A NEW SULFUR-CONTAINING AMINO-ACID ISOLATED FROM THE HYDROLYTIC PRODUCTS OF PROTEIN.*

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(Received for publication, March 15, 1923.)

During an investigation of the cultural requirements of certain bacteria, the writer isolated from the hydrolytic products of casein, a fraction of material which was apparently required by hemolytic streptococci for satisfactory growth, and which was found on examination to contain a considerable amount of sulfur (1). The sulfur was apparently not in the form of cystine since it did not give the customary reactions (lead blackening and sodium nitroprusside) which are given by the sulfur in this compound. Since the participation, as growth factors under the experimental conditions, of any of the known amino-acids had been ruled out, it was thought possible that this new sulfur compound might be the factor which was being sought from the casein. An attempt was, therefore, made to isolate the material in sufficient quantity and purity to determine its chemical nature; and although the final separation in pure form proved exceedingly difficult, it was found that the sulfur compound lost its activity as a growth-inducing substance when it became even relatively pure. It is not yet clear whether this is due to some kind of chemical change in the sulfur compound, or simply to the elimination of a separate active substance during the purification. In view, however, of the general impression that there was present in protein one or more other sulfur-containing amino-acids, besides cystine, it seemed to be important to carry through the isolation of the

* During the summer of 1922, a portion of the work on purification was carried out in the Biochemical Laboratory, Cambridge University, Cambridge, England, and the writer wishes to express his indebtedness to Prof. F. Gowland Hopkins for this courtesy, as well as for valuable advice and suggestions.
material if it could be done, as a problem of some biochemical importance with possible bacteriological bearing.

The literature on the non-cystine protein sulfur need not be extensively reviewed in this place, beyond referring to the paper of Osborne (2) in which the existence of other forms of sulfur than cystine in the protein molecule has been fairly definitely indicated.

An amino-acid, agreeing closely with the formula $C_3H_{13}SNO_2$, has been obtained as a result of the work. During the progress of the investigation it has been necessary to modify the original method of preparation in many ways, and each modification has resulted in some increase in the yield. However, the writer feels that the present method is by no means quantitative, and that a still greater portion of the sulfur present in protein hydrolysates than that actually isolated can be accounted for on the basis of the compound to be described. It would seem wise, however, to present this compound as a primary constituent of the protein molecule with a good deal of conservatism. It will be shown that there is considerable evidence pointing toward the preexistence of the compound, as such, and while there is no direct evidence to the contrary, the writer does not wish to state definitely that he is convinced that the amino-acid is not formed by some secondary reaction during the hydrolysis or separation. Assurance on this point may well be withheld until more work has been done on the structure and physiological properties of the compound.

The yield obtained by the method described below varies from about 1 to 2 gm. from a pound of casein; i.e., from 0.2 to 0.4 per cent. This represents about ten times the quantity obtained by earlier investigations. Because of this small yield, it has been necessary to use large quantities of casein and three preparations of from 30 to 50 pounds each have been put through. The final purification of the compound has been a matter of much difficulty because of the presence of two or three other amino-acids, particularly phenylalanine and glutamic acid, which are

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1 The writer is indebted to Prof. Ralph McKee of the Department of Chemical Engineering, Columbia University, to Dr. Frederick Zinsser of Zinsser & Company, Hastings-on-Hudson, New York, and to Prof. J. C. Olsen of the Brooklyn Polytechnic Institute for the use of large scale apparatus and equipment necessary for handling this quantity of material through the preliminary stages.
J. H. Mueller

found in considerable quantity in the Ba(OH)₂ extract of the HgSO₄ precipitate. While the latter acid can be separated without a great deal of difficulty by precipitation with barium hydroxide and alcohol, the phenylalanine cannot be removed by recrystallization, nor, quantitatively, by the distillation of the ethyl esters. Before the presence of phenylalanine had been recognized in the mixture, it was found that a constant composition seemed to be reached on recrystallization, and from this mixture which has since been shown to contain phenylalanine and one other unidentified impurity with the sulfur compound, the formula C₁₁H₂₂SN₂O₄ was erroneously deduced, and presented, tentatively, before the Society of Experimental Biology and Medicine in New York last year (3). By the distillation, in vacuo, of the ethyl esters prepared from such a mixture, it developed that the ester of the sulfur compound distilled in the same fraction with that of phenylalanine, and further, that it shared the property of the latter in being relatively insoluble in water and soluble in ether. A small amount of material was, however, obtained in this preparation from the aqueous solution of the esters, which proved to be fairly pure, contaminated only with a small amount of phenylalanine, and from this preparation the formula here presented for the sulfur compound was first calculated.

A review of the earlier modifications of the method and of the various unsuccessful efforts at purification need not be presented here. The method as now used is as follows:

*Preparation of the Compound.*

Casein is hydrolyzed by boiling for 18 hours with six times its weight of water, and from two to three times its weight of concentrated sulfuric acid. The solution is cooled, diluted somewhat with water, and neutralized by the addition of sodium hydroxide solution, or, better, on a large scale with commercial soda ash. The neutral mixture is allowed to cool and, if necessary, is decanted from sodium sulfate which separates if the material is not sufficiently diluted before neutralization. It is then precipitated by a solution of mercuric sulfate containing 10 per cent of HgSO₄ and 5 per cent, by volume, of concentrated H₂SO₄. After adding the reagent, the mixture is neutralized with strong sodium hydroxide solution, to litmus paper. The neutralization brings down a much more bulky precipitate, but it approximately doubles the yield of sulfur compound. It is essential to use care not to carry the neutralization too far, because the compound to be isolated is quite readily soluble in dilute alkali solution. After standing
New Sulfur-Containing Amino-Acid

over night, the precipitate is filtered by suction, or on a filter press, and
the precipitate is washed, thoroughly, with water, resuspending and filter-
ing each time, for at least three or four washings, until the greater part of
the sodium sulfate and unprecipitated amino-acids have been removed.
Filtration is slow and as large a filter as possible should be used. The
precipitate is then extracted with hot 2 per cent barium hydroxide solution,
using approximately a liter of the solution for each pound of casein repre-
sented in the original preparation. In making the extraction, the precipi-
tate is first suspended in water and treated with hot saturated Ba(OH)₂
until the reaction is faintly alkaline to litmus, and then 2 per cent additional
Ba(OH)₂ is added. The mixture is heated for ½ hour, either on the water
bath or with a steam coil, and is then filtered by suction or on a press. It is
necessary to repeat the extraction altogether about four times, in order to
obtain the maximum yield, but after the first extraction, the precipitate is
suspended directly in a 2 per cent Ba(OH)₂ solution. The united extracts
are heated to about 60°, a solution of barium sulfide in water is added to
precipitate the mercury, and sulfuric acid is added to remove the barium.
After filtering, the filtrate is evaporated in an open dish, heated directly on a
gas plate in front of an electric fan, to a volume of about 200 cc., for each
original pound of casein. It is then freed of an excess of either barium or
sulfate, and is precipitated with a mercuric chloride solution. The solution
is first brought to boiling, and then a boiling saturated solution of mercuric
chloride is added, using about 30 gm. of the reagent for each pound of
protein in the preparation. The precipitate will begin to separate in the
hot solution, sometimes as a sticky syrup, and, occasionally, as a semi-
granular material, and it is allowed to stand, best, in the ice box, for 24
hours before filtration. The precipitate will now be found to be either a
coursely granular material or a brittle homogeneous mass on the bottom
and sides of the beaker, depending on the purity of the preparation. The
supernatant fluid is decanted or filtered off, the precipitate washed once or
twice with cold water, and ground in a mortar with distilled water to break
up the lumps. The mercury is removed by adding hot saturated barium
sulfide solution in slight excess, and after stirring for a time the barium is
largely removed by acidifying with sulfuric acid. The precipitated mer-
curic sulfide and barium sulfate are filtered off, and washed by grinding again
in a mortar two or three times with more water, adding each time a small
amount of barium sulfide and sulfuric acid to insure the complete decom-
position of organic mercury derivatives. The combined filtrates are freed
from barium or sulfuric acid, and are evaporated to dryness, in vacuo, to
remove the excess of hydrochloric acid which is formed. The residual
chlorides are taken up in water and treated with fresh silver oxide suspen-
sions until the reaction becomes slightly alkaline; the silver chloride is
filtered off and the filtrate freed from silver with H₂S. After removing the
silver sulfide, the filtrate is evaporated to crystallization, in vacuo, and
then heated on a boiling water bath to bring the crystalline material into
solution. Finally, 3 or 4 volumes of boiling 95 per cent alcohol are added,
and, upon standing, the sulfur compound will separate as shining crystals
and may be filtered off, washed with alcohol, and dried; the mother liquors are concentrated further and a second group of crystals is removed in the same way. The yield of crystalline material varies from 1.5 to 2.5 gm., for each pound of casein, and will be found to be of varying degrees of purity, depending on factors which it has not yet been found possible to define or control. A sulfur determination will indicate the degree of purity, the theoretical being 21.5 per cent, and the crystals should be from 75 to 90 per cent pure. They may be rendered completely pure by carrying out a second mercuric chloride precipitation, exactly as described above, starting with a hot 10 per cent solution of the crystalline material in water, and adding about eight to ten times the weight of mercuric chloride. There is, however, an appreciable loss on reprecipitation, as well as in the first precipitation, and it has not proved possible, so far, to recover, at all quantitatively, the whole of the sulfur compound which is present in the extracts.

The resulting crystals are white and not unlike leucine or phenylalanine in appearance. Under the microscope they are found to be made up of hexagonal plates, often massed together. They are easily soluble in cold water, although when first added, they are moistened by it with some difficulty.

Combustions carried out by the Dennstedt method, permitting simultaneous determination of sulfur, gave the following results.

<table>
<thead>
<tr>
<th>Weight of compound</th>
<th>Volume of water</th>
<th>Differential thermometer reading</th>
<th>Conversion factor</th>
<th>Rise</th>
<th>Molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>gm.</td>
<td>cc.</td>
<td>mm.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2041</td>
<td>0.1384</td>
<td>0.3013</td>
<td>0.3229</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2060</td>
<td>0.1382</td>
<td>0.3061</td>
<td>0.3259</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Nitrogen determinations by the micro Kjeldahl method resulted as follows:

<table>
<thead>
<tr>
<th>Weight of compound</th>
<th>Volume of water</th>
<th>Differential thermometer reading</th>
<th>Conversion factor</th>
<th>Rise</th>
<th>Molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>gm.</td>
<td>cc.</td>
<td>mm.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.20158</td>
<td>0.1384</td>
<td>0.3013</td>
<td>0.3229</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


Found. C 40.27, 40.43; H 7.59, 7.50; N 9.53, 9.40; S 21.73, 21.72.

Molecular weight determinations by the ebullioscopic method, using Menzies, apparatus, with water as a solvent, gave the following results.

Barometric reading, corrected, 760 mm.

<table>
<thead>
<tr>
<th>Weight of compound</th>
<th>Volume of water</th>
<th>Differential thermometer reading</th>
<th>Conversion factor</th>
<th>Rise</th>
<th>Molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>gm.</td>
<td>cc.</td>
<td>mm.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.3345</td>
<td>29.3</td>
<td>16.9</td>
<td>0.002507</td>
<td>0.04389</td>
<td>140.5</td>
</tr>
<tr>
<td>0.4760</td>
<td>29.3</td>
<td>22.8</td>
<td>0.002507</td>
<td>0.05920</td>
<td>148.2</td>
</tr>
<tr>
<td>0.6834</td>
<td>28.5</td>
<td>37.7</td>
<td>0.002505</td>
<td>0.09005</td>
<td>149.8</td>
</tr>
</tbody>
</table>

Average molecular weight found .................. 144.2
Calculated " " .................................. 149.17
Heated in an open capillary, the crystals begin to turn brown and shrink at 278°, and melt with decomposition quite sharply at 283°. In a sealed capillary, slight browning and shrinking of the material occurs at 274°, and it melts with decomposition at 280–281°. The readings are uncorrected.

0.4439 gm. of the compound in 16 cc. of water in a 2 dm. tube rotated the plane of polarized light $-0.4°$.

$$\left[\alpha\right]_D^{\circ} = -7.2°$$

It is possible that the compound is partially racemized by the extraction with hot barium hydroxide solution.

**Preparation of the Naphthol Isocyanate Derivative.**

0.75 gm. of pure sulfur compound was dissolved in 30 cc. of water containing 5 cc. of $\text{n KOH}$, and 1 cc. of $\alpha$-naphthol isocyanate was added. The mixture was shaken for about 5 minutes and allowed to stand for about 1 hour with occasional shaking. It was then filtered by suction and the filtrate acidified with hydrochloric acid. A heavy curdy precipitate separates which was filtered by suction, washed with cold water, and dissolved in about 50 cc. of boiling alcohol. Boiling water was then added until crystallization commenced (2 to 3 volumes), and on cooling a heavy crop of short needles separated. After filtering and washing with cold water, the crystals were dried *in vacuo* over $\text{H}_2\text{SO}_4$ at room temperature. The yield was 1.0 gm. of material which lost no weight on further drying at 110° in an air oven.

The crystals were moderately soluble in cold acetone and 95 per cent alcohol, more so on boiling. They were not appreciably soluble in cold or hot water, benzene, chloroform, or ether.

Melting point (uncorrected) 186°.

<table>
<thead>
<tr>
<th></th>
<th>calculated</th>
<th>(Kjeldahl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2056 gm.</td>
<td>0.1488 gm.</td>
<td>BaSO$_4$</td>
</tr>
<tr>
<td>0.2004 &quot;</td>
<td>0.1436 &quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>0.0463 &quot;</td>
<td>neutralized 14.68 cc. 0.02 n NaOH (Kjeldahl)</td>
<td></td>
</tr>
<tr>
<td>0.1272 &quot;</td>
<td>39.71 &quot; 0.02 n &quot;</td>
<td></td>
</tr>
</tbody>
</table>

$$\text{C}_{14}\text{H}_{18}\text{SN}_2\text{O}_3 = \left(\text{C}_{16}\text{H}_7\cdot\text{NH}\cdot\text{CO}\cdot\text{NH}\cdot\text{CH}\left(\text{COOH}\right)\right).$$

Calculated. N 8.83, S 10.08.

Found. " 8.89, 8.75; S 9.94, 9.84.
Composition of the Mercuric Chloride Precipitate.

The compound produced by mercuric chloride is fairly soluble in boiling water, but separates on cooling. Prepared from a fairly pure specimen of the amino-acid, it separates from the hot solution as a sticky, oily material on the sides and bottom of the beaker, which becomes brittle as the solution cools. From a more dilute solution, it separates as a coarse, granular material, which under the microscope is made up of minute spherules. It has not proved possible to prepare it in crystalline form. The composition appears to be highly complex, corresponding, roughly, in the only specimen analyzed, to the formula (C₆H₁₅SNO₂)₂Hg₅Cl₆.

\[
\begin{align*}
0.1058 \text{ gm.} &: 0.0799 \text{ gm. AgCl.} \\
0.1223 \text{ "} &: 0.0878 \text{ " HgS.} \\
1.0125 \text{ "} &: 0.3077 \text{ " BaSO₄.}
\end{align*}
\]

(C₆H₁₅SNO₂)₂Hg₅Cl₆. Calculated. Cl 17.9, S 4.05, Hg 63.3. Found. " 18.7, " 4.18, " 61.9.

Preparation of Copper Salt.

From the purified sulfur compound, a crystalline copper salt can be easily prepared by treating the hot solution with either copper acetate solution or copper hydroxide or carbonate. The copper salt separates quickly as minute hexagonal plates, light blue in color, which are almost insoluble in cold water, and only moderately soluble in boiling water. This salt is not suitable for purification of the amino-acid, however, since the latter, in the presence of phenylalanine, yields mixed crystals of the copper salts of both amino-acids.

Some of the copper salt prepared as described, using copper acetate, was dried for analysis at 110°.

\[
\begin{align*}
0.1759 \text{ gm.} &: 0.2233 \text{ gm. BaSO₄ and 0.0466 gm. CuS.} \\
(C₆H₁₅SNO₂)₂Cu. \text{ Calculated. S 17.82, Cu 17.66.} \\
& \text{ Found. " 17.44, " 17.61.}
\end{align*}
\]

Preparation of the Compound by Alkali Hydrolysis.

10 lbs. of casein were hydrolyzed by heating with 42 liters of 18 per cent NaOH in a stone jar under slight pressure (varying from 1 to 5 lbs. per sq. in.) in an autoclave for 14 hours. It was then cooled and neutralized with H₂SO₄, and after standing over night the tyrosine which had separated...
was filtered off, and the filtrate precipitated by 10 lbs. of mercuric sulfate as already described, except that after the addition of the mercuric sulfate reagent, the mixture was not again neutralized, but precipitation was allowed to take place in the acid condition. It may be noted here again that under these conditions the yield is approximately half that obtained from a neutral solution, but the precipitate is less bulky and filters more easily. The precipitate, after thorough washing, was extracted ten times with 10 liters of 1 per cent \( \text{Ba(OH)}_2 \) solution in the cold. (The use of hot \( \text{Ba(OH)}_2 \) of 2 per cent concentration has been found to give a much quicker and more complete extraction.) After concentrating, and removing glutamic acid, etc., by a baryta-alcohol precipitation, which has since been found unnecessary, the material was crystallized and later purified by two precipitations with mercuric chloride as already described. The yield was about 3 gm. of pure material. The gross and microscopic appearance is practically the same as that of the material prepared by \( \text{H}_2\text{SO}_4 \) hydrolysis, except that the platelets are somewhat larger and more compact.

Combustions by the Dennstedt method, and micro Kjeldahl determinations of nitrogen resulted as follows:

0.2024 gm.: 0.2984 gm. \( \text{CO}_2 \), 0.1352 gm. \( \text{H}_2\text{O} \), and 0.3175 gm. \( \text{BaSO}_4 \).

0.1995 " : 0.2960 " " 0.1344 " " 0.3153 " "

0.02140 " neutralized 7.14 and 7.19 cc. 0.02 N \( \text{H}_2\text{SO}_4 \).

0.01002 " : (Van Slyke) 1.641 and 1.697 cc. N at 19° and 787 mm.

\( \text{C}_4\text{H}_{11}\text{SNO}_2 \). Calculated. C 40.24, H 7.43, N 9.39, S 21.50.

Found. " 40.18, 40.46; H 7.47, 7.54; N (total) 9.35, 9.41 and N (amino) 9.45, 9.26; S 21.54, 21.70.

Heated in an open capillary the substance melts with decomposition at 265–266°.

In a sealed capillary it melts and decomposes at 262–264°.

0.3133 gm. dissolved in 16 cc. water in a 2 dm. tube did not rotate the plane of polarized light.

**Preparation from Egg Albumin.**

10 lbs. of dry commercial egg albumin were hydrolyzed with 30 liters of water and 30 lbs. \( \text{H}_2\text{SO}_4 \) by heating in a stone jar, placed in an autoclave without pressure, for 20 hours. The resulting solution was neutralized with \( \text{Na}_2\text{CO}_3 \) and precipitated with 10 lbs. mercuric sulfate, and the mixture neutralized with \( \text{NaOH} \). The resulting precipitate, after washing, was extracted seven times with about 14 liters of cold \( \text{Ba(OH)}_2 \) solution, about 1.3 per cent. The extracts after concentrating and precipitating with baryta and alcohol were concentrated and the resulting mixture of amino-acids was recrystallized. The ethyl ester hydrochlorides were prepared by suspending the dry crystals weighing 12.9 gm. in absolute alcohol and saturating with dry \( \text{HCl} \) gas in the usual way. The esters were liberated after distillation of the alcohol, by anhydrous \( \text{Ba(OH)}_2 \) added to an ether
suspension cooled by ice. The ethereal solution was filtered off, the ether distilled in vacuo at room temperature, and the esters were distilled from an oil bath in a vacuum produced by a Geryk pump. A small amount of ester passed over before the bath reached 100°, which has not been identified. More rapid distillation began with the bath at a temperature of 120°, and the vapor at 80–90°. At 90° the distillation was interrupted, the receiver changed, and a fraction collected with the bath between 120 and 160°, and the vapor between 92 and 116°. About half the material had not passed over, and the distillation was carried no further.

The first fraction of distillate (vapor up to 90°) contained only a small amount of sulfur and was not examined further. The second fraction (vapor 92–116°) contained much sulfur, as did the distillation residue. The latter on cooling set to a mass of whitish semicrystalline material. This was insoluble in water and moderately soluble in hot ethyl acetate from which it crystallized on cooling. The resulting crystals apparently did not represent a pure compound, as analysis showed them to have the following composition.

Diketopiperazine of C_{10}H_{16}SNO_{2}, C_{10}H_{16}S_{2}N_{2}O_{3}.

The esters distilling between 92 and 116°, weighing 6.8 gm. were poured into about 20 cc. of water, in which the greater part appeared to dissolve. This was extracted with an equal volume of ether. The aqueous phase was run off and the ether washed twice with 1 to 2 cc. of water, the washings were added to the first aqueous solution. The combined aqueous solution was now washed twice with 3 to 4 cc. of ether to remove as much remaining phenylalanine as possible. The solution was heated on a water bath under an air condenser for 4 hours until the alkaline reaction had disappeared, evaporated with the addition of a little alcohol to facilitate crystallization, and recrystallized once. The yield was only 0.35 gm. The ether solution contained the greater part of the sulfur compound ester mixed with the phenylalanine ester.

Analysis of the 0.35 gm. lot gave the following results.

0.2002 gm.: 0.3019 gm. CO_{2}, 0.1314 gm. H_{2}O, and 0.3039 gm. BaSO_{4}.
0.0199 " neutralized 6.56 cc. H_{2}SO_{4}.
0.0202 " " 6.71 " " The H_{2}SO_{4} was approximately 0.02 N, of which 1.0 cc. was equivalent to 0.2788 mg. N.
Found. C 41.44; H 7.34; N 9.19, 9.26; S 20.85.
It was from this preparation that the formula $C_5H_{12}SNO_2$ was first deduced for the compound. It agrees quite well with that calculated for a mixture containing 97 per cent $C_5H_{11}SNO_2$ and 3 per cent phenylalanine.

**Preparation from Other Proteins.**

No attempt has been made to compare, by the method given above in detail, the yield of sulfur compound obtained from other sources than casein. As already stated, it has been prepared from egg albumin, the yield being roughly the same as from casein. It has also been prepared in an impure form from edestin and wool, the yield being about the same as from casein, and from gelatin, although from the latter protein very little resulted. In the case of wool, which contains most of its sulfur in the form of cystine, it seemed desirable to find out if possible just how much of the sulfur could be accounted for as cystine. A small sample of the same lot of wool which had been used for preparing the new amino-acid was carefully washed with water, alcohol, and ether and dried, and the total sulfur determined by burning 1.242 gm. in the Dennstedt furnace.

0.3030 gm. BaSO$_4$ corresponding to 3.35 per cent sulfur was obtained.

Through the courtesy of Dr. J. M. Looney of the Biochemical Laboratory, Harvard Medical School, the cystine was determined on part of the same washed wool, and found to be 9.1 per cent, corresponding to 2.43 per cent of sulfur. Roughly, 0.9 per cent of sulfur, therefore, remained not accounted for, a part of which may have been in the form of SO$_4$.

**Evidence as to the Existence of the Compound in the Protein Molecule.**

It has been known for some time that the sulfur which is in the protein molecule is not entirely in the form of cystine. Osborne (2) has reviewed the matter thoroughly and has made a careful quantitative study of the proportion of lead blackening to firmly bound sulfur. Whether the amino-acid described in the present paper can be regarded as accounting for a part of the firmly bound sulfur cannot be regarded as definitely established at present, although the evidence furnishes strong indication for so believing.
If it has been produced through a secondary reaction following hydrolysis, the sulfur in the compound must result either from the reagents used, or from some other sulfur-containing nucleus in the protein. In regard to the latter possibility, it has at least been shown that wool, which contains much cystine, gives no larger yield of the compound than does casein, in which cystine, if present at all, is in minimal amounts. As far as sulfur from the reagents is concerned, the evidence is more direct. Sulfuric acid can be definitely ruled out since sodium hydroxide can be used equally well for hydrolysis, and while the precipitation is carried out in the presence of neutral sulfate, it is highly improbable that a compound of the type described could result from such a source. The compound has also been prepared from "aminoids," a commercial protein hydrolysate prepared by enzymes. Sulfur from H$_2$S which may be used in the removal of mercury in place of BaS, has been eliminated in one experiment by the use of hydrogen selenide, which is just as effective in separating the mercury, and while the resulting crystals were not freed from phenylalanine, they corresponded in all their properties to similar mixtures obtained in the usual way.

Structure of the Compound.

The definite structure of the amino-acid has not yet been determined. The type of sulfur linkage is particularly puzzling. From the proportion of hydrogen to carbon, a ring of some sort is less probable than an aliphatic structure. A hydrogen replaceable by metals is present, since the copper salt corresponding to the formula (C$_5$H$_{10}$SN0$_2$)$_2$Cu can be readily formed. The replaceable hydrogen is probably attached to a COOH group, and not an SOOH group, since heating in a dry tube leads to an evolution of CO$_2$. Moreover, at the same time a sulfur-containing complex is split off, having an odor suggesting boiled cabbage, which gives a strong reaction with sodium nitroprusside in alkaline solution. The nitrogen is present in the NH$_2$ form, probably in the $\alpha$ position, since it is given off quantitatively in the Van Slyke amino nitrogen apparatus in 3 minutes. An asymmetric carbon atom is indicated by the optical rotation of the product of acid hydrolysis. Sufficient material has not been available to carry out satisfactory oxidation experiments.
It was suggested to me by Dr. Stewart of the Biochemical Laboratory at Cambridge University, that the compound might be ethyl cysteine, a thio ether, having the structure

$$\text{C}_2\text{H}_5\text{S}-\text{CH}_2\text{CH}-\text{COOH}$$

$$\quad \text{NH}_2$$

This compound was prepared by Brenzinger (4) and by Neuberg and Mayer (5) during the study of the structure of cystine. Its properties as described by them, correspond in some, but not in all, points with the compound here described. Preparation of the ethyl cysteine by the method of Brenzinger, from cystine, proved a simple matter, and the resulting crystals were identical in gross appearance and crystal form with the new compound, but the melting point was definitely lower. The composition of the ethyl cysteine was found to be correct by complete analysis. The chemical properties of the two substances, however, are quite different, since ethyl cysteine, on boiling with even fairly weak NaOH solution (2 to 3 per cent) is broken up with an evolution of ethyl mercaptan and ammonia, while the new compound treated in the same way is apparently quite stable, and, therefore, obviously has a different structure.

The writer hopes to be able to carry out further work on the structure and possible synthesis in the near future.

CONCLUSIONS.

A new amino-acid, which apparently has the formula $\text{C}_3\text{H}_7\text{SNO}_2$, has been isolated from the sulfuric acid hydrolysis products of several proteins, and from casein also after hydrolysis with sodium hydroxide. The yield from casein varies from 0.2 to 0.4 per cent, and is probably not quantitative.

While the writer wishes to be extremely conservative in presenting this compound as a primary cleavage product of protein, there is a certain amount of evidence to indicate that it is not a secondary decomposition product, but is present as such in the protein, and will account for at least a part of the non-lead blackening, or firmly bound sulfur.

The structure has not yet been determined.
BIBLIOGRAPHY.

A NEW SULFUR-CONTAINING AMINO-ACID ISOLATED FROM THE HYDROLYTIC PRODUCTS OF PROTEIN

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