LXXXII. THE IDENTIFICATION OF AMINO-ACIDS BY MEANS OF 3:5-DINITROBENZOYL CHLORIDE.

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(Received March 1st, 1934.)

THE present investigation arose as a result of an attempt to prepare an easily obtainable crystalline derivative of oxidised glutathione. The author [1933] has already shown that oxidised glutathione fails to give a dinaphthalenesulphonyl derivative with naphthalene-2-sulphonic chloride, the latter being reduced to naphthalene-2-sulphinic acid.

An attempt was made to prepare the *N*-dibenzoyl derivative of oxidised glutathione by shaking it with benzoyl chloride and alkali according to the usual Schotten-Baumann method. On acidifying the product, a sticky mass was obtained together with a copious evolution of hydrogen sulphide.

It was obvious that the peptide was being decomposed by the alkali, and hence it seemed advisable to cut down the time of benzoxylation to a minimum, a condition which might be accomplished by using 3 : 5-dinitrobenzoyl chloride. Both on the Lapworth theory of alternating polarities [Lapworth and Shoosmith, 1922] and on the more modern electronic theories this should behave as a highly reactive acid chloride. Unfortunately no product could be isolated in the case of GSSG, but this method of benzoxylation using 3 : 5-dinitrobenzoyl chloride was extended to many other amino-acids with success.

It was found to be an exceedingly rapid and efficient method, pale yellow or colourless crystalline derivatives, having sharp melting-points, being usually obtained. The method consists in dissolving the amino-acid in excess of alkali and adding the calculated quantity of finely powdered 3 : 5-dinitrobenzoyl chloride. The mixture is shaken vigorously for about two minutes. The dinitrobenzoyl chloride dissolves almost instantaneously and on acidification the 3 : 5-dinitrobenzoyl derivative separates. In most cases practically no 3 : 5-dinitrobenzoic acid is formed provided that the acid chloride is not in excess.

All the monoamino-monocarboxylic acids examined gave crystalline *N*-3 : 5-dinitrobenzoyl derivatives, which on hydrolysis with hydrochloric acid gave 3 : 5-dinitrobenzoic acid and the original amino-acid.

The behaviour of the monoamino-dicarboxylic acids was rather unexpected. In the case of aspartic acid no product was isolable, but a considerable quantity of 3 : 5-dinitrobenzoic acid was produced. A formaldehyde titration of the filtrate showed that much of the aspartic acid remained unchanged. Glutamic acid also reacted only partially with 3 : 5-dinitrobenzoyl chloride, but in this case a certain amount of the 3 : 5-dinitrobenzoyl derivative was actually isolated. As previously stated no product could be isolated from GSSG.

It seems from this that the NH_2 group in the monoamino-monocarboxylic acids is highly reactive since it reacts rapidly and more or less completely with

3:5-dinitrobenzoyl chloride in the presence of sodium hydroxide. The mono-amino-dicarboxylic acids (and some other "acidic" amino-acids such as tyrosine) appear to have a much less reactive NH_2 group since much (or all) of the 3:5-dinitrobenzoyl chloride is hydrolysed before any condensation can be effected.

This observation is substantiated by the fact that whereas aspartic acid gives no product, and glutamic acid reacts only partially, asparagine and glutamine (which are less acidic) react completely with one¹ molecule of 3:5-dinitrobenzoyl chloride giving high yields of the corresponding mono-dinitrobenzoyl derivatives, practically no 3:5-dinitrobenzoic acid being formed. Furthermore *mesodiamino-succinic* acid gives a theoretical yield of the corresponding di-3:5-dinitrobenzoyl derivative.

The apparent lower reactivity of the NH_2 group in the "acidic" amino-acids compared with the reactive NH_2 group in the "neutral" amino-acids towards 3:5-dinitrobenzoyl chloride in alkaline solution may perhaps be due to the different state of ionisation of this group at the p_{H} employed. As the reaction is a very rapid one such differences might be detected, whereas in the slower ordinary benzoylations using benzoyl chloride, there is opportunity for an equilibrium to be disturbed.

Whatever the cause, this difference in behaviour has permitted of a smooth and complete separation of certain classes of amino-acids. For instance, glycine has been separated from a mixture of aspartic acid and glycine, the latter reacting preferentially with 3:5-dinitrobenzoyl chloride. The separation was not impaired by the presence of large quantities of sodium salts. It is advisable, but not necessary, to remove ammonium salts. They do not interfere with the reaction between 3:5-dinitrobenzoyl chloride and glycine, but some of the acid chloride may be wasted as 3:5-dinitrobenzamide. The latter, however, owing to its insolubility in sodium hydroxide does not contaminate the product.

It has not yet been definitely established whether there is any reaction between 3:5-dinitrobenzoyl chloride and gelatin in alkaline solution, but large quantities of the protein interfere with the reaction between the acid chloride and glycine. A weight of gelatin equal to the weight of glycine can however be present without any deleterious effect.

Glycine has also been separated from glutamic acid and asparagine from aspartic acid. Although glutamic acid does form a derivative, the glycine derivative is formed preferentially.

An efficient separation has also been effected between glycine and leucine, owing to the fact that 3:5-dinitrobenzoylleucine is precipitated from alkaline solution by dilute acetic acid, whereas 3:5-dinitrobenzoylglycine is not. Other possibilities in this direction become evident from details given in the experimental part.

Cystine reacted very rapidly with 3:5-dinitrobenzoyl chloride to give di-(3:5-dinitrobenzoyl)-cystine which was characterised by the formation of a gel in aqueous or alcoholic solutions.

One would expect a different type of derivative in the case of the thiolamino-acids. To determine the reaction of the SH group alone thiolacetic acid was used. A rapid reaction involving the SH group took place as shown by the disappearance of the nitroprusside reaction. On acidification an oil was obtained, which solidified on long standing and from its reactions appeared to be *S*-3:5-dinitrobenzoylthiolacetic acid. Wertheim [1929] was the first to use 3:5-dinitrobenzoyl chloride for the identification of simple mercaptans. His method however was

¹ There is no reaction between 3:5-dinitrobenzoyl chloride and the acid amide group as shown by absence of reaction with acetamide.

different from the above in that he used small quantities of pyridine as a catalyst instead of sodium hydroxide and found that it was necessary to fuse the reactants. It is obviously an advantage to use sodium hydroxide as the amino-acids are usually soluble in this medium. It should be pointed out that he did not use 3 : 5-dinitrobenzoyl chloride in conjunction with the amino group.

3 : 5-Dinitrobenzoyl chloride reacts with cysteine to produce what appears to be the *S*-derivative containing a free NH_2 group. Unlike the *N*-derivatives it does not possess a sharp melting-point. It is a light brown powder which is not easily recrystallised and cannot be recommended for identification purposes.

No reaction could be effected between tyrosine and 3 : 5-dinitrobenzoyl chloride. Neither phenol nor salicylic acid reacted with this reagent in alkaline solution.

Guanidine gives an almost theoretical yield of di-(3 : 5-dinitrobenzoyl)-guanidine. This derivative is insoluble in NaOH and so does not interfere with the identification of the amino-acids by this method.

The three aminobenzoic acids react almost instantaneously with 3 : 5-dinitrobenzoyl chloride, but homogeneous products are not obtained in the case of the *o*- and *p*-compounds. This problem will be discussed elsewhere.

The 3 : 5-dinitrobenzoyl derivatives cannot be analysed satisfactorily by the micro-Kjeldahl method, low results being obtained. Correct results were obtained by the micro-Dumas method.

Buehler *et al.* [1933] have recently described the 3 : 5-dinitrobenzoates of certain amines; these are salts and are distinct from the derivatives described above. The author was unable to obtain the 3 : 5-dinitrobenzoates of glycine and several other amino-acids, a result to be expected.

An attempt was made to prepare the 3 : 5-dinitrobenzoyl derivatives of the amino-acids using pyridine instead of NaOH as the condensing agent. On acidification in each case the same compound was obtained, which proved to be a very stable acid pyridine salt of 3 : 5-dinitrobenzoic acid ($2\text{C}_6\text{H}_3(\text{NO}_2)_2\text{COOH} \cdot \text{C}_5\text{H}_5\text{N}$). This was confirmed by dissolving 3 : 5-dinitrobenzoic acid in excess of pyridine and acidifying, the same product being obtained. This compound had previously been prepared by Pfeiffer [1914].

3 : 5-Dinitrobenzoic acid and all the 3 : 5-dinitrobenzoyl derivatives here described gave an intense violet coloration with acetone and NaOH. This test could be used qualitatively to determine whether the 3 : 5-dinitrobenzoyl residue had entered the amino-acid molecule. The nature of this colour change is now being investigated.

EXPERIMENTAL.

3 : 5-Dinitrobenzoylglycine. 0.75 g. (1 mol.) of glycine was dissolved in 20 ml. *N* NaOH (2 mols.) and 2.32 g. (1 mol.) of finely powdered 3 : 5-dinitrobenzoyl chloride were added. The mixture was shaken vigorously in a stoppered bottle, whereupon the acid chloride dissolved almost immediately, the solution becoming deep red. The shaking was continued for about 2 minutes, the solution filtered if necessary and acidified with dilute hydrochloric acid. The dinitrobenzoyl derivative crystallised out immediately¹. Yield 2.1 g. (80 % theoretical). It was recrystallised from water in long colourless silky needles: m.p. 179°. (Found: C, 37.6; H, 3.08; N, 14.66 %. $\text{C}_6\text{H}_7\text{O}_7\text{N}_3$, H_2O requires: C, 37.63; H, 3.13; N, 14.63 %. Dried at 100°. Found: H_2O 6.0 %. Calc. 6.27 %.)

¹ If an excess of 3 : 5-dinitrobenzoyl chloride were used, this appeared as 3 : 5-dinitrobenzoic acid in the precipitate produced on acidification and could be removed by shaking the precipitate in the cold with a mixture of five volumes of 50–60° light petroleum and two volumes of alcohol. The glycine derivative is insoluble in this mixture. If the acid chloride were not used in excess, it was usually found unnecessary to carry out this treatment.

Hydrolysis of 3:5-dinitrobenzoylglycine. The compound was boiled with HCl (2 vols. HCl, 1 vol. water) for 1½ hours. On cooling 3:5-dinitrobenzoic acid crystallised out and was recrystallised from water: m.p. 207°. (Found: N, 13.1%; $C_7H_4O_6N_2$ requires: N, 13.2%.)

A formaldehyde titration of the filtrate showed that the hydrolysis to glycine was complete.

The 3:5-dinitrobenzoyl derivatives of the monoamino-monocarboxylic acids described below were prepared in a manner similar to the above. Each derivative dissolved in strong alkali to give a dark red colour discharged by acids. Dilute HCl was used for precipitation from the alkaline solution unless otherwise stated.

3:5-Dinitrobenzoyl-dl-alanine. Recrystallised from water in minute pale yellow needles: m.p. 177°. (Found: N, 14.88%. $C_{10}H_9O_7N_3$ requires: N, 14.84%.)

3:5-Dinitrobenzoyl-l-leucine. Precipitated by dilute acetic acid. Only slightly soluble in boiling water, from which however it can be recrystallised in long colourless needles. Recrystallised more readily from 50% aqueous alcohol: m.p. 186–7°. (Found: C, 48.06; H, 4.6; N, 12.62%. $C_{13}H_{15}O_7N_3$ requires: C, 48.0; H, 4.62; N, 12.92%.)

3:5-Dinitrobenzoyl-d-valine. Precipitated by dilute acetic acid. Recrystallised from water in colourless feathery masses: m.p. 157–8°. (Found: N, 12.78%. $C_{12}H_{13}O_7N_3$, H_2O requires: N, 12.76%. Found: H_2O , 5.5%. Calc. 5.47%.)

3:5-Dinitrobenzoyl-l-phenylalanine. Precipitated by dilute acetic acid. In order to purify it, it was dissolved in alcohol, diluted with water until just cloudy and placed in the ice-chest to crystallise. Short colourless needles: m.p. 93°. (Found: N, 11.54; H_2O , 2.9%. $C_{16}H_{13}O_7N_3$, 0.5 H_2O requires: N, 11.69; H_2O 2.45%.)

3:5-Dinitrobenzoyl-l-asparagine. Asparagine reacts with one molecule of 3:5-dinitrobenzoyl chloride to give the above compound using the method already described. Yield = 90%. Recrystallised from water in very thin flat glistening leaflets: m.p. 196°. (Found: N, 17.19%. $C_{11}H_{10}O_8N_4$ requires: N, 17.18%.)

3:5-Dinitrobenzoyl-d-glutamine. Formed from one molecule of glutamine and one molecule of 3:5-dinitrobenzoyl chloride. Yield, 95%. Recrystallised from water in colourless radiating needles: m.p. 217°. (Found: N, 16.38%. $C_{12}H_{12}O_8N_4$ requires: N, 16.47%.)

N-3:5-Dinitrobenzoyl-S-benzylcysteine. 1.05 g. of *S*-benzylcysteine were dissolved in 10 ml. of *N* NaOH and 1.15 g. of 3:5-dinitrobenzoyl chloride added. The mixture was shaken until all the acid chloride had dissolved. A strong mercaptanic odour was noticeable, owing presumably to the partial hydrolysis of the *S*-benzylcysteine by the alkali. The solution was filtered and acidified with dilute acetic acid, a white feathery crystalline precipitate being formed. Recrystallised from aqueous alcohol in long slender needles: m.p. 92°. It was subsequently found advantageous to carry out the reaction at 0°, in which case no mercaptanic odour was perceptible. The reaction was complete in a few minutes at this temperature. Yield, 90%. (Found: N, 9.69; S, 7.26%. $C_{17}H_{15}O_7N_3S$, 2 H_2O requires: N, 9.52; S, 7.26%.)

N-3:5-Dinitrobenzoyl-S-ethylcysteine. As for *S*-benzylcysteine. Precipitated by acetic acid. Recrystallised from aqueous alcohol in colourless needles: m.p. 65°. Hygroscopic. (Found: N, 12.26; S, 9.03%. $C_{12}H_{13}O_7N_3S$ requires: N, 12.24; S, 9.33%.)

3:5-Dinitrobenzoyl-l-methionine. Unlike *S*-ethyl- and *S*-benzyl-cysteine, methionine could be acylated at room temperature without any decomposition. Recrystallised from aqueous alcohol in slender silky needles: m.p. 94–5°; anhydrous substance has m.p. 150°. Very soluble in cold alcohol. (Found: C, 40.76; H, 4.08; N, 12.14; S, 8.98%. $C_{12}H_{13}O_7N_3S$, 0.5 H_2O requires: C, 40.91; H, 3.98; N, 11.93; S, 9.09%.)

3:5-Dinitrobenzoyl-l-tryptophan. 1 mol. of tryptophan reacted with 1 mol. of 3:5-dinitrobenzoyl chloride. An orange precipitate was produced on the addition of dilute acetic acid to the alkaline solution. Recrystallised from alcohol as an orange crystalline powder. Insoluble in boiling water. m.p. 233° (decomp.). (Found: C, 54.3; H, 3.54%. $C_{18}H_{14}O_7N_4$ requires: C, 54.27; H, 3.52%.)

Di-(3:5-dinitrobenzoyl) mesodiaminosuccinic acid. 1 mol. of mesodiaminosuccinic acid reacted with 2 mols. of dinitrobenzoyl chloride in the presence of 4 mols. of *N* NaOH. On the addition of dilute acetic acid to the alkaline solution a crystalline precipitate slowly formed. The filtrate gave no precipitate with HCl showing that no 3:5-dinitrobenzoic acid had been produced. The precipitate was insoluble in boiling water, alcohol, acetone, benzene and glacial acetic acid. It dissolved in alkali with the production of a deep red colour. In order to purify it, it was dissolved in NaOH,

water was added until the red colour was just discharged and dilute acetic acid was added to reprecipitate it as a colourless crystalline powder. Melted with decomp. 254° . (Found: N, 16.03 %. $C_{18}H_{12}O_{14}N_6$ requires: N, 15.67 %.)

Di-(3 : 5-dinitrobenzoyl)-d-arginine. 1 mol. of arginine hydrochloride reacted with 2 mols. of the acid chloride. Precipitated from the alkaline solution by dilute acetic acid. The product so obtained was boiled with alcohol and filtered from a small quantity of tarry residue. On the addition of water a colourless crystalline powder gradually separated. M.P. indefinite, shrinks and softens about 150° . (Found: C, 41.9; H, 3.69; N, 18.95; H_2O , 1.4 %. $C_{20}H_{10}O_{12}N_8$, 0.5 H_2O requires: C, 42.03; H, 3.51; N, 19.61; H_2O 1.58 %.)

Di-(3 : 5-dinitrobenzoyl)-l-cystine. 0.48 g. of cystine (1 mol.) dissolved in 8 ml. *N* NaOH reacted with 0.93 g. of the acid chloride (2 mols.). On acidifying the alkaline solution with dilute HCl a gummy mass was precipitated which became friable on standing. This was filtered off and purified by dissolving in hot alcohol, filtering and adding water until just turbid. The liquid was then warmed until just clear and allowed to stand. The whole mass set to a gel which was broken up, filtered and dried in a desiccator, giving a powder which gradually turned brown in the course of a few days. Darkened at 154° , melted with decomposition about 180° . (Found: N, 12.41, 12.54; S, 9.38, 9.67 %. $C_{20}H_{16}O_{14}N_6S_2$, 2 H_2O requires: N, 12.66, S, 9.64 %. Loss of H_2O at 100° , 4.4 %. Cal. 5.1 %. Found for anhydrous substance N, 13.13 %. Calc. 13.37 %.)

The dry powdered product again formed a gel when dissolved in alcohol and diluted with water. A gel was also formed from aqueous acetone, dilute HCl and dilute acetic acid. Insoluble in benzene. The nitroprusside test was given after reduction with Zn and HCl.

Di-(3 : 5-dinitrobenzoyl)guanidine. 0.54 g. of guanidine sulphate was dissolved in 10 ml. *N* NaOH, 1.15 g. of powdered 3 : 5-dinitrobenzoyl chloride added and the mixture shaken for about 2 minutes. The guanidine derivative (insoluble in NaOH) was filtered off and recrystallised from alcohol in radiating clusters of colourless needles. Insoluble in water, moderately soluble in acetone, slightly soluble in benzene: M.P. 280° with blackening (shrinks about 215°). The filtrate from the reaction gave no precipitate with HCl, showing that all the acid chloride had reacted. (Found: N, 22.05 %. $C_{15}H_8O_{10}N_7$ requires: N, 21.93 %.) The substance was hydrolysed by boiling with 1 : 1 HCl for 1 hour. On cooling 3 : 5-dinitrobenzoic acid crystallised out.

Reaction between d-glutamic acid and 3 : 5-dinitrobenzoyl chloride. 0.73 g. (1 mol.) of glutamic acid was dissolved in 15 ml. of *N* NaOH. 1.15 g. (1 mol.) of powdered 3 : 5-dinitrobenzoyl chloride were added and the mixture shaken for 2 minutes. Nothing was precipitated on the addition of dilute acetic acid. Acidification with HCl gave an oil which crystallised on standing. After some time the crystalline precipitate was filtered off and the filtrate made up to 100 ml. It still contained unchanged glutamic acid. Using Sørensen's formaldehyde titration, 10 ml. of this solution required 2.1 ml. of *N*/10 NaOH. This indicated that 42 % of the glutamic acid was unattacked.

The precipitate was dissolved in cold alcohol (in which it was very soluble). Water was added until just cloudy, and the mixture was allowed to stand, whereupon 0.17 g. 3 : 5-dinitrobenzoic acid separated. The alcohol was evaporated from the filtrate and 3 : 5-dinitrobenzoylglutamic acid crystallised out. It was recrystallised from water as colourless needles. The substance softens and shrinks at $98-99^{\circ}$. (Found: C, 41.24; H, 3.26; N, 11.89 %. $C_{12}H_{11}O_9N_3$, 0.5 H_2O requires: C, 41.14; H, 3.28; N, 12.0 %. Loss in weight when dried at 100° , 2.4 %. Calc. 2.6 %. Found for anhydrous substance: N, 12.45 %. Calc. 12.32 %.)

It was subsequently found that a better separation of 3 : 5-dinitrobenzoic acid from the glutamic acid derivative could be obtained as follows. The reaction was carried out as above except that 1.5 g. of sodium acetate were added before the addition of the dinitrobenzoyl chloride. On acidifying with HCl, 3 : 5-dinitrobenzoic acid separated in crystalline form (0.53 g.). The filtrate on standing for a few minutes deposited 3 : 5-dinitrobenzoylglutamic acid (0.54 g.). This was then recrystallised from hot water.

Reaction between aspartic acid and 3 : 5-dinitrobenzoyl chloride. 0.66 g. of aspartic acid was dissolved in 15 ml. of *N* NaOH and 1.15 g. of powdered 3 : 5-dinitrobenzoyl chloride added and the mixture shaken for 2 minutes. No precipitate was produced on acidification with dilute acetic acid, but 0.5 g. 3 : 5-dinitrobenzoic acid separated at once on addition of dilute HCl. Formaldehyde titration showed that 50 % of the aspartic acid remained unchanged.

Separation of glycine from aspartic acid. A mixture of 0.38 g. of glycine and 0.66 g. of aspartic

acid was dissolved in 20 ml. of *N* NaOH. 1.3 g. of 3 : 5-dinitrobenzoyl chloride were added (an excess for the glycine present). The mixture was shaken, filtered and acidified with dilute HCl. The precipitate, which consisted of a mixture of the glycine derivative and 3 : 5-dinitrobenzoic acid, was dried and shaken with light petroleum-alcohol (5 : 2) mixture in the cold. The residue was recrystallised from water: m.p. 178°. The presence of large quantities of sodium acetate and other sodium salts did not impair the separation.

In the following mixtures the first named component was separated by a method similar to the above: asparagine from aspartic acid; glycine from gelatin (an equal weight); glycine from glutamic acid.

In presence of gelatin an excess of alkali was required. Also the product on acidification was usually oily, but crystallised if allowed to stand for some time. For the mixture of glycine and glutamic acid it is an advantage to have as little excess of 3 : 5-dinitrobenzoyl chloride as possible (calculated for the glycine).

Separation of leucine and glycine. A mixture of 0.33 g. of leucine and 0.19 g. of glycine was dissolved in 10 ml. of *N* NaOH. 1.15 g. of 3 : 5-dinitrobenzoyl chloride were added and the mixture shaken. On acidification with acetic acid 3 : 5-dinitrobenzoylleucine crystallised out. After standing for some time (to ensure complete precipitation) this was filtered off and recrystallised from aqueous alcohol: m.p. 186°.

Dilute HCl was added to the filtrate to precipitate the glycine derivative. Recrystallised from water: m.p. 178–179°.

Reaction between thiolacetic acid and 3 : 5-dinitrobenzoyl chloride. 0.92 g. of thiolacetic acid was dissolved in 20 ml. of *N* NaOH and shaken with 2.30 g. of 3 : 5-dinitrobenzoyl chloride. The nitroprusside test became negative almost immediately. On acidification either with dilute acetic or hydrochloric acid an oil was produced which solidified on long standing. The substance proved difficult to recrystallise. (Found for unrecrystallised substance: N, 10.3; S, 12.0 %. Calc. for $C_9H_9O_7N_2S$: N, 9.8; S, 11.19 %.) Melts with decomp. about 135°. Very soluble in alcohol and acetone, slightly soluble in benzene. When recrystallisation was attempted by dissolving in alcohol and adding water, an interchange of radicals evidently took place as the substance which crystallised out contained no sulphur and proved to be ethyl 3 : 5-dinitrobenzoate.

The product of the condensation was undoubtedly *S*-3 : 5-dinitrobenzoylthiolacetic acid. It was completely hydrolysed by boiling with 5*N* HCl for 30 minutes. On cooling 3 : 5-dinitrobenzoic acid separated out, and the filtrate gave a strong nitroprusside reaction.

Reaction between cysteine and 3 : 5-dinitrobenzoyl chloride. 0.79 g. of cysteine hydrochloride (1 mol.) was dissolved in 15 ml. of *N* NaOH and shaken with 1.15 g. (1 mol.) of the acid chloride. The nitroprusside test became negative almost immediately. The solution was filtered and a brown powder precipitated by the addition of dilute acetic acid. The product was purified by dissolving in alkali and reprecipitating with acetic acid. Melted with decomp. about 160°. Very soluble in alcohol and acetone, slightly soluble in water, insoluble in benzene. Hygroscopic. (Found: N, 13.31; S, 9.96 %. $C_{10}H_9O_7N_3S$ requires: N, 13.33; S, 10.1 %.)

On carrying out the reaction with 2 mols. of the acid chloride the same substance appeared to be produced. The reaction was also carried out in an atmosphere of nitrogen with identical results.

SUMMARY.

1. 3 : 5-Dinitrobenzoyl chloride is recommended as a reagent for the rapid identification of many amino-acids, crystalline derivatives usually being obtained in good yield. The condensation is almost instantaneous in alkaline solution.

2. The acidic amino-acids appear to be less reactive towards this reagent. Under the conditions cited in the experimental part aspartic acid does not give a derivative. In this way glycine and several other amino-acids have been separated from aspartic acid.

Although glutamic acid does give a derivative, 3 : 5-dinitrobenzoylglycine is formed preferentially and a separation has been based on this fact.

No condensation could be effected with tyrosine in alkaline solution.

3. The 3 : 5-dinitrobenzoyl derivatives of certain amino-acids are precipitated from alkaline solution by dilute acetic acid. Others are not precipitated by this reagent, but only on the addition of dilute HCl. In this way glycine and leucine have been separated.

4. Guanidine gives a good yield of crystalline di-(3 : 5-dinitrobenzoyl) guanidine, which is insoluble in alkali.

5. Thiolacetic gives *S*-3 : 5-dinitrobenzoylthiolacetic acid by the above method. Cysteine also gives an *S*-derivative which is not, however, suitable for identification purposes.

I wish to thank Sir F. G. Hopkins for the kind interest that he has shown in this work.

REFERENCES.

- Buehler, Currier and Lawrence (1933). *Ind. Eng. Chem. (Anal.)* **5**, 277.
Lapworth and Shoesmith (1922). *J. Chem. Soc.* **121**, 1931.
Pfeiffer (1914). *Ber. deutsch. chem. Ges.* **47**, 1581.
Saunders (1933). *Biochem. J.* **26**, 397.
Wertheim (1929). *J. Amer. Chem. Soc.* **51**, 3661.