TECHNOLOGY OF THE ISOLATION OF CYTISINE FROM THERMOPSIS ALTERNIFLORA

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Cytisine [1] is produced from the seeds of the plant Thermopsis lanceolata R. Br. [2]. The epigeal part of Thermopsis alterniflora Rgl. contains about 3% of total alkaloids [3]. This plant is widely distributed in Central Asia [4] and is more economically promising than T. lanceolata. We have established that by treating the epigeal part of T. alterniflora the technological cycle is greatly simplified. Consequently, it is appropriate to organize the production of cytisine from the epigeal part of T. alterniflora.

The plant collected in the budding period was ground and extracted by percolation. Organic solvents, water, and aqueous solutions of acids of various concentrations at various temperatures were used as extractants. On extraction with chloroform, the yield of cytisine was 1.2% and that of pachycarpine 0.25% of the weight of the raw material.

In the subsequent experiments, pure water, water containing 0.01% of the surfactant "Tween-80", 0.5% and 1% aqueous solutions of hydrochloric acid, and 1% and 3% solutions of sulfuric acid were used as extractants. The alkaloids from the extracts were adsorbed on KU-1 and KU-2 ion exchangers. They were desorbed with 90% alcohol containing 1.5% of ammonia.

The best results were obtained by extraction with 1% aqueous hydrochloric acid and 2% aqueous sulfuric acid at 55° C with adsorption on KU-1 cation exchanger in the hydrogen form, which gave a yield of cytisine of 1.0% and of pachycarpine of 0.2%, respectively, on the weight of the raw material.

The experiments carried out show that the most useful method for the industrial production of cytisine from T. alterniflora is extraction with 1% aqueous hydrochloric acid or 2% aqueous sulfuric acid at 50–60° C with subsequent adsorption on KU-1 cation exchanger.

The industrial method for the production of cytisine is being developed further.

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STRUCTURE OF ROBUSTININE

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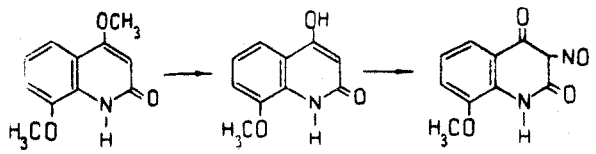
Continuing the separation of the total alkaloids of the roots of Haplophyllum robustum Bge. (family Rutaceae) [1], we have isolated a crystalline base with mp 232°–233° C having the composition $C_{11}H_{11}O_3N$ and the analytical formula $C_9H_5ON(OCH_3)_2$.

Chromatography of the alkaloids on a thin fixed layer of alumina in the butan-1-ol–pyridine–acetic acid–water (45:30:9:6) system gave a single spot with a R_f value of 0.60. We have called the alkaloid robustinine (I). This compound is optically inactive and readily soluble in organic solvents.

The UV spectrum (in alcohol) is very similar to that of derivatives of quinol-2-one (maxima at 270 and 282 mμ). [2]. The IR spectrum of robustinine has a strong absorption band with a maximum at 1630 cm^{-1} showing the presence of a –NH–CO– group [3].

When robustinine was heated with alcoholic hydrochloric acid, the methoxy group in the γ position was saponified with the formation of norrobustinine with mp 245°–246° C (II). This gave a nitroso derivative with mp 226°–227° C (III).

A direct comparison of the substances has shown that norrobustinine is identical with 4-hydroxy-8-methoxycarbostyryl [4]. A mixture of the nitroso derivatives of these substances gave no depression of the melting point. Thus, robustinine has the structure 4, 8-dimethoxycarbostyryl (I).



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AMINOETHYLATED PEPSIN

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In the investigation of the primary structure of proteins, hydrolysis with trypsin is first used, this specifically breaking the peptide bonds formed by the carboxy groups of arginine or lysine. Where the content of these amino acids in the protein is low, it is desirable to modify its molecule in such a way that new points for the attack of trypsin appear in the peptide chain. When the thiol groups appearing on reduction of the disulfide bonds are blocked with ethyleneimine by Raftery and Cole's method [1], the cysteine residues are converted into S-(β-aminoethyl) cysteine residues. S-(β-Aminoethyl) cysteine is an isoster of lysine, since its sidechain differs from the side chain of lysine only by a sulfur atom in place of a CH_2 group. This similarity leads to the situation that the peptide bonds formed by the carboxy group of S-(β-aminoethyl) cysteine are hydrolyzed by trypsin, as well as those formed by lysine and arginine.

The molecule of porcine pepsin is characterized by a very low content of basic amino acids (out of 340 amino acid residues, there are only two arginine residues and one lysine residue). In order to increase the number of peptide bonds capable of hydrolysis by pepsin, we have carried out the aminoethylation of pepsin whose disulfide bonds had been preliminarily cleared by reduction with mercaptoethanol. 207 mg of porcine pepsin inactivated with phenol was dissolved in 15 ml of 8 M aqueous urea, with the addition of concentrated LiOH to pH 8.5. 0.42 ml of mercaptoethanol was added to the pepsin solution, and the pH was brought to 8.5 with 1 N LiOH. Argon was passed through the reaction mixture for 5 min, and the solution was left in a closed vessel in an atmosphere of argon at 37° C. After 4 hr, 3.1 ml of ethyleneimine was added to the mixture. Aminoethylation was carried out in an autotitrator for 30 min in a current of argon, the pH being maintained at 8.6 with a 4 M solution of urea in 3 N hydrochloric acid. After the end of the reaction, the reduced aminoethylated pepsin (aminoethylpepsin) was precipitated with a fivefold volume of cold ethanol–1 N hydrochloric acid (39:1), and the precipitate was separated off by centrifuging and was washed with cold alcohol, then it was suspended in 50 ml of water and dissolved at pH 10.3 by the addition of concentrated LiOH, and the aminoethylpepsin was precipitated by the addition of 6 N hydrochloric acid to pH 5.5. The mixture was left at 4° C for 16 hr, after which it was centrifuged, and the precipitate was washed with water, suspended in 30 ml of water, and lyophilized. The yield of aminoethylpepsin was 179 mg. By means of an amino acid analyzer, 1 mole of aminoethylpepsin was found to contain 5.5–5.9 moles of S-(β-aminoethyl)cysteine (taking into account the degree of destruction of this amino acid on alkaline hydrolysis).

The aminoethylpepsin was subjected to triptic hydrolysis at pH 10. A determination of the N-terminal amino acids by dinitrophenylation showed that during the trypsin decomposition a considerable rise in the number of N-terminal amino acids took place (in comparison with the hydrolysis of carboxymethylpepsin [2]). The fractionation of the triptic hydrolyzate of aminoethylpepsin on Sephadex G-50 (4 M urea in triethylammonium carbonate buffer; pH 10) showed a marked decrease in the size of the peak corresponding to the fraction with the high-molecular-weight fragments and an increase in the low-molecular-weight fractions (as compared with the hydrolyzate of carboxymethylpepsin). Trypsin can be used