1314. *2-Iodoethoxycarbonyl Chloride. A New Amine-protecting Group of Possible Value in Peptide Synthesis*

By J. Grimshaw

The application of 2-iodoethoxycarbonyl chloride as a protecting group in peptide synthesis is examined. This reagent reacts with the amino-function to give derivatives of the type (IV) from which the amino-group can be regenerated by the action of zinc and methanol.

The formation of an olefin by simultaneous removal of the halogen and hydroxyl groups from a 2-halogeno-alcohol has occasionally been used as a synthetic procedure. Fischer,[1] for example, first used this reaction to prepare glycal derivatives, whilst more recently it has been employed [2] in the stereoselective synthesis of squalene. At the start of the work described here it was intended to explore the possibilities of using this reaction to form a novel amine-protecting group. A useful reagent has been found but some of its derivatives appear to be very toxic and we do not intend to examine them further.

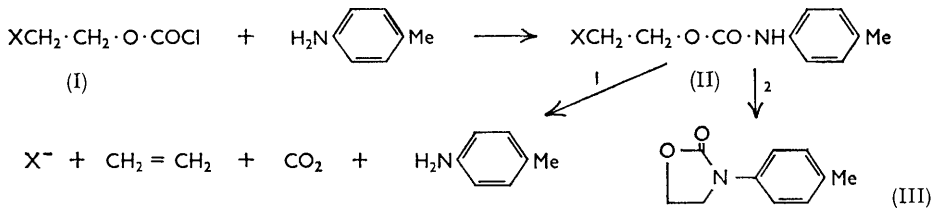
The protecting reagent envisaged was one of the 2-halogenoethoxycarbonyl chlorides (I). In preliminary experiments to investigate the reactivity of the halogenoethoxy-group, *p*-toluidine was used as a test amine; it reacted with the chloroformates (I) to give the corresponding urethanes (II). Of these derivatives, only the iodo-compound (II; X = I) reacted sufficiently rapidly (see Table) with zinc dust in methanol for the purpose

[1] E. Fischer, *Ber.*, 1914, **47**, 196.
[2] J. W. Cornforth, R. H. Cornforth, and K. K. Mathew, *J.*, 1959, 112. 2539.

Action of zinc on compounds (II)

p-Tolylurethane from:	After 10 min. Recovered (II) (%)	After 10 min. *p*-Toluidine (%)	After 20 min. Recovered (II) (%)	After 20 min. *p*-Toluidine (%)	After 80 min. Recovered (II) (%)	After 80 min. *p*-Toluidine (%)	After 6 hr. Recovered (II) (%)	After 6 hr. *p*-Toluidine (%)
$ICH_2 \cdot CH_2 \cdot OH$	25	65	10	85	—	—	—	—
$BrCH_2 \cdot CH_2 \cdot OH$	—	—	85	10	45	35	—	—
$ClCH_2 \cdot CH_2 \cdot OH$	—	—	—	—	—	—	80	5

in hand. This reaction was complete after 30 min. at 60°, and *p*-toluidine could be recovered in excellent yield. 2-Iodoethoxycarbonyl chloride (I; X = I) was prepared by the action of phosgene on 2-iodoethanol and is moderately stable though it probably could not be kept unchanged for long periods.



(III)

Reagents: 1, Zn + MeOH; 2, KOH

A number of unwanted reactions of the iodoethyl group could be expected. Cyclisation of the urethane (II) in the presence of a base [3] to give the compound (III) is an example of one possibly disadvantageous reaction. 2-Iodoethoxycarbonyl-*p*-toluidine was largely unaffected by prolonged boiling with neutral ethanol and by 0·5N-ethanolic potassium hydroxide at room temperature for 20 min. but cyclisation to give compound (III) proceeded rapidly at the boiling point of 0·5N-ethanolic potassium hydroxide. This cyclisation was not then expected to interfere with peptide synthesis provided that the reaction used to form the peptide bond was one that exposed the materials to alkaline conditions for a short time only. Accordingly, in the peptide-forming experiments, the mixed anhydride procedure with isobutyl chloroformate [4] was employed, using triethylamine as the base. In these experiments there was no great loss of product such as would lead one to suspect quaternary salt formation, a second possible side-reaction. In some experiments ethyldi-isopropylamine, which does not readily form quaternary salts,[5] was employed as the base with no significant increase in yield of the protected dipeptide.

Amino-acids reacted readily in alkaline solution with 2-iodoethoxycarbonyl chloride, and the corresponding derivatives [*e.g.*, (V) from glycine] were precipitated on acidification. The derivatives of L-valine, L-leucine, and L-proline could not be obtained crystalline.

The glycine derivative was converted into the anilide in good yield by first preparing a mixed anhydride with isobutyl chloroformate in the presence of triethylamine and then treating this anhydride with aniline. Removal of the protecting group by reaction with zinc dust in refluxing methanol for 20 min. gave glycylaniline which, being an ether-soluble base, could be readily separated from inorganic salts and was characterised as its benzoyl derivative.

$ICH_2 \cdot CH_2 \cdot O \cdot CO \cdot NH \cdot CH_2 \cdot CO_2H$ (IV)

$ICH_2 \cdot CH_2 \cdot O \cdot CO \cdot NH \cdot CH_2 \cdot CO \cdot NH \cdot CH_2 \cdot CO_2Et$ (V)

$ICH_2 \cdot CH_2 \cdot O \cdot CO \cdot NH \cdot CHMe \cdot CO \cdot NH \cdot CH_2 \cdot CO_2Et$ (VI)

Derivatives (V) and (VI) of the dipeptides glycylglycine and alanylglycine, respectively, were also prepared using the mixed anhydride procedure. In order to obtain the free

[3] R. Adams and J. B. Segur, *J. Amer. Chem. Soc.*, 1923, **45**, 785.
[4] J. R. Vaughan and R. L. Osato, *J. Amer. Chem. Soc.*, 1952, **74**, 677.
[5] S. Hünig and M. Kiessel, *Chem. Ber.*, 1958, **91**, 380.

dipeptide from (V), the amine-protecting group was first removed by reaction with zinc dust in methanol. The reaction mixture was then acidified and filtered, and the filtrate was made sufficiently alkaline to hydrolyse the ester function. Iodide ions were then precipitated with an excess of silver oxide. The filtrate from this procedure, which contained only the dipeptide and excess sodium hydroxide, was neutralised with Amberlite IRC-50 ion-exchange resin in the acid form. Evaporation of the eluent to dryness gave glycylglycine in 50% yield.

Experimental

As iodoethanol is physiologically active, great care should be exercised when handling it and its derivatives. Some of the derivatives described here are believed to have a powerful effect on the central nervous system. The molecular weights of the acid chlorides were determined by titration, with 0·1N-sodium hydroxide, of the hydrochloric acid liberated after gently boiling a sample with aqueous ethanol for 1 min. M. p.s were taken using a Kofler hot-stage apparatus calibrated with standard substances. The light petroleum had b. p. 60—80°.

2-Halogenoethoxycarbonyl Chlorides.—Phosgene gas was passed into the corresponding 2-halogenoethanol until more than the theoretical increase in weight was observed. Excess of phosgene was then removed in a current of nitrogen and the product distilled. 2-Chloroethoxycarbonyl chloride had b. p. 153—154°/758 mm. (lit.,[6] 152·5°/752 mm.); *2-bromoethoxycarbonyl chloride* was a fuming liquid, b. p. 72—74°/28 mm. (Found: *M*, 189. $C_3H_4BrClO_2$ requires *M*, 187); *2-iodoethoxycarbonyl chloride* was obtained as a colourless fuming liquid, b. p. 82—84°/15 mm. (Found: *M*, 235. $C_3H_4ClIO_2$ requires *M*, 234); samples not specially purified, although brown, were suitable for subsequent operations.

p-*Tolylurethane Derivatives* (II).—*p*-Toluidine in benzene was stirred with N-sodium hydroxide (1 equiv.) and cooled in ice during the addition of the appropriate 2-halogenoethoxycarbonyl chloride (1 equiv.). The benzene layer was then separated, washed with dilute hydrochloric acid, and water, dried (Na_2SO_4), and the solvent removed. The residue crystallised from light petroleum or ethyl acetate–light petroleum. 2-Chloroethoxycarbonyl-*p*-toluidine separated as plates, m. p. 58—59° (lit.,[3] 61°); *2-bromoethoxycarbonyl-p-toluidine* formed needles, m. p. 77—78° (Found: C, 46·65; H, 4·7; N, 5·7. $C_{10}H_{12}BrNO_2$ requires C, 46·5; H, 4·65; N, 5·4%); *2-iodoethoxycarbonyl-p-toluidine* formed needles, m. p. 114—115° (Found: C, 39·6; H, 3·8; N, 4·6. $C_{10}H_{12}INO_2$ requires C, 39·4; H, 3·95; N, 4·6%). The bromo-compound gave the iodo-compound in quantitative yield on refluxing with sodium iodide in acetone for 2 hr.

2-Halogenoethoxycarbonyl Derivatives of Amino-acids.—The amino-acid was dissolved in 2N-sodium hydroxide (2 equiv.), cooled in ice, and the appropriate 2-halogenoethoxycarbonyl chloride (1 equiv.) added with stirring. After 20 min., the solution was washed with ether and then acidified with concentrated hydrochloric acid. The product precipitated (yield 70% or more) and was crystallised from benzene or ethyl acetate–light petroleum. *2-Chloroethoxycarbonylglycine* separated from benzene as needles, m. p. 87—88° (Found: C, 33·3; H, 4·6; N, 7·7. $C_5H_8ClNO_4$ requires C, 33·1; H, 4·4; N, 7·7%). *2-Bromoethoxycarbonylglycine* was obtained as plates from benzene, m. p. 92—93° (Found: C, 26·6; H, 3·7; N, 6·4. $C_5H_8BrNO_4$ requires C, 26·6; H, 3·55; N, 6·2%). *2-Iodoethoxycarbonylglycine* crystallised from ethyl acetate–light petroleum as plates, m. p. 127—128° (Found: C, 21·8; H, 2·95; N, 5·2. $C_5H_8INO_4$ requires C, 22·0; H, 2·9; N, 5·2%); (±)-*2-iodoethoxycarbonylalanine*, needles from benzene, m. p. 126—127° (Found: C, 25·5; H, 3·3; N, 4·8. $C_6H_{10}INO_4$ requires C, 25·2; H, 3·5; N, 4·9%); L-*2-iodoethoxycarbonylalanine*, needles from either water or benzene, m. p. 105—106° C, 25·5; H, 3·8; N, 5·1%); L-*2-iodoethoxycarbonylphenylalanine*, needles from ethyl acetate–light petroleum, m. p. 61—62° (Found: C, 39·6; H, 3·8; N, 4·2. $C_{12}H_{14}INO_4$ requires C, 39·7; H, 3·85; N, 3·85%).

Stability of 2-Iodoethoxycarbonyl-p-toluidine.—This urethane was recovered in 85% yield after refluxing for 4 hr. with ethanol, and in 90% yield after standing for 20 min. in 0·5N-ethanolic potassium hydroxide. At the boiling point this latter solution rapidly precipitated potassium iodide and afforded the compound (III) on dilution with water, m. p. 91—92° (lit.,[3] 91°).

Stability of 2-Iodoethoxycarbonylglycine.—A solution of the acid in water was neutralised with 0·1N-sodium hydroxide using Thymol Blue as indicator. At 30°, the titre increased by less than 5% after 1 hr. At 60° the titre had increased by 5% after 10 min.

[6] V. Nekrasov and Ya. F. Komissarov, *J. prakt. Chem.*, 1929, [2], **123**, 163.

Action of Zinc on the 2-Halogenoethoxy-p-toluidines.—Zinc dust was washed before use with 2% hydrochloric acid, water, and methanol, and then rapidly dried at 100°. The appropriate *p*-toluidine derivative (0·5mM) in methanol (6 ml.) was heated under reflux in a thermostat at 60° and treated with zinc dust (1 g.). Samples were withdrawn at appropriate times, acidified with dilute hydrochloric acid, and separated, using ether, into neutral and basic fractions. *p*-Toluidine was identified as its azo-2-naphthol derivative, m. p. and mixed m. p. 130—131°.

2-Iodoethoxycarbonylglycylaniline.—2-Iodoethoxycarbonylglycine (0·5 g.) and triethylamine (0·25 ml.) were dissolved in tetrahydrofuran (10 ml.) and cooled to −10°; isobutyl chloroformate (0·24 ml.) was added with stirring. After 15 min., aniline (0·4 ml.) was added and the mixture left at room temperature for 15 min., poured into excess of dilute hydrochloric acid, the product isolated with ether, washed with sodium hydrogen carbonate, and water, and dried (Na_2SO_4) and the solvent removed. The residual *anilide* crystallised from ethanol as needles (0·42 g., 66%), m. p. 173—174° (Found: C, 37·7; H, 3·5; N, 8·1. $C_{11}H_{13}IN_2O_3$ requires C, 38·0; H, 3·75; N, 8·05%).

2-Iodoethoxycarbonylglycylglycine Ethyl Ester (V).—2-Iodoethoxycarbonylglycine (0·5 g.) and triethylamine (0·25 ml.) were dissolved in chloroform (5 ml.) and cooled to −10°; isobutyl chloroformate (0·24 ml.) was added with stirring. After 10 min. a slurry of glycine ethyl ester hydrochloride (0·25 g.) and triethylamine (0·25 ml.) in chloroform (2 ml.) was added and the mixture left at room temperature for 10 min., washed with dilute hydrochloric acid, sodium hydrogen carbonate solution, and water, dried (Na_2SO_4), and the solvent removed under reduced pressure. The residual *2-iodoethoxycarbonylglycylglycine ethyl ester* crystallised from ethyl acetate–light petroleum as needles (0·47 g., 72%), m. p. 121—122° (Found: C, 30·0; H, 4·4; N, 8·0. $C_9H_{15}IN_2O_5$ requires C, 30·2; H, 4·2; N, 7·85%).

(±)-*2-Iodoethoxycarbonylalanylglycine Ethyl Ester* (VI).—(±)-2-Iodoethoxycarbonylalanine (2·0 g.) and triethylamine (0·74 g.) were dissolved in tetrahydrofuran (20 ml.) and cooled to −5°; isobutyl chloroformate (0·96 g.) was added. After 10 min. a slurry of glycine ethyl ester hydrochloride (1·0 g.) with triethylamine (0·74 g.) and chloroform (8 ml.) was added. The resulting mixture was worked up as for the previous experiment, to give (±)-*2-iodoethoxycarbonylalanylglycine ethyl ester* (1·3 g., 50%) which crystallised from ethyl acetate–light petroleum as needles, m. p. 93—94° with resolidification and m. p. 102—103° (Found: C, 32·5; H, 4·8; N, 6·9. $C_{10}H_{17}IN_2O_5$ requires C, 32·3; H, 4·6; N, 7·5%).

Glycine Anilide.—2-Iodoethoxycarbonylglycylaniline (0·30 g.) in methanol (12 ml.) was refluxed with zinc dust (2 g.) for 15 min. and the mixture was filtered and the filtrate diluted with water, acidified, and evaporated under pressure. The small residue was made alkaline with dilute sodium hydroxide and extracted continuously with ether during 5 hr. The ether extract was evaporated, finally over potassium hydroxide *in vacuo*, leaving an oil which was dissolved in ethanolic hydrogen chloride. Addition of ether precipitated glycine anilide hydrochloride as plates (0·10 g., 77%), m. p. 200—203° (rapid heating) (lit.,[7] 190—195°) (Found: Cl, 18·8. Calc. for $C_8H_{11}ClN_2O$: Cl, 19·1%). This hydrochloride was shaken wih benzoyl chloride and N-sodium hydroxide, to give benzoylglycine anilide which crystallised from ethanol as laths, m. p. 213—214° undepressed on admixture with an authentic sample (lit.,[8] 214°).

Glycylglycine.—2-Iodoethoxycarbonylglycylglycine ethyl ester (1·0 g.) was dissolved in methanol (15 ml.) and refluxed with zinc dust (2 g.) for 15 min., and the mixture was cooled, diluted with water, acidified with N-hydrochloric acid (6 ml.), and filtered. The filtrate was made alkaline with N-sodium hydroxide (18 ml.), left for 1 hr. at room temperature, and filtered from zinc oxide. The filtrate was stirred for 1 hr. with silver oxide [from silver nitrate (3·5 g.)], filtered, and the solution passed through a column of Amberlite IRC-50 (acid form). Evaporation of the eluent, now neutral, to dryness, left a residue of glycylglycine which crystallised from water–ethanol as needles (0·18 g., 50%). The method of Nefkens *et al.*,[9] was used to convert this material into the phthaloyl derivative, m. p. 231—232° undepressed on admixture with phthaloylglycylglycine (lit.,[10] 231—232°).

DEPARTMENT OF ORGANIC CHEMISTRY,
QUEEN'S UNIVERSITY, BELFAST, NORTHERN IRELAND. [*Received, February 8th,* 1965.]

[7] A. J. Hill and E. B. Kelsey, *J. Amer. Chem. Soc.*, 1920, **42**, 1706.
[8] M. Bergmann, L. Zervas, and J. S. Fruton, *J. Biol. Chem.*, 1936, **115**, 593.
[9] G. H. L. Nefkens, G. I. Tessler, and R. J. F. Nivard, *Rec. Trav. chim.*, 1960, **79**, 688.
[10] F. E. King, J. W. Clark-Lewis, R. Wade, and W. A. Swindin, *J.*, 1957, 873.