Separation of the Racemate of Mandelic Acid by Electrophoresis on the Starch Block

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The possibility of electrophoretic separation of D,L-mandelic acid on starch as optically active supporting medium was examined. The obtained results did not exceed the limit of separation achieved by other methods. Under the conditions of high-voltage electrophoresis in acid buffer, mandelic acid moved in the opposite direction than it did under the conditions of low-voltage electrophoresis, *i.e.*, from anode towards cathode; however, fractions were enriched by optical antipodes only partially.

The selective or preferential adsorption of one optically active component of a racemate on the surface of optically active solid substance has drawn attention of many investigators for a long time. This phenomenon, designated as the "asymmetric adsorption", was investigated by different methods and many theories were declared explaining it on the basis of the accumulated facts.

At the beginning of the fifties already, *Dalgliesh* [1] elaborated a so-called "three-point" rule on the basis of experiences gained in separation of racemates by paper chromatography. It was found later that chromatography on columns of some optically active compounds resulted in separation also of such racemates which did not comply with the mentioned rule. Thus, for example, solutions of D,L-mandelic acid were partially separated on the white rabbit hair [2]. So far, the best separation of racemates was achieved on columns of starch grains [3—9]. It was found to be an adsorption chromatography on the surface of starch which proceeded according to the Langmuir isotherm. Adsorption on the individual components of starch (amylose and amylopectin) is approximately the same [4—6, 8]. Considering the particular sensitivity of asymmetric adsorption towards the structure of the adsorbent, the conformation of starch macromolecules as well as the experimental conditions of the separation have dominant effect on the course of adsorption [3, 10].

We have decided to examine the separation of racemate by preparative electrophoresis on the block of starch grains since remarkable results in the separation of proteins have been achieved by this method recently [11]. In the mentioned work, the authors call attention to the fact that when the potential is increased from usual 6—8 V/cm to so-called "critical voltage" 12—15 V/cm, a new unexpected sharp separation of components to further fractions takes place. We focused our attention on the separation of racemic modification of mandelic acid mainly because its antipodes had high values of optical rotations.

Experimental

Chemicals and methods

The used chemicals were anal. grade. The potato starch was a product of Bohemian Starchproducing Factory, Brno — Dornych, extra fine quality.

Optical rotations of solutions were measured on a Bendix—Ericsson, type 143 A automatic polarimeter at room temperature. The content of mandelic acid in solution was determined alkalimetrically and the amylose content of starch was determined by iodometric titration with an amperometric indication [12].

Apparatus

The used apparatus consisted of the power source and the electrophoretic equipment itself. The source of the unidirectional current (with adjustable potential from 0 to 5,000 V and maximum demand 150 mA) was produced by Development Workshop, Czechoslovak Academy of Sciences, Prague.

The electrophoretic equipment constructed with Lucite had a double bottom with labyrinth barriers where the cooling water of a constant temperature circulated. The dimensions of the block of starch grains were 300 × 125 × 5 mm with broadened sections at both ends. In these sections of the block, platinum electrodes were placed and were electrically connected with the voltmeter indicating the proper voltage of the block. There was also the outlet of Whatman No. 3 cardboards by which the electrophoretic section of the apparatus was electrically connected with the power source through troughs filled with electrolyte.

Procedure

The space of the electrophoretic equipment including the broadened sections was filled with suspension of potato starch grains in 0.1 N acetic acid similarly as in [11]. The racemate of mandelic acid (200 mg) dissolved in 2 ml of used electrolyte was applied to the proper place of the prepared block of starch grains. The block was then covered by a plastic foil and the current of an appropriate voltage was switched on for a desired time under cooling the apparatus with water. On the basis of the experiences gained so far, the place of application of mandelic acid was chosen near cathode in the case of low-voltage electrophoresis (7 V/cm) and in the case of high-voltage electrophoresis (17 V/cm) near anode.

The apparatus was adjusted to such a height that the starch block was *ca.* 20 cm above the level of electrolyte (0.1 N acetic acid) in troughs so that the excess amount of liquid could not penetrate the starch block during electrophoresis. In the reverse case, the diluted starch suspension would flow over the apparatus.

During electrophoresis, the potential of the starch block was maintained constant by gradual elevation of the current voltage from the power source (probably the resistance of the Whatman cardboards was increasing). Approximately in 3-hour intervals, the content of troughs was exchanged by fresh electrolyte.

Mandelic acid on the starch block was detected by spraying it with 1% solution of ferric chloride (yellow colour) at the end of electrophoresis. The starch block was sliced transversely to four equal sections which were extracted separately by methanol in Soxhlet extractor. Methanolic extracts were filtered and evaporated under vacuum. The distillation residue was dissolved in water and the content of mandelic acid as well as its optical rotation were determined.

After extraction, the amylose content in starch grains was determined.

Results and Discussion

We have examined the separation of D,L-mandelic acid in acidic, neutral, and alkaline media, respectively. There were difficulties with reliable detection in neutral and alkaline media. Moreover, in alkaline medium, the starch grains became partially gelatinous and

the extraction of mandelic acid with organic solvents (also after acidification) was not quantitative.

Considering these reasons, we turned attention to the electrophoretic separation in acidic medium. Under these conditions, the polysaccharides of starch were partially hydrolyzed. We discovered it on determining the amylose content in starch which dropped to zero value during electrophoresis. However, the potato starch grains were not markedly different from the originally used ones when viewing under optical microscope.

The typical movement of mandelic acid towards anode at low-voltage electrophoresis (7 V/cm) was changed to movement towards cathode in the case of high-voltage electrophoresis (17 V/cm). This could be explained by electroosmotic transfer of water from the anolytic section to the catholytic section [11] and consequently by that of mandelic acid as well, since it was soluble in water.

The racemate of mandelic acid was not separated into individual fractions during electrophoresis, but it was evident from the detection that mandelic acid appeared in a wide band similarly as on the starch column chromatography [3—8]. During 48 hours of low-voltage electrophoresis, the forehead of the mandelic acid band moved 21 cm while the backside of the band only 3 cm from the place of sample application. At high-voltage electrophoresis (reverse course from anode towards cathode) it moved 19 cm and 1 cm, respectively, during the same time.

That part of the band which moved farthest from the origin was enriched by D-(—)-isomer of mandelic acid, while the backside of the band contained also L-(+)-isomer. The forehead of the mandelic acid band was enriched by 5% of the appropriate isomer in the case of low-voltage electrophoresis after the mentioned time and by 9% in the case of high-voltage electrophoresis. Similarly, the backside of the band was enriched by the appropriate isomer of mandelic acid.

In our experiments we achieved but similar separation of the mandelic acid racemate as by the chromatography on starch column [3—9], though the amount of applied racemate was chosen so that the capacity of the migration system was not exceeded markedly. Since optical rotation was not measurable at lower concentration, the weight of the sample could not be smaller. It was not possible to increase the amount of the supporting medium either, because in the described procedure the constant physicochemical conditions could not have been ensured.

References

1. Dalgliesh C. E., *J. Chem. Soc.* ***1952***, 3940.
2. de Vreker R., Lontie R., *Colloq. St. Jans Hosp.*, Brugge (Belg.), 3ᵉ Colloq. ***1955***, 185; *Chem. Abstr.* ***51***, 14554*b* (1957).
3. Krebs H., Wagner J. A., Diewald J., *Chem. Ber.* ***89***, 1875 (1956).
4. Lautsch W., Steinicke D., *Kolloid. Z.* ***153***, 103 (1957).
5. Lautsch W., Steinicke D., *Kolloid. Z.* ***154***, 1 (1957).
6. Ohara M., Fujita I., Kwan T., *Bull. Chem. Soc. Jap.* ***35***, 2049 (1962).
7. Ohara M., Ohta K., Kwan T., *Bull. Chem. Soc. Jap.* ***37***, 76 (1964).
8. Ohara M., Chen C. Y., Kwan T., *Bull. Chem. Soc. Jap.* ***39***, 137 (1966).
9. Ohara M., Chen C. Y., *Bull. Chem. Soc. Jap.* ***39***, 1440 (1966).
10. Ohara M., Watanabe T., Kwan T., *Z. Phys. Chem.* (Frankfurt) ***56***, 314 (1967).
11. Pilz W., *Z. Anal. Chem.* ***243***, 587 (1968).
12. Holló J., Szejtli J., *Brauwissenschaft* ***13***, 348 (1960).

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