α -Amino Isobutyric Acid as a Constituent Amino Acid of Protein

(Studies on Muscle Proteins. Part 14)

Sir:

α -Amino isobutyric acid as a constituent amino acid of protein was at first reported by T. Yabuta¹⁾ and his collabolates in 1938 and isolated as 5,5'-dimethylhydantoin in the dry distillate of pupae of silkworm.

Thereafter, few reports have appeared concerning this fact until Y. Oshima²⁾ et al. in 1953 recognized the presence of this amino acid in the acid-hydrolysate of casein by paper chromatography.

M. Kandatsu³⁾, one of the authors, confirmed the production of acetone from the acid-hydrolysate of rabbit muscle proteins by the oxidation of hypochlorite and suggested its presence in the protein, because α -amino isobutyric acid produces acetone exclusively by the oxidation of hypochlorite among known amino acids.

Continuing our investigations to clear up its presence in muscle protein, we could now

isolate this amino acid from both of the acid- and pepsin-hydrolysates of horse muscle proteins which were prepared from the hind leg.

A sample of 700 g of horse muscle proteins was hydrolysed by 10-fold 20% HCl solution under reflux for 20 hrs., then humin was filtered off, and concentration in vacuum repeated to remove as much Cl' as possible. The hydrolysate was converted to copper salts with CuCO_3 and $\text{Cu}(\text{OH})_2$ by the usual method.

The hydrolysate decomposed by enzyme was prepared from 100 g of muscle proteins by the usual method with concentrated pepsin at pH 2.0-2.2 and 39°C, for 40 hrs. and after adding 3% CCl_3COOH solution, the coagulants were filtered off. With the filtrate, copper salts were formed as described above.

The copper salts were fractionated with water and dry methanol, and the water soluble and methanol insoluble fractions were

	Acid-hydrolysate	Pepsin-hydrolysate	Calculated for $\text{C}_4\text{H}_6\text{NO}_2$
C (%)	46.71	46.06	46.60
H	8.95	8.07	8.74
N	13.59	13.86	13.60
Sublimating point (°C)	275-	281-	280
R_F (phenol)	0.58		0.58
Picrate			
m. p. (°C)	194	194	193-4
C	36.61		36.14
H	4.11		3.61
N	16.82		16.87
Picrolonate			
m. p.	232-3		236
C	45.84		45.78
H	4.22		4.63
N	19.44		19.07

1) T. Yabuta et al, *Bulletin of the Institute of Physical and Chemical Research*, **17**, 1241 (1938).

2) Y. Oshima et al, *J. Agr. Chem. Soc. Japan.*, **27**, 102 (1953).

3) M. Kandatsu, *J. Agr. Chem. Soc. Japan*, **22**, 7 (1948).

collected.

After removal of copper by H_2S , diamino and dicarboxylic acids were removed by

phosphotungstic acid and $\text{Ba}(\text{OH})_2$ -ethanol, respectively. The filtrate was again treated as mentioned above to form copper salts, then the water soluble and methanol insoluble fractions were collected. After the removal of copper, the mono-amino mono-carboxylic fraction was concentrated under vacuum and treated by silica-gel chromatography with phenol: H_2O (8:2). The elute which contained this amino acid only was shaken with ether to remove phenol and added with absolute ethanol to crystallize the amino acid.

By repetition of recrystallization with ethanol, about 140 mg of white prisms crystalline was obtained. (Including about 20 mg from enzyme hydrolysate)

The properties of this crystalline are summarized in the table.

The appearance of sublimation, results of

elementary analysis, are consistent with theoretical values and m.p., elementary composition of picrate and picrolonate and also infra-red absorption spectrum of this amino acid are identical with a synthetic sample.

Accordingly, it is confirmed by us that α -amino isobutyric acid is one of the constituent amino acids of the muscle protein.

The distribution of this amino acid in various proteins and its metabolism in higher animals is now under study.

Details of this experiment will appear in this journal.

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