PURIFICATION AND SEPARATION OF ANTHRACENE DERIVATIVES ON A POLYSTYRENE—DIVINYLBENZENE COPOLYMER

ROB DENEE and HINDRIK J. HUIZING*

Laboratory for Pharmacognosy and Galenic Pharmacy, Antonius Deusinglaan 2, 9713 AW, Groningen, The Netherlands

ABSTRACT.—Extracts of drugs containing anthracene derivatives were enriched by XAD-2 column chromatography; elution was carried out with water-ethanol mixtures. By means of gradient elution, a fractionation of the glycosides and aglycones from *Rhamnus purshiana* extracts was obtained.

The method was also adaptable for *Frangula alnus*, *Rheum palmatum* and *Senna* preparations.

Crude extracts of anthracene derivatives containing drugs such as *Rhamnus purshiana*, *Frangula elnus*, *Cassia* spp., and *Rheum palmatum* are still widely used as purgatives. The anthraquinone glycosides from these extracts travel to the large intestine where they are hydrolyzed; the aglycones which are liberated act as stimulators of the colon.

Separation of anthracene derivatives (aglycones and glycosides) by paper and thin layer chromatography (tlc), paper electrophoresis, column chromatography, counter-current distribution, and gaschromatography have been described. Separation on silica gel or Sephadex LH-20 columns is preferable for separation of larger amounts of anthracene derivatives (1). A rapid and fractional preparative separation of glycosides and aglycones from crude extracts containing, amongst others, salts and sugars nevertheless remains troublesome. XAD-2 is a polystyrene-divinylbenzene copolymer which can be used as an adsorbant for organic molecules from an aqueous solution. Recently it was shown that pyrrolizidine alkaloids could be separated by elution with acidified methanol-water gradients. More hydrophilic substances showed less adsorption than less hydrophilic ones, resulting in a reversed phase type of chromatography on elution by aqueous solutions (2). This phenomenon was the reason we investigated the separation of hydrophilic anthraquinone glycosides from lesser hydrophilic anthraquinone aglycones by XAD-2 column chromatography.

EXPERIMENTAL

EXTRACTS.—Extractum Rhamni Purshianae Aquosum Siccum was supplied by Lehner A.G. (Muttenz/Basel, Switzerland).

Extractum Rhamni Frangulae Aquosum Siccum and Extractum Rhei Siccum were prepared according to the Dutch Pharmacopoeia (3).

Extractum Sennae Siccum was obtained from Fa. Finzelberg (Andernach, G.F.R.).

COLUMN PARAMETERS.—For clean-up and enrichment of extracts from *Rhamnus purshiana*, a 30 x 3 cm. ID column (A) filled with 200 g (wet weight) of XAD-2 was used. The clean-up and enrichment of extracts of Senna was done on a 25 x 1 cm ID column filled with 25 g (wet weight) of XAD-2.

Analytical separations were performed by use of a 26 x 1.3 cm ID column (C) filled with 40 g of XAD-2.

XAD-2 was purchased from Serva (Heidelberg, G.F.R.) as 50–100 μm. beads. Columns were packed in methanol by the slurry method. Each column was washed with water prior to being loaded with extracts.

ENRICHMENT OF EXTRACTS WITH XAD-2.—A 10 g portion of *Rhamnus purshiana* extract was taken up in the smallest possible volume of water and placed onto the top of column A. Elution with 600 ml of water, 2500 ml of 15% ethanol, and 1500 ml of 94% ethanol afforded, respectively, fractions 1, 2 and 3.

For the procedure of clean-up of 1 g of Senna extract on column B, three fractions were collected after elution with 200 ml of water, 200 ml of 15% ethanol, and 200 ml of 94% ethanol.

The flow of the eluent was adjusted to 0.5 ml/min and controlled by the application of compressed air to the eluent reservoirs.

ANALYTICAL SEPARATION OF GLYCOSIDES AND AGLYCONES.—Gradients increasing in ethanol content from 0–96 v/v % were developed as previously described (2).

Aqueous suspensions derived in general from 1 g of dry extract were submitted to column C. Fractions showing one single spot on tlc were pooled.

ASSAY OF ANTHRACENE DERIVATIVES IN *Rhamnus purshiana* EXTRACTS.—The assay for mono- and diglycosides and free anthraquinones was done according to the method described by the Joint Committee of the Pharmaceutical Society and the Society for Analytical Chemistry (3, 4).

ASSAY OF ANTHRACENE DERIVATIVES IN EXTRACTUM SENNAE AQUOSUM SICCUM.—The assay and the calculation of the percentage of anthracene derivatives as sennoside B were done according to the European Pharmacopoeia (5).

THIN LAYER CHROMATOGRAPHY.—Silica gel 60 F 254 tlc plates (Merck, G.F.R.) were used throughout. Eluents used for the separation of carbohydrates, anthraquinone glycosides, and aglycones were, respectively: (1) acetic acid-ether-acetone-isopropanol-water (10:30:35:20:5); (2) ethylacetate-methanol-water (77:13:10); (3) petroleum ether (40–60°)-ethylacetate-acetic acid (75:24:1).

The detection reagents were as follows. (1) Carbohydrates: Spray with a solution consisting of nine parts of 2% 1,3-dihydroxy-naphtalene in 96% ethanol and one part of 85% phosphoric acid, heat after spraying for 30 minutes at 100°; (2) Anthracene derivatives: a. Spray with a solution of 5% potassium hydroxide in 50% ethanol, heat for 15 minutes at 100°. b. Place the tlc plate in an atmosphere saturated with ammonia. Survey both plates in daylight and under ultraviolet light at 254 and 365 nm.

RESULTS AND DISCUSSION

Rhamnus purshiana EXTRACT.—After elution of 10 g of the extract over column A according to the method described on page 4, three fractions were collected. The first fraction, obtained after elution with water, contained the sugars and salts (compare fig. 1). Fraction 2 contained, after evaporation of the eluent, 3.55 g of dry matter with 2.1% monoglycosides and 58.9% diglycosides; no anthraquinone aglycones, sugars, or salts could be detected. A two-and-a-half fold enhancement of the diglycoside content was obtained by the clean-up procedure in comparison with crude extracts, as can be concluded from table 1. The third fraction yielded the free anthraquinones together with comparatively small amounts of mono- and diglycosides.

TABLE 1. Purification of anthracene derivatives from Extractum Rhamni Purshianae Aquosum Siccum on XAD-2 (50–100 μm). Column parameters: 30 x 3 cm ID, 200 g of XAD-2.

	Extract[a]	Fraction 1[a]	Fraction 2[a]	Fraction 3[a]	Recovery in %
Eluent		600 ml water	2.5 liters 15% ethanol	1.5 liters 94% ethanol	
Dry weight[b]	10.00	2.70	3.55	3.63	98.8
Free anthraquinones	0.01			0.01	⩽100
Monoglycosides	0.23		0.07	0.15	95.7
Diglycosides	2.41		2.09	0.31	99.6

[a]The values in this column give the weights in grams of the amount of extract loaded on the column or of the dry matter in the fractions, as well as the computed amounts of respectively the free anthraquinones, the mono- and the diglycosides derived according to the assays reported.

[b]The weights of the dry matter in the collected fractions were obtained after evaporation of the eluent and drying of the residue until constant weight.

EXTRACTUM SENNAE AQUOSUM SICCUM.—In a similar experiment, the enhancement of the anthraquinone glycoside content calculated as sennoside B was about six-and-a-half fold as is shown in table 2. In fraction 2, no anthraquinone aglycones were detected. The remaining anthraquinone aglycones in both described procedures could be eluted with 94% ethanol. After this stripping, columns could be used for subsequent purifications by passing three times a bedvolume of water and loading the column again with extract.

TABLE 2. Purification of anthracene derivatives from Extractum Sennae Aquosum Siccum on XAD-2 (50–100 μm). Column parameters: 25 x 1 cm ID, 25 g of XAD-2.

	Extract[a]	Fraction 1[a]	Fraction 2[a]	Fraction 3[a]	Recovery in %
Eluent		200 ml water	200 ml 15% ethanol	200 ml 94% ethanol	
Dry weight[b]	1.003	0.605	0.104	0.143	84.9
Anthraquinones	0.078	0.008	0.052	0.020	102.6

[a]The values in this column give the weights in grams of the amount of extract loaded on the column or of the dry matter in the fractions, as well as the computed amount of the anthraquinones which was derived from the assayed percentage (sennoside B), according to the reported method.

[b]See footnote 'b' in table 1.

In several experiments identical to those described above, the effects of the quantity of loaded extract, the ethanol content of the eluent, and their volumes to be passed were tested on yields of purified glycosides. It was concluded that maximally 0.05 x 1 g of extract could be loaded onto a column containing 1 g of XAD-2. On elution with 3 x 1 ml of water, sugars and salts were removed. On elution with 12.5 x 1 ml of 15% ethanol, the anthraquinone glycosides were separated most effectively. Elution with 12.5 ml. of 94% ethanol yielded all free anthraquinones.

ANALYTICAL SEPARATIONS.—On stepwise elution of anthraquinone derivatives from crude extracts, the glycosides and aglycones could be isolated and purified. Linear gradient elution starting with water and increasing in ethanol content up to 96% afforded single compounds (by the control) from a *Rhamnus purshiana* extract. In figures 1 through 3, the thin layer chromatographic separation (tlc) of several fractions eluted from column C is shown. Each fraction represented a number of pooled fractions which appeared identical on preliminary tlc survey. The total gradient volume was 500 ml, and the elution rate was 0.5 ml./min. Tlc of the sugars (fig. 1) which eluted first from the column yielded three spots. The main spot was sucrose, and the two minor spots were glucose and fructose by comparison with references. Figure 2 shows the results of the tlc of all eluted anthracene derivatives, and figure 3 shows the additional tlc of the anthraquinone aglycones. On elution of the column, only the anthraquinone aglycones migrated as visible yellowish bands.

Separation of anthracene derivatives from extracts from *Frangula alnus*, *Rheum* and *Senna* was done by the same procedure also and gave comparable results.

From the above results, it is concluded that XAD-2 columns can be used for clean-up procedures by stepwise elution with water, ethanol, or a mixture

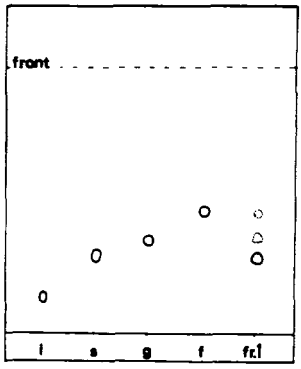

FIG. 1. Tlc of carbohydrates from pooled fractions which eluted first after linear gradient separation of *Rhamnus purshiana* extract over XAD-2. l=lactose; s=sucrose; g=glucose; f=fructose. For separation parameters and detection see "experimental".

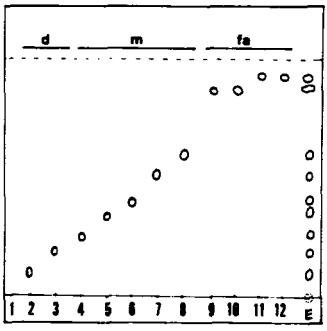

FIG. 2. Tlc of diglycosidic-, monoglycosidic- and free anthraquinones (resp. D, M and FA) from pooled fractions after linear gradient elution of *Rhamnus purshiana* extract over XAD-2. E=original extract. For separation parameters and detection see "experimental".

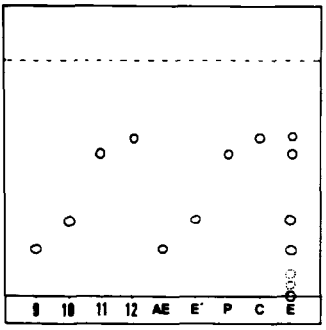

FIG. 3. Tlc of free anthraquinones from pooled fractions 9–12. Compare with fig. 2. AE=aloe-emodin; E'=emodin; P=physcion; C=chrysophanol; E=original extract. For separation parameters and detection see "experimental".

of both. Single anthracene derivatives could be obtained by linear gradient elution with increasing ethanol contents. The ease by which columns could be regenerated makes the procedure excellent and inexpensive for the preparation of standardized extracts. In general the elution behavior of the anthracene derivatives and for the pyrrolizidine alkaloids could be described as being from the reversed-phase type.

ACKNOWLEDGMENTS

We wish to express our gratitude to Dr. J. H. Zwaving for supplying anthraquinone aglycones, for reading the manuscript, and for his critical remarks.

Received 29 April 1980.

LITERATURE CITED

1. J. H. Zwaving, Onderzoek naar de kwalitatieve en kwantitatieve samenstelling van de anthraceenderivaten van Rheum palmatum L., Ph.D. thesis, University of Groningen, The Netherlands, 1973.
2. H. J. Huizing and Th. M. Malingré, *J. Chromatogr.*, **176**, 274 (1979).
3. Dutch Pharmacopoiea Vol. VI[2], 1966.
4. European Pharmacopoiea Vol. III, 1975.
5. Joint Committee of the Pharmaceutical Society and the Society for Analytical Chemistry, *Analyst*, **93**, 749 (1968).
6. Joint Committee of the Pharmaceutical Society and the Society for Analytical Chemistry, *Analyst*, **98**, 830 (1973).