If a rough estimate of A has been obtained from preliminary measurements we may adjust the setting of the prisms according to this result and we may use a combination of sample concentration and tube length such that $\alpha = 0.87/A$. Doing this we ensure the highest pen deflection (D_{app}) which may be produced by any sample of the substance in question subject to our requirements of resolution.

The exchange of the ordinary light sources of the spectrophotometer for more powerful ones serves the purpose of increasing D_{max}. In our present setup this has enabled us to work with values of Θ as small as 1.5°, *i.e.* a rotation of 0.001° corresponds to an apparent optical density of *ca.* 0.001, and we believe that it will prove difficult to increase this sensitivity very much. (The Perkin-Elmer attachment [8] is operated at $\Theta \geq 10°$).

The accuracy of the values of α obtained is comparable, or a little inferior, to that with which absorption spectra are recorded by the spectrophotometer, *i.e.* α is correct to within *ca.* $\pm$ 1 %, provided $A < 5$. Substances with appreciably higher values of A must be measured in very dilute solutions, and the correspondingly lower values of D_{app} cannot be evaluated with this relative accuracy.

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Isolation of a New Neutral Amino Acid from *Lactarius helvus*

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During chromatographic investigations on the soluble nitrogen compounds in the mushroom *Lactarius helvus* we found relatively large amounts of an amino acid which could not be identified with any knovn amino acid already found to occur in plant tissues. This new substance corresponded to spot A in Fig. 1. When treated with ninhydrin on paper, it was easily recognized by a strong yellow colour at 80°C. The colour soon turned violet brown. On two-dimensional chromatograms the substance moved just above glutamine when phenol-water-ammonia and butanolacetic acid-water were used as solvents. The R_F-values

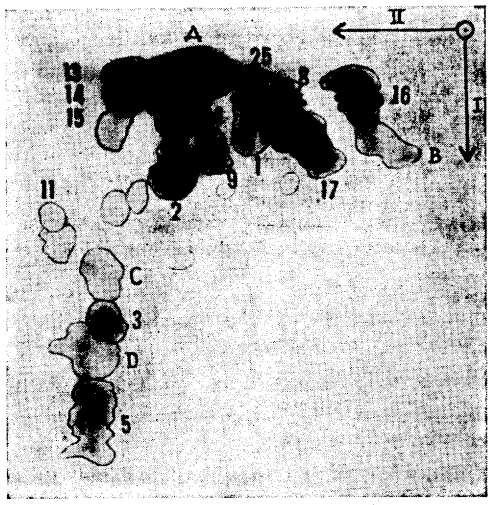

Fig. 1. Two-dimensional paper chromatogram of a 70 % ethanol extract of *Lactarius helvu.*. Spots A, C, and D apparently represent unknown neutral amino acids and spot B an acidic one. 1 gly, 2 ala, 3 val, 5 leu, 8 ser, 9 threo, 11 pro, 13—15 basic, 16 asp, 17 glu, 24 glu-NH_2, 25 asp-NH_2. I butanol-acetic acid-water, II phenol-water-NH_3.

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(Whatman 4) in the two solvents are 0.54 and 0.06, respectively. Additional unknown spots coloured with ninhydrin are also present on the chromatogram. A substance which gives a yellow spot B near aspartic acid, moves with the acidic fraction on paper electrophoresis at pH 5.6. Brown spots C and D were present in smaller amounts than A, moving with phenol-water-ammonia as valine and with butanol-acetic acid-water just above and below valine, respectively. A, C, and D appeared to be neutral.

Isolation of the amino acid A. About 10 kg (fresh weight) of *Lactarius helvus* were extracted with 40 l of ethanol. After partial decolorization with active charcoal (Darco G 60), which absorbs tyrosine and phenylalanine but not substances A, B, C, or D, the extract was concentrated and passed through an Amberlite IR-120 column. Amino acids were eluted from the column with 1 N ammonium hydroxide. The eluate was evaporated to dryness *in vacuo* giving a yellow powder (26 g). From a 10 g portion of this powder dissolved in 70 ml of 0.5 N acetic acid the acidic amino acids were separated from the neutral and basic ones in a Dowex 1 column (4.5 × 75 cm). 300 fractions, 25 ml each, were first taken using 0.5 N acetic acid and then 100 similar fractions using 1 N HCl as solvent. Fractions 19—28 contained the neutral and basic amino acids, 82—101 glutamic acid, 220—239 aspartic acid, and 358—369 the acidic substance partly decomposed during fractionation and giving two additional yellow spots with phenol-ammonia on a paper chromatogram.

The neutral eluate was evaporated to dryness and the residue dissolved in 65 ml of 1.5 N HCl. The solution was applied to a column of Dowex 50 (4 × 100 cm) which had been treated with 1.5 N HCl. The amino acids were eluted firstly with 1.5 N HCl (150 fractions each of 30 ml), then with 2.5 N HCl (150 fractions of 30 ml), and finally with 4 N HCl (100 fractions of 30 ml). Fractions 175—195 contained spots C and D, and fractions 334—384 only spot A. In another separation it appeared possible to elute the new substance from Dowex 50 with 2.5 N HCl, substance A coming between leucine and lysine.

Fractions 334—384 were combined and evaporated to dryness. Attempts to obtain crystals from methanol or ethanol were unsuccessful. Yellow impure crystals were obtained from *iso*propanol, from a small part of the residue. The unknown A was crystallized as hydrochloride from 6 N HCl and 1.75 g of pure, colourless crystals were obtained. This material corresponded to the unknown A and was found free from contaminants on a two-dimensional chromatogram with the usual solvents. The crystals were soluble in water, methanol, and ethanol. The free amino acid could be prepared by removing the HCl with a small bed of Dowex 1. The colourless crystals were moderately soluble in water but almost insoluble in methanol and ethanol.

Melting point determination on A. The new substance did not melt and on heating it began to turn yellow at about 200°C with a little sublimation and continued to darken until 300°C, when the heating was stopped.

On *total hydrolysis* with 6 N HCl for 24 h at 108°C the amino acid was stable, as it was on *alkaline hydrolysis* with saturated $Ba(OH)_2$ for 1 h at 100°C.

On *catalytic reduction with* PtO_2 *and* H_2 about two moles hydrogen were taken up per 1 mole. Spot A disappeared and a new spot A_1 was formed just above alanine on the two-dimensional chromatogram. The spot was immediately coloured violet by ninhydrin.

The free amino acid showed no absorption maxima in the UV region of the spectrum.

A micro test for the aldehyde or keto group (with 2,4-dinitrophenylhydrazine) was negative. Isatin spraying (proline and its homologue) gave no colour.

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