SHORT COMMUNICATIONS

Synthesis of L-Pyroglutamyl-L-glutaminyl-L-glutamine

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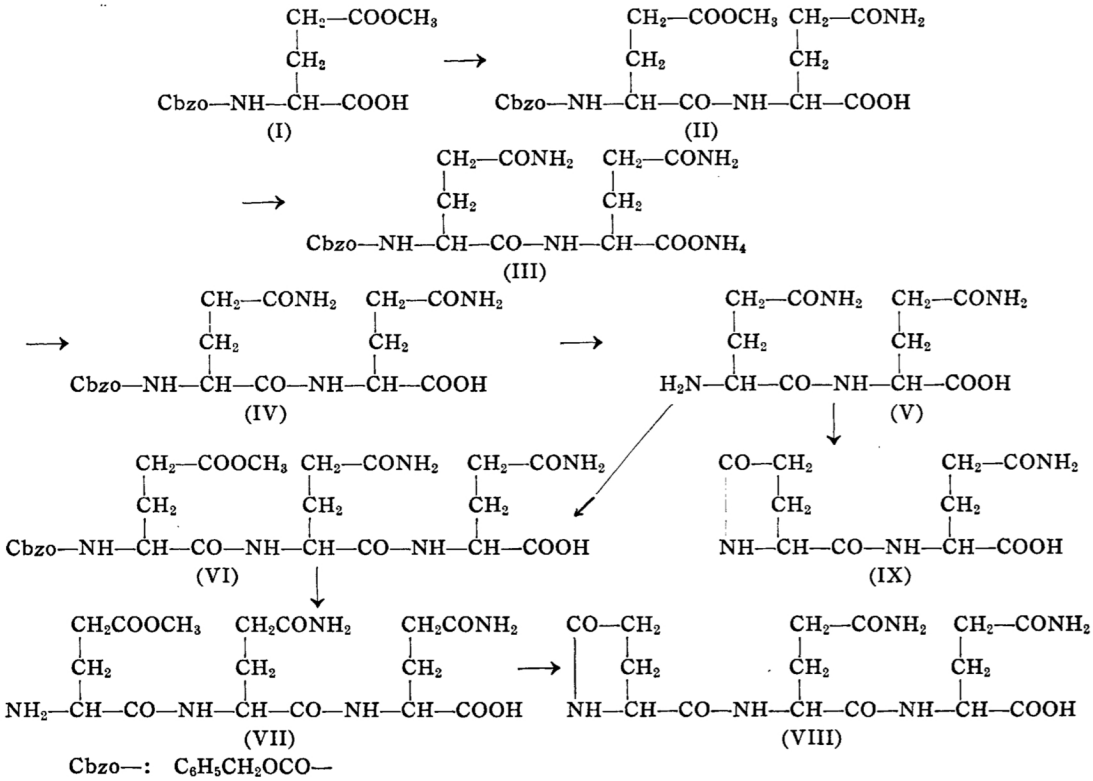
The synthesis of eisenine[1] isolated from a brown marine alga *Eisenia bicyclis* Setchell had been recently performed by us[2]. Eisenine is a tripeptide having the structure L-pyroglutamyl-L-glutaminyl-L-alanine. In 1949 a tripeptide fastigiatine* was isolated by J. S. Fruton and his collaborators[3] from American brown alga *Pelvetia fastigiata*. In the present investigation L-pyroglutamyl-L-glutaminyl-L-glutamine proposed by J. S. Fruton for the structure of fastigiatine was synthesized according to the following scheme similar to the synthetic route of eisenine.

A mixed anhydride from γ-methyl-carbobenzyloxy-L-glutamate (I) and ethyl chloroformate was coupled with L-glutamine to yield carbobenzyloxy-L-(γ-methyl ester)-glutamyl-L-glutamine (II) (37%), $C_{19}H_{25}O_8N_3 \cdot 2H_2O$, m. p. 184.5—185°C**. Compound (II) was converted by methanolic ammonia into amide ammonium salt (III) (77%), m. p. 195—196°C (dec.), which was heated at 100°C over phosphorous pentoxide in vacuo to give carbobenzyloxy-L-glutaminyl-L-glutamine (IV), m. p. 177—178°C. Carbobenzyloxy diamide (IV) was



1) T. Ohira, *J. Agr. Chem. Soc. Japan* (*Nogei Kakaku Zassi*) **15**, 370 (1939).
2) T. Kaneko, T. Shiba, S. Watarai, S. Imai, T. Shimada and K. Ueno, *Chem. & Ind.* (London), **1957**, 986.
3) C. A. Dekker, D. Stone and J. S. Fruton, *J. Biol. Chem.*, **181**, 719 (1949).
4) A. Meister, "Biochemistry of the Amino Acids", Academic Press Inc., Publishers, N. Y. (1957), p. 56.

* For this tripeptide, J. S. Fruton has expressed in the term of "peptide from *Pelvetia fastigiata*". While the name "pelvetine" was used by us early, A. Meister[4] employed the nomenclature "fastigiatine". Now, we use fastigiatine instead of pelvetine to avoid the confusion of these nomenclatures.

** All melting points were uncorrected.

hydrogenated using palladium charcoal to L-glutaminyl-L-glutamine (V) (78%), $C_{10}H_{18}O_5N_4$ $1\frac{1}{2}H_2O$, m. p. 208—209°C (dec.). $[\alpha]_D^{29}$ +24.54° (*c*, 1.74, in H_2O). Furthermore diamide (V) and γ-methylcarbobenzyloxy-L-glutamate (I) were combined through the mixed anhydride method as described above, to yield carbobenzyloxy tripeptide derivative (VI) (53%), m. p. 175—180°C (dec.), which was hydrogenated to compound (VII) (61.3%), m. p. 221°C (dec.). Compound (VII) was cyclized in absolute methanol saturated with ammonia, followed by treatment with Dowex 50 to obtain L-pyroglutamyl-L-glutaminyl-L-glutamine (VIII)*** (80%), m. p. 203—204°C (dec.), $[\alpha]_D^{28}$ −36.24° (*c*, 2.02, in H_2O), pK_a' 3.5.

Anal. Found: C, 46.75; H, 5.81; N, 18.47; equiv. 427. Calcd. for $C_{15}H_{23}O_7N_5$: C, 46.75; H, 6.02; N, 18.17%; equiv. 385.

While this substance failed to give a color with ninhydrin, it gave a strong biuret reaction. From the results obtained above, however, it is not necessarily concluded that this synthetic product was identical with natural fastigiatine, since the latter has m. p. 190—195°C and $[\alpha]_D$ −43.7∼−46.5° (in H_2O).

The cyclization of a γ-amide to a pyroglutamyl derivative can also be successfully produced by boiling the aqueous solution of the former. For example, L-glutaminyl-L-glutamine (IV) was converted by this procedure into L-pyroglutamyl-L-glutamine (IX) (73%), m. p. 178—180°C (dec.), $[\alpha]_D^{15}$ −22.14° (*c*, 1.65 in H_2O).

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*** After this synthesis had been accomplished, a private communication from Professor J. Rudinger to Professor S. Akabori indicated that Professor Rudinger and his collaborators have also succeeded in the synthesis of the same peptide independently of ours.