STUDY OF THE STRUCTURE OF CHEMICAL COMPOUNDS, METHODS OF ANALYSIS, AND PRODUCTION CONTROL

ISOLATION OF GLYCYRRHETINIC ACID BY PAPER ELECTROPHORESIS

I. A. Murav'ev and L. N. Savchenko

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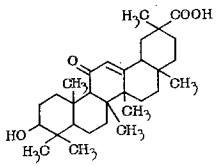
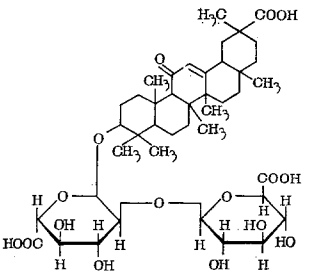
Glycyrrhetinic acid (I) is an aglycone of the glycyrrhizic acid (II), the principal pharmacologically active compound of licorice (*Glycyrrhiza glabra* L., *G. uralensis* Tirsh., *G. kurshinskyi* brig.)



Glycyrrhetinic acid (I)



Glycyrrhizic acid (II)

Compound II is used in the form of a monoammonium salt (glycyrrham) for treating bronchial asthma and other illnesses related to the disturbance of adrenal gland activity. But I also was found to be pharmacologically active. Its sodium salt, the glycyrrhenate, has been approved for medical use as an antiinflammatory and trichomonacidal agent [1-3].

During the production of glycerrhenate (the starting material II), I is quantitatively determined at separate stages, and from the results, the required amount of alkali is further determined. The quantitative determination of I is carried out spectrophotometrically. Since the products tested, besides I, contain impurities, before the quantitative determination, I must be isolated in a pure state. For this purpose, up to now various chromatographic methods have been proposed: paper [3-5], or thin-layer chromatography on aluminum oxide [6], silica gel [8-10] and polyamide [6].

The main drawback of paper chromatography is its long duration (20-40 h), since the molecule of I is only slightly mobile.

On thin-layer chromatograms on silica gel and aluminum oxide, the spots of I are difficult to detect, since I appears in UV light in the form of dark spots at 254 nm only.

We took the above drawbacks into account, and developed a more simple method for isolating I by the method of paper electrophoresis. We propose to use this method for a stagewise analytical control of the content of I during the production of glycyrrhenate.

Apparatus and Materials. The electrophoresis was carried out on the PEF-3 apparatus; the paper for the electrophoresis was brand FN-3 (GDR). As a buffer solution, we used: 1) veronal–medinal buffer pH 8.6 (10.32 g of medinal, 1.84 g of veronal, water to 1000 ml); 2) veronal–acetate buffer pH 8.6 (8.71 g of veronal, 1.89 g of sodium hydroxide, 6.48 g of sodium acetate, 60 ml of 0.1 M hydrochloric acid, water to 1000 ml); 3) borate buffer pH 8.8 (11.5 ml of 0.05 M solution of borax, 50 ml of 0.2 m solution of boric acid, water to 200 ml); 4) veronal buffer pH 8.8 (4 ml of 0.2 M hydrochloric acid, 50 ml of 0. 2 M solution of veronal, water to 200 ml); 5) citrate buffer pH 4.6 (25.5 ml of 0.1 M citric acid, 24.5 ml of 0.1 sodium citrate solution, water to 100 ml); 6) citrato–phosphate buffer pH 5.6 (21.0 ml of 0.1 M solution of citric acid, 29.0 ml of 0.2 M solution of disodium phosphate, water to 100 ml); 7) hydrochloric acid–potassium chloride buffer pH 2.2 (50.0 ml of 0.2 M solution of potassium chloride, 6.7 ml of 0.2 M solution of hydrochloric acid, water to 200 ml).

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TABLE 1. Electrophoretic Investigation of I

No.	Buffer solution	pH of buffer solution	Operational conditions: voltage, V	Operational conditions: current intensity, μA	Duration of electrophoresis, h	Distance moved by I, cm	Distance moved after 1 h, cm
1	Veronal–medinal	8,6	250	10	3,5	4,5	
			350	15	6,5	5,8	0,9—1,3
2	Veronal–acetate	8,6	200	8	3,5	3,5	
			300	12	6,5	4,8	0,7—1,0
3	Borate	8,8	250	10	3,5	4,5	
			400	20	6,5	6,2	0,95—1,3
4	Veronal	8,8	250	10	3,5	3,0	
			300	12	6,5	5,1	0,8—0,9
5	Citrate	4,6	250	10	3,5	0	
			350	15	6,5	0	0
6	Citrate–phosphate	5,6	200	8	3,5	0	
			300	12	6,5	0	0
7	Hydrochloric acid – potassium chloride	2,2	250	10	3,5	0	
			350	15	6,5	0	0

The prescriptions given for the buffer solutions Nos. 1-4 are given in optimal values, at which the highest mobility of the spots of I was observed. The buffer solutions Nos. 5-7 are given at one of the pH values showing the composition of the buffer solution.

The method is based on the fact that under the influence of a stationary electric field, the molecules of I with an electric charge move on a paper strip wetted with the buffer solution at a rate depending on the value of the charge, potential gradient, pH and type of the buffer solution, thickness of paper, temperature at which the electrophoresis takes place, and other factors. The optimal time for the electrophoresis is chosen experimentally.

EXPERIMENTAL

For the electrophoresis, paper strips (2.5 × 30 cm) are used, in the middle of which the starting line is marked, and the solutions of I and samples of the developer (0.1% alcohol solutions) are deposited by a capillary tube. Before charging the strips with the deposited substances into the electrophoresis chamber, they are wetted with the given buffer solution (apart from the place where the samples are deposited), and then dried between sheets of filter paper and placed in the electrophoresis chamber with cuvettes filled with buffer solutions. Care must be taken to ensure that the ends of the paper strips submerged in the buffer solutions are regularly drawn. The chamber is covered by a lid, connected to the power supply system, the working voltage is established, and the electrophoresis is carried out. At the end of the electrophoresis, the paper strips are extracted from the chamber and dried in an exciccator at 80°C for 20 min (fixation). When dry, the strips are placed in an enameled cuvette for 15 min with a coloring solution: 0.1% solution of methyl violet or 0.1% aqueous solution of rhodamine G. The phoregrams become dark-violet (when methyl violet is used) and red (with rhodamine G), and then are dried in an exciccator at 80°C for 10-12 min. The phoregrams are then submerged in a cuvette with a 1% solution of trichloroacetic acid for washing the dye which is not bound to I. Usually, the phoregrams are washed by several portions (3-4) of trichloroacetic acid solution, until their background becomes colorless. The phoregrams are freed from the trichloroacetic acid solution by washing them with distilled water, and then are dried in an exciccator at 80°C for 10-15 min.

The dried phoregrams are colorless strips with clear dark-violet (coloration with methyl violet) and red (coloration with rhodamine G) spots of I. The distance, in centimeters, moved by the spots of I is measured. The results of the measurements are listed in Table 1.

It is seen that in buffer solutions Nos. 1-4, the spots of I move the greatest distance, from 3.0 to 6.2 cm during 3½-6½ h, at a voltage from 200 to 400 V and a current density of 5-20 mA in the direction of the cathode (Fig. 1). The direction of the movement towards the cathode is probably due to the fact that under the above conditions I and II form complex compounds with a positive charge.

With decrease or increase in the pH of the above buffer solutions Nos. 1-4, compound I is always either stationary (the spot remains at the starting line) or moves a minimal distance.

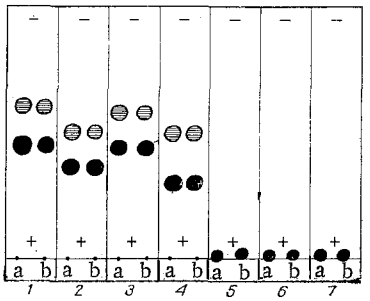

Fig. 1

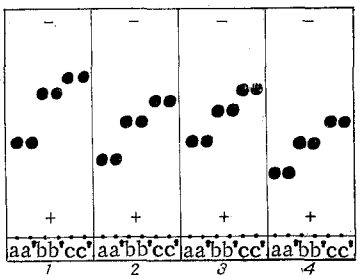

Fig. 2

Fig. 1. Electrophoresis of I in buffer solutions Nos. 1-7: a) developer; b) compound I studied; dark circles — spots of I after 3½ h of electrophoresis; crosshatched circles — spots after 6½ h of electrophoresis.

Fig. 2. Electrophoregrams of I, glycyrrham and II in buffer solutions Nos. 1-4 (voltage 400 V, current intensity 20 μA, time 3½ h). a) Compound I studied; a') developer for I; b) compound II studied; b') developer for II; c) glycyrrham; c') developer for glycyrrham.

In buffer solutions Nos. 5-7, compound I is electrophoretically stationary, its spots remain at the starting line at different values of the pH of the solution, the applied voltage, current intensity, and time of electrophoresis.

To isolate I from a mixture of compounds (II, glycyrrham) obtained in the course of the industrial production of glycyrrhenate, we used the conditions we selected. Studies by the above procedure with 0.1% solutions of glycyrrham and II in buffer solutions Nos. 1-4 showed that their molecules have a higher mobility than that of I. They move to a greater distance on the electrophoregrams (Fig. 2). Figure 2 shows that during the electrophoresis it was possible to obtain sharp separation of the spots of I, II and glycyrrham, so that I could be determined quantitatively, directly from the electrophoregrams. For this purpose, the spots of I were cut out and eluted with alcohol, and the optical density of the solutions obtained was determined on the SF-4A spectrophotometer at 248 nm.

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