

On the Structure of the New Neutral Amino Acid A Isolated from *Lactarius helvus*

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Some characteristics of the amino acid A isolated from *Lactarius helvus* in this laboratory were presented in a previous paper by Casimir and Virtanen¹. We have continued the investigations on the chemical composition and the structure of this amino acid. Some results of these studies are presented in this paper.

The hydrochloride of the new amino acid (Dr. Casimir's preparation) was dissolved in 0.5 M acetic acid and then passed through a column containing Dowex 1 in the acetate form. The resulting solution was evaporated to dryness *in vacuo* and the residue was dissolved in water. White crystals (needles) separated in an ice box. They were recrystallized from water three times. In accordance with the behaviour of the amino acid on a Dowex 1 column the result of paper electrophoresis showed that it is neutral.

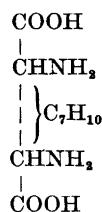
Elementary analysis: Found: C 54.28; H 7.52; N 11.53; O 26.70 (determined). Calc. for $C_{11}H_{15}N_2O_4$: C 54.55; H 7.50; N 11.57; O 26.45. This preparation was used in the present work. An estimation of the molecular weight by isothermal distillation according to Childs² gave the value 251 (average of three determinations). The formula of the amino acid is accordingly $C_{11}H_{15}N_2O_4$.

Estimation of α -amino and carboxyl groups. The formation of CO_2 and NH_3 by ninhydrin was determined according to Linko³. From 2.562 mg of substance 0.895 mg or 34.9 % of CO_2 was formed, which is 96.9 % of the theoretical value assuming two carboxyl groups in one amino acid molecule. 0.4199 mg of NH_3 or 96.4 % of the total N was found. These results suggest that the amino acid contains two α -amino groups and two carboxyl groups.

The estimation of amino nitrogen by van Slyke's method gave 99.5 % of the total N to be amino N. This result confirms the presence of two α -amino groups in the new amino acid.

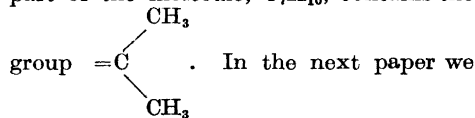
By titration of the carboxyl groups in 90 % ethanol, 2.331 mg of substance con-

sumed 1.387 ml of 0.01195 N KOH. This corresponds to 86.2 % of the carboxyl groups, assuming two carboxyl groups in one molecule. Under the same conditions 83.8 % of the carboxyl groups in α,ϵ -diaminopimelic acid were titratable. Accordingly the titration confirms the presence of two carboxyl groups in the new diamino acid. All the findings presented above suggest that the new amino acid is an aliphatic diamino-dicarboxylic acid



The positions of seven C atoms and ten H atoms in the molecule have thus still to be elucidated. Our knowledge about this part of the molecule is at the moment as follows:

As described in the paper of Casimir and Virtanen, the amino acid takes up 2 moles of H_2 per mole of amino acid on catalytic hydrogenation. The bromine uptake was estimated according to Rossmann⁴ and it was found that 1.1075 mg of the amino acid absorbed 0.2055 mg, or 1.9 mole of bromine per molecule of amino acid. Both the uptake of hydrogen and bromine suggest that the amino acid contains two double bonds. The rate of decolorization of $KMnO_4$ in alkaline solution by the amino acid confirms the presence of double bonds in the latter molecule. The amino acid is also attacked by ozone. Acetone, oxalic acid, and acetic acid could be found among the ozonization products. On the basis of the formation of acetone the unknown part of the molecule, C_7H_{10} , contains the



hope to be able to present more detailed information about this part of the molecule.

1. Casimir, J. and Virtanen, A. I. *Acta Chem. Scand.* **13** (1959) 2139.
2. Childs, C. E. *Anal. Chem.* **26** (1954) 1963.
3. Linko, P. *Suomen Kemistilehti B* **28** (1955) 96.
4. Rossmann, E. *Ber.* **65** (1932) 1847.

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