chlorides of the tertiary alcohols is not a satisfactory reaction, since the purely aliphatic alcohols do not react.

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THE FORMOL TITRATION OF CERTAIN AMINO ACIDS

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The formol titration method of Sörensen[1] has been found to be of great usefulness. Therefore, it seemed desirable, if not imperative, to learn as much as possible about its accuracy or inaccuracy.

The primary cleavage products which proteins yield under the influence of acids, alkalies, enzymes or micro-organisms, are ordinarily referred to as amino acids, for the sake of convenience. Actually, however, they represent a variety of compounds, namely *amino acids* proper, that is, compounds which contain in their molecule one amino and one carboxyl group, such as leucine and alanine, *diamino acids* which contain two amino and one carboxyl groups, such as lysine and ornithine, *hydroxy-amino acids*, such as serine, *imino acids* which contain an imino group instead of the amino group, as is the case with proline and hydroxyproline, *amino-dicarboxylic acids* which contain one amino and two carboxyl groups, such as aspartic and glutaminic acids, *cyclic* amino acids such as tryptophan, and amino acids which contain groups other than those enumerated above, like arginine (guanidine group). Of these compounds, as was pointed out in a previous paper,[2] only the genuine amino acids, containing one amino and one carboxyl group, can accurately be estimated by the formol titration method. It may be added here that also the formol titration of hydroxy-amino acids as well as of amino-dicarboxylic acids yields accurate results, while the other "amino" acids show a greater or less deviation from calculated results. In this paper the titration of several amino acids according to Sörensen's method is given, the formol titration of which has as yet not been reported in the literature as far as the writer is aware. For details of the operations incidental to the formol titrations made, which cannot be given here, the reader is referred to the papers of Sörensen[1] and his associates.

Experimental Part

Cystine is the disulfide of cysteine which is analogous to serine, the latter having the hydroxyl group instead of the sulfhydryl (SH) group.

[1] Sörensen, *Biochem. Z.*, **7**, 45, 407 (1907); *Z. physiol. chem.*, **63**, 27 (1909); **64**, 120 (1910).

[2] Jodidi, THIS JOURNAL, **40**, 1031 (1918).

Inasmuch as serine[3] can accurately be determined by the formol titration method, while the sulfur group, if anything, can have but an acidifying influence on the cystine molecule, it was anticipated as we did, that the formol titration of cystine will give an accurate result. This was corroborated by the direct experiment. For the formol titration l-cystine, a preparation from the research laboratory of Eastman Kodak Company, was used. On purification by crystallization it showed 11.50% of nitrogen. The formula requires 11.66%. Since l-cystine is very slightly soluble in water, sodium hydroxide solution was used as solvent.

Ten milli-equivalents (1.2013 g.) was dissolved in 75 cc. of 0.2 *N* sodium hydroxide solution and the mixture made up with water to 100 cc. Thus, a 0.1 *N* solution was obtained, to each cubic centimeter of which 0.75 cc. of 0.2 *N* sodium hydroxide solution was added in advance. Twenty-cc. portions of this solution formol titrated, with phenolphthalein as indicator, required for their neutralization 5.10 and 5.14 cc. of 0.2 *N* hydrochloric acid, respectively. Since the 20 cc. of cystine solution received in advance 15 cc. of 0.2 *N* sodium hydroxide solution, the titrations secured indicate 99.0 and 98.6% of cystine.

The *hippuric acid* (benzoyl-glycine) used was a preparation obtained from the University of Illinois. Several concordant analyses (Kjeldahl) showed that it had a nitrogen content of 7.75%. The formula requires 7.82%.

Being difficultly soluble in cold water, the hippuric acid was dissolved in alkali, namely, 25 millimoles (4.4775 g.) was dissolved in 187.5 cc. of 0.2 *N* sodium hydroxide solution and the mixture made up with water to 250 cc. To two 20-cc. portions of this 0.1 *N* solution, 10 cc. of neutralized formaldehyde (containing phenolphthalein) was added and titrated. They required 4.92 cc. and 4.91 cc. of 0.2 *N* hydrochloric acid for their neutralization. Taking into consideration that the 20-cc. solutions received in advance 15 cc. of 0.2 *N* sodium hydroxide solution, it means that the results obtained indicate, respectively, 100.8 and 100.9% of hippuric acid.

Two other 20-cc. portions required 4.90 cc. and 4.96 cc. of 0.2 *N* hydrochloric acid for their neutralization. Addition of neutralized formaldehyde to these neutralized solutions had no effect on the end-point of the titrations, which thus indicated 101.0 and 100.4% of hippuric acid, or an average of 100.77% for the four formol titrations. It is true that Henriques and Sörensen[4] have already reported the formol titration of hippuric acid in urine. They did it, however, after its *hydrolysis* to glycine and benzoic acid.

Inasmuch as in the hippuric acid molecule the imino group is neutralized by the C_6H_5CO group, leaving the COOH group free, the hippuric acid as such should be capable of being titrated like any fatty acid, which is actually the case. Two 20-cc. portions of the solution described above required, each, for their neutralization 4.92 cc. of 0.2 *N* hydrochloric acid, thus indicating in each case 100.80% of hippuric acid. These titrations

[3] Sörensen, *Biochem. Z.*, **7**, 74 (1907–1908).

[4] Henriques and Sörensen, *Z. physiol. Chem.*, **64**, 135 (1910).

agree very well with those given above and show that hippuric acid can be estimated by the usual titration as accurately as by the formol titration.

Since methylene-hippuric acid resulting from the treatment with formaldehyde is a stronger acid than the hippuric acid itself, it was hoped that the formol titration of hippuric acid contained in more or less *colored* solutions might give a sharper end-point than the ordinary titration and, hence, be preferable to the latter. To test this point, 20-cc. portions of the solution described above were colored with the same quantity of Bismarck brown solution, whereupon they were formol titrated, or titrated in the usual way. The results of the titrations were practically identical, but the formaldehyde-treated solution was somewhat darker, contrary to what was expected. It is needless to say that compounds similar to hippuric acid, such as aceturic acid, undoubtedly behave in like manner.

Tryptophan, which has an amino group in the side chain and an imino group in the indole ring, should behave, as far as formol titration is concerned, like histidine and proline, which have a similar constitution. Since the formol titration yields about 89% of histidine and 80% of proline, one should expect the formol titration to indicate a percentage of tryptophan in the neighborhood of those values. The experimental results secured were not far in error.

For the formol titration we have used a preparation of the Special Chemicals Company. After repeated recrystallization, it showed a nitrogen content of 13.02%.[5] The formula requires 13.73%.

Owing to the slight solubility of tryptophan in cold water, five millimoles (1.0207 g.) was dissolved in 35 cc. of 0.2 *N* sodium hydroxide solution and the mixture made up with water to 50 cc. Thus a 0.1 *N* solution was secured, each cubic centimeter of which received 0.70 cc. of 0.2 *N* sodium hydroxide solution in advance. Ten-cc. portions of this solution, formol titrated with phenolphthalein as indicator, required for their neutralization 2.62 and 2.66 cc. of 0.2 *N* hydrochloric acid. Considering that the 10-cc. portions received in advance 7 cc. of 0.2 *N* sodium hydroxide solution, the results secured indicate 87.6 and 86.8% of tryptophan, respectively.

Summary

Cystine can be estimated accurately by the formol titration method.

Titration of hippuric acid with standard alkali yields a result as accurate as that obtained by the formol titration, there being no advantage in the application of the latter method.

The formol titration apparently indicates about 87% of tryptophan.

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[5] A further purification could not be effected for lack of material.