XXXIX. A NEW SYNTHESIS OF METHIONINE AND A SCHEME RELATING CERTAIN α -AMINO-ACIDS.

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THE constitution of methionine has been fully established both by analysis [Barger and Coyne, 1928] and by synthesis [Barger and Coyne, 1928; Windus and Marvel, 1930; Barger and Weichselbaum, 1931]. Moreover, the process whereby it was originally isolated by Mueller [1923] from protein hydrolysates has been so modified [Pirie, 1932; 1933; Hill and Robson, 1934] that it is now one of the more readily accessible of the amino-acids.

The synthesis of methionine about to be described is of interest in that it reveals a possible relationship between certain of the known α -amino-acids, to which, so far as we are aware, attention has not previously been drawn.

The new synthesis originated in the observation that during Kjeldahl analysis of methionine there occurred a vigorous evolution of gas having the odour of a volatile sulphur compound. Since the odour was noticeable soon after heat was applied to the reaction mixture, it was thought that the sulphur compound resulted from a comparatively simple reaction and that it was probably dimethyl disulphide, formation of the latter arising by oxidation of the methyl mercaptan liberated from the methionine.

Such a non-hydrolytic liberation of methyl mercaptan from methionine appeared to us necessarily to involve simultaneous formation of α -amino- γ -butyrolactone:

At a later stage of our work we learned in fact that Butz and du Vigneaud [1932], in discussing the breakdown of methionine under the action of hot 18*N* H_2SO_4 , concluded that it was split partly into methyl mercaptan and α -amino- γ -hydroxybutyric acid and partly into methyl alcohol and homocysteine, from the oxidation of which latter compound homocystine arose. In this connection it should be added that, from the work of Fischer and Blumenthal [1907], who synthesised both α -amino- γ -hydroxybutyric acid and α -amino- γ -butyrolactone, it appears that the former compound is incapable of existing in acid solution since it reverts to the lactone in such a medium. In any case, it is an open question whether the decomposition, as we regard it, necessarily involves a molecule of water.

A further study of the probable course of the reaction raised the interesting question of its possible reversibility; in other words can methionine be synthesised from methyl mercaptan and α -amino- γ -butyrolactone?

Selecting α -benzamido- γ -butyrolactone as the most suitable starting-point, we synthesised this compound according to the method of Fischer and Blumenthal [1907] as follows:

the only modifications being the preparations of (1) phenoxyethyl bromide in quantity by the admirable method of Marvel and Tanenbaum [1933] and of (2) ethyl phenoxyethylmalonate as described by Leuchs [1911].

We first attempted to synthesise methionine by direct combination of methyl mercaptan with α -benzamido- γ -butyrolactone, but in this direction we have so far been unsuccessful. An alternative route however was available and the success attained along these lines makes it appear that a direct synthesis such as we have suggested above may be possible.

Treatment of a suspension of α -benzamido- γ -butyrolactone (I) in absolute alcohol with a rapid stream of dry hydrogen chloride gave ethyl α -benzamido- γ -chlorobutyrate (II),

an oil which gradually solidified on standing. The latter compound, dissolved in absolute alcohol, immediately reacted with a hot alcoholic solution of sodium methyl mercaptide, as evidenced by the separation of sodium chloride, to give the ester of benzoylmethionine (III), which in turn, on alkaline hydrolysis, gave benzoylmethionine (IV) in good yield and excellent purity. The removal of the benzoyl group by boiling hydrochloric acid completed the synthesis.

The synthesis of methionine from α -amino- γ -butyrolactone raises several questions. One of the more interesting of these is whether the aminolactone is the immediate precursor of methionine in nature. In this connection moreover we cannot overlook the fact that the lactone is closely related chemically to several of the naturally occurring α -amino-acids. On the one hand, it is, for instance, but a short step to the

lactone from aspartic acid, shown by Dunn and Fox [1933] to be derivable from fumaric acid. On the other hand the basic reaction whereby α -butyrolactone is converted into γ -cyanobutyric acid may be regarded as a possible basis of the conversion of the aminolactone into α -amino- γ -cyanobutyric acid (VI) from which one might proceed either by hydrolysis to glutamic acid (VII) or by reduction to ornithine (VIII), the latter compound being the direct precursor of arginine. The relationship might be extended in other directions but it will suffice to trace but one more connection at this point. Recently Garforth and Pyman [1935] have shown that α -amino- γ -butyrolactone is an excellent starting-point for the synthesis of substituted glyoxalines and it is conceivable that the natural synthesis of histidine may lie along some such path.

The main objection at the present time to the scheme which we have advanced above lies in the fact that neither α -amino- γ -butyrolactone nor α -amino- γ -hydroxybutyric acid has been found in nature either in the free state or as part of a larger molecule. Nevertheless, their relationship to other amino-acids is so close that we believe that a search for them might profitably be undertaken especially among plant products. In this connection it may be added that

Schryver and Buston [1926] reported the isolation of a hydroxylated α -amino-butyric acid from the acid hydrolysate of "oat-protein", but it was apparently not identical with the α -amino- γ -hydroxybutyric acid of Fischer and Blumenthal to which the authors make reference in their communication.

For purposes of record, the carbamido- and hydantoin derivatives of methionine have been made and their melting points and solubilities determined.

EXPERIMENTAL.

Ethyl α -benzamido- γ -chlorobutyrate. Through a solution of α -benzamido- γ -butyrolactone (10 g.) (prepared according to Fischer and Blumenthal with the modifications mentioned above) in absolute alcohol (150 ml.) was passed a rapid stream of hydrogen chloride until the temperature, which rose during the preliminary stage of the reaction, began to fall. Next morning the mixture was concentrated under reduced pressure and the residue extracted with dry ether. The insoluble portion was again esterified, the reagents removed as before, and the residue extracted with ether. The ethereal extracts were combined, the ether evaporated, and the residual oil poured into a crystallising basin where it rapidly solidified. The product melted at 45° and weighed 10.2 g. (78 % of theory).

Benzoylmethionine ethyl ester and benzoylmethionine. Methyl isothiocarbamide sulphate (6 g.) prepared according to the method of Arndt [1921] was hydrolysed with 5*N* NaOH (20 ml.) and the methyl mercaptan evolved directly absorbed in a solution of sodium (0.6 g.) in absolute alcohol (20 ml.). To the solution of the mercaptide was added a solution of ethyl α -benzamido- γ -chlorobutyrate (5 g.) in absolute alcohol (15 ml.) and the mixture refluxed for 4 hours. Next morning the alcohol was distilled off and water added to the residue; needles immediately commenced to separate. When crystallisation was completed, the solid (1.6 g.) was removed at the pump and the filtrate acidified. A further precipitate (2.4 g.) was thrown down and was seen to consist of long narrow plates. Examination of the two sets of crystals showed that the former consisted of ester, the latter of acid, partial hydrolysis of the ester apparently having occurred owing to the presence of the slight excess of sodium mercaptide in the reaction mixture.

The ester, without further purification, was hydrolysed with boiling 2 % NaOH (40 ml.) and the acid obtained on acidifying the cold hydrolysate added to that already isolated. The total yield of benzoylmethionine was 3.4 g. (72 % of theory). A portion of the product recrystallised from water melted at 151° (Windus and Marvel give 143 – 145°). (Found: N, 5.50 %. $C_{12}H_{15}O_3NS$ requires N, 5.54 %.)

Methionine. Benzoylmethionine (1.8 g.) was refluxed with 20 % HCl (50 ml.) for 18 hours. The hydrolysate when cold was extracted with ether to remove benzoic acid and the aqueous solution concentrated *in vacuo* almost to dryness. The residue was dissolved in absolute alcohol and the solution made just alkaline to bromophenol blue with pyridine. On standing for 48 hours the methionine separated. This was removed at the pump, washed with alcohol and ether and dried. It weighed 0.6 g. (55 % of theory). (Found: N, 11.85 %. $C_5H_{11}O_2N$ requires N, 11.96 %.)

Carbamidomethionine. Methionine (4 g.) was heated on a water-bath with a solution of potassium cyanate (2.7 g. = 1.25 mols.) in water (50 ml.). When cold, the solution was neutralised with HCl and evaporated to dryness under reduced pressure. The residue was extracted with rectified spirits, the extract being

again evaporated to dryness under reduced pressure. A portion of the carbamido-acid so obtained (the yield was theoretical) was recrystallised from water, after which it melted at 171–172°. Carbamidomethionine is very soluble in water and alcohol but insoluble in ether.

Methionine hydantoin. Carbamidomethionine (3 g.) was boiled with dilute HCl (10 ml.) for 5 min. and the solution evaporated to a small volume and cooled. Methionine hydantoin crystallised out. The solid was removed at the pump. A further crop of the hydantoin was obtained by concentrating the filtrate. The product (2.5 g.) after recrystallisation from water melted at 117–118°. (Found: N, 16.00 %. $C_6H_{10}O_2N_2S$ requires N, 16.09 %.)

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