810. *Peptides. Part XVI.*[1] *Experiments Related to Acyl Cyanides*

By D. S. Jones, G. W. Kenner, and R. C. Sheppard

Phthaloylglycyl cyanide reacts smoothly with esters of glycine and α-methylalanine. A small yield of phthaloylglycyl chloride is obtained from phthaloylglycine and 2-chlorobenzoxazole, but 2-cyanobenzoxazole is inert.

The azide method of peptide synthesis is unique in never having been implicated in racemisation,[2] and consequently it retains its place of prime importance despite competition from newer methods. However, there are disadvantages, notably the formation of ureas through Curtius rearrangement, and it is still worth examining other mixed anhydrides as possible alternatives. Racemisation is known to be catalysed by strong bases and by chloride ions.[3] The reason for the immunity of acyl azides is still unknown, but weak, volatile acids may generally be the most suitable partners in mixed anhydrides of peptides, and we decided to investigate acyl cyanides.[4]

Benzoyl cyanide has been reported to give a 48% yield of ethyl hippurate from glycine ester,[5] and therefore our first task was to discover whether an acceptable yield of a simple peptide could be obtained from a well-defined α-amidoacyl cyanide. For this purpose the α-amidoacyl cyanide could be prepared by a method which would not actually be useful in peptide synthesis. Phthaloylglycyl chloride was an obvious starting material, but the attempted reactions with cuprous cyanide [6] or hydrogen cyanide and pyridine [7] were unsuccessful. Following the report that acyl bromides are better reagents, particularly in the aliphatic series,[8] we prepared phthaloylglycyl bromide and thence the crystalline, rather unstable phthaloylglycyl cyanide. It gave very high yields of the dipeptide esters from glycine ethyl ester and even α-methylalanine methyl ester, which couples with tosylglycyl chloride in only moderate yield.[9] Any broad generalisation from these results would be unjustified because the phthaloylglycyl residue is such a favourable case, but at least these results encouraged efforts to discover a method of preparing acyl cyanides from *N*-protected peptides with an optically active *C*-terminal residue.

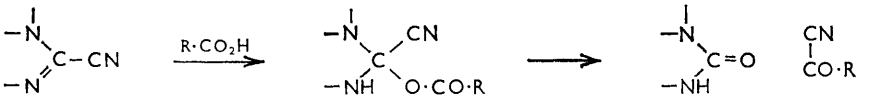
Cyanide ion is quite a strong base, and consequently it is unlikely that acyl cyanides could be prepared by a mixed anhydride or carbodi-imide procedure, except under conditions likely to cause racemisation. Moreover, the instability of phthaloylglycyl cyanide shows that it would not be possible to isolate the acyl cyanide from such reactions, and therefore there would be no advantage over a straightforward coupling with the mixed anhydride

[1] Part XV, M. Bethell and G. W. Kenner, *J.*, 1965, 3850.
[2] N. A. Smart, G. T. Young, and M. W. Williams, *J.*, 1960, 3902; M. W. Williams and G. T. Young, *J.*, 1963, 881; F. Weygand, A. Prox, L. Schmidhammer, and W. König, *Angew. Chem.*, 1963, **75**, 282.
[3] M. W. Williams and G. T. Young, *J.*, 1964, 3701.
[4] J. Thesing, D. Witzel, and A. Brehm, *Angew. Chem.*, 1956, **68**, 425.
[5] A. Dornow and H. Theidel, *Chem. Ber.*, 1955, **88**, 1267.
[6] J. S. Oakwood and C. A. Weisgerber, *Org. Synth.*, Coll. Vol. III, 1955, 112.
[7] L. Claisen, *Ber.*, 1898, **31**, 1023.
[8] W. Tschelinzeff and W. Schmidt, *Ber.*, 1929, **62**, 2210.
[9] M. T. Leplawy, D. S. Jones, G. W. Kenner, and R. C. Sheppard, *Tetrahedron*, 1960, **11**, 39.

or carbodi-imide adduct. A further complication is that cyanide ion readily converts acyl cyanides into dimers through attack at the carbonyl group;[4] admittedly these dimers are also acylating agents.

Thionylbisimidazole is a potent reagent for converting carboxylic acids into *N*-acylimidazoles,[10] and we thought that thionyl cyanide might similarly give acyl cyanides accompanied only by innocuous sulphur dioxide and hydrogen cyanide. Thionyl cyanide was described a century ago as, rather surprisingly, a crystalline solid, prepared simply from thionyl chloride and silver cyanide.[11] The sole later reference which we can find is a summary of a lecture,[12] including the statement that thionyl cyanide decomposes to sulphur dioxide, sulphur, and cyanogen. We were able to obtain small amounts of thionyl cyanide by the published method,[11] but in our hands it was too unstable for satisfactory analysis. (Good analyses are recorded.[11]) Benzoic acid did not react with thionyl cyanide unless triethylamine was added, when the product was benzoic anhydride. Experiments with benzyloxycarbonylglycine and toluene-*p*-sulphonylalanine were unsuccessful. Thionyl bisimidazole can be used for direct coupling (one-step process) of acids and esters,[13] but only a very low yield of benzyloxycarbonylglycylglycine ethyl ester could be obtained from thionyl cyanide and this yield was only slightly increased by inclusion of triethylamine. Although our experiments do not in themselves cast any doubt on the structure of " thionyl cyanide," confirmation by physical methods is desirable; it might, for example, be polymeric or be an isocyanide. In any case this compound does not seem to be of any value in peptide synthesis. Carbonyl cyanide, although rather difficult to prepare,[14] was a possible alternative, and Dr. M. T. Leplawy generously provided a sample but the results were no better. Carbonyl cyanide is readily polymerised by a trace of triethylamine, and there was no reaction with benzyloxycarbonylglycine in absence of base.

A different approach to the problem of forming acyl cyanides under non-racemising conditions was based on the hypothesis that a cyanoformamidine, having the general structure (I), might be sufficiently basic to form with a carboxylic acid an ion-pair, in which the cationoid character of the central carbon atom would be sufficient for combination with the carboxylate anion. The resulting adduct (II) might then decompose to the urea and acyl

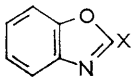


(I) (II)

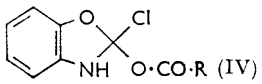
cyanide. The driving force of the whole process would be formation of a urea from a potent reagent, as in the carbodi-imide method, and the advantage would be the maintenance of virtually neutral conditions. As we expected, *NN'*-diphenylcyanoformamidine, being a weak base, was inert to acetic acid, and therefore aliphatic cyanoformamidines were required. 4-Cyanoformimidoylmorpholine is said to have m. p. 90—92°,[15] and we obtained unstable material, m. p. 93—96°, from reaction between morpholine and cyanogen, but it lacked infrared absorption in the 4·5-μ region. Reaction between ethylenediamine and cyanogen, which is reported to take a different course,[16] was also tried unsuccessfully. As substitutes for the cyanoformamidines, systems containing an oxygen atom instead of one nitrogen atom were considered. 2-Cyanobenzoxazole (IIIa) was prepared for this purpose, but there was no sign of reaction between this compound and phthaloylglycine in concentrated solution in tetrahydrofuran, even when catalytic amounts of toluene-*p*-sulphonic acid or trifluoroacetic acid were added. Low basicity of 2-cyanobenzoxazole

[10] H. A. Staab and K. Wendel, *Angew. Chem.*, 1961, **73**, 26.
[11] F. Gauhe, *Annalen*, 1867, **143**, 263.
[12] H. Gall, *Z. angew. Chem.*, 1928, **41**, 683.
[13] T. Wieland and K. Vogeler, *Angew. Chem.*, 1961, **73**, 435.
[14] O. Achmatowicz and M. T. Leplawy, *Roczniki Chem.*, 1958, **32**, 1375.
[15] T. K. Brotherton and J. W. Lynn, *Chem. Rev.*, 1959, **59**, 855.
[16] H. M. Woodburn and J. R. Fisher, *J. Org. Chem.*, 1957, **22**, 895.

may be a factor in preventing reaction, and indeed we were unable to prepare the quaternary salt, which we envisaged as partner in reaction with a salt of phthaloylglycine, from methyl toluene-*p*-sulphonate. On the other hand 2-chlorobenzoxazole (IIIb) did give a small yield of the acid chloride from phthaloylglycine when the reagents were kept at room temperature *without solvent*. The adduct (IV) may have been an intermediate.



(IIIa: X = CN).
(IIIb: X = Cl)



(IV)

Our conclusion is that acyl cyanides may yet become useful intermediates in peptide synthesis, but there can be no further progress until a mild method for their preparation is discovered.

Experimental

Phthaloylglycyl Bromide.—Phthaloylglycine (1·00 g.) was gradually dissolved in thionyl bromide (8·00 g.) at 120—130° during 1 hr. The excess of thionyl bromide was evaporated under reduced pressure, and crystallisation of the residue from benzene–light petroleum afforded phthaloylglycyl bromide (1·22 g., 93%), m. p. 74—75° (Found: C, 45·1; H, 2·3; N, 5·45. $C_{10}H_6BrNO_3$ requires C, 44·8; H, 2·3; N, 5·2%).

Phthaloylglycyl Cyanide.—Phthaloylglycyl bromide (0·54 g.) and cuprous cyanide (0·36 g.) were finely ground together, and the mixture was then kept at 130° for 2 hr. The cold solid was extracted with cold benzene (10 ml.), which was then evaporated, to give pale yellow *phthaloylglycyl cyanide* (0·22 g., 51%), m. p. 140—143° (decomp.) after two recrystallisations from benzene (Found: C, 61·7; H, 2·9; N, 13·15. $C_{11}H_6N_2O_3$ requires C, 61·7; H, 2·8; N, 13·1%); $\nu_{max.}$ (Nujol) 2217, 1780, 1718, 1350, 1300, 1190, 1109, 1073, 935, 787, 717, and 714 cm.$^{-1}$. Benzoyl cyanide [6] has $\nu_{max.}$ (film) 2222 and 1690 cm.$^{-1}$, whereas "bisbenzoyl cyanide," m. p. 96°, has $\nu_{max.}$ (Nujol) 1730 cm.$^{-1}$ and lacks absorption in the nitrile region. Phthaloylglycyl cyanide deteriorates even when kept over phosphoric oxide for a few days, and it decomposes easily during crystallisation. Phthaloylglycine anhydride [$\nu_{max.}$ (Nujol) 952 cm.$^{-1}$] is a common contaminant and it is also formed in the preparation. This anhydride was the main product of reaction between phthaloylglycyl chloride and hydrogen cyanide in ether with pyridine at 0° (a satisfactory method of preparing benzoyl cyanide [7]).

Phthaloylglycylglycine Ethyl Ester.—A solution of phthaloylglycyl cyanide (0·107 g., 0·5 mmole) and glycine ethyl ester (0·052 g., 0·5 mmole) in dry tetrahydrofuran (14 ml.) was boiled for 40 min. The neutral product was phthaloylglycylglycine ethyl ester (0·142 g., 98%), m. p. 194—195° (lit.,[17] 194—195°) (Found: C, 57·8; H, 4·7; N, 9·7. Calc. for $C_{14}H_{14}N_2O_5$: C, 57·9; H, 4·9; N, 9·65%). In another experiment the solution was kept at room temperature and the product started to crystallise after 20 min.; after 40 min. the dipeptide derivative was obtained in 95% yield.

Phthaloylglycyl-α-methylalanine Methyl Ester.—A solution of phthaloylglycyl cyanide (0·107 g., 0·5 mmole) and α-methylalanine methyl ester [9] (0·059 g., 0·5 mmole) in dry tetrahydrofuran (5 ml.) was kept at room temperature for 1 hr. The neutral product was *phthaloylglycyl-α-methylalanine methyl ester* (0·143 g., 97%); crystallised from ethyl acetate–light petroleum this had m. p. 179—180° (Found: C, 59·1; H, 5·1; N, 8·9. $C_{15}H_{16}N_2O_5$ requires C, 59·2; H, 5·3; N, 9·2%).

Thionyl Cyanide.—Finely ground silver cyanide (11 g.) was added slowly to thionyl chloride (2·5 ml.) in a flask which was cooled in ice. After the addition was complete, the solid darkened and fumes were evolved. Colourless needles were formed on the sides of the flask and amongst the solid mass. The flask was kept in ice for 10 min. and then at room temperature for 1 hr. Extraction of its contents with dry ether (25 ml.) gave a colourless solid, which rapidly turned yellow and was crystallised twice from carbon tetrachloride forming platelets (0·52 g., 14%), m. p. 64—66° (Found: C, 26·7; N, 26·5; S, 35·7. Calc. for C_2H_2OS: C, 24·0; N, 28·0; S, 32·0%); $\nu_{max.}$ (Nujol) 2175, 693, and 675 cm.$^{-1}$ (no other absorption outside the Nujol regions). The compound turned yellow quickly at room temperature or during a few days at −78°; it was freshly prepared for reactions. Attempts to identify the volatile products of reaction were inconclusive. Thionyl bromide behaved like thionyl chloride in reaction with silver cyanide.

[17] R. A. Boissonnas, *Helv. Chim. Acta*, 1951, **34**, 874.

2-*Chlorobenzoxazole.*—Benzoxazole-2-thione [18] was suspended in dry, ice-cooled chloroform, through which chlorine was bubbled until no more was absorbed. The solution was initially blue-green and finally, when all the thione had dissolved, yellow. The 2-chlorobenzoxazole (70%), n_D^{20} 1·5680 (lit.,[19] 1·5678) (Found: N, 8·8. Calc. for $C_7H_4ClNO_4$: N, 9·1%) was obtained by washing the chloroform solution with water, sodium hydroxide, and water before distillation; it had b. p. 200—205°.

2-*Cyanobenzoxazole.*—A mixture of 2-chlorobenzoxazole (0·614 g., 4 mmoles), dried, powdered sodium cyanide (0·216 g., 4·4 mmoles), and dimethyl sulphoxide (1 ml.) was kept at 90—100° for 1 hr. The cooled mixture was separated between water and chloroform, which was washed with dilute hydrochloric acid, sodium hydrogen carbonate solution, and water before being dried and evaporated to a partly crystalline residue. Pure 2-*cyanobenzoxazole* (0·134 g., 23%), m. p. 103° (Found: C, 66·3; H, 2·95; N, 19·7. $C_8H_4N_2O$ requires C, 66·7; H, 2·8; N, 19·4%); $\nu_{max.}$ (Nujol) 2232 cm.$^{-1}$, was obtained by sublimation at 80°/0·1 mm.

Benzoxazole-2-carboxamide.—This *amide*, m. p. 175—176° after crystallisation from ethanol (Found: C, 58·9; H, 3·8; N, 17·2. $C_8H_6N_2O_2$ requires C, 59·3; H, 3·7; N, 17·3%), was obtained from the acid chloride [20] by passing ammonia gas into the ethereal solution. It resisted dehydration by toluene-*p*-sulphonyl chloride in pyridine.

We thank Monsanto Chemicals for a studentship (D. S. J.) and Dr. M. T. Leplawy for a sample of carbonyl cyanide.

THE ROBERT ROBINSON LABORATORIES,
UNIVERSITY OF LIVERPOOL. [*Received, February 25th,* 1965.]

[18] J. A. Van Allan and B. D. Deacon, *Org. Synth.*, 1963, Coll. Vol. IV, 570.
[19] H. N. McCoy, *Amer. Chem. J.*, 1899, **21**, 111.
[20] S. Skraup and M. Moser, *Ber.*, 1922, **55**, 1080.