Titrimetric Determination of α-Amino Acids via Formation of Dithiocarbamates

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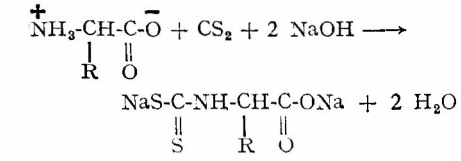
A titrimetric method for the determination of α-amino acids via formation of dithiocarbamates has been developed. The acids have been titrated in aqueous *t*-butanol with sodium hydroxide using phenolphthalein as indicator. The results are found to be accurate.

SORENSEN formol titration[1] for the estimation of amino acids is used extensively in biochemical investigations. However, the method suffers from certain inaccuracies[2]. Thus, accurate results are obtained in the case of such acids which contain amino and carboxyl groups only and in the case of diamino acids, imino acids and those amino acids which contain phenolic hydroxyl or guanido groups inaccurate results are obtained. The Van Slyke procedure[3], though accurate, is tedious. Certain amino acids react abnormally[4] owing to side reactions and yield more than their theroretical amounts of nitrogen. Pope and Stevens[5] developed a method in which the amino acid is complexed with Cu(II) by adding a suspension of copper phosphate. The solution is filtered and Cu(II) is estimated iodometrically. The procedure has, however, been reported to give erratic results[6]. Amino acids may be estimated by non-aqueous titrimetry but the precision and therefore accuracy is not so good as in aqueous medium. The development of a titrimetric procedure based on the following reaction was, therefore, undertaken and the results are reported in this note.



The reaction was rapid and quantitative in the case of amino as well as imino acids. An imino group forming part of an aromatic ring system did not undergo this reaction. The acids were titrated in aqueous *t*-butanol with sodium hydroxide using phenolphthalein as indicator. The end points were stable for about 15 min and the results were accurate even in the case of diamino acids and the amino acids which contain phenolic hydroxyl groups. The results are recorded in Table 1. Due to the development of an intense yellow colour in the case of cystine and cysteine hydrochloride, the titrations were unsatisfactory. In the case of aspartic acid, glutamic acid and arginine monohydrochloride the results were inaccurate. Methanol, ethanol, isopropanol and dioxane were also used as solvents but none was found better than *t*-butanol.

Procedure — To a solution of amino acid (50-100 mg) dissolved in minimum quantity of water (by warming if necessary) were added carbon disulphide (5 ml) and *t*-butanol (50 ml or less so that the amino acid did not precipitate out). The contents were shaken for a few minutes and then titrated with 0·1*N* sodium hydroxide using phenolphthalein (*t*-butanol solution) as indicator. A little more of *t*-butanol may be added during the titration if the solution becomes cloudy at any stage. In order to prevent a local excess of sodium hydroxide from accumulating in the titration medium the solution was stirred. The end point should be stable for at least 5 min. A blank determination was carried out similarly.

TABLE 1 — DETERMINATION OF AMINO ACIDS VIA FORMATION OF DITHIOCARBAMATES*

Amino acid	Added mg	Found mg	Deviation mg
Glycine	107·4	107·9	+0·5
DL-Alanine	101·0	100·5	−0·5
DL-Serine	111·5	111·4	−0·1
DL-2-Amino-*n*-butyric acid	101·8	102·4	+0·6
DL-Threonine	95·2	94·43	−0·77
L-Proline	81·4	80·71	−0·69
DL-Valine	108·5	108·2	−0·3
L-Hydroxyproline	59·1	59·57	+0·47
DL-Methionine	95·8	96·79	+0·99
L-Ornithine monohydrochloride	97·6	98·01	+0·41
L-Lysine monohydrochloride	102·6	102·3	−0·3
DL-Leucine	71·0	71·18	+0·18
DL-Isoleucine	110·5	110·2	−0·3
DL-Norleucine	86·4	86·92	+0·52
L-Histidine monohydrochloride	95·0	94·45	−0·55
DL-3-Phenylalanine	73·8	73·56	−0·24
L-Tyrosine	106·0	105·4	−0·6
DL-3,4-Dihydroxyphenylalanine	45·0	45·15	+0·15
DL-Tryptophan	43·2	42·88	−0·32

*Average of two determinations.

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Estimation of Cu(II) with Fe(II) in Phosphoric Acid Medium in Presence of Thiocyanate Using Cacotheline, Methylene Blue & Thionine as Indicators

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Cu(II) has been estimated by visual titration with Fe(II) in H_3PO_4 (9-10·5*M*) and in presence of potassium thiocyanate (0·25-0·60 ml of 10%) using cacotheline, methylene blue or thionine as indicator. The method has been extended for the determination of copper in Cu-Ni alloy and brass.