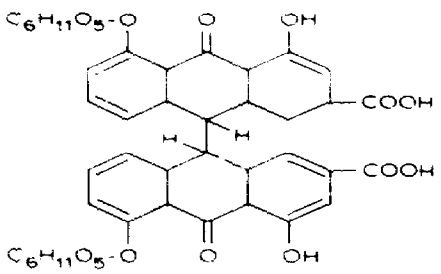
Notes

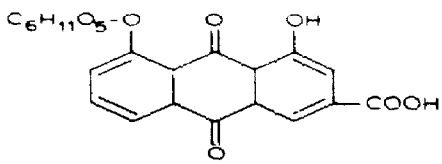
CHROM. 3528

Separation of dianthrone glycosides and anthraquinone glycosides in senna and rhubarb on columns of Sephadex LH-20

Both senna leaf (*Cassia angustifolia*) and rhubarb root (*Rheum palmatum*) contain dianthrone glycosides and anthraquinone glycosides[1,2]. The separation of these



Dianthrone glycoside
(Sennoside A)



Anthraquinone glycoside
(Rhein monoglycoside)

two groups of glycosides is important inasmuch as they possess a different pharmacological activity[3]. Separation is possible with counter-current distribution[2], paper chromatography[2] and thin-layer chromatography[1,2]. HORHAMMER, WAGNER AND LEUE[6] reported the separation of anthraquinone glycosides in rhubarb on a polyamide column. Using this method, however, we found that the dianthrone glycosides were retained on the column and it was impossible to elute them without destroying them. The many aromatic rings which are present per molecule of dianthrone glycoside are responsible for the strong adsorption on the polyamide. We next tried separation on Sephadex since the two groups of glycosides differ considerably in molecular size. On Sephadex G-10, sennoside B ($K_{av} = 0.18$) and rhein monoglycoside ($K_{av} = 0.23$) could be separated with water as eluent. The glycosides of senna and rhubarb extracts, which contain more and other dianthrone glycosides and anthraquinone glycosides than the two tested, could only be partly separated. A strong adsorption of the greater part of the glycosides present was observed on Sephadex G-10. This is a well-known effect of aromatic substances containing phenol groups on the more cross-linked dextran gels[7].

Attempts to separate the glycosides with Sephadex LH-20, which allows the use of polar solvents, were more successful and the results will be described in this communication.

Experimental

Materials

Sephadex LH-20 (Pharmacia),

Sennoside A, B and C (Sandoz),

Rhein monoglycoside (from our laboratory stock; chromatographically pure by PC and TLC),

Rhubarb and senna extracts were prepared by boiling 100 mg of the drugs with 20 ml 70 % methanol and drying 3 ml of this solution *in vacuo* below 30°.

Chromatographic procedure

Column chromatography. Sephadex LH-20 is allowed to swell in 70 % methanol before packing the column. Bed dimension: 20 × 1 cm; flow rate 0.1 ml/min. The extracts were dissolved in 70 % methanol and applied on the top of the column. 70 % methanol was used as an eluent. Zones eluted from the column were evaporated to dryness *in vacuo* and investigated by paper and thin-layer chromatography.

Paper chromatography. Paper: Schleicher and Schüll 2043 b. Solvent: *n*-propanol–ethyl acetate–water (4:3:3)[6].

Thin-layer chromatography. Adsorbent: Kieselgel G (Merck). Solvent: *n*-propanol–ethyl acetate–water (4:4:3)[4,5].

Detection. Paper and thin-layer chromatograms are observed under U.V. light with the following results: Dianthrone glycosides give a dull ochre colour; anthraquinone glycosides and aglycones give an orange colour; flavonoids give a grey-brown colour, which turns to bright yellow under ammonia vapour. In daylight, dianthrone glycosides give a yellow colour, which changes to red with ammonia vapour or on spraying with a 5 % solution of KOH in 50 % MeOH.

Anthraquinone glycosides give a faint yellow colour, which intensifies with ammonia vapour or alcoholic KOH; flavonoids give a yellow colour, which intensifies with ammonia vapour or alcoholic KOH.

Results and discussion

The extracts develop a number of well-separated, yellow-coloured zones on the column during elution. The best separation was obtained using 70 % methanol as eluent. Other eluents which were tried, such as methanol–water mixtures and acetone–water mixtures of different composition, gave an inferior separation and, in some cases, a strong adsorption of the glycosides was observed.

Tables I and II show the results of the fractionation of the extracts investigated.

Dianthrone glycosides with large molecular size are eluted first, followed by the anthraquinone glycosides, which are about half the molecular size of the dianthrone glycosides. Anthraquinone aglycones, which have the smallest molecular dimensions, are eluted last from the Sephadex. Zones 7 and 8 of senna extract remain strongly adsorbed and could only be eluted with 70 % acetone. This fractionation confirms the theory of gel filtration. However, the aromatic adsorption effect also plays a role in the elution pattern, as is shown by the large effluent volumes which contain most of the anthraquinone glycosides and aglycones.

In addition it will be seen from Tables I and II that, in senna, aloeemodin monoglycoside (together with a flavonoid) is eluted before rhein monoglycoside, while in rhubarb, rhein monoglycoside (together with aloeemodin diglycoside) is eluted before aloeemodin monoglycoside. Although it is not possible to obtain a separation

TABLE I

FRACTIONATION OF AN EXTRACT FROM SENNA LEAF ON A SEPHADEX LH-20 COLUMN

Zone	*Colour*	*Effluent volume (ml)*	*Components*	*R_F value*	
				PC	*TLC*
1	faint yellow	8.0– 8.5	Sennoside B	0.13	0.08
2	faint yellow	8.5–10.5	Sennoside A	0.25	0.16
3	light brown	11.0–13.0	Sennoside D +	0.28	0.25
			Sennoside C +	0.51	0.39
			aloeemodin dianthrone glycoside	0.54	0.54
4	bright yellow	14.5–15.8	Aloeemodin mono glycoside +	0.81	0.67
			flavonoid (glycoside)	0.72	0.55
5	bright yellow	20.5–27.0	Rhein monoglycoside +	0.36	0.33
			flavonoid (glycoside)	0.76	0.62
6	bright yellow	35.0–41.5	Rhein aglycone +	0.65	0.45
			aloeemodin aglycone	0.98	0.85
7	green	—	Chlorophyll	0.98	0.87
8	yellow	—	Flavonoid (aglycone)	0.98	0.89

TABLE II

FRACTIONATION OF AN EXTRACT FROM RHUBARB ROOT ON A SEPHADEX LH-20 COLUMN

Zone	*Colour*	*Effluent volume (ml)*	*Components*	*R_F value*	
				PC	*TLC*
1	faint yellow	8.5– 9.0	Sennoside B	0.13	0.08
2	faint yellow	9.0–10.5	Sennoside A	0.25	0.16
3	faint yellow	10.5–13.5	Sennoside D +	0.28	0.25
			Sennoside C +	0.51	0.39
			rhein diglycoside	0.21	0.15
4	yellow	14.0–17.5	Rhein monoglycoside +	0.36	0.33
			aloeemodin diglycoside	0.53	0.42
5	bright yellow	20.0–29.0	Chrysophanol diglycoside	0.59	0.40
6	faint red	29.0–31.5	Emodin monoglycoside	0.83	0.74
7	yellow	33.5–38.0	Aloeemodin monoglycoside	0.81	0.67
8	bright yellow	38.5–43.0	Chrysophanol monoglycoside +	0.88	0.75
			physcion monoglycoside	0.88	0.75
9	light yellow	50.0–56.0	Aglycones: rhein	0.65	0.45
			chrysophanol, physcion, aloeemodin, emodin	0.98	0.85

of all the different glycosides present in senna and rhubarb, the dianthrone glycosides and the anthraquinone glycosides in senna can be separated. In rhubarb, only rhein diglycoside cannot be separated from the dianthrone glycosides.

Acknowledgements

The author is grateful to Prof. Dr. F. H. L. VAN Os for his critical interest. He also wishes to thank Miss A. R. RICHTERS for skilled technical assistance and Sandoz A.G., Basel, for the generous gift of Sennoside A, B and C.

Laboratory of Pharmacognosy, State University, Groningen (The Netherlands) J. H. ZWAVING

1 J. K. CRELLIN, J. W. FAIRBAIRN, C. A. FRIEDMANN AND H. H. RYAN, *J. Pharm. Pharmacol.*, 13 (1961) 639.
2 J. H. ZWAVING, *Planta Med.*, 13 (1965) 474.
3 J. W. FAIRBAIRN, *Pharm. Weekblad*, 100 (1965) 1493.
4 L. HÖRHAMMER, H. WAGNER AND G. BITTNER, *Pharm. Ztg.*, 108 (1963) 259.
5 R. ANTON AND P. DUQUÉNOIS, *Ann. Pharm. Franc.*, 25 (1967) 589.
6 L. HÖRHAMMER, H. WAGNER AND H. J. LEUE, *Deut. Apotheker Ztg.*, 99 (1959) 1043.
7 B. GELOTTE, *J. Chromatog.*, 3 (1960) 330.

Received March 25th, 1968

J. Chromatog., 35 (1968) 562–565

CHROM. 3532

Improved flow rate with Sephadex superfine G-200 in column chromatography

Quite superior resolution has been experienced in our laboratory and in others, on columns packed with fine grade of G-200 Sephadex as compared with separation on the usual G-200 product, owing to the presentation of more "plates" per unit of column volume and the narrower range of dry sphere volumes. We have used 2.5 × 100 cm columns and TRIS (0.1 *M*)–HCl buffer at pH 8.0 (containing 0.2 *M* NaCl for antiswelling control). Our particular area of interest was aqueous extract of epithelium, in which three peaks of materials smaller than serum albumin could be detected only by the superfine product.

Irregular and low flow rates were obtained, however, in successive packings of the Superfine G-200 product. While the first packing, for unknown reasons, gave excellent resolution and permitted an upwards flow rate of 8–10 ml/h (peristaltic pump), later packings, made similarly or with variant conditions, were unsuccessful, with flow rate always less than 5.0 ml/h (descending system).

We have been successful in improving the product by differential flotation of the dry powder in cold, dry diethyl ether. The removal of only a small amount (*ca.* 2–3 %) of "fines"—two-thirds ranging from 12–5 μ in diameter—has allowed repeated packings of 2.5 × 100 cm columns with equilibrium flow rates of 7–8 ml/h (descending