643. *Cytotoxic Compounds. Part VI.*[1] *Some* N′-[α-(*Acylamino*)-*acyl*]-NN-*di*-2′-*chloroethyl*-p-*phenylenediamines.*

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Syntheses of fourteen compounds of this type, designed to undergo enzymic activation *in vivo*, are described.

It is known that acylation of the free amino-group in *NN*-di-2′-chloroethyl-*p*-phenylenediamine (I) deactivates the " nitrogen mustard," with the result that the halogen atoms in the derivative are more resistant to hydrolysis and the general toxicity is reduced. If the acyl group is sensitive to fission by an enzyme which is relatively more abundant in tumour cells, administration of the derivative leads to facilitated release of the parent compound (I) at the site where it is required, some selectivity of action thereby being achieved.[2-4] This principle of enzymic activation has motivated the synthesis and biological examination of many compounds in which the amino-group has been modified by acylation,[3,5,6] or in other ways,[3,6-9] and has also been applied to other types of nitrogen mustards.[10]

Danielli [2] has pointed out that increased selectivity should result if the action of a drug is made dependent upon a larger number of cell variables. This might be achieved if the release of the active nitrogen mustard required the successive action of two enzymes, and he suggested to us that α-(acylamino)acyl derivatives (II; Y = acyl) would be interesting because the enzyme aminopeptidase can function only if a free α-amino-acyl group is present; [11] consequently the acyl group Y would have to undergo enzymic removal first, after which the aminopeptidase could attack the intermediate (II; Y = H) to break the amide linkage and liberate the mustard (I).

p-$(CH_2Cl{\cdot}CH_2)_2N{\cdot}C_6H_4{\cdot}NH_2$ (I) p-$(CH_2Cl{\cdot}CH_2)_2N{\cdot}C_6H_4{\cdot}NH{\cdot}CO{\cdot}CHX{\cdot}NHY$ (II)

The only compounds of type (II) which have hitherto been described are some benzyloxycarbonyl derivatives (II; Y = $CO_2{\cdot}CH_2Ph$) prepared as intermediates by Bergel and Stock,[5] two of which they converted into the free α-aminoacyl derivatives (II; Y = H). In the present work the compounds listed in the Table were obtained by condensation of the amine (I) with the appropriate α-*N*-acylamino-acid, either by the use of dicyclohexylcarbodi-imide in a variety of solvents, or by the mixed carbonic-anhydride procedure in dimethylformamide. In general, the former method gave products which were cleaner and more easily purified. The amino-acids used were glycine, DL-methionine, DL-ethionine, the γ-benzyl ester of L-glutamic acid, and L-tyrosine; for each, the α-*N*-acyl groups, introduced by conventional methods, were one or more of the following: formyl, acetyl, fluoroacetyl, dichloroacetyl, methoxycarbonyl. Ethionine was included because, as an antimetabolite, it might act as an additional cytotoxic agent when enzymically liberated alongside the nitrogen mustard; similar reasoning prompted the use of the *N*-fluoroacetyl group. The α-*N*-dichloroacetyl compounds were made because of the anti-tumour

[1] Part V, Benn, Creighton, Johnson, Owen, and White, preceding Paper.
[2] Danielli, *Nature*, 1952, **170**, 863; Ciba Foundation Symposium, " Leukaemia Research," Churchill, London, 1954, p. 263.
[3] Ross, Warwick, and Roberts, *J.*, 1955, 3110.
[4] Hebborn and Danielli, *Nature*, 1956, **177**, 25; *Biochem. Pharmacol.*, 1958, **1**, 19.
[5] Bergel and Stock, *J.*, 1959, 97; Bergel, J. M. Johnson, Wade, Everett, and Mitchley, *Ann. Reports British Empire Cancer Campaign*, 1962, **40**, 4; J. M. Johnson, Ph.D. Thesis, Univ. of London, 1963, p. 205.
[6] Benn, Creighton, Owen, and White, *J.*, 1961, 2365.
[7] Everett and Ross, *J.*, 1949, 1972.
[8] Everett, Roberts, and Ross, *J.*, 1953, 2386; Ross and Warwick, *J.*, 1956, 1364, 1724.
[9] Popp and Kirsch, *J. Org. Chem.*, 1961, **26**, 3858.
[10] Ross, " Biological Alkylating Agents," Butterworths, London, 1962.
[11] Boyer, Lardy, and Myrbäck, " The Enzymes," 2nd edn., Academic Press Inc., New York, 1960, **4**, 7.

IC No.	Compound (II) X	Compound (II) Y	Method [a]	Solv.[b]	Cryst.[c]	Yield (%)	M. p.	Found C	Found H	Found Cl	Found N	Formula	Requires C	Requires H	Requires Cl	Requires N
187	H	CO·H	D M	E–F F	A–C	65 61	163°			22·5	12·9	$C_{13}H_{17}Cl_2N_3O_2$			22·4	13·1
175	H	Ac	D M	E F	A	50 64	172	50·65	5·75		12·7	$C_{14}H_{19}Cl_2N_3O_2$	50·6	5·8		12·65
196	H	$CO·CHCl_2$	D	G	B–C	65	171	42·4	4·5	35·1	10·7	$C_{14}H_{17}Cl_4N_3O_2$	41·9	4·3	35·3	10·5
178	H	CO_2Me	M	F	B–C	77	169	48·3	5·5	20·3	12·1	$C_{14}H_{19}Cl_2N_3O_3$	48·3	5·5	20·4	12·1
174	$CH_2·CH_2·SMe$ [d]	CO·H	M	F	A–B	*f*	160	49·1	6·1			$C_{16}H_{23}Cl_2N_3O_2S$	49·0	5·9		
172	$CH_2·CH_2·SMe$ [d]	Ac	M	F	B–C	60	181			17·75	10·4	$C_{17}H_{25}Cl_2N_3O_2S$			17·5	10·35
197	$CH_2·CH_2·SMe$ [d]	$CO·CH_2F$	D	E	A–B	63	160	48·1	6·1		9·9	$C_{17}H_{24}Cl_2FN_3O_2S$	48·1	5·7		9·9
191	$CH_2·CH_2·SMe$ [d]	$CO·CHCl_2$	D	H	A–B	*f*	172	43·4	5·0	29·8	8·7	$C_{17}H_{23}Cl_4N_3O_2S$	43·0	4·9	29·8	8·8
181	$CH_2·CH_2·SMe$ [d]	CO_2Me	D	E	A–B	39	141			16·7	9·8	$C_{17}H_{25}Cl_2N_3O_3S$			16·8	9·9
190	$CH_2·CH_2·SEt$ [d]	CO·H	D	C	B–C	26	143	50·2	5·9		10·65	$C_{17}H_{25}Cl_2N_3O_2S$	50·25	6·2		10·35
192	$CH_2·CH_2·SEt$ [d]	CO_2Me	D	E	B–C	33	110	48·95	6·0	16·4	10·1	$C_{18}H_{27}Cl_2N_3O_3S$	49·5	6·2	16·2	9·7
180	$CH_2·CH_2·CO_2·CH_2Ph$ [e]	CO·H	M	F	A–B	*f*	151	57·8	5·8	14·5	8·8	$C_{23}H_{27}Cl_2N_3O_4$	57·5	5·7	14·7	8·8
194	$CH_2·C_6H_4·OH$-*p* [e]	CO·H	D	F	A	*f*	192	56·65	5·6		10·1	$C_{20}H_{23}Cl_2N_3O_3$	56·6	5·5		9·9
200	$CH_2·C_6H_4·O·CO_2Me$-*p* [e]	CO_2Me	D	E	B–C	50	168			13·9	8·3	$C_{23}H_{27}Cl_2N_3O_6$			13·8	8·2

[a] D, dicyclohexylcarbodi-imide; M, mixed anhydride. For procedure, see Experimental section.
[b] Reaction carried out in: C, chloroform; E, dichloromethane; F, dimethylformamide; G, acetonitrile; H, nitromethane.
[c] Recrystallised from: A, acetone; B, light petroleum; C, chloroform.
[d] DL-form.
[e] L-form.
Final yield of pure product very small.

properties of *N*-dichloroacetylserine [12] (though more recently it has been suggested [13] that the real activity is due to *O*-dichloroacetylserine, formed by rearrangement *in vivo*). The α-amino-group was protected as the methylurethane in four of the compounds (IC 178, 181, 192, and 200) in the hope that their biological action might be capable of potentiation; this possibility arises from the observation [14] that pretreatment with isopropyl *N*-phenylcarbamate induces the formation of an adaptive enzyme capable of splitting the urethane linkage.

Although it has been reported [15] that the mixed carbonic anhydride derived from *N*-formylglycine and ethyl chloroformate reacts abnormally with ethyl *p*-aminobenzoate, to give only the *N*-ethoxycarbonyl derivative of the amino-ester, such behaviour was not encountered when this mixed anhydride reacted with the aromatic amine (I), the yield of the required amide (IC 187; see Table) being about the same as that obtained by the other method. Attempts to remove the benzyl ester group in the *N*-formylglutamyl derivative (IC 180) by hydrogenolysis were unsuccessful; a similar difficulty was experienced by Bergel and Stock [5] with the *N*-benzyloxycarbonyl derivatives mentioned above.

Experimental

The *N*-formyl derivatives of glycine, DL-methionine, and DL-ethionine were prepared by Sheehan and Yang's general method.[16] *N*-Acetyl-,[17] *N*-dichloroacetyl-,[18] and *N*-methoxycarbonyl-glycine;[19] *N*-acetyl-,[20] and *N*-dichloroacetyl-DL-methionine;[18] γ-benzyl *N*-formylglutamate;[21] anhydrous *N*-formyl-L-tyrosine;[22] and *NO*-bismethoxycarbonyl-L-tyrosine,[23] were prepared according to the methods in the references cited.

N-*Methoxycarbonyl*-DL-*methionine*.—Methyl chloroformate (10·5 c.c.) was added slowly to a stirred solution of DL-methionine (14·9 g.) in 4N-sodium hydroxide (50 c.c.) at 0°. The solution was set aside overnight, then acidified and extracted with chloroform to give the derivative as an oil (16·3 g.). It was characterised by reaction with *NN*-diethyl-*p*-phenylenediamine and dicyclohexylcarbodi-imide in chloroform, by the general method outlined below, to give N′-(N-*methoxycarbonyl*-DL-*methionyl*)-NN-*diethyl*-p-*phenylenediamine*, m. p. 122° (Found: N, 12·2; O, 13·3. $C_{17}H_{27}N_3O_3S$ requires N, 11·9; O, 13·6%).

N-*Fluoroacetyl*-DL-*methionine*.—Similarly, fluoroacetyl chloride (3 c.c.), DL-methionine (3 g.) and 8N-sodium hydroxide (5 c.c.) gave the derivative as an oil (2·1 g.) which was used without further purification.

N-*Methoxycarbonyl*-DL-*ethionine*.—Similar treatment of DL-ethionine (3·2 g.) in 4N-sodium hydroxide (10 c.c.) with methyl chloroformate (1·6 c.c.) gave an oil (3·8 g.), which was characterised by reaction with aniline and dicyclohexylcarbodi-imide in dichloromethane to give the *anilide*, m. p. 143° (Found: N, 9·55; O, 16·2; S, 10·5. $C_{14}H_{20}N_2O_3S$ requires N, 9·45; O, 16·2; S, 10·8%).

Acylation of NN-*Di*-2′-*chloroethyl*-p-*phenylenediamine*.—(i) *Carbodi-imide method.* A solution of the α-*N*-acylamino-acid (0·01 mole) in the chosen solvent (*ca.* 20 c.c.) was added to a mixture of *NN*-di-2′-chloroethyl-*p*-phenylenediamine hydrochloride [7] (2·7 g., 0·01 mol.) and triethylamine (1·4 c.c., 0·01 mol.) in the same solvent (20 c.c.), followed by *NN*′-dicyclohexylcarbodi-imide (2·1 g., 0·01 mole). The mixture was shaken for 1—2 days and then filtered. Acetic acid (0·7 c.c.) was added to the filtrate, and after 2 hr. the solution was washed with 2N-hydrochloric acid, then with water, and concentrated. The residue, usually a solid, was purified by chromatography (alumina, chloroform) and then recrystallised.

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[13] Levi, Weed, Laflamme, and Koller, *Canad. J. Chem.*, 1961, **39**, 2491.
[14] Danielli, *Ann. Reports British Empire Cancer Campaign*, 1959, **37**, 575.
[15] King, Clark-Lewis, Kidd, and Smith, *J.*, 1954, 1039.
[16] Sheehan and Yang, *J. Amer. Chem. Soc.*, 1958, **80**, 1154.
[17] Herbst and Shemin, *Org. Synth.*, Coll. Vol. II, 11.
[18] Ronwin, *J. Org. Chem.*, 1953, **18**, 1546.
[19] Leuchs, *Ber.*, 1906, **39**, 857.
[20] Birnbaum, Levintow, Kingsley, and Greenstein, *J. Biol. Chem.*, 1952, **194**, 455.
[21] Borek and Waelsch, *J. Biol. Chem.*, 1953, **205**, 459.
[22] Fischer, *Ber.*, 1907, **40**, 3704.
[23] Havestadt and Fricke, *Ber.*, 1924, **57**, 2051.

(ii) *Mixed anhydride method.* Ethyl chloroformate (2·0 c.c., 0·02 mole) was added to a solution of the α-*N*-acylamino-acid (0·02 mole) and triethylamine (2·8 c.c., 0·02 mole) in dry dimethylformamide (15—20 c.c.) at 0°. After 20 min., a solution of *NN*-di-2′-chloroethyl-*p*-phenylenediamine hydrochloride (5·4 g., 0·02 mole) and triethylamine (2·8 c.c., 0·02 mole) in the same solvent (30 c.c.) was added, and the mixture was stored at *ca.* 4°. Next day it was filtered, concentrated under reduced pressure, and then diluted with chloroform. The solution was then washed and worked up as described in the first method.

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