ALKALOIDS OF CORYDALIS SEVERTZOVII

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From the epigeal part of C. severtzovii Rgl., collected in the flowering stage near Tashkent (2.4 kg of air-dry plant material) by ordinary chloroform extraction we have isolated 20 g (0.83%) of total alkaloids. The separation of the latter yielded protopine (0.2 g), cryptopine (0.15 g), and corlumine (4 g). The authenticity of the first two was shown by their comparison with authentic samples and that of the third by the identification of the products of oxidative decomposition under the action of nitric acid.

After the extraction of the alkaloids with chloroform, the plant was treated with methanol. The methanolic extract was evaporated and the residue was diluted with 5% sulfuric acid. The acid solution was washed with ether and made alkaline with ammonia, and the alkaloids were extracted with ether. This gave 0.7 g of total alkaloids, from which a further 0.2 g of corlumine was isolated. When the ether used for washing was evaporated, fumaric acid (0.7 g) crystallized out.

From C. severtzovii (180 g) gathered on the northern slopes of the Turkestan range (upper reaches of the R. Zaamin) we isolated protopine (0.3 g) and a base (0.15 g) with mp 193-195° C (methanol—acetone); $[\alpha]_D^{33}$ −110° (c 0.27; chloroform); UV spectrum, λ_{max} 285 mμ (log ε 3.86). The IR spectrum of the base was identical with that of d-bicuculline. A mixture of the two samples melted at 187-210° C. These results permitted us to consider that the base isolated is the levorotary form of the known alkaloid d-bicuculline, and this is apparently the first time it has been found in a plant.

In addition to protopine and *l*-bicuculline, we isolated small amounts (10 mg) of a base with mp 200-202° C, IR spectrum: 920 cm^{-1}, 940, 1470, 1485, 1505, 2770, 2790, 3400 cm^{-1}. The base contains a methylenedioxide group (reaction with gallic acid).

Protopine, sanguinarine, and α-allocryptopine have previously been isolated from the tubers of C. severtzovii [1].

Thus, the two cases cited show that different alkaloids (with the exception of protopine, found in both plants but in different amounts) have been found in one and the same species. This fact is a striking example of the dependence of the qualitative and quantitative composition of the alkaloids on the growth site of the plant [2].

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AMMONOLYSIS OF PHENYLTHIOHYDANTOIN (PTH) DERIVATIVES OF AMINO ACIDS

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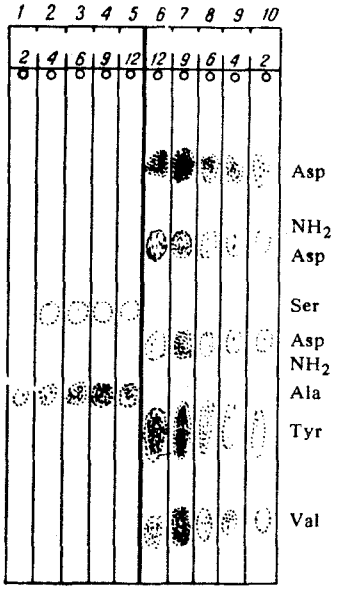
In the determination of N-terminal amino acids in proteins and peptides by the phenyl isothiocyanate method, the amino acids are identified by chromatographing both the PTHs [1] and also the free amino acids obtained by regeneration from the PTHs [2-5]. We have attempted to develop a method of identifying the amino acids by the hydrolysis with ammonia of the corresponding PTHs.

To five 100-γ samples of the PTHs of serine, alanine, aspartic acid, tyrosine, and valine we added 300 μl of 28% ammonia solution. The mixture was heated in a sealed capillary in the boiling water bath for 2, 4, 6, 9, and 12 hr (figure). One-dimensional chromatography was carried out in the water-saturated phenol system; the chromatograms were revealed with 0.25% ninhydrin in butanol.

For hydrolysis with hydrochloric acid, the PTHs must be subjected to prolonged heating at 150° C, which leads to considerable losses of the regenerated amino acids. Hydrolysis with baryta has the same disadvantages, and the necessity for eliminating Ba^{++} after hydrolysis increases the losses of amino acids due to their adsorption on the massive precipitates of $BaSO_4$ or $BaCO_3$, which complicates the process. Consequently, we used ammonia hydrolysis on a boiling water bath. This method enables the hydrolysis to be carried out more rapidly and at a moderate temperature; it is particularly suitable for the identification of tryptophan.

The optimum hydrolysis period, giving the greatest amount of free amino acids, proved to be 9 hours. The possibility of using the method was checked in a determination of the N-terminal amino acids in the peptides of silk fibroin and in mung bean globulin. An ethanolic or ethyl acetate extract of the PTHs was evaporated in vacuum [5] and the residue was repeatedly extracted with cord ammonia and hydrolyzed in a sealed capillary. Completely satisfactory results were obtained (figure).

The new method of the hydrolysis of the PTH-amino acids with ammonia is proposed for the direct identification of the N-terminal amino acids. In this method, serine is almost completely decomposed and aspartic acid is partially converted into the α- and γ-amides.



Chromatogram of the amino acids liberated from the phenylthiohydantoins. 1–5) Hydrolyzates of the PTH derivatives of alanine and serine, 6–10) hydrolyzates of the PTH derivatives of valine, tyrosine, aspartic acid, and asparagine after 2, 4, 6, 9, and 12 hr.

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N-TERMINAL AMINO ACIDS OF HUMAN THYROGLOBULIN

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Thyroglobulin (TG) is a physiologically active protein of the thyroid gland. Its molecular weight is 6.5×10^6 [1] and its sedimentation coefficient 19. In a determination of the N-terminal amino acids of the protein [3–5], nine different amino acids were found, which suggests the presence of nine peptide chains in it.

We have determined the N-terminal amino acids in TG and have therefore found the true number of polypeptide chains.