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Note

Determination of dihydroxydianthrones by densitometry after thin-layer chromatographic separation*

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Medicinal plants such as senna, frangula, cascara and rheum have laxative activity resulting from their component dihydroxydianthrone glycosides. After oral intake of the crude drugs, the anthraglycosides are metabolized in the gastrointestinal tract by bacterial enzymes, and the free aglycones should cause the laxative effect[1]. According to Fairbairn and Moss[2], the sugars should have a transport function, enabling the aglycones to reach the site of action in the large intestine.

In order to study the metabolism of the senna glycosides in the body, where sennidines are formed, a specific method for the determination of dihydroxydianthrones is required. We have tried to determine 1,8-dihydroxydianthrones by densitometry on thin-layer chromatograms. This method has the advantage that in a mixture of aglycones, the dianthrones can be separated from the other anthracene compounds. Because of the specificity of the detection reaction, the method is more suitable for plant extracts than are the methods of Auterhoff and Kinsky[3] and Lawrence and Frei[4], in which interferences from natural compounds can affect the determination. The method is easier to perform than the method of Lemli[5] on paper chromatograms. We have applied the method to the determination of the dianthrone glycosides in senna plants.

EXPERIMENTAL AND RESULTS

Sennidine A was obtained by hydrolyzing a solution of 25 mg of sennoside A in 25 ml of 0.5% sodium hydrogen carbonate solution with 25 ml of 10 *N* sulphuric acid in a glycerine bath at 120° for 15 min. After cooling, the solution was extracted with 100 ml of diethyl ether and a 2-ml volume of the ethereal solution was evaporated to dryness (≡ 0.5 mg of sennoside A). Before use, the residue was dissolved in 2 ml of methanol.

The sennidine solution was spotted with a Hamilton microsyringe on precoated silica gel G (Merck, Darmstadt, G.F.R.) plates and developed twice over a distance of 5 cm with *n*-hexane–benzene–acetic acid (40:40:20). The plates were air

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dried, sprayed with a 30% solution of dimethylformamide in acetone and heated at 120° for 15 min. The plates were then cooled for 15 min. The sprayed plates were examined in the reflection mode using a Zeiss chromatogram spectrophotometer at a wavelength of 530 nm.

Calibration graphs with peak areas obtained using various amounts of the standard solution were plotted. Sennidine A showed a linear relationship between concentration and peak area over the range 0.10–1.5 μg of sennidine (Fig. 1). The colour of the spots was stable for at least 1 h.

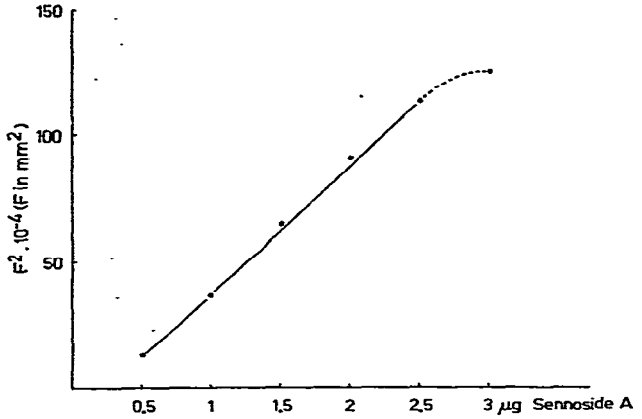

Fig. 1. Relationship between the amount of sennidine A and the corresponding peak area (F), calculated as sennoside A.

Hydrolysis of a senna leaf extract

Senna leaf (150 mg) was extracted with 30 ml of water and the extract was heated under a reflux condenser for 15 min. A 20-ml volume of the centrifuged liquid was transferred into a separating funnel and the aglycones were extracted with chloroform, after the addition of 0.1 ml of 2 N hydrochloric acid. After the addition of 100 mg of sodium hydrogen carbonate, the aqueous layer was shaken and centrifuged, then 10 ml of the liquid was hydrolyzed with 10 ml of 10 N sulphuric acid under a reflux condenser in a boiling water-bath for 15 min and, after cooling, was extracted with 50 ml of diethyl ether. A 20-ml volume of this solution was evaporated and, before use, the residue was dissolved in 2 ml methanol. For chromatography, 8-μl aliquots of this solution were spotted four times between the 4- and 6-μl spots of the standard sennidine A solution. A typical densitometric trace of a hydrolyzed senna leaf extract is shown in Fig. 2.

The order of separation on the plates was sennidine A + B (R_F 0.61) and sennidine C + D (R_F 0.41). Rhein (R_F 0.84) did not develop a colour after spraying with dimethylformamide. Densitometric analysis of senna leaf gave a concentration of 1.77% of sennoside A and B ($n = 6$, $S_{rel.} = 4.18\%$) and 0.52% of sennoside C and D, calculated as sennoside A. These results are in agreement with the results found by Lemli[5]. Spectrophotometric analysis of the total concentration of glycosides on the same senna leaf, following the method of the European Pharmacopea, gave 2.97%, calculated as sennoside B.

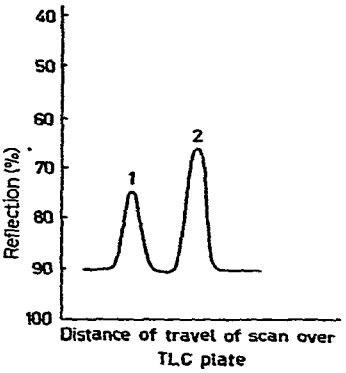

Fig. 2. A densitometric trace of the thin-layer chromatographic separation of sennidine C and D (1) and sennidine A and B (2) in a hydrolyzed extract from senna leaf.

DISCUSSION

The method described offers an approach to the analysis of sennidines and other 1,8-dihydroxydianthrones in mixtures of anthracene derivatives. The reaction of dimethylformamide is specific for 1,8-dihydroxydianthrones (*i.e.*, other dihydroxydianthrones and anthraquinones do not develop a colour with the detection solution). The method can be used to determine the composition of the dianthrone glycosides in laxative plants. This method will be applied in metabolic studies of the sennosides in the organism.

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