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Short Communications

Separation of Polyphenolic Glycosides by Gel Chromatography

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Polyphenolic glycosides extracted from plant material are mostly obtained as complex mixtures, and besides separation from unrelated impurities, group separation is desirable. With the very tightly crosslinked dextran gels (Sephadex G-10 and G-15) now available separation of these glycosides according to molecular size by gel filtration appeared possible. However, attempts to fractionate an aqueous test mixture containing arbutin, chlorogenic acid, rutin, and quercitrin on a Sephadex G-10 column using water or aqueous sodium chloride solution as eluent were unsuccessful, mainly because the glycosides were strongly adsorbed on the column material.

Adsorption of aromatic structures on cross-linked dextran gels was first observed by Gelotte.[1] Phenols are apparently adsorbed very strongly. Thus Bock [2] found that flavonoids and tannins could only be eluted with aqueous alkali. Nilsson [3] was able to separate isoflavones by eluting with ammonia and Woof and Pierce [4] achieved separation of some flavonoids by complexing them with molybdate. Somers [5] found that the adsorptive effects of dextran gels could be eliminated by using aqueous alcoholic eluents.

Separation by molecular sieve action could not be achieved, but it was found that the adsorptive properties of the G-10 gel could be suitably modified by using aqueous-methanolic eluents of varying methanol content and thus obtain fractionation. Using 50 % methanol (v/v) the test mixture was separated into three fractions containing chlorogenic acid and arbutin, rutin, and quercitrin, respectively. Further glycosides were then chromatographed and their elution characteristics in the form of their K_d values [1] are given in Table 1.

Table 1. Approximative K_d values of polyphenolic glycosides and related compounds on a Sephadex G-10 column (1 × 50 cm). Eluent 50 % methanol (v/v).

Compound	K_d
Aesculin	0.8
Chlorogenic acid	0.9
Arbutin	1.4
Quercetin-4′-glucoside	5.8
Phloridzin	6.8
Rhoifolin	7.5
Apiin	8.7
Rutin	9.3
Apigenin-7-glucoside	10.9
Quercitrin	20.4

With the exception of the fact that the highly eluent-soluble simpler phenol derivatives are eluted in front of the flavonoid glycosides there seems to be no clear relationship between order of elution and structure. A worthwhile separation of the glycosides can in any case be achieved quite often, but complete fractionation is restricted by zone broadening. Thus, chromatography of a very complex mixture containing all the polyphenols mentioned in the table results mainly in fractions containing pairs of compounds although the single components could be obtained by suitable fraction cutting. Slightly less zone broadening was observed when elution was started with 30 % aqueous methanol and a linearly rising methanol gradient applied. Such gradient elution is made possible by the fact that the degree of swelling of the

G-10 gel appears to be the same in aqueous methanol and in water, in contrast to Sephadex G-25 and the polyacrylamide gel Bio-Gel P 2, which both shrink in the presence of methanol. These gels are also weaker adsorbers than Sephadex G-10 and no useful separation was observed when they were tested with eluents of constant methanol content (50 %). The above method has been found especially useful for the separation of simpler phenol derivatives from flavonoid glycosides, but quite often separation of flavonoid mono- and diglycosides is also possible.

Experimental. Columns (1 × 50 cm) were prepared either in dilute aqueous sodium chloride solution in which case the column was washed overnight with the eluent, or directly in the elution medium. 0.1–0.2 mg of each glycoside or a mixture containing the same amount of each compound in 1 ml of eluent was applied to the column. Elution was carried out by gravity feed at a flow rate of 5–10 ml/min. 5–6 ml fractions were collected and analysed both spectrophotometrically and chromatographically (PC, solvent: butanol-acetic acid-water, 6:1:2, TLC on silica gel, solvent: ethyl acetate-butanone-formic acid-water, 50:30:10:10). When required a linearly rising methanol gradient was obtained by continually replacing the solvent leaving the solvent reservoir by pure methanol.

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Studies on Synthetic Ascorbigen as a Source of Vitamin C for Guinea Pigs*

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Ascorbigen is known to be a bound form of ascorbic acid chemically synthesized [1] or isolated from cabbage and other plants.[2] The question has arisen whether ascorbigen does act as a preventative and cure of scurvy as a source of vitamin C, since the native vitamin C in vegetables and plants is more stable and active than synthetic L-ascorbic acid in humans and guinea pigs.

Ascorbigen was synthesized chemically from ascorbic acid by conjugation with 3-hydroxymethylindole, which was prepared from 3-indolealdehyde and sodium borohydride, by the method of Virtanen and Kiesvaara.[1] The yield of pure ascorbigen, according to analyses by paper and thin-layer chromatography and estimation by ultraviolet absorption and infrared analysis, was approximately 67 %.

Guinea pigs of a pure albino strain, weighing about 200 to 250 g, were used in the animal experiments. Twenty-four animals were fed on the scorbutic diet and tap water was provided *ad libitum*. On the 10th day of feeding, the animals were divided into four groups as follows: (1) control group on the scorbutic diet, (2) ascorbic acid group (2 mg per day), (3) ascorbigen group (3.5 mg per day), and (4) ascorbigen group (21 mg per day). Each group comprised three female and three male guinea pigs.

The animals in the control group were given 1 ml of 50 % sucrose solution *per os* by pipette daily, and those in groups 3 and 4 were given supplements containing 3.5 and 21 mg ascorbigen, respectively. The animals in group 2 were given 2 mg of ascorbic acid dissolved in 1 ml of 50 % sucrose solution. The animals were and body weight, appetite, movement, and

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