column first. The smaller molecules penetrate into and are retained by the gel; their elution is correspondingly retarded. Consequently, flavonoids with a higher molecular weight pass through the Sephadex and those with a lower molecular weight will be retained. However, experiment has shown that the molecular weight of a flavonoid does not always exert a substantial influence on the rate of passage of the flavonoid through Sephadex. On elution with water, flavone diglycosides (rutin, luteolin diglycoside, and apigenin glucoxyloside) and monoglycosides are readily eluted from the column. Flavanone diglycosides (hesperedin), and also the aglycones (quercetin, luteolin, and hesperetin) are adsorbed and are not eluted by water. As a rule, diglycosides migrate at a greater rate than monoglycosides. When water is replaced by a 0.15 M solution of common salt, as before, the aglycones are not eluted. When a mixture of flavonoids consisting of aglycones, monoglycosides, and diglycosides is eluted with 3% ammonium hydroxide solution, the aglycones (quercetin) are eluted first, and then some monoglycosides and diglycosides, or some compounds may undergo a

change. Thus, for example, the luteolin 7-β-diglycoside from the petals of Colchicum speciosum decomposes.

Flavonols are adsorbed more strongly than flavanones. The presence of an ortho-dihydroxy structure in the lateral phenyl radical has no substantial influence on this.

The virtue of gel filtration is the rapidity of the analysis and the possibility of using the column again for the separation of a new portion of a mixture of mono- or diglycosides. Its disadvantage is that one cannot use high concentrations of the substances or ethanol.

Gel filtration through a column of Sephadex may also be used to separate the products of the alkaline degradation of the flavonoids and phenolic acids of plants. In this case, the adsorption effects depends on the presence of the aromatic nucleus and of phenolic hydroxyls [1] and not on the carboxyl group of the acids.

When Sephadex was used for thin-layer chromatography in an aqueous medium, the rapid separation of rutin from the other flavonoids was observed. The remaining flavonoids separated slowly. The separation of the monoglycosides took place considerably more rapidly in 10% aqueous sodium chloride or 1% aqueous sodium acetate.

EXPERIMENTAL

Sephadexes of types G-25 and G-50, previously swollen in distilled water or in 0.05 M sodium chloride solution, were transferred to a column (50 cm × 10–15 mm). Then various mixtures of flavonoids in the form of aqueous or ethanolic solutions (50–100 mg of flavonoid in 1 ml) were carefully deposited on the surface of the gel. Elution was carried out with distilled water, mixtures of ethanol and water, ammonia solution, or a dilute solution of common salt. In the separation of the products of alkaline degradation, the liquid was neutralized to pH 7.

In the preparation of the thin-layer chromatograms, two parts of Sephadex were mixed with 8 or 10 parts by weight of water. The resulting gel was uniformly deposited on plates with dimensions of 4 × 13 cm. Chromatography was carried out for 2–4 hr by the descending method in chambers 8 cm high, the solvent being fed to the plate by means of a wick made from chromatographic paper.

CONCLUSIONS

The conditions of gel filtration, through Sephadex of 14 different flavonoids have been studied. It has been shown that the structure of the flavonoid compound affects the filtration process.

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