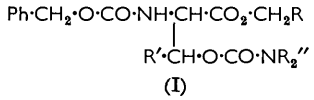
191. *Cyto-active Amino-acids and Peptides. Part VII.*[1] *Derivatives of Serine and Threonine.*

By F. Bergel and Roy Wade.

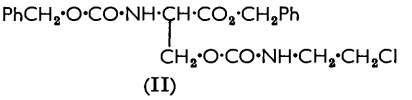
Amino-acid derivatives carrying the " nitrogen-mustard " radical as an amido-group have been synthesised in the form of *O*-[*NN*-di-(2-chloroethyl)-carbamoyl]-DL- and -L-serine and -DL-threonine. A number of other *N*-substituted *O*-carbamoylserines have been prepared. The *N*-benzyloxycarbonyl- and *N*-*p*-nitrobenzyloxycarbonyl-threonine, and to a smaller extent the corresponding serine compounds, produced oxazolidones on treatment with alkali.

In view of the contrasting biological behaviour of the serine and threonine " mustards," the hydrolysis of these two compounds *in vitro* was studied and *iso*propyl *NN*-di-(2-chloroethyl)carbamate was synthesised. This compound, like the threonine derivative, was inactive in anti-tumour tests while the serine and the ethyl carbamate " mustards " showed remarkable activity.

Previous papers in this series[2] and the work of Larionov and Knunyants and their co-workers[3,4] have dealt with the synthesis of α-amino-acid derivatives containing a " nitrogen-mustard " substituent (M) on an aromatic nucleus. Other amino-acid derivatives, carrying M on the α-carbon atom in place of an unsubstituted amino-group, have been described by Ishidate and his co-workers[5] and more recently by Nyhan and Busch.[6] The work of Skinner *et al.*[7,8] showing *O*-carbamoylserine and *O*-(hydrazinocarbonyl)serine to be antagonists of glutamine in various bacterial systems indicates that corresponding derivatives of serine carrying M in the carbamoyl moiety might possess antitumour activity.



(I)



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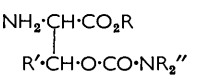

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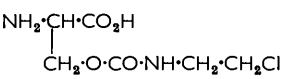


(IV)

This paper describes the preparation of several derivatives of serine and threonine in which a $-CO-$ group is used to provide the link between the α-amino-β-hydroxy-acid and amido-groups with potential cytotoxicity. *N*-Benzyloxycarbonyl-DL- and -L-serine benzyl ester and -DL-serine methyl ester were prepared by slight modification of known methods[7,9-13] and converted into the *O*-chlorocarbonyl derivatives by reaction with carbonyl chloride. These were not characterised but coupled directly in an inert solvent with di-(2-chloroethyl)amine, dipropylamine, or 2-chloroethylamine, a second equivalent of base being used to take up the hydrogen chloride formed in the reaction. The coupling

[1] Parts V and VI, Bergel and Stock, *J.*, 1959, 90, 97.
[2] Part I, Bergel and Stock, *J.*, 1954, 2409; Part II, Bergel, Burnop, and Stock, *J.*, 1955, 1223; Part III, Bergel and Lewis, *J.*, 1957, 1816; Part IV, Bergel and Stock, *J.*, 1957, 4563.
[3] Larionov, Shkodinskaja, Troosheikina, Khoklor, Varina, and Novikova, *Lancet*, 1955, II, 169.
[4] Knunyants, Kil'disheva, and Golubeva, *Izvest. Akad. Nauk S.S.S.R., Otdel. Khim. Nauk*, 1956, 1418.
[5] Ishidate, Sakurai, and Izumi, *J. Amer. Pharm. Assoc.*, 1955, **44**, 132.
[6] Nyhan and Busch, *Fed. Proc.*, 1958, **17**, 639.
[7] Skinner, McCord, Ravel, and Shive, *J. Amer. Chem. Soc.*, 1956, **78**, 2412.
[8] McCord, Ravel, Skinner, and Shive, *ibid.*, 1958, **80**, 3762.
[9] Bergmann and Zervas, *Ber.*, 1932, **65**, 1192.
[10] Ben-Ishai and Berger, *J. Org. Chem.*, 1952, **17**, 1564.
[11] Baer and Maurukas, *J. Biol. Chem.*, 1955, **212**, 25.
[12] Jones and Lipkin, *J. Amer. Chem. Soc.*, 1956, **78**, 2408.
[13] Fölsch and Mellander, *Acta Chem. Scand.*, 1957, **11**, 1232.

of di-(2-chloroethyl)amine with ethyl chloroformate in a similar manner has been described by Childs *et al.*[14] The resulting carbamoyl derivatives (I and II; R′ = H; Table 1, Nos. 1—5) were in general low-melting solids with physical properties similar to those of the starting material. The protecting benzyloxycarbonyl and benzyl ester groups were removed by hydrogenolysis in methanol in presence of palladium–charcoal; uptake of hydrogen was usually rapid and the free amino-acid derivatives and one ester hydrochloride (III; R′ = H; and IV) are described in Table 2.

In the case of the threonine derivatives, *N*-benzyloxycarbonyl-DL-threonine was prepared by the reaction of the amino-acid with benzyloxycarbonyl chloride in aqueous sodium hydrogen carbonate, as carried out by Riley, Turnbull, and Wilson.[15] After use of a stronger alkali as in the conventional Bergmann technique[9] there was no precipitation of the product on acidification; however, extraction of the clear aqueous solution with ethyl acetate and working up the extract gave about 50% yield of a compound which was readily water-soluble, giving a strongly acid solution with a negative ninhydrin test and

TABLE 1.

	Compound (I)				Yield		Found (%)				Required (%)			
No.	R	R′	R″	M. p.	(%)	Formula	C	H	N	Cl	C	H	N	Cl
1	Ph	H	$Cl{\cdot}CH_2{\cdot}CH_2$	70—71° [b]	71	$C_{23}H_{26}O_6N_2Cl_2$	55·55	5·5	5·6	14·3	55·5	5·2	5·6	14·3
2 [a]	Ph	H	,,	54—55 [c]	83	$C_{23}H_{26}O_6N_2Cl_2$	56·3	5·2	5·6	14·1	55·5	5·2	5·6	14·3
3		Compound (II)		102—103 [d]	75	$C_{21}H_{23}O_6N_2Cl$	58·4	5·5	6·4	7·9	58·1	5·3	6·45	8·2
4	Ph	H	Pr^n	56—57 [c]	82	$C_{25}H_{32}O_6N_2$	65·8	7·2	6·2	—	65·8	7·0	6·1	—
5	H	H	$Cl{\cdot}CH_2{\cdot}CH_2$	79—80 [b]	63	$C_{17}H_{22}O_6N_2Cl_2$	48·2	5·3	6·7	17·0	48·5	5·2	6·7	16·9
6	Ph	Me	,,	80—81 [c]	65	$C_{24}H_{28}O_6N_2Cl_2$	56·8	5·4	5·3	14·0	56·4	5·5	5·5	13·9

[a] L-Isomer of No. 1, $[\alpha]_D^{22}$ −13·5° (*c* 1 in EtOH). [b] From ethyl acetate–light petroleum (b. p. 60—80°). [c] From ether–light petroleum (b. p. 40—60°). [d] From ethanol.

TABLE 2.

	Compound (III)					
No.	R	R′	R″	M. p.*	Yield (%)	R_F
1	H	H	$Cl{\cdot}CH_2{\cdot}CH_2$	140°	82	0·53
2 [a]	H	H	,,	127	89	0·53
3		Compound (IV)		180	72	0·43
4	H	H	Pr^n	121	85	0·71
5 [b]	Me	H	$Cl{\cdot}CH_2{\cdot}CH_2$	115—116	95	0·78
6	H	Me	,,	130	95	0·60

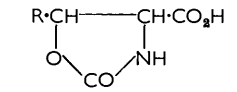
		Found (%)				Required (%)			
No.	Formula	C	H	N	Cl	C	H	N	Cl
1	$C_8H_{14}O_4N_2Cl_2$	35·0	5·3	10·3	25·4	35·2	5·1	10·3	26·0
2 [a]	$C_8H_{14}O_4N_2Cl_2,H_2O$	33·2	5·7	9·6	24·4	33·0	5·5	9·6	24·4
3	$C_6H_{11}O_4N_2Cl$	34·6	5·4	13·1	16·8	34·3	5·2	13·3	16·9
4	$C_{10}H_{20}O_4N_2$	52·0	8·9	12·5	—	51·7	8·7	12·1	—
5 [b]	$C_9H_{17}O_4N_2Cl_3$	33·3	5·1	8·7	32·7	33·4	5·4	8·7	32·9
6	$C_9H_{16}O_4N_2Cl_2,H_2O$	35·7	6·1	9·1	22·9	35·4	5·9	9·2	23·3

* With decomp. [a] L-Isomer, $[\alpha]_D^{22}$ +6·0° (*c* 1 in H_2O), $[\alpha]_D^{22}$ +22·0° (*c* 1 in N-HCl). [b] Isolated as the hydrochloride. [c] Run on Whatman No. 1 paper, ascending-descending technique with butan-1-ol–ethanol–propionic acid–water (10 : 5 : 2 : 5 v/v).

analyses as for $C_5H_7O_4N$. Hydrolysis in concentrated acid solution gave DL-threonine once again, identified by its *N*-benzoyl derivative. The compound is probably 5-methyl-2-oxo-oxazolidine-4-carboxylic acid (V; R = Me) in which the asymmetric carbon atoms have the same configuration as in DL-threonine. Such a cyclisation may well involve the transient formation of the intermediate (VI) followed by elimination of benzyl alcohol. An intermediate of this nature has been suggested previously[16] in cases of $N \longrightarrow O$ acyl migration which was obviously not observed under our alkaline conditions. Gish and

[14] Childs, Goldsworthy, Harding, King, Nineham, Norris, Plant, Selton, and Tompsett, *J.*, 1948, 2174.
[15] Riley, Turnbull, and Wilson, *J.*, 1957, 1373.
[16] Welsh, *J. Amer. Chem. Soc.*, 1949, **71**, 3500.

Carpenter,[17] in their work on the *p*-nitrobenzyloxycarbonyl derivatives of α-amino-acids, noticed that the yields were low with the serine and threonine derivatives when strongly alkaline conditions were used. They reported that these derivatives were rapidly hydrolysed by strong alkali with liberation of *p*-nitrobenzyl alcohol. While these authors [17] did not isolate any product from the amino-acid portion, we found that oxazolidones were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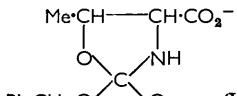
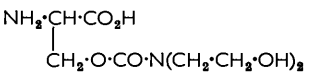
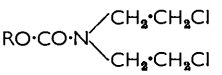

(VI)

indeed formed from *N*-*p*-nitrobenzyloxycarbonyl-DL-threonine (yield 60%) and in much smaller quantities from the corresponding *N*-benzyloxycarbonyl- and *N*-*p*-nitrobenzyloxycarbonyl-serine in addition to the threonine derivative mentioned above.

The last compound, which was not obtained crystalline, was converted into the benzyl ester by the procedure adopted for the analogous serine compound. Treatment of this ester with carbonyl chloride, as before, followed by coupling with di-(2-chloroethyl)amine gave a product which consisted mainly of starting material. Fractionating the mother-liquors on alumina gave only a small yield of chlorine-containing material which was recrystallised and gave the correct analysis for *N*-benzyloxycarbonyl-*O*-[*N*-di-(2-chloroethyl)carbamoyl]-DL-threonine benzyl ester. The preparation was subsequently repeated, the carbonyl chloride being allowed to remain in contact with benzyloxycarbonyl-DL-threonine benzyl ester for a total time of 14 days by introducing more of the chloride to the solution from time to time. From this a better yield of carbamoyl derivative was obtained. When the initial preparation, using carbonyl chloride treatment for 4 hours, was repeated in the presence of one equivalent of dimethylaniline, formation of the chlorocarbonyl derivative was more rapid and this gave finally a 65% yield of the required carbamoyl derivative (I; R = Ph, R′ = Me, R″ = CH_2·CH_2Cl; Table 1, No. 6). The compound was hydrogenolysed in the usual way and the free amino-acid derivative (III; R = H, R′ = Me, R″ = CH_2·CH_2Cl; Table 2, No. 6) was obtained in good yield.

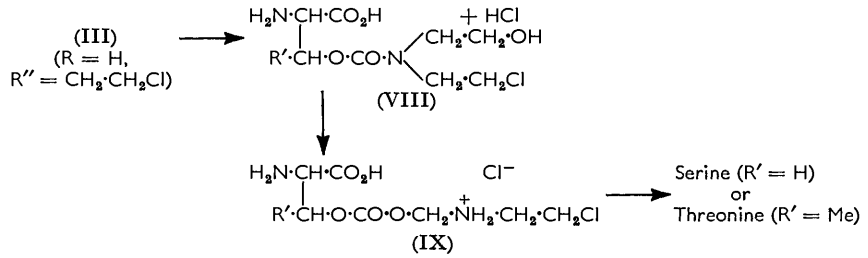

(VII)


(X)

The DL- and L-serine mustards (Table 2, No. 1, CB 3159, and No. 2, CB 3189), compounds of low toxicity, were found to be very effective (the L more so than the DL) as anti-tumour agents against the Walker rat carcinoma 256. CB 3159 was also tested on the very resistant benzopyrene and " August" tumours in rats and caused some hold-up of their growth rates, but the methyl ester hydrochloride (Table 2, No. 5, CB 3178) showed a greater effect on the latter tumour, but no effect on the transplanted mammary tumour in C^- male mice. Both the other serine derivatives examined (Table 2, Nos. 3 and 4) proved to be inactive on the Walker carcinoma, as did the threonine mustard (Table 2, No. 6, CB 3182). This prompted us to compare the hydrolytic behaviour of the serine derivative CB 3159 and the threonine derivative CB 3182: it was found that under *in vitro* conditions, at pH 7·4 and 37°, CB 3182 produced acidity at roughly twice the rate of CB 3159. When the two amino-acids were treated at 100° in aqueous solution (starting pH 5·7) and hydrolysis was followed chromatographically on paper, more rapid breakdown of the threonine derivative was again observed. In addition to this overall rate difference the pathway of degradation of the serine derivative went *via* two ninhydrin-positive, relatively stable intermediates before any free serine was produced. On the other hand, during the hydrolysis of CB 3182, free threonine appeared at an earlier stage, although an analogous sequence of degradation products was observed, implying a greater lability

[17] Gish and Carpenter, *J. Amer. Chem. Soc.*, 1953, **75**, 950.

of the second intermediate. The chemical constitution of each intermediate has not been determined yet with certainty although the possibility that either of them is the dihydroxy-analogue (VII) is ruled out by their different R_F values when chromatographed on paper in phenol. By analogy with the behaviour of di-(2-chloroethyl)amides of carboxylic acids which undergo $N \longrightarrow O$ acyl migration under acid conditions [14] the annexed scheme is tentatively postulated.



The quantitative differences in the hydrolysis of CB 3159 and CB 3182 are seemingly too small to account for the marked difference in biological activity of the two compounds; moreover the evidence so far available, albeit under *in vitro* conditions, does not allow for the formation of di-(2-chloroethyl)amine. The situation is probably different *in vivo*, where enzymic reactions may liberate the cytotoxically active di-(2-chloroethyl)amine or in some other way remove the deactivating influence of the acyl group on the chlorine atoms of the " nitrogen mustard."

The problem is somewhat reminiscent of that obtaining with urethane derivatives (X).

As mentioned above, Childs *et al.*[14] have reported the synthesis of the urethane (X; R = Et). Dr. S. R. M. Bushby [18] has tested this and various homologues for anti-tumour activity against the Walker carcinoma. He found it was active but that replacement of the ethyl by other alkyl radicals diminished considerably, or completely eliminated, this activity. We prepared the compound (X; R = Pr^i) because of its broad analogy with the serine–threonine relationship, and like the threonine mustard (CB 3182) this urethane was inactive.

Experimental

*Benzyloxycarbonyl-*DL*-serine Benzyl Ester.*—Benzyloxycarbonyl-DL-serine was prepared by the method of Bergmann and Zervas [9] in 83% yield. Esterification by Ben-Ishai and Berger's general procedure [10] gave a crude product which was heated to 100—120°/1 mm. for 30 min. to remove excess of benzyl alcohol. The residual gum crystallised from ether on addition of light petroleum, as needles, m. p. 73—74° (lit.,[7,11-13] 72—73° to 74·0—74·4°).

*Benzyloxycarbonyl-*L*-serine Benzyl Ester.*—The protected ester, prepared in the same way as above, crystallised from ether as needles, m. p. 82—83° (lit.,[11-13] 83—84° to 84—85°), $[\alpha]_D^{21}$ −8·8° (*c* 5·0 in EtOH) [lit.,[12] −8·7° (*c* 5 in EtOH)].

N-*Benzyloxycarbonyl-*O-*chlorocarbonyl-*DL- *and* -L-*serine Benzyl Ester.*—Essentially the general procedure as described by Skinner *et al.*[7] was used. Carbonyl chloride was passed into a stirred suspension of benzyloxycarbonyl-DL- or -L-serine benzyl ester (10 g.) in benzene or toluene (200 ml.) at 0° for 20 min. The gas inflow and cooling were then stopped but the stirring was continued for 3 hr. A stream of dry nitrogen was bubbled through the by then clear solution until all the hydrogen chloride and excess of carbonyl chloride had been removed and the solvent was evaporated off under reduced pressure at <40°. No attempt was made to crystallise the residual oil which was used directly in the coupling experiments.

Substituted N-*Benzyloxycarbonyl-*O-*carbamoyl-*DL- *and* -L-*serine Benzyl Ester* (I and II).—Solutions of the chloroformate, prepared as above in benzene (about 3 ml./g.), were stirred and cooled during slow addition of solutions of various bases (2·3 equivs.) in ether (about 5 ml./g.). The solutions were kept at room temperature overnight then washed with dilute acid, sodium hydrogen carbonate solution, and water, dried (Na_2SO_4), and evaporated to

[18] Bushby, personal communication.

dryness under reduced pressure at $>35°$. The *carbamates* crystallised from the solvents indicated in Table 1 (Nos. 1—4).

DL-*Serine Methyl Ester Hydrochloride.*—The ester prepared by Fischer and Suzuki's method [19] had m. p. 134° (decomp.). These authors give m. p. 114° (decomp.), as do Mattocks and Hartung,[20] but King [21] gives m. p. 134° (decomp.).

N-*Benzyloxycarbonyl*-DL-*serine Methyl Ester.*—The preparation was carried out by a method analogous to that used by Riley, Turnbull, and Wilson [15] for the corresponding ethyl ester. N-*Benzyloxycarbonyl*-DL-*serine methyl ester* had b. p. 178—183°/1 mm. (Found: C, 57·1; H, 6·1; N, 5·5. $C_{12}H_{15}O_5N$ requires C, 56·9; H, 6·0; N, 5·5%).

N-*Benzyloxycarbonyl*-O-[NN-*di*-(2-*chloroethyl*)*carbamoyl*]-DL-*serine Methyl Ester.*—*N*-Benzyloxycarbonyl-DL-serine methyl ester (7·0 g.) was converted into the *O*-chlorocarbonyl derivative in the same way as the benzyl ester described above. This was then coupled in benzene (40 ml.) with di-(2-chloroethyl)amine (9·5 g., 2 equivs.) and after being worked up in the usual way the *carbamate* recrystallised as recorded in Table 1 (No. 5).

N-*Benzyloxycarbonyl*-DL-*threonine Benzyl Ester.*—*N*-Benzyloxycarbonyl-DL-threonine was prepared from DL-threonine (10 g.) by the method of Riley *et al.*[15] but was not obtained crystalline and was converted (18·9 g.) into the benzyl ester directly, by boiling benzyl alcohol (18 ml.), benzene (180 ml.), and toluene-*p*-sulphonic acid (0·5 g.), with a Dean and Stark water-separator. After 8 hr. the esterification was complete and the cooled solution was washed with sodium hydrogen carbonate solution and with water and dried (Na_2SO_4). The benzene was evaporated off on the water-pump and the excess of benzyl alcohol on the oil-pump (bath-temp. 100—120°). Trituration of the residual oil with light petroleum caused crystallisation, and recrystallisation of the solid from ether–light petroleum gave N-*benzyloxycarbonyl*-DL-*threonine benzyl ester* as needles, m. p. 63—64° (13·65 g., 49% based on threonine) (Found: C, 66·3; H, 6·1; N, 4·2. $C_{19}H_{21}O_5N$ requires C, 66·5; H, 6·2; N, 4·1%).

Attempted Preparation of Benzyloxycarbonyl-DL-*threonine in Aqueous Alkali.*—As in Bergmann and Zervas's method,[9] DL-threonine (2·4 g.) was dissolved in 4N-sodium hydroxide (5 ml.), the solution cooled ($<5°$), and benzyl chloroformate (3·8 ml.) added portionwise with vigorous shaking. Between each addition 2N-sodium hydroxide was added to maintain pH 8—10. After all the chloroformate had been added, the mixture was shaken for 1 hr., then extracted with ethyl acetate (2 × 20 ml.). Careful addition of concentrated hydrochloric acid gave neither a precipitate nor a turbidity, in contrast to the preparation mentioned above.[15] Extraction with ethyl acetate (4 × 25 ml.) was followed by drying (Na_2SO_4); evaporation of the solvent under reduced pressure gave a solid residue which recrystallised from ethyl acetate–light petroleum as prisms (1·5 g., 52%), m. p. 123—124°, of 5-*methyl*-2-*oxo*-*oxazolidine*-4-*carboxylic acid* (Found: C, 41·6; H, 4·9; N, 9·6. $C_5H_7O_4N$ requires C, 41·4; H, 4·9; N, 9·6%).

Treatment of N-*Benzyloxycarbonyl*-DL-*threonine and* -DL-*serine and* N-p-*Nitrobenzyloxycarbonyl*-DL-*threonine and* -DL-*serine with Alkali.*—The acids (0·01 mol.) were dissolved in 2N-sodium hydroxide (10 ml., 2 equiv.) with shaking at room temperature. After a few minutes each solution became turbid and an oil was formed which in the case of the *p*-nitro-derivatives rapidly crystallised. Extraction with ethyl acetate (3 × 10 ml.) removed the benzyl or *p*-nitrobenzyl alcohol, and the aqueous layer was acidified by the dropwise addition of concentrated hydrochloric acid. The clear aqueous solution was extracted with ethyl acetate (6 × 15 ml.), and the organic layer dried (Na_2SO_4) and evaporated to dryness. Trituration with light petroleum caused the residues to crystallise, and the solids recrystallised from ethyl acetate–light petroleum. The two threonine derivatives gave DL-5-methyl-2-oxo-oxazolidine-4-carboxylic acid, m. p. and mixed m. p. 123—124°. The two serine derivatives gave DL-2-*oxo*-*oxazolidine*-4-*carboxylic acid*, m. p. 125—126° (Found: C, 36·2; H, 3·9; N, 10·7. $C_4H_5O_4N$ requires C, 36·6; H, 3·8; N, 10·7%).

Acid-hydrolysis of 5-*Methyl*-2-*oxo*-*oxazolidine*-4-*carboxylic Acid.*—The acid (500 mg.) was boiled with concentrated hydrochloric acid (10 ml.) for 6 hr. After evaporation to dryness the residue was dissolved in ethanol (5 ml.), and morpholine (0·5 ml.) was added. White crystals separated and were filtered off, washed with a little ethanol, and recrystallised from aqueous ethanol. This gave 350 mg. of threonine, m. p. 236° (decomp.).

The amino-acid was benzoylated (Schotten–Baumann) and the *N*-benzoyl derivative

[19] Fischer and Suzuki, *Ber.*, 1905, **38**, 4193.
[20] Mattocks and Hartung, *J. Biol. Chem.*, 1946, **165**, 501.
[21] King, *J. Amer. Chem. Soc.*, 1947, **69**, 2738.

recrystallised from ethyl acetate–light petroleum to give rods, m. p. and mixed m. p. 144—145° with *N*-benzoyl-DL-threonine.

N-Benzyloxycarbonyl-O-[NN-di-(2-chloroethyl)carbamoyl]-DL-threonine Benzyl Ester.—*N*-Benzyloxycarbonyl-DL-threonine benzyl ester (6·8 g.) was dissolved in dry benzene (70 ml.), dimethylaniline (2·53 ml., 1 equiv.) added, and carbonyl chloride passed through the stirred solution at 5° for 20 min. Stirring was continued for a further 4 hr. at room temperature. Next day dry nitrogen was used to remove excess of carbonyl chloride, and the dimethylaniline hydrochloride removed by washing the solution with dilute acid. The organic layer was dried (Na_2SO_4) and treated with ethereal di-(2-chloroethyl)amine (6·2 g., 2·2 equiv.) in the usual way. Solid material began to separate almost immediately but stirring was continued for 3 hr. After working up, the required *carbamate* was obtained in 65% yield (see Table 1, No. 6).

An attempted preparation by the method successfully applied to the serine derivative (see above) gave only a 3% yield. This was separated from unchanged starting material by passing a benzene solution down an alumina column. The carbamate passed straight through whereas the hydroxy-compound was retarded somewhat.

Repetition of this preparation but with the carbonyl chloride and the hydroxy-compound in contact for 2 weeks gave a 37% yield.

Substituted O-Carbamoyl-DL-and-L-serine and -DL-threonine Derivatives.—The fully protected carbamate derivatives were dissolved in methanol (8—10 g. per 200 ml.) and hydrogenolysed with 5% palladium–charcoal (200 mg.). Uptake of hydrogen was complete in a few hours, the catalyst was filtered off, and the solvent removed under reduced pressure at $\gtrless 40°$. In the case of *N*-benzyloxycarbonyl-*O*-[*N*-di-(2-chloroethyl)carbamoyl]-DL-serine methyl ester, concentrated hydrochloric acid (2 ml.) was added to the methanolic solution before hydrogenolysis and the product isolated as the *hydrochloride*. The products are recorded in Table 2.

O-[*N*-Di-(2-chloroethyl)carbamoyl]-DL-serine methyl ester hydrochloride was also prepared by saturating a suspension of the corresponding amino-acid (Table 2, No. 1) in dry methanol (1 g. per 15 ml.) with hydrogen chloride at 5°. The resulting clear solution was evaporated to dryness and the residue recrystallised from methanol–ether (yield 78%).

Hydrolysis Experiments on O-[NN-*Di*-(2-*chloroethyl*)*carbamoyl*]-DL-*serine* (CB 3159) *and on* O-[NN-*Di*-(2-*chloroethyl*)*carbamoyl*]-DL-*threonine* (CB 3182).—The compound (0·1 mmol.) was dissolved in water (50 ml.) contained in a jacketed vessel fitted with a magnetic stirrer and kept at 37°. Atmospheric carbon dioxide was precluded by releasing a slow stream of argon near the surface. A Pye automatic titrator was used to regulate the addition of 0·01N-sodium hydroxide to adjust the solution to pH 7·4 and maintain this value for 24 hr.

Volumes of 0·01N-alkali required to maintain pH 7·4 for 24 hr. were: CB 3159, 2·75 ml.; CB 3182, 5·35 ml.

TABLE 3. *Aqueous hydrolyses.*

	CB 3159; R_F				CB 3182; R_F			
Time	0·05	0·08	0·10	0·53	0·08	0·11	0·15	0·60
Zero	—	—	—	++++	—	—	—	++++
5 min.	—	+	—	+++	+	+	—	++
15 min.	+	++	—	+	+	++	+	—
30 min.	+	++	—	—	+	++	+	—
45 min.	+	++	+	—	+	+	++	—
1 hr.	+	++	+	—	+	+	++	—
2 hr.	++	+	++	—	+	—	++	—
3 hr.	++	+	++	—	+	—	++	—
5 hr.	+	+	++	—	+	—	+++	—
6 hr.	+	—	+++	—	—	—	++++	—
Amino-acid standard	—	—	++++	—	—	—	++++	—

The relative intensities of the spots are shown by the number of + signs, *e.g.*, ++++ = very strong, etc.

We are indebted to Dr. W. Davis for carrying out these determinations.

In the experiments at 100°, the " mustards " (25 mg.) were boiled in water (5 ml.). Samples (0·01 ml.) were withdrawn from time to time, spotted on paper (W-1), and chromatographed with butanol–ethanol–propionic acid–water (10 : 5 : 2 : 5) as solvent. After 18 hours' running the chromatogram was developed with ninhydrin (0·25% in acetone); the ninhydrin-positive spots are shown in Table 3.

These may be interpreted as showing that, in the case of CB 3159, the " mustard " (R_F 0·53) is decomposed into a first relatively stable intermediate (R_F 0·08) which itself is decomposed to a second intermediate (R_F 0·05) which breaks down to serine (R_F 0·10). Similarly, the threonine " mustard " (R_F 0·60) gives a first intermediate (R_F 0·11), then a second (R_F 0·08), and finally threonine (R_F 0·15).

With phenol (water-saturated) as solvent, serine and the two preceding intermediates have R_F 0·30, and the di-(2-hydroxyethyl)carbamoyl-DL-serine has R_F 0·72.

iso*Propyl* NN-*Di*-(2-*chloroethyl*)*carbamate*.—*iso*Propyl chloroformate [22] (12·3 g.) in benzene (40 ml.) was added dropwise to a stirred solution of di-(2-chloroethyl)amine, prepared from the hydrochloride (36 g.), in ether (200 ml.), the temperature being kept at 20—25°. Next morning the solution was washed with dilute acid, aqueous sodium hydrogen carbonate solution, and water, and dried (Na_2SO_4), the solvent evaporated, and the residual iso*propyl* NN-*di*-(2-*chloroethyl*)*carbamate* (17·0 g., 65%) distilled under reduced pressure (b. p. 121—123°/1 mm.) (Found: C, 42·3; H, 6·5; N, 5·7; Cl, 31·0. $C_8H_{15}O_2NCl_2$ requires C, 42·1; H, 6·6; N, 6·1; Cl, 31·1%).

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[22] Thiele and Dent, *Annalen*, 1898, **302**, 269.