ANALYSIS OF ERYSIMOSIDE

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Erysimoside is the main cardenolide of almost all plants of the genus Erysimum, family Cruciferae that have been studied up to the present time [1]. The seeds of E. diffusum Ehrh. are recommended for the industrial production of erysimoside. In the seeds of this species of erysimum about 10 glycosides have been found, and therefore for the quantitative determination of erysimoside it is necessary to separate it from the accompanying cardenolides and other substances. Previously, thin-layer chromatography (TLC) on a nonfixed layer of alumina in the butan-1-ol–water (1 : 1) system has been used for this purpose. Then the combined glycosides were separated into monoside and bioside fractions; on quantitative analysis, the erysimoside (biosides) and the other glycosides with polarities similar to it were determined, which led to high results [2].

The best separation of erysimoside from accompanying cardenolides is achieved by chromatography on paper in the toluene–butan-1-ol (1 : 1)–water system. For estimating erysimoside in the raw material and for industrial control we have used this type of chromatography, since it permits a sharper separation of all the cardenolides of the erysimum seeds (as compared with TLC), which is very important under industrial conditions when the amount of accompanying cardenolides is considerable.

The erysimoside was eluted from the paper with 70% ethanol. The completeness of the elution of the substance from the paper was checked by comparing the optical densitites of the eluate and of a standard solution. The desorption of the erysimoside from the paper was satisfactory. The amount of erysimoside was determined photocolorimetrically with the Tatje reagent [2]; the relative error of the chromatophotocolorimetric determination was not greater than ±2.5%. The sensititivity of the method was 20 μg of erysimoside.

The proposed method permits the determination of the amount of erysimoside in the raw material and in extracts at all stages of the purification of the extracts, and also in the mother solutions.

As an example, we give figures for the determination of erysimoside at various stages of the industrial process:

Stage of Production of Erysimoside	Amount of Erysimoside, % of the weight of the raw material
Seeds	3,92
Total extract (7 extractions)	3,72
Spent raw material	*0,08**
Extract after precipitation of the sugars	3,62
Precipitated sugars	*0,04*
Ethereal solution	*0,12*
Chloroform extract	*0,013*
Insoluble residue	*0,35*
Aqueous solution of erysimoside:	
before ion-exchange resin	2,80
after ion-exchange resin	2,45
Losses on the ion-exchange resin	*0,35*
Chloroform–isopropanol extraction	2,10
Aqueous residue	*0,27*
Mother solutions:	
1st crystallization	*0,23*
2nd crystallization	*0,30*
Losses unaccounted for	*0,47*
Yield of crystalline erysimoside	1,50

*Losses shown in italics.

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The amount of erysimoside in the seeds of E. diffusum varies according to the season and to the soil and climatic conditions (from 2.5 to 4%).

EXPERIMENTAL

Analysis of the Raw Material. To isolate the erysimoside, 2 g of the comminuted air-dry erysimum seeds that had been passed through a sieve (with 0.5-mm holes) were defatted three times with petroleum ether. The seeds freed from the solvent were covered with 10 ml of 70% ethanol and placed in a vibro-extractor for 6 h. Chromatographic paper of type "C" ("medium") (14 × 55 cm) was divided lengthwise into four strips. The first strip served as background (blank test) in photocolorimetry. and on the second and third strips as they dried out were deposited 0.05 and 0.06 ml, respectively, of filtered ethanolic extract. On the fourth strip was deposited 0.06 ml (300 μg) of a 0.5% standard sample of erysimoside. The paper was dried in the air and chromatography was performed by the descending method in the n-butanol–toluene (1 : 1)–water system. Chromatography was continued until the layer of solvent had travelled 40 cm (4 h). After drying, the 3rd and 4th strips of paper were cut off and the spots were revealed by spraying first with a 5% solution of m-dinitrobenzene in benzene and then with a 10% solution of caustic potash in 75% methanol. The cardenolides appeared in the form of blue-violet spots. From the position of the spot of the erysimoside (R_f about 0.35), its zones of the third and second strips and the corresponding background zone on the 1st strip were marked. The correctness of the cutting out of the section of paper with the substance was checked by the appearance of a residual band on the paper. The erysimoside was eluted from the paper with 15 ml of 70% ethanol with shaking for 2 h. The amount of erysimoside in the filtered eluate was determined on the FÉKM photocolorimeter with a yellow filter in cells 1 cm thick using as the reagent a 0.075% solution of 2,4-dinitrodiphenyl sulfone in ethanol and a 0.1 N aqueous solution of caustic potash [2].

In parallel, under the same conditions, 0.06 ml (300 g) of a 0.5 solution of standard erysimoside was chromatographed and photometered. The amount of erysimoside in the erysimum seeds relative to the standard erysimoside was calculated by means of a well-known formula [3].

Analysis of the Intermediates in the Isolation of Erysimoside. Seven extracts were needed for the complete extraction of 95% ethanol from the erysimum seeds. Figures for the analysis of the extracts from 20 kg of seeds are given below.

Extract No.	Amount of extract deposited on a chromatograph	Amount of erysimoside, found, % on the weight of the raw material
1	0,01	1,22
2	0,01	1,04
3	0,03	0,51
4	0,08	0,36
5	0,15	0,24
6	0,20	0,20
7	0,25	0,15
Total		3,72

For the analysis of the spent raw material, 20 g of it (accurately weighed) was extracted with 95% ethanol in a Soxhlet apparatus for 8 h, and then the extract was evaporated to a volume of 30 ml and 0.1 ml was deposited on paper.

To take into account the losses of glycosides in the precipitation of the sugars, the ethanolic extract was concentrated and treated with acetone. The sugars that deposited from the acetone solution were dissolved in water, and 0.1 ml of the resulting solution was deposited on a chromatogram. The concentrated extract after the precipitation of the sugars was treated with ether to eliminate residues of fatty oils and resinous substances. The individual glycosides usually do not dissolve in ether, but when adsorbed on oil and resinous substances they pass into diethyl ether. The ethereal solution (0.2 ml) was analyzed chromatographically. The extract after treatment with diethyl ether was dissolved in water; the aqueous solution was treated with chloroform to eliminate monosides and 0.25 ml of the resulting solution was deposited from a chromatogram.

In the extraction by chloroform of the aqueous solution, a water-insoluble precipitate deposited. This was separated off by decantation and dried. Then it was dissolved in 95% ethanol, and 0.05 ml of the ethanolic solution was deposited on a chromatogram. After treatment with chloroform, the aqueous solution was freed from ballast substances on ion-exchange resins. To determine the loss of erysimoside at this stage, the aqueous solution was analyzed before and after the ion-exchange purification. The erysimoside was extracted from the purified aqueous solution with a mixture of chloroform and isopropanol and 0.04 ml of the extract was chromatographed.

A total of two recrystallizations of the technical erysimoside from isopropanol was performed. In each case, 0.01 ml of the mother solution was deposited on a chromatogram.

CONCLUSIONS

A method for the chromatophotocolorimetric determination of erysimoside in raw material is given which has been made the basis of the analytical control of its production.

LITERATURE CITED

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