262. *The Identification of Amino-acids by Means of* 3 : 5-*Dinitrobenzoyl Chloride. Part II.*

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The method of identifying amino-acids by means of 3 : 5-dinitrobenzoyl chloride has been extended to other amino-acids, peptides, etc.

The previously recorded high activity of this reagent towards neutral and basic ampholytes has again been observed. The hydroxyl group in an acidic molecule shows little tendency to react.

In Part I (Saunders, *Biochem. J.*, 1934, **28**, 580), 3 : 5-dinitrobenzoyl chloride was recommended as a reagent for the rapid identification of certain classes of amino-acids. The method is simple and consists in dissolving the amino-acid in N-sodium hydroxide and shaking the solution with finely powdered 3 : 5-dinitrobenzoyl chloride : the latter dissolves immediately, and on acidification, the 3 : 5-dinitrobenzoyl derivative is precipitated.

With neutral and basic ampholytes, highly crystalline derivatives were usually obtained in good yield; acidic ampholytes, on the other hand, were less reactive; *e.g.*, no derivative was isolated from aspartic acid under these conditions (although there was evidence that the reaction had taken place to a limited extent), and so it was possible to separate glycine and other monoamino-monocarboxylic acids from aspartic acid by using 3 : 5-dinitrobenzoyl chloride.

The range of usefulness of this reagent has now been further examined, and the results obtained with a variety of amino-acids and other compounds of biological interest are recorded below. The theoretical deductions arising therefrom will be the subject of a future communication.

The peptides glycylglycine and diglycylglycine reacted similarly to glycine towards the reagent. Monoamino-acids other than α-amino-acids, *e.g.*, β-alanine and ε-aminohexoic acid, also reacted readily, giving highly crystalline derivatives. It is to be noted that the derivatives of these two amino-acids could be precipitated from the alkaline

solution by dilute acetic acid as well as by dilute hydrochloric acid.* Several derivatives (*e.g.*, that of glycine) can be precipitated only by dilute mineral acid. Hence a separation of amino-acids can often be based on this difference (cf. the separation of glycine and leucine, *loc. cit.*).

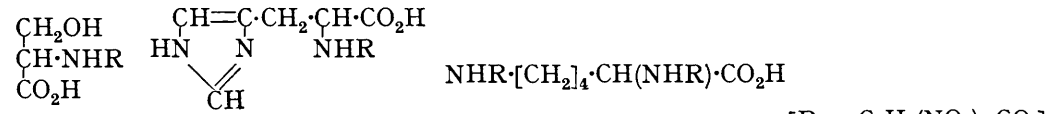
Sarcosine gave a yield of 84% compared with an 80% yield from glycine. *dl*-Serine gave the N-*derivative* (I), but in only a 20% yield. It thus appears that one carboxyl group in the molecule reduces the reactivity of the hydroxyl group towards the acid chloride very considerably, but does not affect the amino-group. Fischer and Jacobs (*Ber.*, 1906, **39**, 2942) also obtained an *N*-derivative of serine by using *p*-nitrobenzoyl chloride. Two carboxyl groups, however, appear to be necessary to reduce the reactivity of the amino-group (*e.g.*, aspartic and glutamic acids; Saunders, *loc. cit.*).

In confirmation, it was found that lactic acid would not condense with 3 : 5-dinitrobenzoyl chloride under the above conditions. Ordinarily, the hydroxyl group in a " neutral " molecule will react with the acid chloride in the presence of alkali, but experiments now in progress appear to show that such a group is less reactive than a similarly situated amino-group.

Histidine reacted immediately with 3 : 5-dinitrobenzoyl chloride, but the process of identification was less simple than with other amino-acids. If 2 g.-mols. of the acid chloride and the calculated quantity of sodium hydroxide were used, the acid chloride dissolved, and almost immediately the sodium salt of the 3 : 5-dinitrobenzoyl derivative of histidine separated in needles. The filtrate on acidification with hydrochloric acid gave 1 g.-mol. of dinitrobenzoic acid. An aqueous solution of the sodium salt when carefully acidified with dilute acetic acid gave 3 : 5-*dinitrobenzoylhistidine*.

On treating histidine with 1 g.-mol. of the acid chloride in the presence of the calculated quantity of sodium hydroxide, no separation of the sodium salt occurred, but after careful acidification with dilute acetic acid, 3 : 5-dinitrobenzoylhistidine slowly separated. Only a very small quantity of dinitrobenzoic acid was produced.

It might have been anticipated that histidine, being a basic ampholyte, would give a diacyl derivative. In order, therefore, to investigate the possible effect of the glyoxaline part of the histidine molecule, the reaction between glyoxaline itself and the acid chloride in the presence of sodium hydroxide was examined. It was found that 3 : 5-dinitrobenzoic anhydride was produced immediately, no dinitrobenzoyl derivative being formed. Pyridine behaved similarly (cf. Wedekind, *Ber.*, 1901, **34**, 2070). Hence, glyoxaline is acting solely in the capacity of a tertiary base. Histidine, therefore, probably converts part of the acid chloride into the anhydride, and may then react with the latter, giving the acyl derivative. The monoacyl derivative is thus assumed to possess formula (II).



(I.) (II.) (III.)

Lysine in alkaline solution reacted with 2 mols. of the acid chloride, and gave the *sodium* salt, from which free *bis*-3 : 5-*dinitrobenzoyl-lysine* (III) was obtained on acidification.

A comparison of the behaviour of taurine and sulphanilic acid towards 3 : 5-dinitrobenzoyl chloride is interesting. All the acid chloride dissolved when added to an alkaline solution of taurine, and the only product on acidification was a small quantity (15%) of pure 3 : 5-dinitrobenzoic acid. Estimation of taurine in the filtrate by Sørensen's method showed that 80% of the amino-acid had reacted, and on concentration of the filtrate, crystals of *sodium* 3 : 5-*dinitrobenzoyltaurate* separated.

On adding 3 : 5-dinitrobenzoyl chloride to sulphanilic acid dissolved in the calculated

* 3 : 5-Dinitrobenzoic acid is precipitated from alkaline solution by dilute hydrochloric acid, but not by dilute acetic acid. Hence derivatives precipitated by acetic acid are entirely free from dinitrobenzoic acid. Derivatives precipitated only by hydrochloric acid, provided they are derived from " reactive " amino-acids, usually contain, however, only small amounts of dinitrobenzoic acid, which is generally completely removed during recrystallisation.

quantity of N-sodium hydroxide (2 mols.), a solid remained which was a mixture of unchanged acid chloride and *sodium* 3 : 5-*dinitrobenzoylsulphanilate* (23·5% yield). Examination of the filtrate showed that not more than 50% of the sulphanilic acid had reacted. With sulphanilic acid dissolved in 4 g.-mols. of N-sodium hydroxide, no solid remained, and acidification of the solution with dilute acetic acid gave a 27% yield of the above salt. Examination of the filtrate again showed that the reaction had proceeded about 50% to completion. The smaller yield from sulphanilic acid than from taurine may perhaps be connected with the weakness of sulphanilic acid as a base.

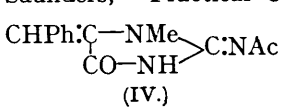
The reaction of the acid chloride towards the aminobenzoic acids was also examined. When anthranilic acid dissolved in 4 g.-mols. of N-sodium hydroxide was shaken with 3 : 5-dinitrobenzoyl chloride, the *sodium* salt of 3 : 5-*dinitrobenzoylanthranilic acid* separated immediately as a gelatinous precipitate, which on treatment with acetic acid gave the colourless *acid*. The filtrate from the sodium salt gave on acidification with dilute acetic acid the bright yellow salt of anthranilic acid and 3 : 5-dinitrobenzoic acid. This salt had been prepared directly from aqueous solutions of its constituents (Buehler *et al.*, *Ind. Eng. Chem., Anal.*, 1933, **5**, 277), and the author has now prepared it by mixing alkaline solutions of these acids and then acidifying with acetic acid, thus accounting for its production in the above benzoylation.

When anthranilic acid was dissolved in only 2 g.-mols. of N-sodium hydroxide, the course of the reaction was different : the acid chloride dissolved immediately, and almost at once the free dinitrobenzoylanthranilic acid separated (the p_H being very nearly 7). The filtrate on acidification with acetic acid gave the yellow salt, and addition of hydrochloric acid gave a negligible weight of 3 : 5-dinitrobenzoic acid.

m- and *p*-Aminobenzoic acids when dissolved in 2 g.-mols. of N-sodium hydroxide and treated as above gave the free *dinitrobenzoyl* derivatives. A comparison of yields is given below :

Acid.	3 : 5-Dinitrobenzoyl derivative, %.	3 : 5-Dinitrobenzoate, %.	3 : 5-Dinitrobenzoic acid, %.
o-Aminobenzoic	62	37	—
m-Aminobenzoic	83	—	14
p-Aminobenzoic	62	35	—

Creatine failed to give a derivative with 3 : 5-dinitrobenzoyl chloride, but in presence of sodium hydroxide, creatinine and the chloride gave an intense violet coloration, which slowly faded to dark brown. This falls into line with the observation (Mann and Saunders, " Practical Organic Chemistry," pp. 189, 225) that substances containing a $-CH_2\cdot CO-$ group, which is capable of enolisation, give immediately an intense violet coloration (which often fades) with sodium hydroxide and 3 : 5-dinitrobenzoic acid.* On acidifying the filtered solution with acetic acid, an amorphous brown solid was obtained which was not readily purified : the substance was probably a salt, but was valueless for identification purposes and was not further examined. Benzylidenecreatinine and benzylideneacetylcreatinine (IV; Ing, J., 1932, 2047) gave neither colorations nor derivatives when treated as above



(IV.)

In Part I it was shown that 3 : 5 dinitrobenzoylglycine could be hydrolysed by boiling with diluted hydrochloric acid for 1½ hours, but it has now been found that 70% sulphuric acid effects hydrolysis in 5 minutes.

Experimental.

3 : 5-*Dinitrobenzoylglycylglycine.*—Glycylglycine (0·61 g.; 1 mol.) was dissolved in N-sodium hydroxide (10 c.c.; 2 mols.), and finely powdered 3 : 5-dinitrobenzoyl chloride (1·15 g.; 1 mol.) added. The mixture was shaken vigorously in a stoppered bottle; the acid chloride dissolved immediately and the solution, which at once acquired a deep red coloration, was filtered from traces of unchanged acid chloride, and acidified with dilute hydrochloric acid. The 3 : 5-*dinitrobenzoyl* derivative, which crystallised out immediately, was filtered off and washed with

* It was found (Part I, *loc. cit.*) that all 3 : 5-dinitrobenzoyl derivatives gave an intense violet coloration with sodium hydroxide and acetone. This test therefore provides a ready means of ascertaining whether a compound has reacted with 3 : 5-dinitrobenzoyl chloride.

water. Yield of dried material 1·23 g.; 75%. Recrystallised from water, it formed clusters of fine colourless needles, m. p. 210° (Found: C, 38·2; H, 3·6; N, 16·0; loss at 110°, 5·1. $C_{11}H_{10}O_8N_4,H_2O$ requires C, 38·3; H, 3·5; N, 16·3; H_2O, 5·2%).

The following 3 : 5-dinitrobenzoyl derivatives were prepared similarly to the above unless otherwise stated, quantities of the order of 0·3—0·5 g. being used.

3 : 5-*Dinitrobenzoyldiglycylglycine.*—Yield 65%. It recrystallised from water in colourless needles, m. p. 236° (decomp.) (Found: C, 40·5; H, 3·4. $C_{13}H_{13}O_9N_5$ requires C, 40·7; H, 3·4%).

3 : 5-*Dinitrobenzoyl*-β-*alanine.*—The β-alanine was obtained from succinimide ("Organic Syntheses," XVI, 1). The *derivative* was precipitated from alkaline solution by dilute acetic acid. Yield 64%. Recrystallised from water, it formed clusters of long, fine, colourless needles, m. p. 202·5° (Found: C, 42·6; H, 3·3; N, 15·0. $C_{10}H_9O_7N_3$ requires C, 42·4; H, 3·2; N, 14·8%).

ε-3 : 5-*Dinitrobenzamidohexoic Acid.*—The ε-aminohexoic acid was obtained from *cyclo*hexanoneoxime (*op. cit.*, XVII, 7). The *derivative* was obtained as an oil, which soon crystallised, by acidifying the alkaline solution with dilute acetic acid. Yield 65%. Recrystallisation from boiling water, in which it is only moderately soluble, afforded colourless needles, m. p. 129—131° (Found: C, 48·0; H, 5·0; N, 12·6. $C_{13}H_{15}O_7N_3$ requires C, 48·0; H, 4·9; N, 13·0%).

3 : 5-*Dinitrobenzoylsarcosine.*—Yield 84%. This recrystallised from water in radiating tufts of needles, m. p. 153·5°, when the temperature was raised very slowly at about 100°: if the temperature was raised quickly, the compound dissolved in its water of crystallisation and gave a low m. p. (Found: C, 39·6; H, 3·85; N, 14·4; loss at 100°, 5·8. $C_{10}H_9O_7N_3,H_2O$ requires C, 39·9; H, 3·65; N, 14·0; H_2O, 6·0%). The *derivative* was not decomposed by dehydration, since the anhydrous substance could be recrystallised from water, again producing the hydrated form.

3 : 5-*Dinitrobenzoyl*-dl-*proline* was precipitated by dilute hydrochloric acid as a gum which soon solidified. Yield 80%. It recrystallised from acetone in small, well-formed prisms, m. p. 217° (Found: C, 46·8; H, 3·6; N, 14·0. $C_{12}H_{11}O_7N_3$ requires C, 46·9; H, 3·6; N, 13·6%).

N-3 : 5-*Dinitrobenzoyl*-dl-*serine.*—Dilute hydrochloric acid precipitated 3 : 5-dinitrobenzoic acid, the filtrate from which slowly deposited the derivative in needles. Yield about 20%. This recrystallised from water in silky needles, m. p. 94—95° (Found: C, 37·6; H, 3·6; N, 13·1. $C_{10}H_9O_8N_3,H_2O$ requires C, 37·9; H, 3·5; N, 13·2%). It was not possible to determine the water of crystallisation owing to decomposition at 100°.

Reaction between Lactic Acid and 3 : 5-*Dinitrobenzoyl Chloride.*—(A) An experiment was carried out as for glycylglycine, the quantities used being: lactic acid (0·9 g.), N-sodium hydroxide (30 c.c.), acid chloride (2·3 g.). The last dissolved immediately: no precipitate was produced with dilute acetic acid, but acidification with dilute hydrochloric acid gave 1·88 g. of 3 : 5-dinitrobenzoic acid (*i.e.*, 90% yield). Recrystallised from water, it had m. p. 208°.

(B) Excess of lactic acid (2 c.c.) and 3 : 5-dinitrobenzoyl chloride (1·15 g.) were heated together on the water-bath for 3 minutes. The acid chloride first dissolved and then the mass became solid. This, washed with cold water, filtered off, and dried, was identified as 3 : 5-dinitrobenzoic acid (0·92 g.; 87%). In these circumstances ethyl alcohol reacts with the acid choride.

Reaction between l-*Histidine and* 3 : 5-*Dinitrobenzoyl Chloride.*—(A) Histidine hydrochloride monohydrate (0·52 g.) was dissolved in N-sodium hydroxide (10 c.c.; 4 mols.), 3 : 5 dinitrobenzoyl chloride (1·15 g.; 2 mols.) added, and the mixture shaken. Almost all the chloride dissolved, giving a deep red solution, which was filtered; it rapidly became nearly colourless, and then slowly deposited pale yellow needles (0·55 g.) of the sodium salt of the derivative. The filtrate gave a negligible precipitate with dilute acetic acid, but with dilute hydrochloric acid gave 0·4 g. of 3 : 5-dinitrobenzoic acid, m. p. 207° (*i.e.*, 76% recovery of 1 g.-mol. of the acid chloride). The sodium salt was dissolved in water, and dilute acetic acid added until the solution was very faintly acid to litmus; the precipitate which slowly separated was recrystallised several times from water in which it is rather soluble, forming radiating tufts of short needles, m. p. 189° (softens about 183°) (Found: C, 42·8; H, 3·8; N, 18·7. $C_{13}H_{11}O_7N_5,H_2O$ requires C, 42·5; H, 3·5; N, 19·1%).

(B) The monohydrate (0·52 g.) was dissolved in N-sodium hydroxide (7·5 c.c.; 3 mols.), and treated with the acid chloride (0·58 g.; 1 mol.). No sodium salt separated, but when the mixture was carefully acidified with dilute acetic acid and set aside, the derivative slowly crystallised out (0·4 g.); it recrystallised from water in tufts of short needles, m. p. 188° (Found:

C, 42·5; H, 3·6; N, 18·95%). The original filtrate, when acidified with hydrochloric acid, gave 0·14 g. of 3 : 5-dinitrobenzoic acid (20% recovery).

Reaction between Glyoxaline and 3 : 5-*Dinitrobenzoyl Chloride.*—Glyoxaline (0·68 g.) was dissolved in N-sodium hydroxide (10 c.c.) and shaken with the acid chloride (2·3 g.). A deep red coloration was produced, and a solid separated (0·83 g.). Addition of hydrochloric acid to the filtrate precipitated 3 : 5-dinitrobenzoic acid (1·2 g.; 59%). The solid which separated from the cold alkaline solution was soluble in excess of alkali, and was shown by the following tests to be 3 : 5-dinitrobenzoic anhydride. It was recrystallised from benzene, and then had m. p. 219—221° (Adams *et al., J. Amer. Chem. Soc.*, 1918, **40**, 428 give 109°; ? misprint). Recrystallisation from alcohol afforded ethyl 3 : 5-dinitrobenzoate, m. p. 92° (Found : C, 45·0; H, 3·3. Calc. for $C_9H_8O_6N_2$: C, 45·0; H, 3·2%), insoluble in alkali.

When glyoxaline and the acid chloride were fused together in the absence of sodium hydroxide, a dark product was obtained, but it was again possible to isolate the anhydride by extraction with benzene.

Bis-(3 : 5-*dinitrobenzoyl*)-d-*lysine.*—*d*-Lysine dihydrochloride (0·55 g.) was dissolved in N-sodium hydroxide (13 c.c.; 5 mols.) and shaken with the acid chloride (1·15 g.; 2 mols.). The latter dissolved, producing a deep red coloration which soon faded with the simultaneous separation of a precipitate (1 g.). The filtrate gave a negligible precipitate with acetic acid, but gave 0·1 g. of dinitrobenzoic acid on acidification with hydrochloric acid, thus indicating that 2 mols. of the acid chloride had reacted. The precipitate which separated from the alkaline solution was a *sodium* salt, a very small quantity of which was recrystallised from dilute sodium carbonate solution (Found : N, 14·2, 14·4. $C_{20}H_{17}O_{12}N_6Na,2H_2O$ requires N, 14·2%). The remainder of the sodium salt was dissolved in hot water, filtered, and dilute hydrochloric acid added; the *bis*-3 : 5-*dinitrobenzoyl* derivative was then precipitated. It recrystallised from alcohol in minute colourless needles, m. p. 169° (softening about 160°) (Found : N, 14·5; H_2O, 6·2. $C_{20}H_{18}O_{12}N_6,2H_2O$ requires N, 14·7; H_2O, 6·3%). The *anhydrous* substance was obtained by 1 hour's heating at 115° (Found : C, 45·0; H, 3·4; N, 15·95. $C_{20}H_{18}O_{12}N_6$ requires C, 44·95; H, 3·4; N, 15·7%).

Sodium 3 : 5-*Dinitrobenzoyltaurate.*—Taurine (0·63 g.) was dissolved in N-sodium hydroxide (10 c.c.), and the powdered acid chloride (1·15 g.) added : a deep red coloration was produced, which rapidly faded to pale yellow. No precipitate was produced with acetic acid, but with dilute hydrochloric acid 3 : 5-dinitrobenzoic acid was produced (0·16 g.; 15%). The solution was filtered and made up to 50 c.c. The amino-acid present was estimated by Sørensen's method : 10 c.c. of the solution required 1 c.c. of 0·1 N-sodium hydroxide. Thus only 20% of the taurine remained unchanged.

In a second experiment, the filtrate from the dinitrobenzoic acid was concentrated to about one-third of its bulk and allowed to cool, large colourless crystalline plates of the *sodium dinitrobenzoyltaurate* separating (1 g.; 60%). These recrystallised from aqueous alcohol in large, almost colourless, feathery plates (Found : N, 12·0. $C_9H_8O_8N_3SNa$ requires N, 12·3%).

Reaction between 3 : 5-*Dinitrobenzoyl Chloride and Sulphanilic Acid.*—Sulphanilic acid (2·09 g.) was dissolved in N-sodium hydroxide (40 c.c.; 4 mols.) and shaken with the acid chloride (2·3 g.; 1 mol.). The latter dissolved : the solution was quickly filtered, and acidified with dilute acetic acid, a precipitate of *sodium* 3 : 5-*dinitrobenzoylsulphanilate* being obtained (0·91 g.; 23·5%). This recrystallised from water in colourless needles (Found : N, 9·3, 9·5; H_2O, 12·3. $C_{13}H_8O_8N_3SNa,3H_2O$ requires N, 9·5; H_2O, 12·2%). Dilute hydrochloric acid was added to the original filtrate : at this p_H, the dinitrobenzoic acid separated out first (1·05 g., representing a 50% recovery of the acid chloride used), and on concentration of the mother-liquor sulphanilic acid crystallised out (0·94 g.; 45% recovery).

In a second experiment, the sulphanilic acid (2·09 g.) was dissolved in 2 mols. of N-sodium hydroxide (20 c.c.) and treated as above. A solid residue was left from which 1·04 g. of the sodium salt were obtained (27% yield). Acetic acid produced no precipitate, but on the addition of hydrochloric acid 0·6 g. of dinitrobenzoic acid was obtained (0·5 g. was also recovered from insoluble residue; total recovery = 1·1 g., or 52%). Concentration of the filtrate gave 1 g. of sulphanilic acid (48% recovery).

Reaction between 3 : 5-*Dinitrobenzoyl Chloride and Anthranilic Acid.*—(i) Anthranilic acid (1·36 g.) was dissolved in N-sodium hydroxide (20 c.c.; 2 mols.) in a mortar, and ground with the acid chloride (2·3 g.). At first the latter dissolved, and a clear solution was momentarily obtained. After 1—2 seconds, a heavy precipitate (*A*, 2·05 g.) was produced. On adding dilute acetic acid to the filtrate, a bright yellow precipitate (*B*, 1·3 g.) was obtained. On adding dilute hydrochloric acid to the filtrate from *B*, a negligible amount of 3 : 5-dinitrobenzoic

acid was produced. The substance *A* proved to be the free *dinitrobenzoyl* derivative and not the sodium salt as might be inferred. The p_H of the solution from which the derivative was precipitated was about 7. Other experiments showed that if *A* were dissolved in alkali and dilute acid added, complete precipitation took place while the solution was still alkaline to bromophenol-blue. The compound *A* recrystallised from acetone in needles, m. p. 278° (softens at 269°) (Found: C, 51·1; H, 3·0; N, 13·0. $C_{14}H_9O_7N_3$ requires C, 50·8; H, 2·7; N, 12·7%).

A substance identical with this was produced by mixing a solution of anthranilic acid (1·36 g.) in dry benzene (30 c.c.) with a solution of the acid chloride (2·3 g.) also in dry benzene (10 c.c.). A colourless precipitate (2·6 g.) was produced immediately in the cold; when boiled with water and recrystallised several times from acetone, it had m. p. 279—280° (Found: C, 51·1; H, 2·8; N, 12·5%).

The product *B* recrystallised from water in beautiful, deep yellow, radiating needles, m. p. 208° (Found: C, 48·7; H, 3·2; N, 12·1. Calc. for $C_7H_7O_2N,C_7H_4O_6N_2$: C, 48·3; H, 3·2; N, 12·0%); it dissolved in hot water, giving a colourless solution, from which yellow crystals separated on cooling. It also dissolved in cold alcohol, giving a colourless solution, from which yellow crystals were again obtained on addition of water. The yellow crystals dissolved in hydrochloric acid, giving a colourless solution from which crystals of 3 : 5-dinitrobenzoic acid separated.

(ii) Under the same conditions as in (i), but with 40 c.c. (4 mols.) of N-sodium hydroxide, a somewhat gelatinous precipitate of *sodium* 3 : 5-*dinitrobenzoylanthranilate* (*C*, 0·7 g.) separated from the alkaline solution. On addition of dilute acetic acid to the filtrate, the compound *B* (2 g.) was obtained, and after its removal the filtrate gave 3 : 5-dinitrobenzoic acid (0·34 g.) when acidified with hydrochloric acid. The salt *C* separated from hot water as a gel, which very slowly changed to fine yellow needles. The needles were obtained more readily by recrystallising from dilute sodium carbonate solution (Found: N, 11·7. $C_{14}H_8O_7N_3Na$ requires N, 11·9%). A solution of *C* in hot water when acidified with dilute acetic acid gave the free dinitrobenzoyl derivative, *A*, in quantitative yield.

Reaction between 3 : 5-*Dinitrobenzoyl Chloride and* m-*Aminobenzoic Acid.*—Experiment (i), above, was repeated, but with *m*-aminobenzoic acid. In this case also, the free *dinitrobenzoyl* derivative (2·76 g.) separated without acidifying; it recrystallised from aqueous alcohol in radiating rosettes of pale yellow needles; m. p. 270° (softening slightly about 240°) (Found: C, 51·1; H, 2·8. $C_{14}H_9O_7N_3$ requires C, 50·8; H, 2·7%). A negligible precipitate was obtained on acidifying the original filtrate with acetic acid, and on adding dilute hydrochloric acid, dinitrobenzoic acid (0·26 g.) separated.

Reaction between 3 : 5-*Dinitrobenzoyl Chloride and* p-*Aminobenzoic Acid.*—Under conditions as in (i) above, this acid afforded the free *dinitrobenzoyl* derivative (2·05 g.) without acidification; it recrystallised from aqueous alcohol in clusters of short needles, unmolten at 290° (Found: N, 12·8. $C_{14}H_9O_7N_3$ requires N, 12·7%). On acidifying the original filtrate with dilute acetic acid, the orange-coloured *p*-aminobenzoic acid 3 : 5-dinitrobenzoate (1·2 g.) separated; this recrystallised from water in long orange needles, m. p. 195° (Found: N, 12·0. Calc. for $C_7H_7O_2N,C_7H_4O_6N_2$: N, 12·0%). Further acidification with dilute hydrochloric acid produced a negligible weight of 3 : 5-dinitrobenzoic acid.

Hydrolysis of 3 : 5-*Dinitrobenzoylglycine.*—The derivative (0·9 g.), m. p. 179°, was gently boiled with 70% (by weight) sulphuric acid (4 c.c.) for 5—7 mins. The mixture was then diluted with water, thoroughly cooled, and the 3 : 5-dinitrobenzoic acid filtered off, m. p. 206° (0·66 g.; 100%). The filtrate was made up to standard bulk, and analysis by Sørensen's method showed that 85% of the theoretical amount of glycine was present in solution.

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