Journal of Chromatography, 97 (1974) 109–111

CHROM. 7664

Note

High-voltage paper electrophoresis for the separation of 1,8-dihydroxyanthracene derivatives in senna and rhubarb

J. H. ZWAVING

Laboratory of Pharmacognosy, State University Groningen, Antonius Deusinglaan 2, Groningen (The Netherlands)

(Received April 9th, 1974)

A large number of 1,8-dihydroxyanthracene derivatives are present in senna leaf (*Cassia senna* L. or *Cassia angustifolia* Vahl) and rhubarb root (*Rheum palmatum* L.)[1]. The activities of all of these constituents have not yet been investigated, but it is known that particularly dianthrone glycosides based on rhein (the sennosides) and rhein glycosides have a high purgative activity[2].

Numerous methods have been described for the separation of these glycosides[1]. Low-voltage paper electrophoresis has been applied before[3–10], but no reports are available concerning the application of high-voltage paper electrophoresis in this field.

This paper describes our studies on low-voltage paper electrophoresis and the results obtained by using high-voltage paper electrophoresis for the separation of dianthrone glycosides and rhein glycosides in senna and rhubarb.

EXPERIMENTAL

Apparatus and materials

A Camag high-voltage electrophoresis apparatus, consisting of an electrophoretic cell, protection chamber and power supply (0–5000 V d.c., 200 mA) was used, with Schleicher and Schüll 2043 bMgl (40 × 20 cm) paper.

For the separation of anthraquinone aglycones, the following buffers were used:

I: pH 6.0; 0.1 *M* citric acid, 36.85 ml; 0.2 *M* sodium phosphate to 100 ml.

II: pH 7.0; 0.1 *N* sodium hydroxide, 29.63 ml; 0.2 *M* sodium diphosphate, 25.00 ml; water to 100 ml.

III: pH 8.0; 0.1 *N* sodium hydroxide, 46.80 ml; 0.2 *M* sodium diphosphate, 25.00 ml; water to 100 ml.

IV: pH 9.0; 0.1 *N* sodium hydroxide, 21.30 ml; boric acid–potassium chloride (12.37 g of boric acid and 14.91 g of potassium chloride per litre), 25.00 ml; water to 100 ml.

For the separation of anthraglycosides, the following buffers were used:

A: pH 9.4; 0.05 *M* sodiumdiborate, 64.3 ml; 0.05 *M* sodium carbonate, 35.7 ml.

B: pH 6.0: 0.1 *N* sodium hydroxide, 5.0 ml; 0.1 *M* potassium diphosphate, 50 ml.

C: pH 8.6: barbital, 0.184 g; sodium barbital, 1.03 g; water to 100 ml.

Operating conditions

For high-voltage paper electrophoresis, the following conditions were used:
Buffers I–IV: 3500–4000 V; 100–120 mA; V4A steel electrode; 30 min.
Buffers A and B: 3700 V; 160 mA; V4A steel electrode; 45 min.
Buffer C: 4500 V; 35 mA; platinum electrode; 50 min.
For low-voltage paper electrophoresis, the following conditions were used:
Buffers A and B: 130 V; 10 mA; V4A steel electrode; 24 h.
Buffer C: 220 V; 10 mA; platinum electrode; 16 h.

Detection

When buffers with a pH greater than 7 are used, spots of anthraquinone aglycones are red and spots of anthraquinone glycosides are orange on the electropherograms. Dianthrone glycosides give a yellow colour, which turns brown after 24 h. Under UV light (360 nm), anthraquinone aglycones and glycosides show an orange fluorescence and dianthrone glycosides a dull ochre colour. Using buffer B, all anthracene derivatives give a yellow colour; after spraying with a 5% solution of potassium hydroxide in 50% methanol, anthraquinones change to red and dianthrones to yellow.

RESULTS AND DISCUSSION

The investigation was started with low-voltage paper electrophoresis. Anthraquinone aglycones could not be separated completely in this way. Rhein migrated 6.7–12 cm, depending on the buffer used, emodin migrated about 1 cm in all buffers, while the other aglycones (chrysophanol, physcion and aloe-emodin) did not migrate.

The dianthrone glycosides sennoside A, B and C could be separated from each other and also from rhein and rhein mono- and diglycoside. All of these compounds contain a carboxyl group in their molecule. Anthraglycosides without a carboxyl group did not migrate. However, the spots obtained by low-voltage electrophoresis are not clearly defined because of diffusion which is produced by the long duration of the runs. In particular, ions with a small molecular weight (< 1000) show this phenomenon of diffusion. In order to reduce this undesirable transport of ions, the duration of the separation should be shortened. This can be achieved by applying high-voltage electrophoresis, as the electrophoretic mobility is a linear function of the field strength. When high-voltage paper electrophoresis was applied to the separation of the anthracene derivatives, no diffusion of the spots occurred. However, with this technique, again no complete separation of the anthraquinone aglycones was obtained. In buffers I–IV, physcion and chrysophanol did not migrate. Also with buffers I, II and III aloe-emodin remained at the start. Rhein showed the greatest migration in all of the buffers used.

The best separation of anthraquinone aglycones was obtained in buffer IV: the migration distances were rhein 8.5 cm, emodin 1.7 cm, aloe-emodin 0.7 cm and

TABLE I

MIGRATION DISTANCES OF 1,8-DIHYDROXYANTHRACENE DERIVATIVES FROM SENNA AND RHUBARB

Migration distances are given in centimetres.

Compound	*Buffer*		
	A	*B*	*C*
Rhein diglycoside	19.5	17.5	13.5
Sennoside B	15.0	14.5	11.0
Sennoside A	15.0	14.5	9.0
Rhein monoglycoside	12.5	10.0	6.0
Sennoside C	8.5	7.0	5.0
Rhein	4.0	2.5	4.0

physcion and chrysophanol 0.0 cm.

The separation of anthraglycosides gave better results. From Table I, it can be seen that these glycosides and the aglycone rhein can be separated in buffers A and B, with the exception of sennosides A and B. When buffer C is used, these two stereoisomeric glycosides also can be separated. Although anthraglycosides without a carboxyl group do not migrate, it is possible to separate the glycosides that contain a carboxyl group from senna and rhubarb by high-voltage paper electrophoresis.

These last named anthracene derivatives are also the constituents which show a high laxative activity.

ACKNOWLEDGEMENT

The author is grateful to Sandoz A.G., Basle, Switzerland, for kindly providing sennoside A, B and C.

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