223. *Acyl* iso*Thiocyanates. Part II.* Reactions of Aroyl* iso*Thiocyanates with Amines and Amino-acids in Aqueous Solution.*

By D. T. Elmore and J. R. Ogle.

Hydrolysis of benzoyl *iso*thiocyanate affords benzamide, dibenzoylamine, and benzoic acid in amounts which depend on pH; a mechanism is advanced to account for the observations. With amino-acids in aqueous dioxan at alkaline pH, benzoyl *iso*thiocyanate in general affords the *N*-benzoyl derivative. Addition may compete or even predominate if (i) the distance between the amino- and the carboxylate group is large (*e.g.*, as in 6-aminohexanoic acid), (ii) the amino-group is attached to an aromatic ring, or (iii) substitution is sterically hindered. 2 : 4 : 6-Tribromobenzoyl *iso*thiocyanate gives exclusively addition products.

In continuation of earlier work,[1] and as a variation of Edman's method [2] of stepwise degradation of peptides from the *N*-terminus, it was of interest to attempt the synthesis of *N*-acylthiocarbamoylamino-acids and -peptides by the interaction of acyl *iso*thiocyanates and amino-acids and peptides in aqueous solvents at alkaline pH. The observation that condensation between methyl *N*-acetyldithiocarbamate and some amino-acids and peptides proceeded slowly was an additional incentive. We have already indicated [3,4] that the reactions of acyl *iso*thiocyanates are complex, since addition to the ·N:C:S system and nucleophilic substitution at the carbonyl-carbon atom may compete with one another. The rates of these reactions depend on factors such as basic strength of nucleophilic reagent, solvent polarity, structure of acyl *iso*thiocyanate, and temperature. Since aroyl *iso*thiocyanates generally react additively (for exceptions see refs. 5, 6), we decided to examine the reactions between benzoyl *iso*thiocyanate and amino-acids in aqueous solvents.[3]

The mode of hydrolysis of benzoyl *iso*thiocyanate depends on pH. In water, benzamide was formed in over 80% yield, presumably through the intermediate thiocarbamic acid, together with a trace of benzoic acid, thus confirming earlier work.[6,7] On the other hand, Dixon and Taylor [6] found that N-sodium hydroxide liberated thiocyanate ion almost quantitatively. We have found that reaction of benzoyl *iso*thiocyanate in aqueous dioxan at pH 8·5 and pH 6·3, followed by acidification, affords a mixture of benzamide, dibenzoylamine, and benzoic acid (Table 1). Dibenzoylamine, which has not previously been reported

Table 1. *Hydrolysis of benzoyl* iso*thiocyanate.*

Solvent	pH	Alkali uptake (mole/mole *iso*thiocyanate)	Yield (%) of mixed amides	Yield (%) of benzoic acid
Water–ether	—	—	81·5[a]	3·0
Water–ether (5 : 1)	6·3	0·45	73·5	34·4
Water–dioxan (3 : 2)	8·5	1·20	52·8	43·8
Water–dioxan (1 : 1)	8·5	1·02	51·2[b]	47·9

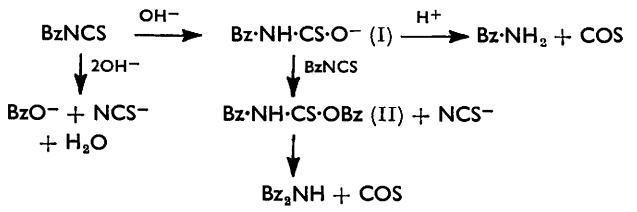
[a] Pure benzamide. [b] Composed of benzamide 13·9% and dibenzoylamine 37·3%.

as a product of hydrolysis of benzoyl *iso*thiocyanate, presumably arises from nucleophilic attack by *N*-benzoylthiocarbamate ion (I) on benzoyl *iso*thiocyanate followed by loss of carbon oxysulphide from the resultant unsymmetrical acid anhydride (II). As expected, the yields of benzamide and dibenzoylamine were lower at the higher pH.

* Part I, *J.*, 1956, 4458.

[1] Elmore and Toseland, *J.*, 1954, 4533; 1957, 2460.
[2] Edman, *Acta Chem. Scand.*, 1950, **6**, 283.
[3] Elmore and Ogle, *Proc. Chem. Soc.*, 1957, 289.
[4] Elmore, Ogle, Fletcher, and Toseland, *J.*, 1956, 4558 and references cited therein.
[5] Wheeler, *Amer. Chem. J.*, 1901, **26**, 345; Hoggarth, *J.*, 1949, 1160.
[6] Dixon and Taylor, *J.*, 1908, **93**, 684.
[7] Miquel, *Ann. Chim.*, 1877, **10**, 289.

Although hydrolysis of benzoyl *iso*thiocyanate was quite fast, in general it did not seriously compete with the reactions involving amines, amino-acids, and peptides, and the combined yields of *N*-benzoyl and *N*-benzoylthiocarbamoyl derivatives usually accounted for more than 70% of the base used (Table 2). Notable exceptions were the reactions involving methylamine, γ-aminobutyric acid, 6-aminohexanoic acid, and 1-amino*cyclo*hexanecarboxylic acid. The high pK_a values of the first three (10·64, 10·43, and 10·75 respectively at 25°) probably explain the low yields, since the small degree of dissociation would allow hydrolysis of *iso*thiocyanate to compete seriously. The pK_a of 1-amino*cyclo*-



hexanecarboxylic acid has not been measured, but it is likely to be at least as high as that of α-amino*iso*butyric acid (10·21 at 25°). The uptake of alkali (Table 2) provides only a rough indication of the extent and direction of reaction; although addition and nucleophilic substitution require up to 1 and 2 moles of alkali per mole of base respectively, paucity of knowledge of pK_a values of amines and amino-acids in aqueous dioxan together with the competing hydrolysis of benzoyl *iso*thiocyanate preclude any quantitative assessment of the results.

TABLE 2. *Reactions of benzoyl* iso*thiocyanate with amines and amino-acids.*

Reactant	pH	Alkali uptake (mole/mole of amine)	*N*-Benzoyl compound %	*N*-Benzoyl compound M. p.	*N*-Benzoylthiocarbamoyl compound %	*N*-Benzoylthiocarbamoyl compound M. p.
Glycine	8·5	2·23	84	186·5—187·0°	0	—
Methylamine	8·5	1·79	9	79	5	147·0—147·5°
α-Alanine	8·5	1·63	90	162·5—163·0	0	—
β-Alanine	9·5	1·69	78	132·0—132·5	0	—
γ-Aminobutyric acid	8·5	1·60	17	88·5—89·0	0	—
6-Aminohexanoic acid	8·5	1·65	49	80—81	5	127·5—128·0
Norleucine	8·5	1·57	73	132—133	0	—
Glycylglycine	7·0	1·64	76	204·5—206·0	7	221 *
p-Aminobenzoic acid	8·5	0·07	0	—	~100	226—227 *
Anthranilic acid	8·5	0·10	0	—	~100	160—161
N-Phenylglycine	8·5	0·23	0	—	~100	168·5—169·0
1-Amino*cyclo*hexanecarboxylic acid	8·5	1·03	0	—	25	194·0—194·5
*cyclo*Hexylamine	8·5	0·65	21	147—148	53	71—72
,, (in C_6H_6)	—	—	0	—	76	71—72

* Decomp.

A nucleophilic substitution involving benzoyl *iso*thiocyanate and an amine should be favoured (i) in a highly polar solvent such as water, and (ii) by using an amine of high pK_a, providing that it is appreciably dissociated at the pH of the reaction. Our results, as well as experiments recorded by Dixon and Taylor,[6] indicate that both factors must be operative for benzoylation to occur. Thus, for example, no *N-cyclo*hexylbenzamide was detected when benzoyl *iso*thiocyanate and *cyclo*hexylamine interacted in benzene, but 21% was isolated after reaction in aqueous dioxan at pH 8·5. Further, in aqueous dioxan anthranilic acid, *p*-aminobenzoic acid, and *N*-phenylglycine, which are weak bases, reacted exclusively by addition.

There are several indications that a carboxylate ion in proximity to the amino-group favours acylation, but it is not known whether this results from acceleration of substitution

or retardation of addition. Thus, α-amino-acids, with the exception of 1-amino*cyclo*hexanecarboxylic acid, gave no *N*-benzoylthiocarbamoyl derivatives in aqueous dioxan at pH 8·5, whereas 6-aminohexanoic acid afforded a small amount. The formation of a little *N*-benzoylthiocarbamoylglycylglycine from the peptide may result from this effect or from the relatively low pK_a of the amino-group. The presence of benzoate ion did not favour benzoylation, since in parallel experiments with *cyclo*hexylamine and benzoyl *iso*thiocyanate in aqueous dioxan at pH 8·5, addition of benzoic acid gave a slightly inferior yield of *N-cyclo*hexylbenzamide. It is also instructive to compare the results of experiments with glycine and methylamine under comparable conditions. As mentioned above, most of the methylamine was unchanged, but *N*-benzoyl-*N'*-methylthiourea (5%) was isolated in addition to *N*-methylbenzamide (9%), whereas glycine yielded hippuric acid (84%) only. A similar attempt to compare the reactions of benzoyl *iso*thiocyanate with *n*-butylamine in aqueous dioxan with that involving norleucine failed, because the products from the amine were oils which could not be satisfactorily purified. This is not surprising, since *N-n*-butylbenzamide and *N*-benzoyl-*N'-n*-butylthiourea both have low m. p.s and inconveniently high solubilities in organic solvents. A surprising anomaly is seen in the experiments involving *cyclo*hexylamine and 1-amino*cyclo*hexanecarboxylic acid. The good recovery of amide and thiourea, which resulted from the reaction of *cyclo*hexylamine with benzoyl *iso*thiocyanate in aqueous dioxan, was not unexpected since, although the pK_a of *cyclo*hexylamine in water is 10·64, the apparent pK_a in 50% aqueous ethanol is 9·83. The only product isolated, albeit in low yield, from the reaction of 1-amino*cyclo*hexanecarboxylic acid with benzoyl *iso*thiocyanate, however, was 1-(*N*-benzoylthiocarbamoyl)amino*cyclo*hexanecarboxylic acid.

It is clear that benzoyl *iso*thiocyanate is an unsuitable reagent for procuring the stepwise degradation of peptides through their *N*-benzoylthiocarbamoyl derivatives. It seemed possible, however, than aroyl *iso*thiocyanates with bulky substituents in the *ortho*-positions might react preferentially by addition, owing to steric retardation of nucleophilic substitution at the carbonyl-carbon atom. It is noteworthy that the methanolysis of benzoyl chloride [8] at 0° is 142 times faster than that of 2 : 4 : 6-tribromobenzoyl chloride at 25°. Several attempts to isolate the acyl *iso*thiocyanate after reaction between potassium thiocyanate and 2 : 4 : 6-tribromobenzoyl chloride in acetonitrile were unsuccessful, although potassium chloride was precipitated quantitatively. The product was usually an insoluble, amorphous, yellow solid with indefinite m. p. and was probably polymeric. The existence of the acyl *iso*thiocyanate in solution was established, however, by isolation, in good yield, of its addition products with aniline and *cyclo*hexylamine. Addition also occurred rapidly with glycine and glycylglycine in aqueous acetonitrile at alkaline pH, and the corresponding *N*-2 : 4 : 6-tribromobenzoylthiocarbamoyl derivatives were isolated in approximately 80% yield. The value of this and similar 2 : 6-disubstituted aroyl *iso*thiocyanates as reagents for peptide degradation remains to be ascertained.

Experimental

Peroxide-free dioxan [9] was used. Experiments at constant pH were performed with a Radiometer type TTTl pH-stat and magnetic valve. Benzoyl *iso*thiocyanate was prepared by heating benzoyl chloride (56·5 g.) and potassium thiocyanate (45 g.) in dry acetone (200 c.c.) under reflux for 1 hr. After cooling and filtration, benzene (300 c.c.) was added to the filtrate, and the mixture kept for 1 hr., filtered, and evaporated under reduced pressure below 40°. Distillation through a short column of glass helices furnished the pure product (30—40 g.), b. p. 58—62°/0·03 mm., which solidified during storage in a desiccator at −20°.

N-*Benzoyl*-N'-cyclo*hexylthiourea*.—Reaction of benzoyl *iso*thiocyanate (0·81 g.) and *cyclo*hexylamine (0·5 g.) in benzene (10 c.c.) afforded an oil which soon solidified. After crystallisation from ethanol, the product (1·02 g.) had m. p. 71—72° (Found: C, 63·9; H, 6·6; N, 11·2. $C_{14}H_{18}ON_2S$ requires C, 64·0; H, 6·9; N, 10·7%).

[8] Norris and Young, *J. Amer. Chem. Soc.*, 1935, **57**, 1420.
[9] Eigenberger, *J. prakt. Chem.*, 1931 **130**, 75.

N-*Benzoyl*-N′-n-*butylthiourea*.—This *compound* (68%), prepared as above, separated after addition of much light petroleum (b. p. 40—60°) and storage at −20°. Recrystallised from light petroleum (b. p. 40—60°), it had m. p. 51—52° (Found: C, 61·0; H, 6·7; N, 12·2; S, 13·1. $C_{12}H_{16}ON_2S$ requires C, 61·0; H, 6·8; N, 11·8; S, 13·6%).

N-*n*-Butylbenzamide was obtained as a colourless oil from the interaction of benzoyl chloride and *n*-butylamine in 2N-sodium hydroxide. After storage at 0° and 10^{-2} mm. over phosphoric oxide and paraffin wax, it solidified and was recrystallised from *cyclo*hexane–light petroleum (b. p. 40—60°) or ethyl acetate–light petroleum (b. p. 40—60°) (1 : 19); it had m. p. 39·5—40·5°. This compound has been variously described as an uncrystallisable oil,[10] and as a solid [11, 12] with m. p. 41—42° or 68—70°.

Hydrolysis of Benzoyl iso*Thiocyanate*.—(i) Agitation of a mixture of benzoyl *iso*thiocyanate, water, and ether for 3 weeks, followed by fractionation with aqueous sodium hydrogen carbonate, afforded benzamide (81·5%) and benzoic acid (3%).

(ii) Experiments at constant pH are summarised in Table 1. At the end of the reaction (12—18 hr.), the mixture was acidified to pH 2 and concentrated under reduced pressure. The insoluble fraction was collected, and the filtrate was extracted continuously with ether overnight. The insoluble fraction and the ether extract were combined and shaken with saturated sodium hydrogen carbonate. Benzoic acid was isolated from the aqueous phase. After evaporation of the ether extract, the residue was fractionally crystallised from aqueous ethanol. Purity of the amides was checked by m. p. alone and in admixture with authentic specimens, by examination of infrared spectra and by paper chromatography in benzene–acetic acid–water (1 : 1 : 1) and *cyclo*hexane–propan-2-ol–90% acetic acid (65 : 15 : 20) followed by detection with a low-pressure mercury-vapour lamp (2537 Å). Alternatively, as in the last experiment in Table 1, dibenzoylamine was isolated by evaporation of the alkaline reaction mixture under reduced pressure. Acidification furnished a mixture of benzamide and benzoic acid, which was resolved by partition between ether and saturated sodium hydrogen carbonate.

Reactions of Benzoyl iso*Thiocyanate with Amino-acids and Amines*.—Generally, the amino-acid (0·01 mole) and benzoyl *iso*thiocyanate (0·010—0·011 mole) were allowed to react in 50% aqueous dioxan at the desired pH. After acidification with hydrochloric acid to pH 1, the solution was concentrated under reduced pressure, and the product was collected and recrystallised. The results of these experiments are summarised in Table 2; experiments which afforded two products or new compounds are described in more detail below.

(i) 6-*Aminohexanoic acid*. Reaction of 6-aminohexanoic acid (1·31 g.) and benzoyl *iso*thiocyanate (1·63 g.) gave a solid, which was separated by fractional crystallisation first from ethyl acetate–light petroleum (b. p. 40—60°) and then from aqueous ethanol into 6-benzamidohexanoic acid (1·01 g.), having m. p. and mixed m. p. 80—81°, and 6-(N-*benzoylthiocarbamoyl*)*aminohexanoic acid* (0·14 g.), m. p. 127·5—128·0° (Found: C, 57·2; H, 6·1; N, 9·5. $C_{14}H_{18}O_3N_2S$ requires C, 57·1; H, 6·2; N, 9·5%).

(ii) *Glycylglycine*. Reaction of the peptide (1·32 g.) and benzoyl *iso*thiocyanate (1·63 g.) afforded hippuroylglycine (2·09 g.), m. p. and mixed m. p. 204·5—206·0°, and *N*-benzoylthiocarbamoylglycylglycine (0·21 g.), m. p. 221° (decomp.) undepressed by admixture with an authentic sample and having an identical infrared spectrum. The insolubility of the latter compound in hot ethanol afforded a ready method of separation.

(iii) 1-*Amino*cyclo*hexanecarboxylic acid*. The amino-acid (1·43 g.) and benzoyl *iso*thiocyanate (1·63 g.) afforded 1-(N-*benzoylthiocarbamoyl*)*amino*cyclo*hexanecarboxylic acid* (0·77 g.), which had m. p. 194·0—194·5° after two recrystallisations from aqueous ethanol (Found: C, 59·0; H, 6·1; N, 9·6. $C_{15}H_{18}O_3N_2S$ requires C, 58·8; H, 5·9; N, 9·1%).

(iv) *Methylamine*. After completion of the reaction between methylamine and benzoyl *iso*thiocyanate, the solution was evaporated under reduced pressure, diluted, and filtered. The gummy residue, twice recrystallised from aqueous ethanol, afforded *N*-benzoyl-*N*′-methylthiourea (5%), m. p. and mixed m. p. 149—150°. The filtrate was evaporated under reduced pressure and twice evaporated with ethanol. An ethanolic extract of the residue was filtered from sodium chloride and evaporated. Ether-extraction left a residue of unchanged methylamine hydrochloride (79%). Removal of ether furnished *N*-methylbenzamide (9%), m. p. and mixed m. p. 79° after two recrystallisations from benzene–light petroleum (b. p. 40—60°).

[10] Coleman and Howells, *J. Amer. Chem. Soc.*, 1923, **45**, 3084.
[11] Grimmel, Guenther, and Morgan, *ibid.*, 1946, **68**, 539.
[12] Braun and Weismantel, *Ber.*, 1922, **55**, 3165.

(v) cyclo*Hexylamine*. After interaction of this base and benzoyl *iso*thiocyanate in 66% aqueous dioxan, acidification and evaporation under reduced pressure gave an oil, which solidified after storage at 0°. Fractional crystallisation first from benzene–light petroleum (b. p. 90—120°) and then from aqueous ethanol yielded *N-cyclo*hexylbenzamide (21%), m. p. and mixed m. p. 147—148°, and *N*-benzoyl-*N'-cyclo*hexylthiourea (53%), m. p. 71—72° alone and in admixture with the sample described above.

N-cyclo*Hexyl*-N'-2 : 4 : 6-*tribromobenzoylthiourea*.—A solution of 2 : 4 : 6-tribromobenzoyl chloride (0·36 g.) and dry potassium thiocyanate (0·11 g.) in acetonitrile (5 c.c.) was heated under reflux for 30 min., cooled, and filtered. The residue was washed with acetonitrile, and *cyclo*hexylamine (0·10 g.) was added to the combined filtrates. After 2 hr., solvent was removed under reduced pressure, and the residue was leached with hot ethyl acetate. The extract was evaporated and kept at −20°; the *product* (0·36 g.), after recrystallisation from ethyl acetate–benzene (1 : 9), melted unsharply at 250—265°, resolidified, and decomposed above 280° (Found: C 34·1; H 3·2; N, 5·8. $C_{14}H_{15}ON_2Br_3S$ requires C, 33·7; H, 3·0; N, 5·6%).

N-*Phenyl*-N'-2 : 4 : 6-*tribromobenzoylthiourea*.—Similarly prepared in theoretical yield, this *compound* had m. p. 213·5—214·5° after recrystallisation from aqueous acetone (Found: C, 34·0; H, 2·1; N, 5·8. $C_{14}H_9ON_2Br_3S$ requires C, 34·1; H, 1·8; N, 5·7%).

N-2 : 4 : 6-*Tribromobenzoylthiocarbamoylglycine*.—A solution of 2 : 4 : 6-tribromobenzoyl *iso*-thiocyanate (0·01 mole) in acetonitrile (40 c.c.), prepared as above, was allowed to react with glycine (0·005 mole) in water (20 c.c.) and acetonitrile (20 c.c.) at pH 8·5 (alkali consumption 0·0054 mole). The solution was acidified and concentrated to small bulk, and the residue was partitioned between saturated sodium hydrogen carbonate and ethyl acetate. The aqueous layer, after two further washes with ethyl acetate and acidification, afforded N-2 : 4 : 6-*tribromobenzoylthiocarbamoylglycine* (1·9 g.), m. p. 228—230° (Found: C, 26·5; H, 2·1; N, 6·0. $C_{10}H_7O_3N_2Br_3S,\frac{1}{2}C_2H_5{\cdot}OH$ requires C, 26·5; H, 2·0; N, 5·6%).

N-2 : 4 : 6-*Tribromobenzoylthiocarbamoylglycylglycine*.—This was prepared from glycylglycine by the method described for the foregoing compound, except that the reaction was conducted at pH 7 (alkali consumption 0·92 mole/mole of peptide). The *product* had m. p. 214·5—215·5° (from ethanol) (Found: C, 27·3; H, 2·2; N, 8·4. $C_{12}H_{10}O_4N_3Br_3S$ requires C, 27·0; H, 1·9; N, 7·9%).

The authors thank Mrs. W. Fletcher for technical assistance, and Imperial Chemical Industries Limited for financial assistance.

STAVELEY RESEARCH LABORATORIES,
THE UNIVERSITY, SHEFFIELD, 10. [*Received, November 12th*, 1957.]