Acetylenedicarboxylic acid (0.50 g) in a mixture of glacial acetic acid (40 ml) and acetic anhydride (10 ml) was ozonised for 90 min at 10°C. The solvent was removed *in vacuo* and the oily residue crystallised only with difficulty. Recryst. four times from nitromethane it had m.p. 130°. (Found: C 37.9; H 3.8; act. O 7.3. Calc. for $C_7H_8O_8$: C 38.2; H 3.6; act. O 7.3). Titrated rapidly in the cold: E = 74.7. If alkali was added in excess and retitrated E = 57.0. Calc. for $C_7H_8O_8$: E tribasic 73.3, E tetrabasic 55.0. $C_7H_8O_8$ (59.5 mg) was refluxed in water for 10 h. The reaction mixture smelled somewhat of acetic acid. After removing the solvent, the residue was dried over potassium hydroxide (37.5 mg, calc. 36.8 mg). It contained active oxygen and had m.p. 120°C, which was undepressed in mixture with hydroperoxymalonic acid.

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## On the Structure of the New Amino Acid A, 2-Methylenecycloheptene-1,3-diglycine, Isolated from the Mushroom *Lactarius helvus*

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In an earlier paper by Casimir and Virtanen [1] the occurrence of four new amino acids (A, B, C, and D) in the mushroom *Lactarius helvus* was described. All these amino acids give on the paper chromatogram with ninhydrin characteristic yellow or yellow-brown spots which after a while turn violet. Komamine and Virtanen,[2] using the method of Linko,[3] showed that the amino acid A contained two α-amino and α-carboxyl groups. On catalytic hydrogenation two moles of hydrogen, or on bromination two moles of bromine, were consumed per mole of the amino acid, showing the presence of two double bonds. The double bonds were not conjugated. Elemental analysis of the compound A gave an empirical formula of $C_{11}H_{18}N_2O_4$.[2]

In continued investigations with a larger amount of material it was observed that the so-called amino acid A was a mixture of three, perhaps isomeric compounds ($A_1$, $A_2$, and $A_3$). By crystallization of this mixture several times from water, the amino acid $A_1$ most difficultly soluble in cold water was obtained in pure from. Its IR-spectrum showed it to be identical with the amino acid A prepared by Komamine and Virtanen.[2] On catalytic hydrogenation, amino acid $A_1$ gave two dihydrogenation products, indicating that a new asymmetric carbon atom was formed in the molecule and that the carbon chain must be branched at the position of the double bond. The NMR spectrum of the amino acid $A_1$ showed that it contained no methyl group. Two ring olefinic and two terminal olefinic protons were, however, evident. After catalytic hydrogenation a well defined signal of a secondary methyl group H–C–$CH_3$ was found. The amino acid $A_1$ must therefore contain a >C=$CH_2$ group, from which the new asymmetric carbon atom is formed on hydrogenation.

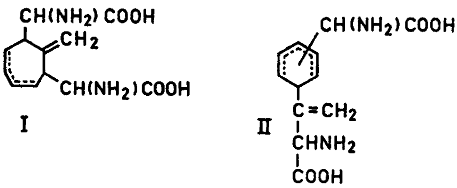
By oxidation of the amino acid $A_1$ with ninhydrin followed by steam distillation only traces of a volatile compound were obtained. The mass spectrum of this compound was typical of a dialdehyde having a molecular weight of 164, indicating the formula $C_{10}H_{12}O_2$ for this dialdehyde. Its 2,4-dinitrophenyl-hydrazone showed an absorption maximum at 355 mμ, which is characteristic of aldehydes having no α,β-double bonds. Therefore the double bonds in the dialdehyde molecule are not conjugated with the carbonyl groups.

By ninhydrin oxidation of the hydrogenated amino acid the corresponding saturated dialdehyde (mol. wt. 168, $C_{10}H_{16}O_2$)

was obtained in much better yield. This saturated dialdehyde gave with silver oxide a dicarboxylic acid $C_{10}H_{16}O_4$ (Found: C 60.34; H 8.10. Calc. for $C_{10}H_{16}O_4$: C 59.98; H 8.05.), from which the corresponding dimethyl ester (mol.wt. 228. Found: C 63.35; H 8.73. Calc. for $C_{12}H_{20}O_4$: C 63.13; H 8.83.) was obtained with diazomethane.

On the basis of these results the true formula of the original amino acid $A_1$ must be $C_{12}H_{18}N_2O_4$. In addition the molecule must contain a ring, since only two double bonds are present.

The nuclear magnetic resonance studies of the original amino acid $A_1$, its diethyl ester and that of the dimethyl ester $C_{12}H_{20}O_4$ lead to the following possibilities (I and II) for the structure of the amino acid $A_1$:




The structure II would give on ninhydrin oxidation an $\alpha,\beta$-unsaturated aldehyde. This is, however, not in agreement with the experimental results. This being the case the structure I, 2-methylenecyclohept-(4,5 or 6)-ene-1,3-diglycine, corresponds best with the before-mentioned facts. The position of the second double bond remains still in doubt.

Full details of these investigations will be published later.

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## The Near IR Absorption of Metal Complexes with Flavin Free Radicals

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It has been demonstrated by Hemmerich[1] and coworkers that flavin free radicals are rather good chelating agents. The affinity of d-metal ions towards the radical is much higher than towards the oxidized and reduced forms of flavin. The importance of these complexes for the catalytic action of metalloflavoproteins is obvious.

We are since some time concerned with the study of the details of the electron distribution within these radicals and their complexes, *e.g.* with metal ions. A special object of interest in this research is the relationship between ESR (electron spin resonance) and light absorption.

When investigating the metal radical chelates, chloroform solutions of tetraacetyl riboflavin have been used in order to obtain sufficient solubility and to avoid precipitation, which occurs with FMN and FAD, when also the phosphate coordinates to the metal ion. Excess of triethyl-

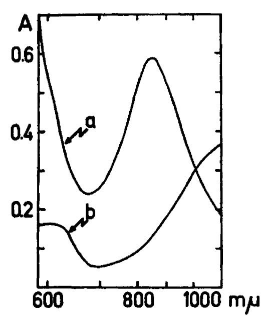


*Fig. 1.* Light absorption of tetraacetyl riboflavin (7 mM) in the near IR region dissolved in $CHCl_3$, 14 mM $(C_2H_5)_3N$, 5.5 M $CH_3CN$: (*a*) with 1.8 mM $Cd(NO_3)_2$ added; (*b*) with no metal ion added. Samples half reduced with dithionite.