CLXXVI. THE AMINO-ACID METHIONINE; CONSTITUTION AND SYNTHESIS.

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As a result of certain bacteriological investigations, Mueller [1923, 1] isolated from caseinogen a new amino-acid, $C_5H_{11}O_2NS$, in a yield of 0.2–0.4 %. About the same quantity was obtained from egg-albumin, and smaller amounts are also present in edestin, wool and gelatin. That the substance is a primary cleavage product of protein, and not foreign to the body, was also indicated by its oxidation after administration to an animal, when the sulphur appears in the urine as inorganic sulphate [Mueller, 1923, 2]. The same amino-acid was subsequently found by Otake [1925]. On working up the residues of a commercial vitamin preparation from 6 tons of yeast, the latter obtained 0.6 g. of the pure amino-acid and published some useful photomicrographs of this substance and its derivatives.

The attention of one of us was called to this subject, in the course of an attempt, in conjunction with Dr A. S. Nayar, to isolate a thiolhistidine as precursor of ergothioneine, afterwards identified in red blood-corpuscles by Newton, Benedict and Dakin [1927] and by Eagles and Johnson [1927]. Whilst we could not obtain thiolhistidine, either from ergot or gelatin, we became involved in an examination of the non-cystine sulphur and prepared Mueller's amino-acid, following his directions. Mueller had already shown that the substance is not ethylcysteine. Since the methylthiol, like the methoxyl, group, is much more common in nature, we applied to the acid Kirpal and Bühn's method [1914] for the determination of CH_3S -groups and found one to be present. This result restricted the possible formulae to derivatives of butyric and isobutyric acids. Since all known cleavage products of protein are α -amino-acids, only four formulae remained, viz. α -, β -, and γ -methylthiol-butyric acids (I–III) and methylthiolisobutyric acid (IV).

Of these (III) seemed much the most probable on account of its relation to cheirolin, a substance isolated from wallflower seeds (*Cheiranthus cheiri*) by Schneider [1910] and shown by him to have the constitution

Cheirolin would thus be the sulphone of a mustard oil, derived from Mueller's amino-acid in the same way as, for instance, phenylethyl mustard oil, $\text{C}_6\text{H}_5.\text{CH}_2.\text{CH}_2.\text{N}:\text{C}:\text{S}$, from the root of *Reseda* [Bertram and Walbaum, 1894], is derived from phenylalanine, via phenylethylamine. We therefore decided to synthesise the racemic substance (III) and indeed found it to be identical with Mueller's acid, except as regards optical activity [cf. Barger and Coyne, 1928]. Later, Dr Mueller informed us that he had himself arrived at the same constitution and had unsuccessfully attempted to prove it by synthesis. Since the amino-acid has a good title to be regarded as a constituent of protein, a shorter name than γ -methylthiol- α -aminobutyric acid seems desirable, and, after consultation with Dr Mueller, we suggest for it the name *methionine*, in allusion to the characteristic grouping.

Before we proceeded to synthesis, we attempted other means of breaking down the amino-acid, by decarboxylation, by oxidation and by exhaustive methylation, but, using only minute amounts of this rather inaccessible substance, we did not arrive at anything definite in this way. The synthesis was first attempted by various modifications of Erlenmeyer's method, from methylthiolacetaldehyde, $\text{CH}_3.\text{S}.\text{CH}_2.\text{CHO}$, which was easily made from bromoacetal. The condensation of the new aldehyde with hippuric acid and with diketopiperazine failed completely, but after prolonged experimentation we obtained a few grams of its condensation product with hydantoin in a state of purity, only to find that we were quite unable to reduce it to methionine; sulphur was eliminated. Thinking we could introduce the methylthiol group last, we attempted the condensation of phenoxyacetaldehyde, $\text{C}_6\text{H}_5\text{O}.\text{CH}_2.\text{CHO}$, with hydantoin, but without success.

We then had recourse to the old Strecker method [1850], from the next higher aldehyde, $\text{CH}_3\text{S}.\text{CH}_2.\text{CH}_2.\text{CHO}$, using a useful modification by Zelinsky and Stadnikoff [1908], and in this way obtained without special difficulty enough synthetic amino-acid to establish its identity with methionine. Whether from caseinogen or by synthesis, methionine is at present a very troublesome substance to prepare. We have tried to improve Mueller's method of isolation, but so far without any real success. Mercuric salts remain so far the only reagents which differentiate methionine somewhat from amino-acids like leucine and phenylalanine. Methionine ethyl ester is unstable when distilled, even in a charcoal vacuum, and a really characteristic derivative, from which methionine might be regenerated, is much to be desired.

EXPERIMENTAL.

Preparation of methionine.

900 g. of gelatin, hydrolysed with hydrochloric acid and freed from the latter as far as possible by evaporation *in vacuo*, were dissolved in 2 litres of water and showed maximum precipitation on adding $1\frac{1}{2}$ volumes of Hopkins's reagent (10 % HgSO_4 in 5 % H_2SO_4). The precipitate, after decomposition with H_2S , was esterified, the fraction boiling at $130\text{--}150^\circ/0.1$ mm. was richest in sulphur, but most of the sulphur remained in the flask (as diketopiperazine?). Only a little impure methionine was obtained from the distillate.

By using Mueller's method, unmodified, we obtained 1–3 g. per kg. from caseinogen, with only a single precipitation with HgCl_2 . The purity, as indicated by the sulphur content, was 85–95 %. A second precipitation with HgCl_2 is very wasteful and was omitted for most purposes.

Mueller's method was examined in greater detail as follows. 2700 g. caseinogen were hydrolysed with sulphuric acid and the filtrate, neutralised with NaOH , was divided into halves. To one half were added 1350 g. of HgSO_4 in 7 litres of 7 % H_2SO_4 . The precipitate (*A*) was filtered off after 24 hours. The filtrate was half neutralised, causing a precipitate (*B*), 4–5 times as bulky as (*A*); neutralisation to Congo red of its filtrate yielded a still larger precipitate (*C*). The second half of the hydrolysate was treated with only one-third of the reagent added to this first half; the resulting precipitate (*D*) was filtered off, an additional third of the reagent was added, giving precipitate (*E*), and to its filtrate the third instalment of the reagent, giving (*F*). All six precipitates were washed twice by decantation, and twice by filtration, and extracted five times, with one litre of 2 % barium hydroxide each time. The five combined extracts were freed from barium hydroxide by sulphuric acid, from mercury by hydrogen sulphide, and the filtrate and washings were evaporated to 500 cc. A boiling saturated solution of 20–50 g. of mercuric chloride (according to the bulk of the original mercuric sulphate precipitate) was added. The mercuric chloride precipitate was freed from mercury by hydrogen sulphide, the filtrate was evaporated *in vacuo*, dissolved in the minimum quantity of boiling water and mixed with 5–10 cc. of pyridine (to remove HCl) and three volumes of boiling alcohol. The following yields were obtained: *A*, 2 g. histidine with traces of methionine; *B*, 0.05 g. methionine; *C* was lost by accident; *D*, *E* and *F* respectively, 0.5, 0.5 and 0.6 g. methionine.

It would seem therefore that the larger quantity of the reagent should be added at once, and that the immediate precipitate, as well as that formed by half neutralisation, may be discarded.

Determination of methylthiol grouping according to Kirpal and Bühn [1914]. The cadmium sulphate bubbler was coloured yellow; hence some sulphur was split off as hydrogen sulphide. The methionine had a sulphur content of 18.9 % and was therefore 88 % pure.

Analysis. 0.2237 g. (crude) = 0.1968 g. (pure) gave 0.2998 g. AgI.

0.1023 g. (crude) = 0.0900 g. (pure) required 5.97 cc. *N*/10 AgNO₃.

Found: CH₃S = 30.5, 31.2; calc. for C₅H₁₁O₂NS: CH₃S = 31.6 %.

In the second estimation the methyl iodide was passed into pyridine, according to Kirpal and Böhn's second method [1915], and, after removal of the excess of pyridine, the methylpyridinium iodide was titrated. Ethyl iodide hardly reacts with pyridine in the cold. We moreover proved the identity of the alkyl iodide by converting the pyridinium salt into mercurichloride and comparing it with authentic specimens of methyl- and of ethyl-pyridinium mercurichloride.

	M.P.
A. Mercurichloride from methionine product ...	160–163°
B. Methylpyridinium mercurichloride ...	161–163°
C. Ethylpyridinium mercurichloride ...	131–133°
Mixture of A and B ...	160–163°
Mixture of A and C ...	110–119°

Hence the presence of one methylthiol grouping was established.

Synthesis of methionine by Strecker's method.

β-Methylthiolpropaldehydediethylacetal, CH₃.S.CH₂.CH₂.CH(OC₂H₅)₂, was prepared by passing excess of methyl mercaptan (2 mol.) into alcoholic sodium ethoxide solution and dropping (1 mol.) *β*-chloropropaldehydeacetal [Wohl, 1898] into it. B.P. 89°/14 mm., 96°/20 mm. Yield 70 % of the theoretical.

Analysis. 0.1124 g. gave 0.1456 g. BaSO₄: S = 17.8 %.

0.1180 g. gave 0.1552 g. BaSO₄: S = 18.0 %.

Calc. for C₈H₁₈O₂S: S = 18.0 %.

For this and other syntheses the methyl mercaptan was prepared by hydrolysis of *S*-methylthiourea [Arndt, 1921]. The latter and the mercaptan were both obtained in a yield of 90 % of the theoretical. It is, however, most convenient to pass the mercaptan at once into sodium ethoxide solution, in a vessel provided with an ice-jacketed condenser, in order to keep back as much as possible of the malodorous mercaptan. Since the chloro- and the methylthiol-acetal have nearly the same boiling-point, a large excess of mercaptan is used in order to make the reaction go completely and avoid the necessity of a separation.

β-Methylthiolpropaldehyde, CH₃.S.CH₂.CH₂.CHO. The acetal was hydrolysed by boiling for half an hour with two volumes of water containing 1–2 cc. of dilute hydrochloric acid. The aldehyde separated on cooling, and was extracted with ether. B.P. 60°/12 mm. Yield, 80 % of the theoretical.

α-Amino-*γ*-methylthiolbutyronitrile, CH₃.S.CH₂.CH₂.CH(NH₂)CN. To a well-cooled mixture of the aldehyde in ether and one equivalent of ammonium chloride in concentrated aqueous solution there was added slowly one equivalent of potassium cyanide in the minimum quantity of water, with frequent shaking. Excess of ammonium chloride (2–3 g.) was then added and the mixture was

shaken for 6 hours. The ethereal layer was separated and the aqueous layer was thoroughly extracted with ether. The total ether extract was dried over sodium sulphate and dry hydrogen chloride was passed into the well-cooled solution. A brown semi-crystalline syrup separated and the ether could be decanted off. Attempts to obtain the pure aminonitrile hydrochloride failed, so the crude product was used.

α -Amino- γ -methylthiolbutyric acid, $\text{CH}_3\cdot\text{S}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{CH}(\text{NH}_2)\text{COOH}$. After pouring off the ether, two volumes of concentrated hydrochloric acid were added, and the solution was boiled for 2 hours, during which the original dark brown colour deepened and a tar separated. The hydrochloric acid was removed as far as possible by vacuum distillation and the semi-crystalline residue, containing a large amount of tar and ammonium chloride, was dissolved in the minimum amount of boiling water. 10 cc. of pyridine were added to remove free hydrochloric acid; on adding three volumes of boiling alcohol the amino-acid separated in clusters of narrow platelets. It was recrystallised from alcohol and melted at 281° with decomposition. The yield was on the average 6 % of the theoretical, calculating from the aldehyde used.

The last reaction is the one unsatisfactory feature of this synthesis. The following improvements were attempted.

(1) Washing the acid solution of the aminonitrile with ether, and boiling with hydrochloric acid, without separating the crude aminonitrile hydrochloride. In this way the large amount of inorganic salt remains in the solution, and the amino-acid has to be precipitated with mercuric chloride; only a trace of the amino-acid was obtained in this manner.

(2) Hydrolysis of the aminonitrile by dilute hydrochloric acid. This did not prevent formation of tar and gave much poorer results than concentrated hydrochloric acid.

(3) Hydrolysis by barium hydroxide. The tar separated on the walls of the flask and the solution remained clear. After 10 hours' boiling, the barium was removed quantitatively and the filtrate was evaporated. Only a little gum remained, which gave the ninhydrin reaction and contained sulphur, but could not be crystallised.

(4) The original condensation was repeated with five equivalents of ammonium chloride and potassium cyanide, but this gave much more tar and only a minute amount of the amino-acid.

(5) A slightly improved yield resulted when the ethereal solution of the aldehyde was added to a concentrated aqueous solution, containing one equivalent each of potassium cyanide and ammonium chloride. The solution was kept ice-cold and stirred vigorously.

Analysis.

5.91 mg. gave 0.551 mg. N (micro-Kjeldahl).

9.38 mg. gave 14.5 mg. BaSO_4 (micro-Carius).

Found: 9.3 % N; 21.3 % S.

Calc. for $\text{C}_5\text{H}_{11}\text{O}_2\text{NS}$: 9.4 % N; 21.5 % S.

α-Naphthylcarbamido-derivative, $\text{CH}_3.\text{S}.\text{CH}_2.\text{CH}_2.\text{CH}(\text{NH}.\text{CO}.\text{NH}.\text{C}_{10}\text{H}_7)\text{COOH}$. 0.1 g. methionine, both synthetic and natural, in 6.8 cc. of *N*/10 sodium hydroxide was mixed with 0.12 g. *α*-naphthyl isocyanate and shaken for one hour, then filtered. The clear filtrate was just acidified with hydrochloric acid and a bulky precipitate was thrown down (excess of acid causes the precipitate to coagulate to a sticky mass which cannot be crystallised). The precipitate was filtered off, thoroughly washed and dried. It crystallised after dissolving in boiling alcohol and adding three volumes of boiling water. On cooling short stout needles separated. Some difficulty was experienced in obtaining good crystals and the product often separated in a semi-amorphous, gelatinous condition, even when an alcoholic solution was left to evaporate at room temperature. Mixtures of benzene with acetone and alcohol are unsuitable. Similar difficulties are not uncommon with naphthylcarbamido-derivatives.

In the case of natural methionine the *α*-naphthylcarbamido-derivative was obtained at the second attempt with the melting-point 187° , that given by Mueller. The derivative of synthetic methionine, however, was only obtained in imperfect crystals, in spite of several attempts, and melted at $181\text{--}2^\circ$. The mixture with the derivative of the natural substance also melted at this temperature. Some other derivatives were therefore made.

Methionine thiohydantoin, $\text{CH}_3.\text{S}.\text{CH}_2.\text{CH}_2.\text{CH}.\text{CO}$. Following Johnson

and Nicolet [1913], 0.1 g. methionine and 0.25 g. ammonium thiocyanate were added to 3 cc. of acetic anhydride containing two drops of glacial acetic acid. The mixture was heated on the water-bath for 5 minutes, when solution was complete. Further heating does not improve the yield. 10 cc. of water were added, when a reddish-brown oil separated. The mother liquor was decanted, and attempts were made to crystallise the oil. Since this failed, 2 cc. of concentrated hydrochloric acid were added and the mixture was evaporated to dryness. This removed acetyl groups and a crystalline residue was obtained. Extraction with hot acetone and concentration gave fine yellow needles. These were recrystallised from hot alcohol, when they became colourless. A small additional crop was obtained by evaporating the aqueous mother liquor to dryness with 3 cc. of concentrated hydrochloric acid and extracting the residue with hot acetone. The total yield was poor. It was later improved by extracting the oil with chloroform.

Analysis.

5.76 mg. gave 0.841 mg. N (micro-Kjeldahl).

8.53 mg. gave 21.06 mg. BaSO_4 (micro-Carius).

Found: 14.6 % N; 33.8 % S.

Calc. for $\text{C}_6\text{H}_{10}\text{ON}_2\text{S}_2$: 14.7 % N; 33.7 % S.

The thiohydantoin of the natural amino-acid, that of the synthetic acid, and the mixture of both, all melted at 146° , proving their identity.

Methionine picrolonate. 0.1 g. of the amino-acid and 0.18 g. picrolonic acid were dissolved in 5 cc. of water; the solution was boiled for 10 minutes and then evaporated on the steam-bath. The residual sticky syrup was dried *in vacuo* over sulphuric acid and then extracted with hot acetone. When thoroughly dried the picrolonate is not very soluble in cold acetone and crystallises from fairly dilute solution in sickle-shaped pale yellow crystals. It is extremely soluble in water and alcohol, and gives a syrup on evaporation of its solution in either solvent.

The picrolonate of natural methionine melted at 178° , that of synthetic at 178° , and their mixture at 177 – 178° . This also proves the identity. A slight darkening occurs at 173° together with softening, but the actual melting and decomposition point is sharply defined at 178° .

Attempted synthesis by Erlenmeyer's method.

This is only of interest in so far as it illustrates the limitations of a favourite way of synthesising amino-acids, which has of late been very useful in connection with thyroxine, 3 : 4-dihydroxyphenylalanine, and other aromatic amino-acids [cf. Harington and McCartney, 1927]. Erlenmeyer [1893, 1899, 1901, 1904] synthesised only two aliphatic amino-acids, leucine and serine, and described in the former case the condensation product of *isobutyraldehyde* and hippuric acid as an oil "welches nach längerem Stehen zu krystallisieren beginnt." The difficulty with aliphatic aldehydes seems to be that the condensation product is so soluble that it does not readily crystallise out and thus inhibits the reaction. The success with aromatic aldehydes can hardly depend on the acidic character of the benzene nucleus, for Pyman made histidine by this method. In the case of methionine hippuric acid and diketopiperazine were quite useless and the preparation of a few grams of the condensation product with hydantoin required much labour.

Methylthiolacetal, $\text{CH}_3 \cdot \text{S} \cdot \text{CH}_2 \cdot \text{CH}(\text{OC}_2\text{H}_5)_2$. This was the first compound of the kind we made. Methyl mercaptan [Arndt, 1921], from 50 g. of *S*-methylthiourea sulphate and 70 cc. of 5 *N* sodium hydroxide, was passed into a solution of 7 g. of sodium in alcohol and then 30 g. of bromoacetal [Fischer and Landsteiner, 1892] were dropped in. The reaction flask and condenser were cooled by ice. Sodium bromide separated at once; next day it was filtered off and after addition of water the acetal was extracted with ether. B.P. 188 – $190^{\circ}/760$ mm. Yield, 80 % of the theoretical.

Analysis.

0.1628 g. gave 0.2307 g. BaSO_4 .

0.1405 g. gave 0.2045 g. BaSO_4 .

Found: S = 19.9 %.

Calc. for $\text{C}_7\text{H}_{16}\text{O}_2\text{S}$: S = 19.5 %.

Methylthiolacetaldehyde, $\text{CH}_3\cdot\text{S}\cdot\text{CH}_2\cdot\text{CHO}$, was prepared by boiling the acetal for half an hour with 1 % HCl, and extraction with ether. The crude aldehyde boiled at 120–140°; yield, 65 %.

Methylthioethylidene hydantoin, $\text{CH}_3\cdot\text{S}\cdot\text{CH}_2\cdot\text{CH}:\text{C} \begin{array}{c} \text{NH}\cdot\text{CO} \\ | \\ \text{CO}\cdot\text{NH} \end{array}$. Using the

ordinary method of condensation with sodium acetate and acetic anhydride, we at first obtained only a very soluble brown oil. Later, following Wheeler and Hoffman [1911], we used glacial acetic acid instead of the anhydride (which causes acetylation) and two equivalents of sodium acetate. After boiling for 2 hours under reflux, water was added, a little tar was removed by ether, the solution was neutralised and extracted with chloroform. The latter on evaporation left a brown syrup which partly crystallised; the crystals were dried on a plate, washed with ether, recrystallised from acetone and then from water. M.P. 156°. Yield, 50 mg. from 2 g. aldehyde or 1 % of the theoretical. Potassium acetate (a better dehydrating agent) increased the yield to 2–3 %. Condensation at 150–200° in a sealed tube was useless. Ultimately we got a yield of nearly 20 % with acetic acid and potassium acetate, by improving the method of extraction. After boiling for 2 hours the acetic acid was removed by vacuum distillation and the semi-solid residue extracted with chloroform. The extract was washed with sodium carbonate and water, dried, and evaporated. It was dissolved in a little chloroform and ether was cautiously added. This caused some of the condensation product to crystallise. The mother liquor was evaporated, the residue was dissolved in acetone and again treated with ether, giving a further yield of crystals, a maximum total of 1 g. from 3 g. aldehyde.

Analysis.

7.25 mg. gave 1.178 mg. N (micro-Kjeldahl).

7.50 mg. gave 1.216 mg. N (micro-Kjeldahl).

0.0690 g. gave 0.0934 g. BaSO_4 .

0.1030 g. gave 0.1403 g. BaSO_4 .

Found: N, 16.3, 16.2 %; S, 18.6, 18.7 %.

Calc. for $\text{C}_6\text{H}_8\text{O}_2\text{N}_2\text{S}$: N, 16.3 %; S, 18.6 %.

Reduction of the hydantoin condensation product. Sodium amalgam in cold absolute alcohol produced no change. Sodium in hot alcohol gave a few crystals, M.P. 210–213°, but mostly an uncrystallisable syrup; judging by the odour, a volatile sulphur compound was given off during the reduction. Zinc dust and 75 % acetic acid gave the same odour, a syrup and some unchanged substance. Aluminium amalgam gave a similar result.

Attempted condensation of hydantoin and phenoxyacetaldehyde. The acetal of the latter was prepared according to Pomeranz [1894] and more conveniently by dissolving one part of sodium in three equivalents of phenol, adding one equivalent of bromoacetal, boiling for 4 hours and then steam-distilling from alkaline solution; yield, 70 %. The free aldehyde, on condensation with hydantoin by various methods, gave much tar and no crystals.

β-Methylthiolpropionic acid, $\text{CH}_3\text{S}\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{COOH}$, was prepared from mercaptan and ethyl-*β*-iodopropionate. The methylthiol ester distilled at $192^\circ/760\text{ mm.}$, $95^\circ/20\text{ mm.}$; yield, 60 % of the theoretical. It was hydrolysed by boiling for 4 hours with *N* hydrochloric acid. The acid was a colourless oil, B.P. $235\text{--}240^\circ/760\text{ mm.}$

Analysis.

0.0961 g. gave 0.1857 g. BaSO_4 .

0.1863 g. neutralised 15.60 cc. 0.1 *N* NaOH.

S = 26.6 %. Equivalent = 119.4.

Calc. for $\text{C}_4\text{H}_8\text{O}_2\text{S}$: S = 26.7 %. Equivalent = 120.

β-Methylsulphonepropionic acid, $\text{CH}_3\cdot\text{SO}_2\cdot\text{CH}_2\cdot\text{CH}_2\cdot\text{COOH}$, was obtained by oxidising 1.7 g. of the methylthiol acid, accurately neutralised with potassium hydroxide, at 0° with 1.5 g. of potassium permanganate in 100 cc. of water. The sulphone acid was extracted, after acidification and evaporation, by means of alcohol and crystallised at 105° as stated by Schneider [1910], who obtained it by oxidation of the amine from cheirolin. This sulphone acid is very stable and boils without decomposition below 200° at 4 mm. We synthesised this acid and its precursor with a view to obtaining them as degradation products of methionine, before the latter was synthesised. For the characterisation of methionine a sulphone may become useful, but, since the oxidation is irreversible, a sulphone derivative would be useless for isolating methionine. A sulfoxide would be preferable.

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