# THE ISOLATION OF ERYSIMOSIDE

M. T. Turakhodzhaev, M.-R. I. Shamsutdinov,
T. T. Shakirov, and G. L. Genkina

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Erysimoside, a steroid glycoside isolated from the seeds of *Erysimum diffusum* [1] and some other species of erysimum [2], is used for the treatment of cardiac insufficiency [3, 4]. Previously existing methods of isolating erysimoside were cumbersome; in view of this, the yield of the drug was comparatively low [5]. We have freed the glycoside from accompanying substances by adsorption of the latter on ion-exchange resins [6, 7]. From the seeds comminuted on a roller mill and defatted with gasoline the glycosides were extracted with 95% ethanol. To investigate the conditions for the precipitation of the sugars, the ethanolic extracts from the erysimum seeds were concentrated to various volumes and the sugars were placed precipitated with various ratios of acetone. The optimum result was obtained when the extract was concentrated to 1/9 of its original volume and was treated with twice its volume of acetone.

To eliminate resinous substances from the extract we used diethyl ether. When a fourfold amount of diethyl ether was added to the viscous extract the insoluble substances (including the glycosides) precipitated. The resinous substances remained in the mother liquor.

The precipitate was dissolved in water, and the monosides were extracted from the solution with chloroform and the last traces of the extractive substances of hydrophilic nature were sorbed on ion-exchange resins. In order to select the ion-exchange resins, the extracts obtained from erysimum seeds (1 kg each) and treated by the procedures described above, were passed through columns each containing 500 g of a particular ion-exchange resin (Table 1).

Table 1 shows that of the anion-exchange resins the best results were given by ÉDÉ-10p, and of the cation-exchange resins the best was KU-2. To neutralize the purified solution, the anion- and cation-exchange resins were used in the form of a mixture. It was established in the experiments that the optimum ratio of the anion- to the cation-exchange resin was 3:1 (by weight). The purification of the extract on the ion-exchange resins is affected by the concentration of the glycosides and extractive substances in the solution, and therefore we investigated the amount of water required to dissolve the precipitated glycosides (Table 2).

It can be seen from Table 2 that with an increase in the amount of water to dissolve the extract the losses of glycoside decrease, but in the subsequent extraction of the erysimoside the consumption of organic solvent rises sharply. Consequently, the optimum amount of water for dissolving the extract is a ratio of 1.5:1 (on the weight of the seeds).

The solvent for extracting the erysimoside from the purified aqueous solution was selected in model experiments. A 2% solution of erysimoside in aqueous methanol (5% methanol) saturated with 10% of common salt was extracted with various mixtures of organic solvents (Table 3). The salt was added to decrease the solubility of the glycosides in water and to improve the separation of the phases.

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TABLE 1

| Type of ion-exchange resin | Amount of ballast substances in extract, g | | Ballast substance sorbed by the resins, % | Amount of glycosides in the extract, g | | Amount of glycosides sorbed by the resins, % |
|---|---|---|---|---|---|---|
| | before purification | after purification | | before purification | after purification | |
| KU-1 | 45 | 3,4 | 92,5 | 34,2 | 19,2 | 44 |
| KU-2 | | 3,2 | 93 | | 26,7 | 22 |
| KB-4 | | 21,2 | 58 | | 25,6 | 25 |
| Wofatit-F | | 28,5 | 37,5 | | 12,0 | 65 |
| EDE-10p | | 0,9 | 98 | | 32,5 | 5 |
| AV-16g | | 18,9 | 58 | | 22,9 | 33 |
| AN-1 | | 24,3 | 46 | | 27,3 | 20 |

TABLE 2

| Expt. No. | Amount of water to dissolve the glycosides, ml | Concn. of erysimoside in soln., mg/ml | | Loss of erysimoside, % on its amount in the initial solution |
|---|---|---|---|---|
| | | before purification | after ion-exchange purification | |
| 1 | 500 | 67 | 40,2 | 40 |
| 2 | 1000 | 33,5 | 23,8 | 29 |
| 3 | 1500 | 22,7 | 20 | 12 |
| 4 | 2000 | 17 | 15,3 | 10 |

TABLE 3

| Expt. No. | Composition of the solvent | Ratio | No. of extractions | Total consumption of solvent, ml | Yield of erysimoside, % of its amount in the solution |
|---|---|---|---|---|---|
| 1 | Chloroform–isopropanol | 1:1 | 9 | 900 | 85 |
| 2 | Chloroform–isopropanol | 2:1 | 9 | 900 | 67 |
| 3 | Chloroform–methanol | 3:1, 4:1 | 9 | 900 | — |
| 4 | Chloroform–ethanol | 2:1 | 9 | 900 | 48 |
| 5 | Chloroform–ethanol | 1:1 | 9 | 900 | 58,4 |
| 6 | Chloroform–butan-1-ol | 1:1 | 9 | 900 | 44 |
| 7 | Dichloroethane–isopropanol | 1:1 | 11 | 1100 | 60 |

Table 3 shows that the best solvent for extracting erysimoside from aqueous methanolic solution is a mixture of isopropanol with chloroform in a ratio of 1:1 (by volume).

## EXPERIMENTAL

Defatting of the Seeds. The comminuted erysimum seeds (50 kg), mixed with 1 kg of wood shavings, were charged into an extractor and defatted with gasoline. The last traces of gasoline were eliminated from the defatted seeds in vacuum.

Extraction of the Glycosides. The glycosides were extracted with 95% ethanol by the steeping method. The process was repeated seven times, and the extracts were combined and evaporated in a vacuum evaporator to 20 liters (temperature of distillation not above 45°C, vacuum 600-700 mm Hg).

Precipitation of the Sugars. To the concentrated extract in a mixer with the stirrer working was added 40 liters of acetone in a thin stream. The acetone solution was separated from the precipitated sugars by decantation and was concentrated to 6-7 liters.

Precipitation of the Glycosides from the Extract. In a mixer with the stirrer working, 15 liters of diethyl ether was added in a thin stream to the concentrated acetone extract. The ether-insoluble glycosides and some hydrophilic impurities precipitated in the form of a viscous mass. The hydrophobic impurities passed into the ether solution. The precipitated mass was washed with five liters of ether and dried.

*Extraction of the Monoglycosides.* The precipitate freed from ether was first dissolved in three liters of methanol in an apparatus with a stirrer, and then 65 liters of water was added. The monosides were extracted with chloroform (4-5 times 10 liters). The chloroform-treated aqueous solution was from residual hydrophobic impurities by the adsorption method.

*Preparation of the Ion-Exchange Resins.* The anion-exchange resin ÉDÉ-10p (6 kg) was converted into the OH form and washed with water until the wash-waters were neutral. The cation-exchange resin KU-2 was converted into the H form and likewise washed to neutrality. The prepared anion- and cation-exchange resins were mixed mechanically, charged into the sorption column (d = 200 mm, h = 500 mm), and washed with 30 liters of water.

*Sorption Purification of the Aqueous Solution.* The aqueous solution was passed through the column from the bottom upwards at the rate of 10 liters/h. The eluent was a clear colorless or faintly yellowish solution.

*Extraction of the Erysimoside.* In an apparatus with a stirrer, 5 g of common salt and 10 liters of a mixture of chloroform with isopropanol (1:1) was added to the purified aqueous solution, and the mixture was stirred for 10-15 min. After the separation of the liquids, the lower chloroformic-alcoholic layer was separated off. Extraction was performed with the same mixture another nine times. The first two extracts contained no erysimoside. The remaining eight extracts were combined and were concentrated until the first crystals appeared. Then the mixture was poured into a crystallizer. The crystals that deposited were filtered off with suction and the mother liquor was treated with a threefold amount of ether. An additional amount of erysimoside precipitated. The total yield of crystals was 1200 g.

*Recrystallization of the Erysimoside.* Recrystallization was performed twice: from isopropanol and from ethanol. The yield of erysimoside was 950-1000 g.

## SUMMARY

1. The conditions for the purification of a crude extract from resinous substances and sugars by organic solvents and from ballast substances by ion-exchange purification, and also the extraction of erysimoside from the purified aqueous solution, have been investigated.

2. The yield of erysimoside by the method described has been increased to 2%* of the weight of the seeds.

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*This value does not correlate with the total yield of erysimoside (1200 g) extracted from 50 kg of the seeds — Publisher.