METHIONINE, a Naturally Occurring Amino Acid containing Sulphur: An Investigation into the Structure and a Synthesis.

by

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I. INTRODUCTION

In 1923 J.H. Mueller (1) published an account of the isolation from casein and other proteins of a new amino acid containing sulphur. He gave the formula as $C_5H_{11}O_2NS$, but was unable to elucidate the structure. The sulphur was not present as a sulphydryl group since the nitroprusside test was negative. There existed a hydrogen atom replaceable by metals since salts could be formed but this hydrogen was thought to be attached to a carboxyl group and not to a sulphinic acid group, because heating the substance in the dry state produced CO_2. The nitrogen was present as an amino group, being given off quantitatively in the Van Slyke amino nitrogen apparatus in three minutes.

The formula suggested ethyl cysteine, $CH_3.CH_2.S.CH_2.CH(NH_2).COOH$, but a comparison of the two substances showed them to be quite dissimilar. For example, in the case of ethyl cysteine the sulphur linkage is broken by boiling for a few minutes with caustic soda and a positive nitroprusside test could then be obtained, while the/

the new amino acid was stable to alkalies.

Two derivatives were prepared, the copper salt and the α-naphthyl isocyanate compound. The melting point of the latter compound was given as 186° C.

In 1925 Sator Odaki (2) described the isolation of a minute amount of the same amino acid from a large quantity of yeast. He obtained 15 grams of crude product from 6000 kilograms of yeast. When purified, this yielded 0.6 grams of pure substance, sufficient only to confirm Mueller's work.

Little is known of the sulphur content of protein and its biological significance. In some proteins the sulphur can be accounted for by cystine but in most cases the amount of cystine is insufficient to account for all the sulphur, and certain proteins which contain no cystine still possess an appreciable content of sulphur. It seemed that Mueller's amino acid accounted for part at least of this discrepancy and further work on its structure and properties appeared to be of some importance. The question of sulphur metabolism is also rather obscure and there was the possibility that new light might be shed on this matter once the particulars of the structure of the amino acid were known./

I. INTRODUCTION

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II. DISCUSSION OF METHODS AND RESULTS

(1) Isolation of amino acid.

Mueller obtained the amino acid by precipitation with mercuric sulphate after hydrolysis of casein. The precipitate was decomposed with baryta, the barium and mercury removed with sulphuric acid and hydrogen sulphide respectively and the solution evaporated to small volume. A second precipitation, this time with mercuric chloride, was brought about, and the precipitate was decomposed by passing hydrogen sulphide into an aqueous suspension. The silver oxide method was used to obtain the free amino acid which was finally crystallised from 75 per cent. alcohol. This method did not give a pure product, the chief contamination being apparently phenyl alanine. By repeating the mercuric chloride precipitation, the substance was obtained pure, but only at the expense of a large loss. The yield of crude product varied from 1-2 grams per lb. of casein. The same method used with gelatin gave smaller yields.

Preliminary experiments were carried out with gelatin/

gelatin for although Mueller obtained smaller yields with it, it was thought that the absence of histidine, tyrosine, and cystine would ensure less bulky precipitates with mercuric sulphate. If so, the time required for the isolation could be considerably shortened as the filtration of the precipitates is extremely slow. About six weeks are required to obtain the final product from about 7 lbs. of casein. Further, the amount of phenyl alanine in gelatin is very much smaller than in casein, and this amino acid appeared to be the chief impurity in the product obtained.

A further modification was introduced in order to dispense with the second precipitation in which the losses are heavy. The product from the first precipitate was esterified and an attempt made to distil the resulting oil. This failed even when charcoal in liquid air was used to give a pressure below 0.1 mm. A portion distilled between 130° and 150° C. but extensive decomposition occurred, a strong odour of volatile sulphur compounds being noted. Sulphur was present both in the distillate and in the residue. Hydrolysis of a little undistilled ester gave only a very small amount of amino acid, so the original method used by Mueller was then adopted.

This work was successful although the yields obtained were smaller than those of Mueller, possibly because of the smaller scale of operations. Later in the research, when more natural product was wanted, experiments were carried out investigating the optimum precipitation with mercuric sulphate. It was found that the amino acid is not appreciably precipitated from acid solution, so that the precipitate which is formed in acid may be removed before neutralisation. At this time also the silver oxide method of removing hydrochloric acid was discarded in favour of the pyridine method (3). This resulted in a much cleaner product and in slightly better yields.

(2) Investigation of structure of amino acid.

The first experimental work carried out with the amino acid was a repetition of Mueller's comparison with ethyl cysteine. The results obtained comfirmed his statement regarding their relative stabilities to alkali.

An attempt was made to obtain a betaine derivative and hence by hydrolysis the corresponding unsaturated acid. This was unsuccessful.

The/

The action of sodium nitrite was also investigated but no appreciable amount of product was obtained pure. Oxidation experiments with potassium permanganate were also tried but in no case was there obtained an amount of product sufficient for identification. As the amount of material available was limited, these experiments were not repeated.

The action of hydriodic acid was investigated since the sulphur atom was probably present as a thio ether, and in that case it was expected that the corresponding alkyl iodide would be liberated. This experiment yielded the first new information regarding the structure of the amino acid since methyl iodide was obtained in almost quantitative yield. The silver iodide gravimetric method was first used to estimate the methyl iodide, and then pyridine was substituted and the pyridinium methyl iodide obtained. This substance being difficult to purify owing to its extreme solubility in water and alcohol, was converted into the mercuric chloride double compound, and compared with a specimen prepared from pyridine amd methyl iodide. The melting point and mixed melting point were identical, thus proving conclusively that the sulphur linkage was that of a methyl thio ether.

Mueller/

Mueller demonstrated the existence of an amino group and a carboxyl group, and if one assumes that the former is attached to the α-carbon atom, as in the majority of naturally occurring amino acids, then only four alternative formulae are possible, viz.

(1) $CH_3.S.CH_2.CH_2.CH(NH_2)\ COOH$

(2) $CH_3.CH(S.CH_3).CH(NH_2).\ COOH$

(3) $CH_3.CH_2.\ C(S.CH_3)(NH_2).COOH$

(4) $CH_3.S.\ CH_2.\ C(CH_3)\ (NH_2).\ COOH$

The third and fourth formulae seemed improbable, and of the other two the normal straight chain structure was favoured, rather than the side-chain alternative, since a closely related substance, Cheirolin, which also occurs naturally, had been described by Schneider[4]. Cheirolin was isolated from the wallflower and its constitution determined and confirmed by synthesis as

$$CH_3.SO_2.CH_2.CH_2.CH_2.N{:}\ C{:}\ S.$$

Amongst/

Amongst the derivatives and intermediates of the synthesis which were obtained by Schneider two were of particular importance as being possible products of (1) decarboxylation and (2) oxidation of the amino acid under consideration. These were:

(1) $CH_3.S.CH_2.CH_2.CH_2.NH_2$

(2) $CH_3.SO_2.CH_2.CH_2.COOH$

An attempt at decarboxylation by heating in diphenylamine proved unsuccessful, and an oxidation experiment yielded only a minute amount of impure substance with a melting point 95-97° instead of 105° C. as required for the sulphone acid. This melting point, however, was not depressed by addition of an equal amount of synthetic sulphone acid. There was not sufficient product from the oxidation to allow of further purification or analysis.

By this time most of the stock of natural amino acid had been used and the indecisive results of the later experiments indicated that a repetition of the isolation from casein on a very large scale would be necessary if sufficient material was to be obtained for further work on the decomposition products/

products. It was therefore considered that an attempt at synthesis on the basis of the suggested formula $CH_3.S.CH_2.CH_2.CH(NH_2).COOH$ would be less costly and probably more fruitful in result.

(3) Synthesis of amino acid.

The methods of synthesis of amino acids used by Erlenmeyer and by Strecker were considered.

Proposed synthesis by Erlenmeyer method.

$$CH_3.CH(OC_2H_5)_2 \rightarrow Br\,CH_2.CH(OC_2H_5)_2$$

$$\rightarrow CH_3.S.CH_2.CH(OC_2H_5) \rightarrow CH_3.S.CH_2.CHO.$$

$$CH_3.S.CH_2.CHO + CH_2\begin{cases} NH-CO \\ CO-NH \end{cases} \rightarrow CH_3.S.CH_2.CH=C\begin{cases} NH-CO \\ CO-NH \end{cases}$$

$$\rightarrow CH_3.S.CH_2.CH_2.CH\begin{cases} NH-CO \\ CO-NH \end{cases} \rightarrow CH_3.S.CH_2.CH_2.CH(NH)_2.COOH$$

Proposed synthesis by Strecker method.

$$CH_2 = CH.CHO \xrightarrow[+\ HCl]{C_2H_5OH} ClCH_2.CH_2.CH(OC_2H_5)_2$$

$$\rightarrow CH_3.S.CH_2.CH_2.CH(OC_2H_5)_2 \rightarrow CH_3.S.CH_2.CH_2.CHO$$

$$\rightarrow CH_3.S.CH_2.CH_2.\underset{NH_2}{\overset{CN}{CH}} \rightarrow CH_3.S.CH_2.CH_2.CH(NH_2)COOH$$

The Erlenmeyer method was first tried although longer since there always appears to be difficulty in avoiding the excessive tarring(and consequent poor yield)which occurs during the hydrolysis of the amino nitrile in the Strecker method.

The synthesis proceeded smoothly until the condensation of the aldehyde with hydantoin was attempted. This did not at first give any result and only after a considerable time and under greatly modified conditions (see Experimental) was it accomplished. Unfortunately all attempts to reduce the product were entirely without success and the method had ultimately to be discarded. It was thought that it might be possible to condense the bromacetaldehyde/

bromacetaldehyde with hydantoin, reduce this compound with hydriodic acid and then introduce the thio methoxy group. This however did not succeed.

A further modification was attempted, that of condensing the corresponding phenoxy-acetaldehyde and hydantoin, with a view to replacing the phenoxy group after reduction. This condensation was also without success.

The method of Strecker was then adopted and although the anticipated trouble during hydrolysis was unavoidable, the desired product was obtained in small yield. Its properties were in all respects identical with these of the natural substance. The same melting point was obtained and no depression occurred with a mixture of the two substances.

(4) Derivatives of amino acid.

The α-naphthyl isocyanate described by Mueller[1] was prepared and the synthetic and natural products were again identical.

Further confirmation was obtained by the preparation of the thio hydantoin and the picrolonate. In each case the products from the synthetic and natural amino acid were identical.

Mueller/

Mueller[1] had determined the optical rotation of the natural product and found it to be very small. This would be expected as the treatment necessary for isolation must certainly produce racemisation. The racemisation is apparently incomplete and will most likely vary in different batches, so that no attempt was made to investigate this point.

The systematic name for this amino acid, α-amino-γ-thio methoxy butyric acid, is rather unwieldy. In such circumstances it is customary to employ a conventional name, which however should be distinctive and to some extent at least descriptive. Professor Barger therefore suggested the name "Methionine", and with the approval of Professor Mueller, this has been adopted.

III. /

III. EXPERIMENTAL.

Attempted isolation of amino acid from gelatin.

2 lbs. of gelatin were hydrolysed by boiling for twelve hours with 6 litres of concentrated hydrochloric acid. The hydrolysate was evaporated to dryness in vacuo to remove as much hydrochloric acid as possible and the residual brown syrup dissolved in 2 litres of water.

A 10% solution of mercuric sulphate in 5% sulphuric acid was added to 5 samples in the ratios 1:2, 1:1.5; 1:1; 1.5:1; 2:1, and the maximum precipitate was obtained at ration 1.5:1. This precipitate is obtained by neutralising to Congo red and is soluble both in acid and in alkali. The mercuric sulphate reagent was therefore added to the bulk of the solution in the ratio of 1.5:1 and a heavy precipitate settled out on neutralising to Congo red. After standing for 48 hours it was filtered, washed thoroughly to remove all sodium sulphate and suspended in water. It was decomposed by passing hydrogen sulphide. The mercuric sulphide was filtered and washed well.

Sulphuric acid was removed quantitatively with baryta/

baryta. The filtrate and washings were then evaporated in vacuo to dryness. Alcohol was added twice and the evaporation repeated in order to remove all water.

300 c.c. of absolute alcohol were added to the residual syrup and dry hydrochloric acid gas passed into the cooled solution. After two hours the evaporation was repeated to remove any water formed. The esterification was then repeated twice and the alcohol removed by distillation. Fresh alcohol was then added and a small amount of insoluble inorganic matter was filtered off. A little ether was added to ensure complete precipitation of inorganic salts.

The free esters were obtained from the hydrochlorides by evaporating the alcohol, suspending the residue in ether, and passing in dry ammonia gas until only ammonium chloride remained insoluble. This was filtered off, and the ether evaporated. A few c.c. of oil remained, and an attempt was made to distil it. At a pressure of 6 mm. distillation occurred, with decomposition, between 130° C. and 150° C. Sulphur was present in the distillate and in the residue, and there was a strong odour of volatile sulphur compounds. On repeating the experiment at a pressure below 0.1 mm. - using

liquid air and charcoal - the same result was obtained.

The remainder of the ester was hydrolysed by refluxing with water until the solution became neutral - about ten hours. On evaporation of the water only a small amount of syrup remained. It contained sulphur and nitrogen and gave a positive ninhydrin reaction. There was insufficient for further work.

Isolation of amino acid from casein by Mueller's Method.(1)

2 lbs. of casein (Laitproto, No. 6) were boiled under a reflux condenser for 40 hours with 6 litres of 25% sulphuric acid. The solution was then neutralised with sodium hydroxide and filtered from the large mass of sodium sulphate which separated on cooling. The filtrate was treated with 15% mercuric sulphate in 7% sulphuric acid, using 1 lb. of mercuric sulphate per lb. of protein.

On neutralising to Congo red and cooling a flocculent precipitate settled and was filtered after standing 24 hours. After thorough washing the precipitate was decomposed by boiling with 2% baryta. It was extracted four times with 2 litres of/

of baryta, after an hour on the steam bath each time. The filtrate was freed from barium and mercury by sulphuric acid and hydrogen sulphide and then evaporated to about 400 c.c.

A second precipitation was brought about with mercuric chloride, by adding 60 grams in boiling saturated aqueous solution. On cooling, a precipitate settled slowly. After standing 24 hours it was filtered, washed thoroughly, suspended in distilled water, and decomposed by passing hydrogen sulphide. After removing the precipitated mercuric sulphide the solution was evaporated in vacuo to remove hydrochloric acid. The residual syrup was dissolved in water and a suspension of silver oxide added to decompose the amino acid hydrochloride. The excess silver was removed with hydrogen sulphide, after filtration of the silver chloride.

On evaporating the clear filtrate to dryness a semi crystalline syrup of crude amino acid remained. This was extremely hygroscopic. It was dissolved in the minimum amount of boiling water and three volumes of boiling alcohol added when the amino acid separated in glistening platelets.

The yield of recrystallised product varied from 0.5 to 1.5 grams per lb. of casein used. In no/

no case was the amino acid obtained pure; after crystallisation it was usually about 85-95% pure, as shown by sulphur content. It was used in this state for qualitative work, since further purification involved a second precipitation with mercuric chloride and an unavoidable loss of much of the material.

Modifications of Mueller's Method.

In order to investigate the method adopted by Mueller, with a view to improving the yield or shortening the time required for isolation - about six weeks - the following modifications were introduced:

After hydrolysis of 6 lbs. of casein the neutralised filtrate was divided into two portions and the precipitation carried out as follows.

First portion.

3 lbs. of mercuric sulphate in 7 litres of 7% sulphuric acid were added and after 24 hours the precipitate was filtered. It was comparatively small, clean, and light brown in colour. Being granular it filtered very readily - precipitate A.

The filtrate was half neutralised and the resulting/

resulting precipitate filtered after 24 hours. It was about 4-5 times the bulk of precipitate A, grey in colour and rather slimy. Filtration was more difficult - precipitate B.

The filtrate was neutralised to Congo red and a third precipitate removed after 24 hours. This was rather larger than precipitate B, dark grey in colour and very slimy. Filtration was extremely slow - precipitate C.

Second portion.

1 lb. of mercuric sulphate in 7% sulphuric acid was added and the solution was neutralised to Congo red. The resulting precipitate was a dirty grey colour, resembling precipitate B in appearance, amount, and filtration time - precipitate D.

The filtrate was treated with 1 lb. of mercuric sulphate as above and the precipitate filtered. It was dirtier and smaller than the previous one, and was extremely slow in filtration - precipitate E.

Another lb. of mercuric sulphate added to the filtrate produced a third precipitate on neutralisation to Congo red. This was almost white in colour but was again very slimy and not easily filtered - precipitate F.

The six precipitates were dealt with separately/

separately in the following way.

Each precipitate was washed four times - twice by decantation after standing 12 hours and twice by filtration. It was then extracted five times with 1 litre of 2% baryta each time, after making the initial suspension just alkaline. The five extracts were combined and a slight excess of sulphuric acid was added to remove all barium. After an hour on the steam bath the excess sulphuric acid was removed by adding a paste of barium carbonate. Hydrogen sulphide was then passed into the hot solution and the precipitate of mercuric sulphide, barium sulphate and barium carbonate removed by filtration. The precipitate was washed thoroughly and all washings added to the filtrate which was then evaporated to about 500 c.c. in an open basin over a gas burner.

A boiling saturated solution of mercuric chloride was then added (20-50 grams of mercuric chloride according to amount of original precipitate) to the hot solution. The precipitate which settled on cooling was filtered after 24 hours, washed thoroughly with cold water, and decomposed by passing hydrogen sulphide into a suspension of the solid in hot water. The mercuric sulphide

was/

was filtered off and washed and the filtrate plus washings evaporated in vacuo to dryness to remove hydrochloric acid. The residue was dissolved in the minimum amount of boiling water, 5-10 c.c. of pyridine added, and then three volumes of boiling alcohol.

The following products resulted:

(a) 2 grams of histidine were obtained, contaminated with traces of sulphur amino acid.

(b) 0.05 grams of sulphur amino acid.

(c) During extraction with baryta the flask broke and the contents fell into the steam bath. An attempt to obtain some product from the 40 litres of water in the steam bath failed - a trace of sulphur amino acid was obtained.

(d) 0.5 grams of amino acid.

(e) 0.5 grams of amino acid.

(f) 0.6 grams of amino acid.

This experiment was not repeated owing to the time required - over two months - but it seems that the extraction would be shortened by proceeding as with Portion 1 and neglecting the first two precipitates. Unfortunately the third precipitate was the one which was lost and no definite conclusions can therefore be drawn.

Preparation/

Preparation of Ethyl Cysteine.

The method used was that of Brenzinger[5] in which the compound is obtained by boiling the mercuric chloride compound of cysteine with ethyl iodide in alcoholic solution.

Cysteine hydrochloride was dissolved in water and a large excess of hot saturated solution of mercuric chloride added. A bulky white precipitate fell down. It was filtered when cold, washed thoroughly and dried. 4 grams of this product were boiled under a reflux with 12 c.c. of ethyl iodide in 20 c.c. of absolute alcohol. The reaction was complete after about six hours on the steam bath. The solution was then poured into water and steam distilled to remove any residual ethyl iodide. After filtration from mercuric iodide, hydrogen sulphide was passed to remove any mercury still present. The filtrate was neutralised with ammonia and evaporated to the point of crystallisation. The yield was about 30% of the theoretical.

Action of alkali on ethyl cysteine and on Mueller's amino acid.

Ethyl cysteine on boiling with dilute caustic soda gives a strong mercaptan like odour in about one/

one minute. The amino acid under investigation withstands prolonged boiling with caustic soda without decomposition. This confirms Mueller's finding.

Attempted preparation of betaine of amino acid.

0.2 grams of amino acid was dissolved in sodium hydroxide and refluxed for two hours with excess methyl iodide in the expectation that an insoluble betaine would be produced. This did not occur, so the reaction mixture was boiled to get rid of any remaining methyl iodide and then refluxed with 40% sodium hydroxide. If a soluble betaine had been formed it would be expected to decompose into trimethyl-amine and an unsaturated acid under this treatment. Trimethyl-amine was evolved and estimated by absorption in standard acid and back titration. Slightly less than 50% of the theoretical amount was obtained. The product was not precipitated on acidification and could not be extracted with ether. The mother liquor failed to yield any of the expected product. The presence of iodine was troublesome and possibly this caused oxidation.

Attempted/

Attempted preparation of hydroxy acid from amino acid.

50 mgm. of amino acid were dissolved in 5 c.c. of water with one equivalent of sodium nitrite. On acidifying with hydrochloric acid a brisk evolution of nitrogen took place. When it finished the solution was warmed on the water bath for an hour and then extracted with ether. Only a microscopic amount of substance was extracted, so the aqueous solution was evaporated to dryness. Extraction of the residue with acetone gave also only a minute amount of material. Boiling alcohol was then used and a little crystalline matter was obtained. This was organic and contained sulphur. It was not very soluble in cold water. There was insufficient for further work.

Action of hydriodic acid.

The method used was that of Zeisel for the determination of methoxyl groups (6) with the modification introduced by Kirpal and Bühn for sulphur compounds (7). About 0.1 gram of amino acid was boiled gently with 10 c.c. of pure hydriodic acid in a stream of carbon dioxide, the alkyl iodide formed being carried through wash-bottles containing red phosphorus in water and cadmium sulphate (to remove iodine and hydrogen sulphide/

sulphide respectively) into an alcoholic solution of silver nitrate. The first wash-bottle was kept in a water bath at 60° C.

About two hours were required to complete the reaction. The precipitate of $AgI.2AgNO_3$ was then washed into about 300 c.c. of distilled water, acidified with dilute nitric acid and left on the steam bath for an hour. The silver iodide was then filtered in a Gooch crucible, dried and weighed.

0.2237 grams of amino acid gave 0.2998 grams silver iodide.

= 85% of theoretical amount.

As the amino acid was known not to be pure a sulphur analysis was carried out.

28.6 mgm. gave 39.45 mgm. of barium sulphate.

= 18.9% sulphur

= 88.0% of theoretical amount.

This showed that the amount of alkyl iodide obtained was equivalent to the amount of sulphur present in the molecule.

The experiment was repeated with ethyl cysteine to see whether ethyl iodide would be obtained quantitatively with the water bath at 60° C. Two attempts/

attempts failed to produce more than 5% of the theoretical amount of alkyl iodide in the silver nitrate absorption bulbs. A third attempt was made with the water bath at 85° C. and about 40% of the theoretical amount of alkyl iodide was obtained. This suggested the existence of a methyl-thio-ether linkage in the molecule.

Confirmation was obtained by repeating the experiment with a further modification of Kirpal and Bühn[8]. By using pyridine to absorb the alkyl iodide instead of silver nitrate a solution of pyridinium alkyl iodide was obtained. Excess pyridine was removed by evaporation on the steam bath, the residue dissolved in water and titrated with N/10 silver nitrate, using potassium chromate as indicator.

0.1023 grams of amino acid --- 5.97 c.c. of N/10 silver nitrate.

= 87% of theoretical amount.

This result was in agreement with the one previously obtained but it had a further significance, in that only methyl iodide reacts quantitatively with pyridine at room temperature.

Samples of pyridinium ethyl and methyl iodides were prepared and their properties examined. They were extremely soluble in water and alcohol so that it/

it was useless to attempt to obtain a pure crystalline product from the Zeisel experiments. The picrate and picrolonates were also very soluble, but it was found that a double compound with mercuric chloride could be prepared and purified easily.

To a solution of the iodide in water, mercuric chloride solution was added until no further precipitate appeared - excess mercuric chloride dissolved the precipitate. The product was filtered and washed with cold water. It re-crystallised from hot water in glistening white platelets. Prolonged boiling should be avoided, as decomposition occurs and mercuric iodide is precipitated.

Melting point of ethyl compound with mercuric chloride	131-133° C. (decomposition)
Melting point of methyl compound with mercuric chloride	161-163° C. "

The product from a repeated Zeisel experiment was then evaporated to dryness on the steam bath and left for several hours to remove all traces of pyridine which itself gives a precipitate with mercuric chloride. The residue was then dissolved in 5 c.c. of water and 2 drops of mercuric chloride solution added. A small

crystalline/

crystalline precipitate separated. It was filtered, washed and recrystallised from hot water. Melting point, 160-163° C.
Mixed melting point with ethyl compound 110-119° C.
Mixed melting point with methyl compound 160-163° C.

This gave conclusive evidence of the existence of a methyl-thio-ether linkage.

Preparation of Methyl Mercaptan.

During the course of this work a number of thio ethers of the general formula $CH_3.S.R$ were prepared by the action of sodium methyl mercaptide on the corresponding halide R.Cl, R.Br or RI. The method used generally was that of passing the methyl mercaptan, as prepared, directly into a solution of sodium ethoxide in alcohol and simultaneously adding the halogen compound drop by drop.

The method described by Arndt (9) for the preparation of the mercaptan was found most convenient, i.e. the hydrolysis of the methylation product of thio urea.

$$C(=S)(NH_2)_2 \rightarrow C(=NH)(NH_2)-S-CH_3 \rightarrow CH_3SH + CN_2H_2$$

76 grams of thio-urea in 50 c.c. of water were gently warmed on a steam bath with 63 grams of acid-free dimethyl sulphate. A vigorous action commenced and the mixture heated up spontaneously. In a few minutes the reaction was over and a clear solution remained. After boiling for about ten minutes, crystals of S-methyl thio-urea sulphate began to separate. The solution was evaporated on the steam bath to a thick paste and an equal volume of alcohol added. After cooling it was filtered. By a repetition of this process a second crop of crystals was obtained from the mother liquor. The yield was 125 grams (90% of theory).

To obtain the mercaptan 70 grams of this salt were warmed gently with 100 c.c. of N/5 sodium hydroxide in a small flask fitted with a reflux condenser to which was connected a receiver strongly cooled in an ice and salt mixture. The methyl mercaptan which collected was a colourless liquid with an extremely offensive odour. It was extremely volatile. (Boiling point 6° C.). The yield was 22 grams - 90% of theory.

It is advisable not to isolate the mercaptan but to pass it directly into the reaction mixture for which it is required.

Preparation /

Preparation of β-iodo propionic ester.

$$I.CH_2.CH_2.COOH \longrightarrow I.CH_2.CH_2.COOC_2H_5$$

7 grams of β-iodo propionic acid were refluxed for an hour in 50 c.c. of absolute alcohol containing 3% of concentrated sulphuric acid (10). On pouring the solution into 100 c.c. of cold water the ester separated and was extracted with ether, dried, and distilled. Boiling point, 200° C. The yield was theoretical.

Preparation of β-thio methoxy propionic acid.

$$I.CH_2.CH_2.COOC_2H_5 \rightarrow CH_3.S.CH_2.CH_2COOC_2H_5$$
$$\longrightarrow CH_3.S.CH.CH_2.COOH$$

5 grams of S-methyl thio-urea sulphate were hydrolysed by boiling with sodium hydroxide and the resulting mercaptan passed into 30 c.c. of alcohol in which 1 gram of sodium has been dissolved. Through an ice-jacketed condenser above the receiver a solution of 8 grams of β-iodo propionic ester in 50 c.c. of ether was dropped slowly into the solution. When all the ester had been added, the reaction mixture was left for several hours at room temperature and then poured into 100 c.c. of cold water. The sodium iodide went into solution and/

and was removed in the aqueous layer. The ethereal layer was dried and distilled. A strongly smelling oil distilled at 192° under atmospheric pressure without decomposition (95° C. under 20 mm. pressure). The yield was 3 grams - 60 per cent. of theory.

The ester was hydrolysed by boiling for four hours with normal hydrochloric acid. The product was insoluble in water but could be extracted with ether, as a colourless oil with a characteristic "sulphur" smell. It distilled at 235-240° C. under atmospheric pressure without decomposition.

Analysis.

Sulphur. (Carius)

0.0961 grams give 0.1857 grams $BaSO_4$

Obtained 26.54% S

Calculated for $C_4H_8O_2S$ ——— 26.67% S

Equivalent weight.

0.1863 grams require 15.60 c.c. of .1000 N. NaOH

Obtained 119.4

Calculated for $C_4H_8O_2S$ ——— 120

Oxidation/

Oxidation of thio ether to sulphone.

$CH_3.S.CH_2.CH_2.COOH \longrightarrow CH_3SO_2CH_2CH_2.COOH$

1.7 grams of propionic acid methyl thio ether were neutralised by titration with potassium hydroxide and the ice-cold solution there was added drop by drop a solution of 1.5 grams of potassium permanganate in 100 c.c. of water. The potassium permanganate was decolorised at once. After standing for an hour the precipitated manganese dioxide was removed by filtration and washed thoroughly with hot water. The filtrate and washings were then acidified and evaporated to dryness on the steam bath and the crystalline residue extracted with boiling absolute alcohol - 50 c.c. in all. On cooling the alcoholic solution gave a deposit of white crystalline needles, melting point 104-105° C. The product distilled without decomposition under a pressure of 4 mm. with oil bath temperature below 200° C. The melting point of the distillation product was unchanged - 105° C. The melting point given by Schneider (4) is 105° C.

Equivalent weight.

0.2120 grams require 13.95 c.c. of ·1 N. NaOH

obtained 151.6

Calculated for $C_4H_8O_2S$ 152

Oxidation/

Oxidation of amino acid with potassium permanganate.

1 gram of amino acid was oxidised by the theoretical amount of potassium permanganate, calculated for the product $CH_3.SO_2.CH_2.CH_2.COOH$, by warming on the water bath to 40° C. Sixteen hours were required before the decolorisation was complete The precipitated manganese dioxide was filtered and washed as before, and the filtrate concentrated, acidified and evaporated to dryness. The alcoholic extract gave only a very small amount of crystalline material, melting point 95-97° C. The melting point was not depressed by mixing with the synthetic product. There was not sufficient material to carry out the purification of the substance.

Attempted synthesis of S-amino acid by Erleymeyer's Method.

Preparation of Bromacetal.

Pinner (11) originally prepared this substance by the action of bromine on acetal, but got a poor yield due to hydrolysis of the acetal by the acid formed. Fischer and Landsteiner (12) introduced the modification of carrying out the reaction in presence of calcium carbonate. This prevents any accumulation of free hydrobromic acid and leads to a considerable improvement in yield.

$$CH_3\,CH(OC_2H_5) + Br_2 \rightarrow Br.CH_2.CH(OC_2H_5)_2 + HBr$$

46 grams of bromine were added drop by drop over a period of an hour to a well cooled mixture of 35 grams of acetal and 15 grams of precipitated chalk. The latter prevented any accumulation of hydrobromic acid which readily hydrolyses the acetal. After the product had stood at room temperature for about an hour, water was added and sodium hydroxide to remove all traces of acidity. The bromacetal separated as an oil and was removed, dried, and distilled. Yield was 31 grams - 50% of acetal used. Boiling point 168-172° C.

Preparation of thio methoxy acetal.

$$BrCH_2CH(OC_2H_5) \longrightarrow CH_3S.CH_2.CH(OC_2H_5)_2$$

Methyl mercaptan was prepared by hydrolysis of 50 grams of S-methyl thio-urea sulphate with 70 c.c. of 5 N. sodium hydroxide and passed directly into a solution of 7 grams of sodium in alcohol. The solution was cooled in ice and had an ice-jacketed condenser attached to the flask. 30 grams of bromacetal were added drop by drop and sodium bromide immediately began to separate. After standing overnight at room temperature the sodium bromide was filtered off and the alcohol distilled on water bath in vacuo as far as possible. Violent bumping /

bumping due to separation of more sodium bromide prevents complete removal of alcohol. Water was added to dissolve sodium bromide and the acetal extracted with ether. The product was dried and distilled. Boiling point, 188-190° C. Yield was 20 grams - 80% of theory.

Analysis.

0.1628 grams - 0.2307 grams $BaSO_4$ - 19.43% S

0.1405 grams - 0.2045 grams $BaSO_4$ - 19.95% S

Calculated for $C_7H_{16}O_2$ S _____ 19.51% S

The large excess of sodium and of methyl mercaptan are necessary to ensure that no bromacetal is present in the product. Owing to the closeness of the boiling points it is difficult to obtain a good fractional distillation.

Hydrolysis of acetal.

$$CH_3S.CH_2.CH(OC_2H_5)_2 \longrightarrow CH_3S.CH_2CHO$$

Shaking for half an hour at room temperature with 5% hydrochloric acid gave less than 10% hydrolysis, with remainder of acetal unchanged. On repeating with 25% hydrochloric acid a brown oil resulted and distilling produced a tar decomposing at 200° C. The hydrolysis was carried out by refluxing with 1% hydrochloric acid for about half an hour.

The/

The product was extracted with ether, dried, and distilled. A little alcohol came over first, then (when acetal was impure) a mixture of bromaldehyde and thio-aldehyde. The fraction between 120° and 140° C. is free from bromo compound. Yield from 14 grams of acetal was 5 grams - 65% of theory.

Preparation of Hydantoin.

$$NH_2.CH_2.COOC_2H_5 \rightleftharpoons NH_2.CO.NH.CH_2.COOC_2H_5 \rightarrow \begin{matrix} NH & - & CO & -NH \\ | & & & | \\ CO & — & CH_2 & \end{matrix}$$

The method of Harries and Weiss (13) was used. 14 grams of glycine ester hydrochloride and 8.1 grams of pure potassium cyanate were mixed in concentrated aqueous solution and cooled in ice for an hour. After a further hour at room temperature, the hydantoic ester which separated was removed by filtration, dried and extracted with absolute alcohol to get rid of traces of potassium chloride. On evaporation of the alcohol the product was obtained pure in about 90% yield. Melting point 135° C.

The hydrolysis of the ester and closing of the ring were effected simultaneously by evaporation to dryness on the water bath with an excess of concentrated hydrochloric acid. The white crystalline residue was dissolved in boiling alcohol and recrystallised/

recrystallised to obtain a quantitive yield of pure hydantoin. Melting point 218° C.

Condensation of aldehyde with hydantoin.

Reference to the literature on the original condensations of aldehydes with hydantoin and hippuric acid by Erlenmeyer, Jun.[14] showed that in practically all cases the aldehyde was an aromatic one. The exceptions were the cases of leucine and serine. Here some difficulty was experienced in obtaining the condensation product in a crystalline state. In the leucine synthesis the product was a yellow oil "welches nach längerem Stehen zu krystallisiren beginnt". This same trouble was experienced in the work which follows.

$$CH_3S.CH_2.CHO + \begin{matrix} NH - CO \\ CH_2 \quad | \\ CO - NH \end{matrix} \rightarrow CH_3S.CH_2.CH = C\begin{matrix} NH - CO \\ \quad | \\ CO - NH \end{matrix}$$

4.5 grams of aldehyde, 4 grams of anhydrous sodium acetate, and 5 grams of hydantoin were refluxed for forty-five minutes with 7 c.c. of acetic anhydride. On cooling the solution set to a semi-solid mass. Filtration yielded only sodium acetate contaminated with a trace of a sulphur containing compound. The filtrate was dark brown in colour and on evaporation left a brown syrup very soluble in/

in ethyl alcohol, methyl alcohol, acetone, chloroform, benzene, and ethyl acetate. All attempts to crystallise anything from these solvents failed. The addition of water or ether gave an amorphous tarry precipitate which could not be crystallised. An attempt to distil the product gave a small amount of yellow oil at 160° C. and 5 mm. pressure. This contained both sulphur and nitrogen but could not be crystallised.

The product from a repeated condensation gave a brown oil on addition of water. This was extracted with chloroform and attempts were made to crystallise the tarry syrup remaining on evaporation of the chloroform. These failed so the syrup was distilled in vacuo and at 140-180° C. under 5 mm. pressure a few drops of yellow oil came over. The product was left overnight in a vacuum dessicator over lime to remove any traces of acetic acid, and in the morning some crystals had appeared. After another night in the ice-chest, the crystals were plated to remove the syrup and the white crystalline solid dissolved in chloroform and recrystallised. It then melted at 90-95° C. and a second crystallisation from acetone gave a big rise in melting point - 152-154° C. Both sulphur and nitrogen were present but only a minute amount of material was obtained/

obtained.

After the chloroform extraction the aqueous mother liquor was acidified with hydrochloric acid and all acetic acid removed by steam distillation. The solution was then neutralised with sodium carbonate and evaporated in vacuo to dryness. The solid residue was extracted with hot absolute alcohol and a white crystalline substance was obtained. This turned out to be unchanged hydantoin (melting point and mixed melting point). The condensation was therefore incomplete and probably the residual aldehyde or its by-products is largely responsible for the non-crystalline nature of the product.

The condensation was repeated and the aqueous solution extracted with a little ether to remove any unchanged aldehyde and then with chloroform after neutralisation. The product was once more a semi-crystalline syrup.

Condensation with hippuric acid.

This experiment was carried out as above with similar results. The solid separating on cooling was sodium acetate. Addition of water gave a brown oil which was extracted with chloroform but could not be crystallised. Distillation gave a sublimate at/

at 120° C. and 5 mm. pressure. It crystallised nicely from hot water and melted at 120° C. It was benzoic acid.

Preparation of diketo-piperazine.

$$2NH_2.CH_2.COOC_2H_5 \longrightarrow \begin{matrix} NH - CH_2 - CO \\ | \qquad\qquad | \\ CO - CH_2 - NH \end{matrix} + 2C_2H_5OH$$

(15)

The method used was that of Fischer . 50 grams of glycine ester hydrochloride were dissolved in 30 c.c. of water and the solution cooled to -5° C. Over a period of an hour there was added drop by drop with constant stirring a solution of 14.5 grams of sodium hydroxide in 30 c.c. of water. After addition of the alkali the solution was left for two hours at room temperature to allow the reaction to finish. Glycine anhydride began to crystallise out and after standing overnight in the ice-chest was removed by filtration. A trace of colour was removed by boiling with about six times the weight of water in presence of a little charcoal, filtering and cooling. The diketo-piperazine separated in colourless needles, melting point 225° C. The yield was 9 grams.

Condensation with diketo-piperazine.

The experiment was repeated as before using glycine anhydride, with similar results. Distillation of/

of the tarry syrup gave a yellow oil at 140-180° C. and 3 mm. pressure. This crystallised from benzene and melted at 92-94° C. After recrystallising from alcohol as short white needles or prisms it melted at 98-99° C.

Analysis showed less than half the theoretical amount of nitrogen and only a trace of sulphur, so that this product was also useless. Only a small amount was obtained.

Attempted modifications of hydantoin condensation.

As the hydantoin condensation seemed the least unprofitable it was repeated using the acetal itself. The result was the same as with the aldehyde. The syrup distilled at 160-200° C. and gave a few crystals after standing over lime in a vacuum dessicator in the ice-chest for 24 hours. Further experiments with both aldehyde and acetal in sealed tubes at temperatures up to 200° C. were also unsuccessful.

An attempt was then made to isolate the product by means of mercuric chloride. The tarry syrup from another condensation was removed from the water - after neutralising with sodium carbonate to remove all acid - by chloroform extraction, dried over sodium sulphate and the chloroform evaporated.

No/

No crystals being obtained the residual syrup was dissolved in 100 c.c. of absolute alcohol and 10 c.c. of boiling saturated alcoholic mercuric chloride added. An amorphous granular precipitate settled on cooling. It was removed by centrifuging, well washed with alcohol, and then hydrogen sulphide was passed into an alcoholic suspension. The mercuric sulphide was filtered, hydrochloric acid removed by silver oxide and excess silver by again passing hydrogen sulphide. On evaporation of alcohol a little non-crystalline syrup remained.

Further amounts of mercury compound separated from the mother liquor on standing, showing that precipitation is incomplete.

Attempted reduction of crude condensation product.

The syrupy product was dissolved in 200 c.c. of alcohol and 200 grams of 4% sodium amalgam added slowly. The solution was stirred vigorously and dilute sulphuric acid added occasionally to prevent accumulation of sodium hydroxide. After four hours the amalgam had all been added and solution was left overnight. The alcohol was then distilled off in vacuo when a brown oil separated from the aqueous residue. This was extracted with chloroform, dried and evaporated, when a brown non-crystalline syrup remained.

This/

This syrup did not seem to reduce cold potassium permanganate in acetone, so was hydrolysed directly with 5 grams of baryta in 30 c.c. of water by refluxing for 20 hours. Ammonia was observed to come off immediately. After cooling, the baryta was neutralised exactly with sulphuric acid and the barium sulphate filtered and washed thoroughly. On evaporation the aqueous solution left a little sticky brown material containing sulphur and nitrogen and reacting positively to the ninhydrin test. There was insufficient for further tests.

The reduction and hydrolysis were repeated on another portion of condensation product and an attempt made to obtain the amino acid by use of mercuric chloride. The result was not successful, as although a precipitate separated with mercuric chloride, the final product was,as before, a minute amount of non-crystalline substance.

Condensation with hydantoin under modified conditions.

In a general investigation into hydantoin condensation Wheeler and Hoffmann (16) discovered that the use of acetic anhydride is not necessary and may even have an adverse effect. This latter is due to acetylation, resulting in a mixture of products. Further they claimed that two equivalents of sodium acetate are necessary for optimum/

optimum yields.

The condensation was therefore repeated using glacial acetic acid only as solvent and doubling the original amount of sodium acetate. After refluxing for two hours water was added and a little tar which separated removed with ether. The solution was then neutralised with sodium carbonate and extracted with chloroform. On evaporation of the solvent there remained a brown syrup which commenced to crystallise on cooling. After standing overnight in the ice-chest, the mixture of crystals and syrup was plated. Washing with a mixture of acetone and ether removed the syrup and most of the colour. The crystalline residue was then recrystallised from acetone from which it separated in fine white needles. It was very soluble in chloroform, soluble in acetone and alcohol, and very slightly soluble in cold water. A further recrystallisation from water gave a product whose melting point could not be raised. Melting point 156.5° C.

The yield was 50 mgms. from 2 grams of aldehyde - about 1% of theory.

Since it had been noticed that potassium acetate is a better dehydrating agent than sodium acetate, it was used in a repeat experiment and the yield was improved to about 2-3%. Less tarring occurred/

occurred.

Further attempts to improve the yield by using the acetal failed as did experiments in sealed tubes at temperatures of 150-200° C. By modifying the method of extraction, however, a much greater amount of the product was obtained:-

After refluxing for about two hours, the acetic acid was removed as much as possible by vacuum distillation and the semi-solid residue extracted with chloroform. The extract was washed with sodium carbonate and water to remove any traces of acid, dried and the chloroform evaporated. A brown semi-crystalline syrup remained. It was dissolved in chloroform and ether added very slowly, with shaking. By this means a small amount of almost pure substance was obtained in crystalline form. Rapid addition of ether gave an amorphous sticky precipitate which would not crystallise. The mother liquor was evaporated to dryness, the residue dissolved in acetone and ether added again slowly. This gave a further yield of less pure substance. By this method the yield was improved to rather less than 20% of the theoretical amount, i.e. a maximum of 1 gram from 3 grams of aldehyde. Experiments on a larger scale were much less/

less successful.

Analysis (of condensation product).

Melting point 156.5°C.

Micro-Kjeldahl.

7.25 mgm. - (5.99 x 0.1937) mgm. N - 16.26% N

7.50 mgm. (6.18 x 0.1937) mgm. N - 16.21% N

Carius.

0.0690 grams gave 0.0934 grams $BaSO_4$ - 18.55% S

0.1030 " " 0.1403 " " - 18.67% S

Calculated for $C_6H_8O_2N_2S$ - 16.28% N

18.60% S

Attempted Reduction of Condensation Product.

(1) Sodium amalgam.

0.5 grams substance were dissolved in 100 c.c. of absolute alcohol and 15 grams of 3% sodium amalgam added over a period of two hours. The solution was stirred continuously and neutralised occasionally with dilute sulphuric acid. After standing overnight the sodium sulphate was filtered off and the filtrate evaporated to dryness. The solid residue was extracted with hot absolute alcohol from which crystals separated on cooling - melting/

melting point 155° C. Mixed melting point with original substance 155-156° C. No reduction had taken place and 0.45 grams of initial substance were recovered.

(2) Sodium and alcohol.

0.5 grams substance (dried at 100° C.) were dissolved in 30 c.c. of absolute alcohol and to the hot solution 3 grams of sodium were added slowly. The product was then boiled for twenty minutes and 100 c.c. of water were added. The sodium hydroxide was neutralised with hydrochloric acid and extracted with chloroform. On evaporation of the latter there remained a brown syrup which was insoluble in benzene, cold alcohol, cold water, but slightly soluble in acetone. After a night in the ice-chest a few crystals appeared. These were plated and washed with acetone and ether mixture. Only a minute amount was obtained - melting point 210-213° C. The aqueous solution after chloroform extraction contained no crystallisable organic substance. Repeated experiments with varying amounts of sodium failed to give more than a trace of this substance each time. The original substance was not recovered. During the reduction a strong odour of volatile sulphur

compounds/

compounds - similar to but not identical with mercaptan - was noted, indicating decomposition.

An attempt was made to hydrolyse the syrup obtained from a repeat experiment by boiling with baryta for six hours. Ammonia was evolved. The barium was removed with carbon dioxide and the solution evaporated to a small volume. At this atage a ninhydrin test was positive. On evaporating to dryness only a little gummy material remained. This was dissolved in a little boiling water and three volumes of alcohol added. On cooling a small amount of gummy material separated on the walls of the vessel. No crystalline product was obtained.

(3) Zinc and acetic acid.

0.5 grams of substance were dissolved in 15 c.c. of 75% acetic acid and 1 gram of zinc dust added slowly to the hot solution. The odour of sulphur compounds again became noticeable. The solution was cooled, water added, and sodium hydroxide to remove acetic acid. A chloroform extract gave a sticky syrup which partly crystallised in the ice-chest overnight. Once again plating and washing yielded only a trace of crystalline material.

A portion of the original substance was recovered from the aqueous solution. This was recrystallised/

recrystallised from alcohol and water - melting point 154-156° C. Mixed melting point with condensation product 154-156° C.

(4) Aluminium amalgam.

0.5 grams of substance were dissolved in 50 c.c. of hot alcohol and 1 gram of aluminium amalgam added. A slow effervescence took place and in about two hours the reaction was finished. After standing overnight the aluminium hydroxide was filtered off and washed with hot alcohol. On evaporation of the alcohol crystals separated. On recrystallising from hot water these melted at 153-155° C. and mixed with the original substance gave the same melting point. Only a portion of the original substance was recovered. The odour of sulphur compounds was again appreciable.

(5) Hydrogen in presence of palladium.

This experiment was also negative owing to the difficulty of obtaining a proper colloidal solution of palladium in aqueous acetic acid which was the only suitable solvent.

Attempts/

Attempts to condense hydantoin with the aldehyde before introduction of sulphur group.

All attempts to condense bromacetaldehyde or bromacetal with hydantoin failed completely.

Preparation of phenoxyacetal.

By heating equivalent amounts of chloracetal and sodium phenate with 5 volumes of alcohol as solvent, in a sealed tube at 200° C. for 8 hours, C. Pomeranz (17) obtained a 70% yield. Boiling point 257° C.

As no autoclave was available only very small amounts could be prepared in this way. A modification was therefore sought.

$$C_6H_5ONa + BrCH_2CH(OC_2H_5)_2 \rightarrow C_6H_5OCH_2CH(OC_2H_5)_2$$

Using bromacetal, phenol, and only enough alcohol to dissolve the sodium, decomposition occurred at 200° C. and the tube burst violently when opened. At 150° C. a 40% yield was obtained. Refluxing at ordinary pressure gave only a minute yield.

The following method enabled a higher temperature to be obtained and gave satisfactory results:

The/

The sodium was dissolved in three times the equivalent amount of phenol. One equivalent of bromacetal was added and the solution was boiled gently under an air cooled condenser for four hours. Tarring occurred to some extent and the product was isolated by steam distillation from alkaline solution. The product was extracted from the aqueous distillate with ether, dried, and distilled. The yield was 70% of the theoretical and 20% of the bromacetal used was recovered at the same time.

Hydrolysis of phenoxy acetal.

$$C_6H_5OCH_2CH(OC_2H_5)_2 \longrightarrow C_6H_5OCH_2CH(OH)_2$$

10 grams of acetal were refluxed for two hours with 30 c.c. of water containing 2 c.c. of dilute hydrochloric acid. The mixture was then steam distilled and from the cooled distillate white crystals of aldehyde hydrate separated. The yield was poor - owing partly to a considerable amount of tarring and partly to the appreciable solubility of the hydrate in water.

Yield, 3.0 grams - 40% of theoretical.

Dehydration/

Dehydration of hydrate.

Pomeranz (17) obtained the anhydrous aldehyde by distilling the hydrate at 30 mm. pressure. Some difficulty was experienced in repeating this work owing to the amount of steam distillation which occurred, and only very small yields were obtained.

The hydrate did not lose its water on standing in vacuo over phosphorus pentoxide for three days at room temperature.

Attempted condensation of phenoxy-aldehyde with hydantoin.

All attempts to obtain a crystalline product after condensation with (a) anhydrous aldehyde or (b) aldehyde hydrate plus a few drops of acetic anhydride, were quite unsuccessful. The product was extremely tarry.

Strecker method of synthesis.

Preparation of β-chlor-propionacetal.

$$CH_2 = CH.CHO \rightarrow ClCH_2.CH_2.CH(OC_2H_5)_2$$

Wohl (18) prepared this substance by adding acrolein slowly to two volumes of absolute alcohol, saturated with dry hydrochloric acid gas. The solution was kept ice-cold and stirred vigorously. After about an hour an oil separated and was removed. A further yield was obtained by resaturating the alcohol/

alcohol with hydrochloric acid. The product was treated with solid sodium bicarbonate to remove all traces of acid, washed with water, dried and distilled in vacuo. The yield was not good.

Calculation showed that only the theoretical amount of alcohol was used, so it should all be converted into acetal. A third saturation with hydrochloric acid produced only the oil with no layer of alcohol. This third amount was treated as before. The total yield of pure substance obtained was 60% of the theory. Boiling point 82-84° C. at 25 mm. pressure; 60° C. at 7 mm. pressure.

Preparation of β-methyl thio-ether of propionacetal.

$Cl\,CH_2\,CH_2\,CH(OC_2H_5)_2 \rightarrow CH_3\,S.CH_2.CH_2\,CH(OC_2H_5)_2$

This was prepared as before by passing excess methyl mercaptan (2 mol.) into sodium in alcohol and dropping in the chloracetal slowly. Yield 70% of theory. Boiling point 96° C. at 20 mm., 89° C. at 14 mm. pressure.

Analysis of S-acetal.

0.1124 grams gave 0.1456 grams $BaSO_4$ - 17.76% S

0.1180 grams gave 0.1552 grams $BaSO_4$ - 18.03% S

Calculated for $C_8H_{18}O_2S$ ———— - 17.97% S

Hydrolysis /

Hydrolysis of acetal.

The acetal was hydrolysed by boiling for half an hour with two volumes of water containing 1-2 c.c. of dilute hydrochloric acid or sulphuric acid. The aldehyde was soluble in hot water but separated on cooling. It was extracted with ether, dried, and distilled. Yield 80% of theory. Boiling point 60° C. at 12 mm. pressure.

Preparation of amino-nitrile.

The method used originally by Strecker (19) in which the aldehyde is mixed with a concentrated solution of ammonium chloride, and potassium cyanide is added slowly, has the objection that extensive tarring takes place. Zelinsky and Stadnikoff (20) introduced a modified method in which the aldehyde is added in ethereal solution and presumably does not come into such close contact with the potassium cyanide. The ammonia and hydrocyanic acid formed pass into the ethereal layer and react with the aldehyde. This method was used and no trouble was experienced with tar during this reaction.

$$CH_3S.CH_2.CH_2.CHO \longrightarrow CH_3S.CH_2.CH_2.CH\begin{matrix}NH_2\\CN\end{matrix}$$

To a well cooled mixture of aldehyde in ether and one equivalent of ammonium chloride in concentrated/

concentrated aqueous solution there was added slowly one equivalent of potassium cyanide in concentrated aqueous solution, with frequent shaking. Excess ammonium chloride (2-3 grams) was then added and the mixture was left in a mechanical shaker for six hours. The ether layer was then separated and the aqueous solution extracted thoroughly with ether. The total ether extract was dried over sodium sulphate and dry hydrochloric acid gas passed in to the well cooled solution. A brown semi-crystalline syrup separated and the ether could be decanted off. Attempts to obtain a pure crystalline specimen of the amino nitrile hydrochloride failed, so the crude product was used.

Hydrolysis of amino nitrile.

$$CH_3S.CH_2.CH_2.\underset{CN}{\overset{NH_2}{CH}} \longrightarrow CH_3S.CH_2.CH_2.CH(NH_2).COOH$$

After removal of the ether two volumes of concentrated hydrochloric acid were added and the solution was warmed on a steam oven to remove ether. It was then boiled for two 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/

crystalline residue (which contained a large amount of tar and ammonium chloride) was dissolved in the minimum amount of boiling water. 10 c.c. of pyridine were added to remove all hydrochloric acid and give the free amino acid which was obtained by adding three volumes of boiling alcohol. On cooling there separated in clusters narrow platelets of amino acid. This was recrystallised from water and alcohol. Melting point 281° C. (decomposition) The yield was very poor and varied somewhat. The average was about 6% of the theoretical, calculating from the amount of aldehyde used.

The following modifications were attempted in order to improve the yield if possible.

(1) Hydrolysis of aqueous solution after ether extraction. In this case the amino acid could only be obtained by means of mercuric chloride owing to the amount of inorganic salts present. Addition of boiling saturated mercuric chloride to the neutralised hydrolysate brought down only a small precipitate showing presence of traces of amino acid only.

(2) Hydrolysis of ether extract with dilute hydrochloric acid. This did not prevent tarring, and gave much poorer yields.

(3) /

(3) Hydrolysis of ether extract with baryta. In this case the tar separated on to the walls of the flask and the solution remained clear. After ten hours boiling the barium was removed quantitatively with sulphuric acid and the filtrate evaporated. A little sticky matter remained. This gave a positive ninhydrin reaction and contained sulphur. No crystals separated on dissolving in the minimum of hot water and adding alcohol.

(4) The condensation was repeated using five equivalents of ammonium chloride and potassium cyanide, but this only resulted in producing a large increase in the amount of tar. Only a minute amount of amino acid was isolated.

(5) A slight improvement in yield was obtained by adding the ethereal solution of aldehyde to a concentrated aqueous solution of one equivalent, each of potassium cyanide and ammonium chloride. The solution was kept ice cold and stirred vigorously.

Analysis of S-amino acid prepared by Strecker synthesis

Melting point 281° C.

Nitrogen (micro-Kjeldahl).

5.91 mgms. amino acid gave (2.81 x 0.196) mgm. N

- 9.32% N

Theoretical - 9.40% N

Sulphur/

Sulphur (micro-carius)

9.38 mgm. amino acid gave 14.5 mgm. $BaSO_4$

- 21.26% S

Theoretical - 21.47% S

Derivatives of Amino-Acid.

α-naphthol isocyanate.

This derivative was prepared by Mueller (1) who gave the melting point 286° C. Odaki (2) confirmed this, giving the melting point 287° C. A specimen was prepared from both the natural and the synthetic amino acid.

0.1 gram of amino acid was dissolved in 6.8 c.c. of N/10 sodium hydroxide and 0.12 gram of α-naphthol isocyanate added. The mixture was left in a mechanical shaker at room temperature for an hour and then filtered. To the clear filtrate there was added just sufficient hydrochloric acid to acidify and a bulky white precipitate was thrown down. Excess acid causes this precipitate to coagulate into a sticky mass which cannot be crystallised. The precipitate was filtered, washed well and dried. It was recrystallised by dissolving in boiling alcohol and adding about three/

three volumes of boiling water. On cooling, short stout needles separated.

Some difficulty was experienced in obtaining the final product in a pure crystalline state. It came down repeatedly in a semi-amorphous condition, even from an alcoholic solution left to evaporate at room temperature. As the substance is insoluble in aromatic hydrocarbon mixtures of these with acetone and alcohol were used as alternatives to the alcohol-water solution, but the result was the same.

The product from the natural amino acid was obtained at the second attempt, the conditions being as far as could be judged identical with those of the first experiment when the product failed to crystallise properly. With the synthetic amino acid several attempts were made to obtain a crystalline product but each time the substance separated in little rosettes of a semi-amorphous character. The melting point could not be raised to the figure given by Mueller and obtained in the case of the natural substance, but no depression in melting point occurred on mixing the two substances.

Melting point of α-naphthol isocyanate of natural amino acid	187° C.
Melting point of α-naphthol isocyanate of synthetic amino acid	181-2° C.
Mixed melting point	181-2° C.

Thio-hydantoin of amino acid.

In 1913 Johnson and Nicolet[21] described the preparation of thio-hydantoins of a number of amino acids. Klason[22] had originally prepared these substances by heating the amino acid ester hydrochloride with potassium thiocyanate but obtained only minute yields. A modification was effected by Komatsu[23] who used acetic anhydride as solvent. The paper of Johnson and Nicolet, however, showed that a great improvement of yield - in some cases theoretical - could be obtained by using ammonium thiocyanate instead of the potassium salt. As the method was simple, this seemed a convenient derivative to prepare on a small scale.

0.1 gram of amino acid and 0.25 gram of ammonium thiocyanate were added to 3 c.c. of acetic anhydride containing two drops of glacial acetic acid. The mixture was heated on a water bath for five minutes when solution was complete. Further heating does not improve the yield. 10 c.c. of water were then added to the solution when a reddish-brown oil separated. The mother liquor was decanted and attempts made to crystallise the oil. These failing, 2 c.c. of concentrated hydrochloric acid were added and the mixture evaporated/

evaporated to dryness. By this means acetyl groups were removed and a crystalline residue obtained. Extraction with hot acetone and concentration of the solvent gave a yield of fine yellow needles. These were recrystallised from absolute alcohol and the pure product was then colourless. A small additional crop of crystals was obtained by evaporating the aqueous mother liquor to dryness with 3 c.c. of concentrated hydrochloric acid and extracting the residue with hot acetone. The total yield was poor. It was improved in a repeat experiment by extracting the oil with chloroform.

Melting point of derivative from natural amino acid ——— 146° C.

Melting point of derivative from synthetic amino acid ——— 146° C.

Mixed melting point ——— 146° C.

Analysis.

N (micro-Kjeldahl)

5.76 mgms gave (4.29 x 0.196) mgm. N - 14.6% N

Calculated for $C_6H_{10}ON_2S_2$ ——— 14.74% N

S (micro-Carius)

8.53 mgm. gave 21.06 mgm. $BaSO_4$ ——— 33.85% S

Calculated for $C_6H_{10}ON_2S_2$ ——— 33.69% S

Picrolonate /

Picrolonate of S-amino acid.

0.1 gram of amino acid with 0.18 gram of picrolonic acid was dissolved in 5 c.c. of water, and the solution boiled for ten minutes and then evaporated to dryness on the steam bath. The residual sticky syrup was dessicated 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 the form of sickle shaped needles of a pale yellow colour. If the substance is not absolutely dry it cannot be obtained easily in a crystalline condition from acetone. It is extremely soluble in water and alcohol and gives a syrup on evaporation of either of these solvents.

Melting point of natural amino acid picrolonate	178° C.
Melting point of synthetic amino acid picrolonate	178° C.
Mixed melting point	177-178° C.

A slight darkening in colour occurs at about 173° C. together with softening, but the actual melting point at 178° is sharply defined, with decomposition.

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