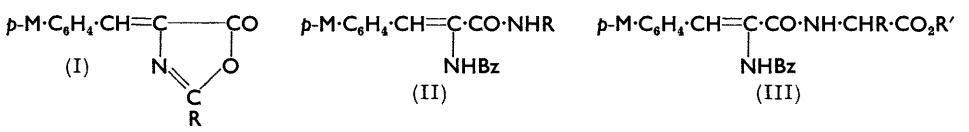
917. *Cyto-active Amino-acids and Peptides. Part IV.*[1] *Synthesis of α-[p-Di-(2-chloroethyl)amino-benzylidene- and -benzyl-]hippuramides and -hippuramido-acids.*

By F. Bergel and J. A. Stock.

4-[*p*-Di-(2-chloroethyl)aminobenzylidene]-2-phenyloxazol-5-one [I; R = Ph; M = $(Cl{\cdot}CH_2{\cdot}CH_2)_2N$] is described. This compound, when treated with ammonia, primary amines, α-amino-acids, or α-amino-acid esters, gave " nitrogen mustard "-substituted unsaturated amides and peptides (as II and III) which were reduced to the corresponding substituted phenylalanine derivatives (as IV and V). An analogous thiazolone has also been prepared.

Syntheses of " nitrogen-mustard " analogues of L-, D-, and DL-tyrosine and of DL-thyronine were described in previous parts of this series,[1] which dealt with potential anti-tumour agents of an α-amino-acid character. We now report an extension of this work aiming at the preparation of related dipeptides.

Although known to be of limited value, Bergmann's azlactone synthesis [2] was employed because of the ready availability of *p*-di-(2-chloroethyl)aminobenzaldehyde.[3] When the latter was heated with hippuric acid in acetic anhydride in the presence of sodium acetate, the azlactone [I; R = Ph, M (in this and all following formulæ) = $(Cl{\cdot}CH_2{\cdot}CH_2)_2N$] was obtained in yields of 60% or more. This reacted readily with ammonia and primary amines to give α-[*p*-di-(2-chloroethyl)aminobenzylidene]hippuramides (II), and with amino-

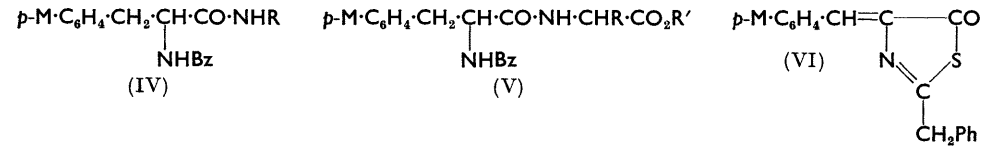


acids and their esters to give the corresponding hippuramido-acids and -esters (III). These compounds may be considered as dehydro-derivatives of N^{α}-benzoylphenylalanine amides and peptides. In order to achieve satisfactory reactions three or more molar equivalents of the amine or amino-acid derivative had to be used. The crystalline compounds prepared in this way are listed in Table 1. They comprise derivatives of ammonia (II; R = H), ethylamine (II; R = Et), and *cyclo*hexylamine (II; R = C_6H_{11}); of glycine (III; R = R′ = H), DL- and L-alanine (III; R = Me, R′ = H), and L-valine (III; R = Me_2CH, R′ = H); and of the ethyl esters of glycine (III; R = H, R′ = Et), L-leucine (III; R = $Me_2CH{\cdot}CH_2$, R′ = Et), and DL-phenylalanine (III; R = $Ph{\cdot}CH_2$, R′ = Et). The azlactone (I; R = Ph) reacted with L-leucine, L-tyrosine ethyl ester, DL-aspartic acid, L-histidine, L-cysteine, and DL-methionine but the products resisted crystallisation. Aniline and *p*-aminobenzoic acid did not react with the azlactone (I; R = Ph) under the conditions outlined in the Experimental section, the azlactone being recovered unchanged.

Conversion of compounds of type (II) and (III) into DL-phenylalanine derivatives (IV) and (V) was first attempted by catalytic hydrogenation. This reduction of the glycine (III; R = R′ = H) and of the DL-alanine derivative (III; R = Me, R′ = H) could be achieved only by using a mixture of Adams platinum oxide, palladium–charcoal, and Raney nickel. Any one or any pair of these catalysts was ineffective. However, it was found that the *cyclo*hexylamide (II; R = C_6H_{11}) could be hydrogenated over Raney nickel. Three other compounds were reduced, not always readily, with the three-catalyst mixture, namely, the derivatives of ammonia (II; R = H), L-alanine (III; R = Me, R′ = H), and DL-phenylalanine ethyl ester (III; R = $Ph{\cdot}CH_2$, R′ = Et).

[1] 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.
[2] Carter, " Organic Reactions," Wiley, New York, 1946, Vol. III, p. 198.
[3] Anker and Cook, *J.*, 1944, 489.

While zinc in glacial acetic acid was not effective, the addition of concentrated hydrochloric acid to this system led to very rapid and efficient reduction, yields being substantially higher than by catalytic hydrogenation (Table 2). The apparent lack of optical activity in the reduced L-alanine and L-leucine ethyl ester derivatives (Table 2; Nos. 6 and 7), the former prepared by either method of reduction, does not indicate that



racemisation had occurred. After hydrolysis of the compounds with 6N-hydrochloric acid, the solutions showed rotations comparable with those to be expected from the presence of an equivalent concentration of L-alanine and L-leucine respectively. Moreover, the m. p. of the L-alanine derivative (Table 2, No. 6) was about 25° lower than that of the corresponding DL-alanine peptide (No. 5).

The methods outlined above thus enabled us to prepare several N^{α}-benzoyl-dipeptides carrying a " nitrogen mustard " group on the phenylalanine moiety. The benzoyl group does not, however, lend itself to removal by means which would preserve the peptide link. We therefore sought to vary the azlactone so as to have ultimately an easily removable *N*-acyl group. To this end we attempted to prepare the azlactones (I; R = Me), (I; R = CF_3), (I; R = Ph·CH_2), and (I; R = Ph·CH_2·O) but failed. We did, however, synthesise the thiazolone (VI) from phenyl(thioacetyl)glycine but this did not give crystalline products when allowed to react with ethylamine or *cyclo*hexylamine. This approach was therefore postponed. However, as indicated in a preliminary communication,[4] we are investigating the use of the readily removable *o*-cyanobenzoyl group in the preparation of peptides related to the compounds described in this paper but possessing a free amino-group. That such an amino-group may be required for powerful anti-tumour activity is illustrated by the apparent ineffectiveness [5] on the Walker carcinoma 256 of the azlactone (I; R = Ph), the thiazolone (VI), of the dehydro-compounds as given on Table 1, and of the glycine peptide (V; R = R′ = H). While none of these compounds has been tested yet up to the limit of tolerance, it seems that pronounced anti-tumour action in this series may depend on the presence of zwitterion systems.

Experimental

4-[p-*Di*-(2-*chloroethyl*)*aminobenzylidene*]-2-*phenyloxazol*-5-*one* (I; R = Ph) (cf. Buck and Ide [6]).—*p*-Di-(2-chloroethyl)aminobenzaldehyde [3] (4·92 g.), hippuric acid (3·58 g., 1·0 mol.), powdered anhydrous sodium acetate (1·64 g., 1·0 mol.), and acetic anhydride (6 ml.) were heated with stirring at about 120°. Complete dissolution occurred within a few minutes. The red solution was then heated on a steam-bath for about 25 min. It deposited red crystals on cooling. Water (7 ml.) was added to the cold mixture. The aqueous layer was poured off after about 15 min., and the solid residue washed with water, collected, and dried. The *oxazolone* crystallised from benzene in bright red prisms (4·89 g., 63%), m. p. 132—135° (with previous softening, and clarification at 142°). Omission of sodium acetate from the reaction mixture resulted in a slightly lower yield. Recrystallisation from benzene raised the m. p. to 135—138° (softening from 122°; clarification and meniscus 142—145°) (Found: C, 64·0; H, 5·0; N, 7·0; Cl, 16·5. $C_{20}H_{18}O_2N_2Cl_2,\frac{1}{2}C_6H_6$ requires C, 64·5; H, 4·9; N, 6·5; Cl, 16·6%). Crystallisation from Cellosolve gave light tan solvent-free prisms of much sharper m. p. (141—142°) (Found: C, 61·5; H, 4·7; N, 7·4; Cl, 18·0. $C_{20}H_{18}O_2N_2Cl_2$ requires C, 61·7; H, 4·6; N,

[4] Bergel and Stock, *Proc. Chem. Soc.*, 1957, 60.
[5] Personal communication from Professor A. Haddow.
[6] Buck and Ide, *Org. Synth.*, Coll. Vol. II, 1943, p. 55.

TABLE 1.

No.	Compound	Isomer	Cryst. from	M. p.	$[\alpha]_D$	Yield (%)	Formula	Found (%) C	H	N	Cl	Required (%) C	H	N	Cl
1	II; R = H	—	A [a]	179—180°	—	95	$C_{20}H_{21}O_2N_3Cl_2$	58·9	5·25	—	17·2	58·9	5·25	—	17·5
2	II; R = Et	—	B [a]	165—166	—	76	$C_{22}H_{25}O_2N_3Cl_2$	—	—	9·25	16·25	—	—	9·7	16·4
3	II; R = *cyclo*hexyl	—	C [b]	177—178	—	75	$C_{26}H_{31}O_2N_3Cl_2$	63·75	6·6	8·3	14·4	63·95	6·35	8·6	14·55
4	III; R = R′ = H	—	B [c]	190—191	—	74	$C_{22}H_{23}O_4N_3Cl_2$	—	—	8·9	14·8	—	—	9·05	15·3
5	III; R = Me; R′ = H	DL	D [a]	176—178	—	81	$C_{23}H_{25}O_4N_3Cl_2$	57·8	5·3	8·4	14·7	57·75	5·2	8·8	14·9
6	III; R = Me; R′ = H	L	E [a]	167—169	+31° ± 1° [d]	82	,,	57·2	5·2	8·3	14·75	57·75	5·2	8·8	14·9
7	III; R = Me_2CH; R′ = H	L	D [c]	166—167	−15 ± 1·5 [e]	79	$C_{25}H_{29}O_4N_3Cl_2$	59·2	5·9	8·0	14·6	59·3	5·7	8·3	14·0
8	III; R = H; R′ = Et	—	F [a]	143—144	—	80	$C_{24}H_{27}O_4N_3Cl_2$	58·8	5·7	8·3	14·4	58·5	5·5	8·5	14·4
9	III; R = Me_2CHCH_2; R′ = Et	L	A [a]	177—178	+20 ± 2 [f]	73 [g]	$C_{28}H_{35}O_4N_3Cl_2$	61·25	6·05	7·9	13·3	61·3	6·4	7·7	13·0
10	III; R = $PhCH_2$; R′ = Et	DL	E [ah]	141—142	—	60	$C_{31}H_{33}O_4N_3Cl_2$	64·0	5·6	7·3	12·15	63·9	5·7	7·2	12·2

A, Aqueous ethanol. B, Methanol. C, Ethanol. D, Aqueous Cellosolve. E, Aqueous propan-1-ol. F, Pentanol. [a] Yellow rods or needles. [b] Colourless needles. [c] Yellow prisms. [d] *c* 1·54 in dioxan at 25°. [e] *c* 0·29 in EtOH at 25°. [f] *c* 1·1 in dioxan at 24°. [g] Calc. on azlactone which reacted; 50% recovered unchanged. [h] Darkened somewhat on storage.

TABLE 2.

No.	Compound	Isomer *	Reduction	Cryst. from †	M. p.	$[\alpha]_D$	Yield (%)	Formula	Found (%) C	H	N	Cl	Required (%) C	H	N	Cl
1	IV; R = H	—	A	D [a]	171—172°	—	53	$C_{20}H_{23}O_2N_3Cl_2$	—	—	9·9	16·9	—	—	10·3	17·4
			B	D [b]	172—173 [c]	—	71	,,	59·0	6·0	10·3	16·9	58·8	5·6	10·3	17·4
2	IV; R = Et	—	B	E [a]	168—169 [d]	—	92	$C_{22}H_{27}O_2N_3Cl_2$	60·5	6·4	—	15·9	60·5	6·2	—	16·3
3	IV; R = *cyclo*hexyl	—	C	F [a]	181 [e]	—	—[f]	$C_{26}H_{33}O_2N_3Cl_2$	63·6	6·85	8·3	—	63·7	6·7	8·6	—
4	V; R = R′ = H	—	A	E [g]	212—213	—	65	$C_{22}H_{25}O_4N_3Cl_2$	56·7	5·3	8·6	—	56·65	5·4	9·0	—
			B	E [g]	211—213 [c]	—	84	,,	—	—	—	—	—	—	—	—
5	V; R = Me; R′ = H	DL	A	D [g]	212—213	—	55	$C_{23}H_{27}O_4N_3Cl_2$	57·0	5·55	8·7	14·6	57·5	5·6	8·75	14·8
			B	E [g]	215 [h]	—	76	,,	—	—	—	—	—	—	—	—
6	V; R = Me; R′ = H	L	A	G [a]	184—187	0° ± 1° [i]	31	,,	—	—	—	—	—	—	—	—
			B	E [a]	186—188 [c]	0 ± 1 [j]	57	,,	57·9	5·6	8·6	—	57·5	5·6	8·75	—
7	V; R = $Me_2CH{\cdot}CH_2$; R′ = Et	L	B	F [a]	149—150	0 ± 1 [k]	68	$C_{28}H_{37}O_4N_3Cl_2$	61·6	6·9	—	13·0	61·1	6·7	—	12·9
8	V; R = $PhCH_2$; R′ = Et	DL	A	E [a]	152—153	—	70	$C_{31}H_{35}O_4N_3Cl_2$	63·8	6·15	6·9	12·1	63·7	6·0	7·2	12·2

* The configuration given is that of the amino-acid attached to the N^{α}-benzoyl-*p*-di-(2-chloroethyl)amino-DL-phenylalanyl group. † All colourless or almost colourless.

A, Catalytic over Pt, Pd, and Ni. B, By Zn–acid. C, Catalytic over Raney Ni alone. D, Methanol. E, Aqueous methanol. F, Aqueous ethanol. G, Aqueous acetic acid. [a] Rods or needles. [b] Plates. [c] Unchanged on admixture with preceding compound. [d] Partial melting and resolidification, 152—156°. [e] Shrinkage and softening at 162°, then resolidification. [f] Not recorded. [g] Prisms. [h] Mixed m. p. with preceding compound, 212—213°. [i] *c* 1·5 in EtOH at 21°. [j] *c* 0·79 in EtOH at 22°. [k] *c* 1·31 in dioxan at 22°.

7·2; Cl, 18·3%). Moistening these with a little benzene caused the colour of the compound to revert to bright red. Both solid forms showed strong fluorescence under ultraviolet light.

2-Benzyl-4-[p-di-(2-chloroethyl)aminobenzylidene]thiazol-5-one (VI) (cf. Abraham *et al.*[7]).—Phenyl(thioacetyl)glycine [8] (2·09 g.), *p*-di-(2-chloroethyl)aminobenzaldehyde [3] (2·46 g., 1·0 mol.), and acetic anhydride (5 ml.) were shaken with gentle warming until dissolution was complete, then kept at 50—60° for 3 hr. After cooling, a solid was deposited; this was collected and washed with ethanol, then light petroleum. The orange rods (2·09 g., 50%), m. p. 153—155°, were recrystallised from benzene–ethanol, then Cellosolve, and yielded the *thiazolone* (1·6 g., 38%), m. p. 157—158° (Found: C, 60·2; H, 5·1; N, 6·5; S, 7·3; Cl, 16·8. $C_{21}H_{20}ON_2SCl_2$ requires C, 60·15; H, 4·8; N, 6·7; S, 7·6; Cl, 16·9%). The use of anhydrous sodium acetate and of a higher reaction temperature offered no advantage.

α-[p-*Di*-(2-*chloroethyl*)*aminobenzylidene*]*hippuramides* (II).—A concentrated aqueous solution of ammonia or of diethylamine (≠3 mol. equiv.) was added to an acetone solution containing the oxazolone (I; R = Ph) (0·5—1·0 g. per 10 ml.). The ammonia reaction was carried out at room temperature and was complete within 15 min. The ethylamine reaction mixture was heated under reflux for a few minutes. Reaction with *cyclo*hexylamine was carried out by heating under reflux a solution of the oxazolone (I; R = Ph) and base (3 mol. equiv.) in ethyl acetate for *ca.* 1 hr. The fading of the initial orange colour of the solution to lemon-yellow was a guide to the rate of reaction in all cases. The mixtures were evaporated to dryness under reduced pressure and the *amides* were crystallised from suitable solvents (Table 1).

α-[p-*Di*-(2-*chloroethyl*)*aminobenzylidene*]*hippuramido-acids and -amido-esters* (III; R′ = H *or* Et).—(a) *Reaction of oxazolone* (I; R = Ph) *with amino-acids.* The oxazolone was dissolved in boiling acetone (*ca.* 10 ml. per mmol. of oxazolone) and treated with a solution of the amino-acid (3 mol.) in N-sodium hydroxide (3 equiv.). The mixture was heated under reflux for 15 min., by which time the colour had usually faded to pale yellow. Most of the acetone was evaporated under reduced pressure, the residual aqueous solution diluted with a few ml. of water, and the product precipitated by addition of dilute hydrochloric acid to pH *ca.* 3. The products which could be purified by crystallisation are tabulated in Table 1.

(b) *Reaction of oxazolone* (I; R = Ph) *with amino-acid ethyl esters.* The oxazolone was refluxed for up to 1 hr. in ethyl acetate with the amino-ester (5 mol.; usually prepared from the ester hydrochloride by treatment with triethylamine in chloroform). The solution was evaporated, and the residue washed with dilute (*ca.* 0·1N-)hydrochloric acid to remove excess of amino-ester. Products obtained crystalline are given in Table 1.

α-[p-*Di*-(2-*chloroethyl*)*aminobenzyl*]-*hippuramides and -hippuramido-acids and -esters* (IV *and* V).—(a) *Catalytic reduction.* The unsaturated compound was shaken in methanol or methanol–ethyl acetate over Adams platinum oxide catalyst, Raney nickel, and 5% palladium–charcoal. The *cyclo*hexylamide (II; R = C_6H_{11}) was successfully reduced in the presence of Raney nickel alone. The results are set down in Table 2.

(b) *Chemical reduction.* The unsaturated compound (1 mmol.) was dissolved in glacial acetic acid (10 ml.), and concentrated hydrochloric acid (3 ml.) was added. Zinc dust (2 g.) was then added in two or three portions to the stirred and cooled solution. Stirring was continued for 10 min., though the colour of the solution generally disappeared within about 3 min. The mixture was filtered, the residual zinc washed with a little acetic acid, and the combined filtrate and washings were diluted with water to about 30 or 40 ml. The solution was brought to pH *ca.* 3 by the addition of N-sodium hydroxide, and the precipitated product was crystallised. The results are given in Table 2.

Hydrolysis of Reduced Peptides of L-*Alanine and of* L-*Leucine Ethyl Ester.*—(a) L-*Alanine derivative* (V; R = Me, R′ = H). The compound (101 mg.) was heated for 3 hr. under reflux in 6N-hydrochloric acid (2 ml.). Colourless needles, presumably benzoic acid, collected in the condenser. The solution was cooled and filtered from benzoic acid, and the filtrate and washings were made up to 2·1 ml. with 6N-acid. Mean α_D^{25} was $+0·10° \pm 0·03°$. Hence, for the alanine released (18·8 mg., assuming complete hydrolysis), $[\alpha]_D^{25} = +11° \pm 3°$ (*c* 0·935 in 6N-HCl). The L-alanine used had $[\alpha]_D^{25} +11° \pm 1°$ (*c* 1·0 in 6N-HCl).[9]

(b) L-*Leucine derivative* (V; R = $Me_2CH{\cdot}CH_2$, R′ = Et). An experiment similar to the above in which the reflux time of the compound (50 mg.) in 6N-hydrochloric acid (1·5 ml.) was

[7] Abraham, Baker, Chain, and Robinson, "Chemistry of Penicillin," ed. Clarke, Princeton Univ. Press, 1949, p. 848.
[8] *Idem, op. cit.*, p. 778.

4 hr. gave a solution (final vol., 1·5 ml.) whose α_D^{25} was $+0{\cdot}09° \pm 0{\cdot}015°$. Hence, for the leucine released (11·9 mg., assuming complete hydrolysis), $[\alpha]_D^{25} = +11{\cdot}5° \pm 2°$ (*c* 0·79 in 6N-HCl). The L-leucine used had $[\alpha]_D^{25} +16° \pm 2°$ (*c* 0·64 in 6N-HCl).[9]

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[9] Cf. Greenstein, Birnbaum, and Otey, *J. Biol. Chem.*, 1952, **204**, 207.