20. *Cyto-active Amino-acids and Peptides. Part VI.*[1] *Synthesis of* N′-α-*Aminoacyl*-NN-*di*-(2-*chloroethyl*)-p-*phenylenediamines.*

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A number of *N*′-[α-(benzyloxycarbonylamino)acyl]-*NN*-di-(2-chloroethyl)-*p*-phenylenediamines and two derived α-aminoacyl compounds are described. Tests of the latter on experimental tumours failed to give promising results.

Ross, Warwick, and Roberts,[2] and Hebborn and Danielli,[3,4] have reported on the chemistry and interesting biological properties of a series of *N*′-acyl-*NN*-di-(2-chloroethyl)-*p*-phenylenediamines. The design of these compounds was based on a working hypothesis of Danielli [5] and of Ross *et al.*[2] for selectivity of biological action. The experimental findings supported the idea that the blocking of the primary amino-group by acylation, and consequent deactivation of the " nitrogen mustard " group, could produce drugs with latent activity. Deacylation by enzymes within the neoplastic cell would then release the active parent compound at the right site. We have extended this work by undertaking the synthesis of some α-aminoacyl analogues of type (II).

The chloroformate mixed anhydride procedure [6,7] was used in the condensation of the benzyloxycarbonyl derivatives of glycine, DL-alanine, L-leucine, DL-methionine, and DL-phenylalanine with *NN*-di-(2-chloroethyl)-*p*-phenylenediamine. The benzyloxycarbonylamino-anilides (I; Nos. 1—5 of the Table) were thus obtained.

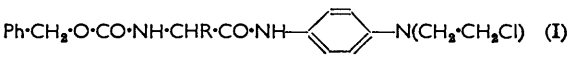
No.	Compound	Isomer	Cryst.¶ from	M. p.	Yield (%)
1	I; R = H	—	EtOH	144—145°	61
2	I; R = Me	DL	Aq. EtOH	126—127	44
3	I; R = $Me_2CH{\cdot}CH_2$	L †	Aq. MeOH	124—125	64
4	I; R = $MeS{\cdot}CH_2{\cdot}CH_2$	DL	A *	94—95	53
5	I; R = $Ph{\cdot}CH_2$	DL	EtOH	146—147	72
6	II; X = Cl, R = H	—	MeOH	250—251 ‡	50
7	II; X = Br, R = H	—	H_2O	232—234 ‡	50
8	II; X = Cl, R = $Ph{\cdot}CH_2$	DL	MeOH–Et_2O	112—114 §	76
9	II; X = Br, R = $Ph{\cdot}CH_2$	DL	H_2O	103—104	66

		Found (%)					Required (%)				
No.	Formula	C	H	N	Cl	S	C	H	N	Cl	S
1	$C_{20}H_{23}O_3N_3Cl_2$	56·9	5·4	9·9	16·4	—	56·85	5·4	9·9	16·7	—
2	$C_{21}H_{25}O_3N_3Cl_2$	57·35	5·7	9·6	15·8	—	57·5	5·7	9·6	16·2	—
3	$C_{24}H_{31}O_3N_3Cl_2$	60·0	6·8	8·9	15·2	—	60·0	6·5	8·8	14·8	—
4	$C_{23}H_{29}O_3N_3Cl_2S$	55·7	6·0	8·3	14·6	6·5	55·3	5·8	8·4	14·3	6·4
5	$C_{27}H_{29}O_3N_3Cl_2$	63·1	5·8	8·1	13·3	—	63·0	5·6	8·2	13·8	—
6	$C_{12}H_{17}ON_3Cl_2,HCl$	—	—	12·8	32·6	—	—	—	12·9	32·6	—
7	$C_{12}H_{17}ON_3Cl_2,HBr$	38·0	5·0	—	—	—	38·6	4·85	—	—	—
8	$C_{19}H_{23}ON_3Cl_2,HCl,MeOH$	52·8	6·15	9·5	23·0	—	53·5	6·3	9·4	23·7	—
9	$C_{19}H_{23}ON_3Cl_2,HBr,2H_2O$	46·2	5·3	8·4	—	—	45·9	5·6	8·45	—	—

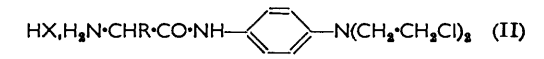
* A, Pentanol–light petroleum. ¶ Colourless or almost colourless rods or needles in each case. † $[\alpha]_D^{20}$ −24·9° ± 1° (*c* 0·92 in MeOH). ‡ With decomp. § Transparent glass; meniscus at 195—200°.

[1] Part V, Bergel and Stock, preceding paper.
[2] Ross, Warwick, and Roberts, *J.*, 1955, 3110.
[3] Hebborn and Danielli, *Nature*, 1956, **177**, 25.
[4] *Idem*, *Biochemical Pharmacology*, 1958, **1**, 19.
[5] Danielli, *Nature*, 1952, **170**, 863; " Ciba Foundation Symposium on Leukaemia Research," Churchill, London, 1954, p. 263; *Brit. Emp. Cancer Camp. Ann. Rep.*, 1954, **32**, 392; 1956, **34**, 398.
[6] Vaughan, *J. Amer. Chem. Soc.*, 1952, **74**, 6137.
[7] Boissonnas, *Helv. Chim. Acta*, 1951, **34**, 874.

Removal of the benzyloxycarbonyl group from each of the five compounds was next attempted. Hydrogenolysis of the phenylalanine derivative (I; R = Ph·CH_2) over Adams's platinum oxide catalyst in methanol failed, while hydrogen chloride in glacial acetic acid [8] had little effect in 48 hours at room temperature. However, deacylation by hydrogen chloride in formic acid or hydrogen bromide in glacial acetic acid [9] gave moderate yields of the hydrohalides of the amides (II; X = Cl or Br, R = H or Ph·CH_2). Deacylation of the alanine and leucine intermediates occurred under these conditions; but, although the hydrohalides became solid when rubbed under ether, they were extremely hygroscopic and rapidly became sticky when exposed to air. Attempts to convert the products into, for example, picrates or reineckates were not successful; a solid reineckate {II; X = $[Cr(NH_3)_2(SCN)_4]$, R = Me} of the alanine derivative was prepared but not analytically pure. The methionine compound (I; R = MeS·CH_2·CH_2) yielded, with hydrogen chloride in formic acid, an intractable hygroscopic gum.



(I)



(II)

As pointed out in the introduction it was hoped that the free *N'*-α-aminoacyl-*NN*-di-(2-chloroethyl)phenylenediamines would be broken down *in vivo* to the active *NN*-di-(2-chloroethyl)phenylenediamine. Neither the phenylalanine (CB 3100) nor the glycine (CB 3132) derivative was consistently effective on the Walker carcinoma 256. It appears, therefore, that the tumour tissue cannot split off the α-aminoacyl residue as readily as it does certain acyl radicals such as trichloro- and trifluoro-acetyl.[2]

Experimental

N'-Benzyloxycarbonylaminoacyl-NN-*di*-(*2-chloroethyl*)-p-*phenylenediamines* (I).—The preparation of the glycine derivative illustrates the general method.[6,7] Ethyl chloroformate (0·19 ml., 2 mmol.) was added to an ice-cooled solution of benzyloxycarbonylglycine (418 mg., 2 mmol.) and triethylamine (0·28 mg., 2 mmol.) in dry dioxan (4 ml.). The flask was stoppered and left in ice-water for 10 min. A freshly prepared solution of *NN*-di-(2-chloroethyl)-*p*-phenylenediamine hydrochloride [10] (538 mg., 2 mmol.) and of triethylamine (0·28 ml., 2 mmol.) in dioxan (2 ml.) and water (1 ml.) was then added, and the mixture set aside for 5 min. at room temperature. Addition of water precipitated the anilide which was then crystallised (see Table, No. 1).

In the preparation of the leucine compound (No. 3), chloroform and *iso*butyl chloroformate were used in place of dioxan and ethyl chloroformate.

N'-Aminoacyl-NN-*di*-(*2-chloroethyl*)-p-*phenylenediamine Hydrohalides* (II).—The benzyloxycarbonyl derivatives (I) were best deacylated by the action of hydrogen chloride in formic acid or by hydrogen bromide in glacial acetic acid.[9] Only the glycine and the DL-phenylalanine compound gave crystalline non-deliquescent salts. Two typical experiments are recorded below.

(*a*) *N'*-Benzyloxycarbonylglycyl-*NN*-di-(2-chloroethyl)-*p*-phenylenediamine (I; R = H) (1·69 g.) was dissolved in a saturated solution of hydrogen chloride in 98% formic acid (20 ml.) and left 2 days at room temperature. The solution was evaporated to dryness under reduced pressure, the residual gum treated with water (1 ml.) (to hydrolyse any deliquescent dihydrochloride) which was then evaporated under reduced pressure with gentle warming, and the *glycine anilide hydrochloride* crystallised (No. 6).

(*b*) *N'*-Benzyloxycarbonyl-DL-phenylalanyl-*NN*-di-(2-chloroethyl)-*p*-phenylenediamine (I; R = Ph·CH_2) (650 mg.) was dissolved in an approximately molar solution of hydrogen bromide in glacial acetic acid (8 ml.) and kept for 16 hr. at room temperature. Addition of

[8] Boissonnas and Preitner, *Helv. Chim. Acta*, 1953, **36**, 875.
[9] Ben-Ishai and Berger, *J. Org. Chem.*, 1952, **17**, 1564.
[10] Everett and Ross, *J.*, 1949, 1972.

ether precipitated a gum which became granular when rubbed under water. The *phenylalaninamide hydrobromide* crystallised as the dihydrate (No. 9).

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