CHEMISTRY.—*Biochemistry by analogy: the sulfur of cystine.*[1]

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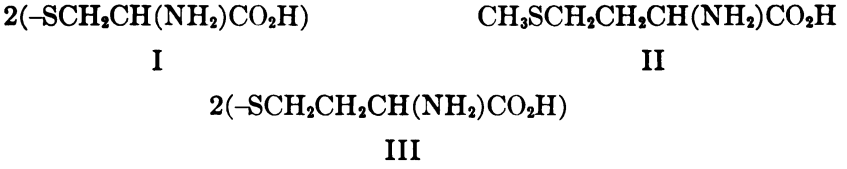
We are, I think, all agreed on the proper way to attack a chemical problem. One should set up a crucial experiment with the substances supposed to be concerned, carry the experiment to completion, and see what happens. But often, particularly in biochemistry, this is hard to do. It may be necessary to carry out an analogous reaction, under supposedly more or less analogous conditions. This is known as a "model" experiment, though it is very far from being a model of what one might wish to do. It frequently amounts to thinking what certain molecules *should* do, and trying to establish, a bit indirectly, whether such a reaction is really plausible. I shall talk to you this evening of "model" experiments, and I intend to see whether I can convince you of the value of the conclusions, as yet incompletely confirmed, which I shall draw from them.

Cystine (I) and methionine (II) are the two best known amino acids containing sulfur which go to make up proteins. Both are readily synthesized by plants, but with regard to animals (including ourselves) both are essential amino acids—or nearly so.

An "essential" amino acid is one which must be supplied in the diet if an animal is to live and grow normally. In other words, the animal body cannot synthesize essential amino acids, or cannot do so in sufficient quantity. According to the latest data,[2] animals can not synthesize methionine under any known conditions. On the other hand, a cystine deficiency can be corrected either by cystine, methionine, or homocystine (III). It is, accordingly, at least very probable that animals *can* synthesize cystine, although only, so far as we yet know, when methionine or homocystine is fed. The problem tonight is, how cystine and methionine get their sulfur, and, in part, how they lose it again.



$2(-SCH_2CH(NH_2)CO_2H)$ — I

$CH_3SCH_2CH_2CH(NH_2)CO_2H$ — II

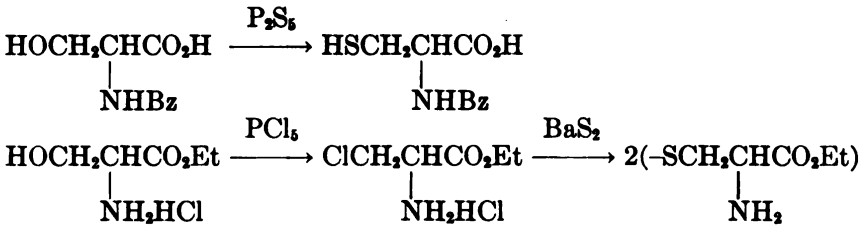
$2(-SCH_2CH_2CH(NH_2)CO_2H)$ — III

There are two syntheses of cystine on record. Erlenmeyer treated benzoylserine with phosphorus pentasulfide. Emil Fischer converted serine ester hydrochloride to β-chloroalanyl ester hydrochloride, and

[1] Address of retiring president, Chemical Society of Washington, January 13, 1938. Received January 28, 1938.

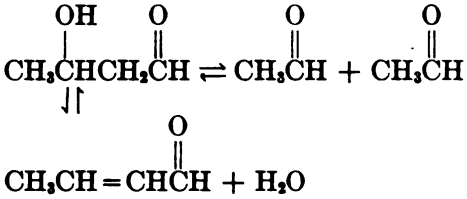
[2] Rose, Science **86**: 298. 1937.

then allowed this to react with barium disulfide. Hydrolysis gave cysteine and cystine, respectively.

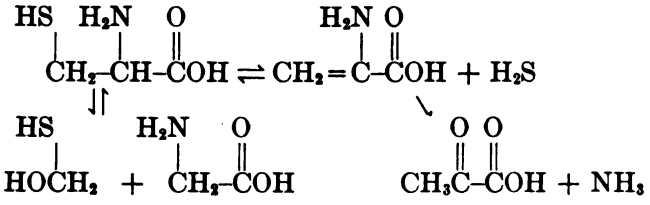


Surely neither of these syntheses approaches the possibility of being a biological synthesis.

A few years ago I talked to you once before[3,4] about cystine. I asked you then to recall the reactions of aldol formation and dehydration, and their reversals. It is particularly necessary to remember that *all* these reactions are reversible.



I presented to you at that time the idea that the desulfurization of cystine by alkali was essentially analogous to the dehydration of an aldol, and should obey the same rules, including reversibility. The work of Dr. H. T. Clarke[5] and his students on the hydrolysis of cystine was entirely in accord with the first part of this notion. It showed very clearly that the chief direction of cystine decomposition by alkali led to hydrogen sulfides, ammonia, and pyruvic acid. In the following equations cysteine is used, for simplicity of presentation, in place of cystine to show the formal analogy to the aldol reactions shown above. Under suitable conditions, reactions analogous to most of these types can be demonstrated.



[3] Nicolet, J. Am. Chem. Soc. 53: 3066. 1931.
[4] Nicolet, J. Biol. Chem. 95: 389. 1932.
[5] Clarke and others, J. Biol. Chem. 94: 541. 1931. 102: 171. 1933. 106: 667. 1934.

At the same time, attention was called to the fact that modification of the cystine molecule in the direction of peptide formation, or, to put it more simply, in the direction of combining the carboxyl group with an amide grouping (as in peptide formation) and the amino group with an acyl group (which *could* be an aminoacyl group) *facilitated* the *removal* of hydrogen sulfide (or disulfide) and *should* facilitate its addition.

It might be argued that the dehydration of an aldol is a very easily occurring reaction, and is due to the activating influence of the aldehyde group. Cystine, on the other hand, offers considerable resistance to alkali; and it is well known that the –CO– grouping present in carboxyl, retains very little of the typical "carbonyl" properties. It was, however, shown quite definitely at that time that mercaptans add very readily to α,β-unsaturated ketones, such as benzalacetone.

$$PhCH=CHCOCH_3 + RSH \rightleftharpoons PhCH(SR)CH_2COCH_3$$

These products lose mercaptans with extreme ease. A quite small fraction of the full "carbonyl" activity would therefore be sufficient to account for the results obtained.

You will perhaps not ask me to repeat my earlier talk further. Let us assume that the reactions eliminating sulfur occur as suggested, and that they are reversible. The simplest reaction by which cystine (or cysteine) could be formed, would be the addition of hydrogen disulfide (or hydrogen sulfide) to α-aminoacrylic acid (IV), which we may also call dehydroalanine, since it represents the removal of two hydrogen atoms from alanine.

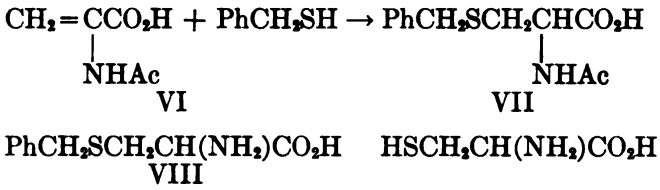
But we shall not expect to be able to demonstrate this reaction, as such, for two reasons. Aminoacrylic acid itself is so unstable that it has never been isolated. And, secondly, we should have much better hope of success if the carboxyl or amino group, or both, were suitably modified. What we should prefer would evidently be a peptide (V) (at least a tripeptide) in which the dehydroalanyl group was not at either end.

$$\underset{\text{IV}}{CH_2=C(NH_2)CO_2H} \qquad NH_2CHR'CO-NH-\underset{\text{V}}{\overset{CH_2}{\overset{\|}{C}}}-CO-NHCHRCO_2H$$

Such a compound was not itself available. For the tentative test, a compromise had to be made. The simplest known derivative of aminoacrylic acid is Bergmann's acetylaminoacrylic acid (VI). It

should not be expected to work very well, but it does work.

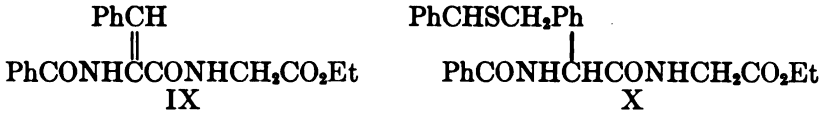
Heated for ten hours at 100° with benzyl mercaptan and a little piperidine as catalyst, it gave a small but definite yield of N-acetyl-S-benzylcysteine (VII). This was hydrolyzed to S-benzylcysteine (VIII).



Dr. Loring was kind enough to apply to this Dr. du Vigneaud's process of reduction by means of sodium in liquid ammonia, for the removal of the benzyl group from sulfur, and obtained a 93 per cent yield of cysteine, as shown by the specific Sullivan method.

This is a new synthesis of cysteine, and therefore of cystine. But certain other requirements must be met if it is to be considered as even a possible model for a biochemical synthesis of these amino acids.

Since a dehydroalanyl tripeptide such as V was not available, benzyl mercaptan was next added to benzoyldehydrophenylalanyl-glycine ester (IX). The resulting mercapto derivative (X) was formed some 50 or 100 times more readily, as estimated by the much better yield obtained in a much shorter time.



Now peptides containing alanine are very common. It is extremely probable that, in their metabolism, they pass, in the presence of suitable enzymes and of suitable hydrogen acceptors (or oxidizing agents), through the stage of dehydroalanine derivatives. I have tried to show elsewhere[6] that this dehydrogenation should take place more readily when the alanine was a component of a peptide chain, and particularly when it was not terminally located. Thus the most commonly formed dehydroalanyl derivatives should be just of the type most suitable for sulfide addition. We have thus acquired, through model experiments, the basis for a picture of the biological synthesis of cystine which is at least somewhat credible.

As a sort of parenthesis, it might well be remarked here that a

[6] Nicolet, Science 81: 181. 1935.

natural synthesis of serine should be based on just the same organic intermediates as the cystine synthesis. It is here merely a case of adding water, instead of sulfide.

Silk proteins contain much more serine than any others. They are also conspicuously richer in alanine than most other proteins, and therefore should offer a richer source of dehydroalanyl derivatives. This is not, I think an accident

I should now like to extend the ideas already advanced to the consideration of the biochemical synthesis of methionine. Only plants can make methionine, for Rose has found it an essential amino acid for animals.

The logical intermediate for its synthesis appears to be methylenepyruvic acid (XI). It is a well known fact that plants have at their disposal for synthetic purposes formaldehyde and pyruvic acid. The condensation of these to form methylenepyruvic acid would be most orthodox. Here again we meet a substance which has never been isolated, presumably on account of its considerable lability.

This is a hindrance to its use by a chemist, but not to its use by a plant. Formaldehyde has been condensed with pyruvic acid by various investigators, and under various conditions. The process has always gone too far, but in such a way as to indicate that methylenepyruvic acid, or possibly hydroxymethylpyruvic acid, which would perhaps serve equally well, has been an intermediate.

$$CH_2O + CH_3COCO_2H \rightleftharpoons \underset{\text{XI}}{CH_2{=}CHCOCO_2H} \rightleftharpoons$$

$$\underset{\text{XII}}{CH_2CH_2(SH)COCO_2H} \rightarrow \underset{\text{XIII}}{CH_2CH_2(SCH_3)COCO_2H} \rightarrow CH_2CH_2(SCH_3)CH(NH_2)CO_2H$$

Whether the addition reaction involves methylmercaptan, or hydrogen sulfide with subsequent methylation, is not at present considered. Plants, in contrast to animals, have a conspicuous capacity for such methylations.

Since the desired substance was not available, recourse was had to another model experiment. Benzalpyruvic acid (XIV) was found to add mercaptans[7] with the greatest ease, under the simplest conditions possible. With no added catalyst whatever, fairly quantitative addition was obtained in five minutes at 100°. It is considered that this is an obviously adequate rate of reaction to justify this stage of the

[7] Nicolet, J. Am. Chem. Soc. 57: 1098. 1935.

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