734. *Cyto-active Amino-acids and Peptides. Part IX.*[1] *Further Studies of* N*-Acyl-dipeptides from Melphalan.*

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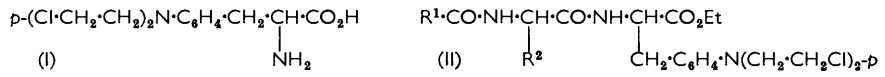
The synthesis of a series of *N*-acetyl-L- and -D-aminoacyl derivatives of melphalan ethyl ester [*p*-di-(2-chloroethyl)aminophenylalanine ethyl ester] is described. The tendency of acetamido-acids to racemise during the mixed-anhydride procedure and the advantage of the azide method for peptide synthesis are confirmed. Biological results are briefly discussed.

In Part VIII [1] of this series the synthesis of several peptide derivatives of melphalan (I) was described. These were prepared during the search for compounds with a chemotherapeutic index more favourable than that of the parent anti-tumour agent.

In a continuation an extended series of *N*-acyl-dipeptide esters (II) has been prepared, with special attention to the configurational purity of the products. In view of the publications by Young and his co-workers,[2] we suspected that the mixed-anhydride procedure, used to prepare the *N*-acetyl-dipeptide esters reported in Part VIII, would, in general, give partially racemised products. Accordingly we compared the *N*-acetyl-dipeptide esters prepared by various methods. We found that the mixed-anhydride or carbodi-imide procedure gave products which showed racemisation when compared with compounds obtained by the azide route. In this connexion we note that Siemion and Nowak [3] prepared optically active azlactones by reaction of formyl- and acetyl-L-leucine with dicyclohexylcarbodi-imide.



(I) (II)

L- and D-*N*-Acetamido-esters were converted into their hydrazides; these were coupled with melphalan ethyl ester by the azide technique. Intermediates not hitherto described in the literature are given in Tables 1 (amino-ester hydrochlorides), 2 (acetamido-esters), and 3 (hydrazides). In addition, the corresponding L- and D-acetamido-acids were coupled with melphalan ethyl ester by the mixed-anhydride technique using isobutyl chloroformate, and three were also prepared from the same starting materials by using dicyclohexylcarbodi-imide. The physical characteristics of the acetamidoacyl-melphalan esters and *N*-formylphenylalanylmelphalan esters prepared by the various routes are listed in Table 4.

The $[\alpha]_D$ values show, as mentioned before, that methods other than that employing an azide caused racemisation of the acylated amino-acid, and also led to mixtures of diastereoisomers. The specific rotations of these products will reflect the combined effects of such racemisation and any fractionation occurring during the recrystallisation of the crude materials, and so should lie between the limits of the L-L- and D-L-isomers, prepared by the azide route. Most of the values in Table 4 are in keeping with this; the few small discrepancies could be due to experimental errors in polarimetry.

The results of biological tests, namely, anti-tumour effects on the Walker rat carcinoma 256 and toxicities, illustrate clearly the following points: (i) for the series as a whole there is a considerable variation in carcinostatic effects and toxicities although the chemical reactivity of the " mustard " group is virtually constant throughout; (ii) within each stereoisomeric pair for which data are available, the L-L- was more active than the D-L-isomer (the melphalan moiety having always the L-configuration) by a factor of at least 100, underlining the importance of the " carrier " portion of the molecule; (iii) on a molar basis the most active compounds had about the same anti-tumour activity as the parent

[1] Part VIII, Bergel and Stock, *J.*, 1960, 3658.
[2] North and Young, *Chem. and Ind.*, 1955, 1597; Smart, Young, and Williams, *J.*, 1960, 3902.
[3] Siemion and Nowak, *Roczniki Chem.*, 1961, **35**, 979.

melphalan; (iv) however as some dipeptides, notably the derivatives with acetyl-L-alanyl-, -L-leucyl, and -glycyl and formyl-L-phenylalanyl residues (Table 4, Nos. 3, 11, 1, and 22, respectively) were relatively less toxic, their approximate chemotherapeutic indices were more favourable than that of melphalan itself.

Experimental

M. p.s were determined on a Kofler block. Analyses were carried out in the Microanalytical Laboratories, the Imperial College of Science and Technology, and by Mr. P. R. W. Baker, the Wellcome Research Laboratories, Beckenham, Kent.

Table 1.

Amino-ester hydrochlorides.

No.	Ester	M. p.*	Yield (%)	$[\alpha]_D$	Temp.	*c* in EtOH
1	D-Val.OEt	107—109°	75	−17·1°	22°	3·3
2	D-Leu.OEt	137·5—139·5	96	−17·6	19	4·9
3	D-Phe.OEt	158—160	74	−34·6	23	3·9
4	L-Phe.OEt	158·5—159·5	84	+34·5	23	4·7
5	L-Phe.OMe	159—161	79	+36·9	22·5	2·9

	Found (%)					Required (%)			
No.	C	H	Cl	N	Formula	C	H	Cl	N
1	46·3	8·9	19·8	8·0	$C_7H_{16}ClNO_2$	46·3	8·9	19·5	7·7
2	49·3	9·5	—	7·1	$C_8H_{18}ClNO_2$	49·1	9·3	—	7·2
3	57·1	7·2	15·0	6·2	$C_{11}H_{16}ClNO_2$	57·5	7·0	15·4	6·1
4	57·2	7·2	15·9	5·9	$C_{11}H_{16}ClNO_2$	57·5	7·0	15·4	6·1
5	56·15	6·7	16·8	6·2	$C_{10}H_{14}ClNO_2$	55·7	6·5	16·4	6·5

* Cryst. from EtOAc–Et_2O, except that no. 5 was from MeOH–Et_2O.

Table 2.

α-Acetamido-esters.

No.	Compound	B. p./mm.	Yield (%)	Specific rotation $[\alpha]_D$	Temp.	*c*	Solvent	n_D^{21}
1	Ac-D-Ala.OEt	91—92°/0·8 *	91 †	+78°	21°	3·1	H_2O	1·4452
2	Ac-D-Val.OEt	100—102/0·75	81	+26·3	22	4·6	EtOH	1·4508
3	Ac-D-Phe.OEt	M. p. 93·5—94·5	74	−13·1	20	2·6	EtOH	—

* M. p. 35°. † Calc. on D-alanine.

	Found (%)				Required (%)		
No.	C	H	N	Formula	C	H	N
1	52·7	8·3	9·15	$C_7H_{13}NO_3$	52·8	8·2	8·8
2	57·9	9·2	7·1	$C_9H_{17}NO_3$	57·7	9·2	7·5
3	66·8	7·3	5·7	$C_{13}H_{17}NO_3$	66·4	7·3	5·95

Table 3.

α-Acetamido-acid hydrazides.

No.	Hydrazide	M. p.	Cryst. from	Yield (%)	$[\alpha]_D$	Temp.	*c* *
1	Ac-D-Val	231—232°	EtOH–Et_2O	58	+46·3°	23°	1·2
2	Ac-L-Val	235—236	,,	80	−46·4	24	1·1
3	Ac-D-Phe	184—185·5	EtOH–Pet	77	−35·1	19	1·1
4	Ac-*p*-NO_2-D-Phe	218—220	EtOH	87	−42·3	20	1·7
5	Ac-*p*-NO_2-L-Phe	219·5—221·5	,,	71	+41·9	21	1·6

	Found (%)				Required (%)		
No.	C	H	N	Formula	C	H	N
1	48·5	8·9	24·3	$C_7H_{15}N_3O_2$	48·5	8·7	24·3
2	48·5	9·0	24·1	$C_7H_{15}N_3O_2$	48·5	8·7	24·3
3	59·7	6·8	19·2	$C_{11}H_{15}N_3O_2$	59·7	6·8	19·0
4	49·4	5·2	20·0	$C_{11}H_{14}N_4O_4$	49·6	5·3	21·0
5	49·3	5·5	19·7	$C_{11}H_{14}N_4O_4$	49·6	5·3	21·0

* In H_2O.

Amino-ester Hydrochlorides.—A suspension of the amino-acid (0·06 mole) in the appropriate alcohol (100 ml.), previously saturated with dry hydrogen chloride, was heated under reflux for 1 hr. Evaporation *in vacuo* gave the *ester hydrochloride* (Table 1) which was purified by crystallisation. In the preparation of D-alanine ethyl ester hydrochloride only a colourless syrup could be obtained which was acylated without further purification.

TABLE 4.

Melphalan peptide derivatives (II).

No.	Compound	R^1	R^2	Method ‖	Yield (%)	M. p.	$[\alpha]_D$	Temp.	*c* in $CHCl_3$
1	Ac-Gly.Mel.OEt	Me	H	*a*	41	93—96° *	58·8°	21°	3·4
2	Ac-L-Ala.Mel.OEt	Me	Me	*a*	71	95—100 *	45·4	19·5	2·0
3	Ac-L-Ala.Mel.OEt	,,	,,	*b*	10	72—74 *	73·0	22·0	2·0
4	Ac-D-Ala.Mel.OEt	,,	,,	*a*	64	131—133 *	48·0	26	2·2
5	Ac-D-Ala.Mel.OEt	,,	,,	*b*	38	134—136 †	49·2	26	1·9
6	Ac-L-Val.Mel.OEt	Me	Pr^i	*a*	61	180—185 ‡	33·2	21	2·1
7	Ac-L-Val.Mel.OEt	,,	,,	*b*	12	197—199 ‡	48·7	24	0·6
8	Ac-D-Val.Mel.OEt	,,	,,	*a*	45	200—202 §	26·7	26	1·7
9	Ac-D-Val.Mel.OEt	,,	,,	*b*	10	203—205 ‡	28·0	25	2·0
10	Ac-L-Leu.Mel.OEt	Me	Bu^i	*a*	72	130—132 *	40·7	21	2·0
11	Ac-L-Leu.Mel.OEt	,,	,,	*b*	61	145—148 §	27·5	19	2·7
12	Ac-D-Leu.Mel.OEt	,,	,,	*a*	80	132—133 *	42·5	21	2·0
13	Ac-D-Leu.Mel.OEt	,,	,,	*b*	64	147—148 §	52·2	20	2·7
14	Ac-L-Phe.Mel.OEt,H_2O	Me	CH_2Ph	*b*	39	164—166 *	36·2	21	2·0
	,, anhydrous	,,	,,	*b*	65	155—157 *	36·0	21	2·1
15	Ac-L-Phe.Mel.OEt	,,	,,	*c*	77	157—159 *	28·3	24	2·2
						168—170 ‡	29·2	23	2·2
16	Ac-D-Phe.Mel.OEt	,,	,,	*a*	66	170—172 ‡	28·3	19	2·3
17	Ac-D-Phe.Mel.OEt	,,	,,	*b*	72	173·5—175·5 *	27·7	21	2·2
18	Ac-D-Phe.Mel.OEt	,,	,,	*c*	79	161—165 *	28·5	23	2·1
						174—175 ‡	29·1	23	2·0
19	Ac-*p*-NO_2-L-Phe.Mel.OEt	Me	††	*b*	50	173—175 *	30·9	21	1·8
20	Ac-*p*-NO_2-D-Phe.Mel.OEt	,,	††	*b*	50	176—178 ¶	54·0	21	0·9
21	H·CO-L-Phe.Mel.OEt	H	CH_2Ph	*a*	66	154—156 *	29·1	21	0·9
22	H·CO-L-Phe.Mel.OEt	,,	,,	*b*	66	157—159 *	32·8	20	2·3
23	H·CO-L-Phe.Mel.OEt	,,	,,	*c*	82	158—159 ‡	29·5	20	2·3

No.	Found (%) C	H	Cl	N	Formula	Required (%) C	H	Cl	N
1	53·2	6·4	16·9	9·6	$C_{19}H_{27}Cl_2N_3O_4$	52·8	6·3	16·4	9·7
2	53·8	6·3	15·9	9·0	$C_{20}H_{29}Cl_2N_3O_4$	53·8	6·5	15·9	9·4
3	54·0	6·5	—	9·6	$C_{20}H_{29}Cl_2N_3O_4$	53·8	6·5	15·9	9·4
4	53·3	6·8	15·7	9·7	$C_{20}H_{29}Cl_2N_3O_4$	53·8	6·5	15·9	9·4
5	54·0	6·8	15·7	8·9	$C_{20}H_{29}Cl_2N_3O_4$	53·8	6·5	15·9	9·4
6	55·9	6·8	15·2	9·1	$C_{22}H_{33}Cl_2N_3O_4$	55·7	7·0	14·9	8·9
7	55·8	7·7	14·8	8·6	$C_{22}H_{33}Cl_2N_3O_4$	55·7	7·0	14·9	8·9
8	55·2	7·4	14·9	9·1	$C_{22}H_{33}Cl_2N_3O_4$	55·7	7·0	14·9	8·9
9	55·5	6·7	14·4	9·1	$C_{22}H_{33}Cl_2N_3O_4$	55·7	7·0	14·9	8·9
10	56·7	6·8	14·7	8·2	$C_{23}H_{35}Cl_2N_3O_4$	56·6	7·2	14·5	8·6
11	56·9	7·6	14·0	8·8	$C_{23}H_{35}Cl_2N_3O_4$	56·6	7·2	14·5	8·6
12	56·8	6·9	14·8	8·8	$C_{23}H_{35}Cl_2N_3O_4$	56·6	7·2	14·5	8·6
13	56·2	6·9	14·3	8·5	$C_{23}H_{35}Cl_2N_3O_4$	56·6	7·2	14·5	8·6
14	58·6	6·6	13·0	7·9	$C_{26}H_{33}Cl_2N_3O_4,\frac{1}{2}H_2O$	58·8	6·5	13·3	7·9
	59·7	6·4	12·8	8·0	$C_{26}H_{33}Cl_2N_3O_4$	59·8	6·4	13·6	8·0
15	61·2	6·3	13·1	7·6	$C_{26}H_{33}Cl_2N_3O_4$	59·8	6·4	13·6	8·0
	59·7	6·3	15·1	8·6	$C_{26}H_{33}Cl_2N_3O_4$	59·8	6·4	13·6	8·0
16	59·8	6·5	13·7	8·0	$C_{26}H_{33}Cl_2N_3O_4$	59·8	6·4	13·6	8·0
17	59·5	6·1	13·3	8·2	$C_{26}H_{33}Cl_2N_3O_4$	59·8	6·4	13·6	8·0
18	60·4	6·4	13·3	8·2	$C_{26}H_{33}Cl_2N_3O_4$	59·8	6·4	13·6	8·0
	59·8	6·4	13·5	8·5	$C_{26}H_{33}Cl_2N_3O_4$	59·8	6·4	13·6	8·0
19	55·0	5·7	12·4	9·8	$C_{26}H_{32}Cl_2N_4O_6$	55·0	5·7	12·5	9·9
20	55·4	5·6	12·4	9·6	$C_{26}H_{32}Cl_2N_4O_6$	55·0	5·7	12·5	9·9
21	59·2	6·3	13·7	8·0	$C_{26}H_{32}Cl_2N_4O_6$	59·1	6·2	14·0	8·3
22	59·3	6·0	13·2	8·2	$C_{25}H_{31}Cl_2N_3O_4$	59·1	6·2	14·0	8·3
23	58·8	6·4	15·3	9·0	$C_{25}H_{31}Cl_2N_3O_4$	59·1	6·2	14·0	8·3

Cryst. from: * aq. EtOH; † EtOAc–light petroleum; ‡ EtOH; § aq. MeOH; ¶ MeOH.
‖ Method: (*a*) mixed anhydride; (*b*) azide; (*c*) dicyclohexylcarbodi-imide. †† $CH_2{\cdot}C_6H_4{\cdot}NO_2$-*p*.

Acetamido-esters.—The amino-acid ester hydrochloride (0·03 mole) was mixed with anhydrous potassium carbonate (0·075 mole) and acetic anhydride (0·3 mole), and the vigorous reaction allowed to subside; the mixture was then heated for 30 min. on the steam-bath. Removal of the excess of acetic anhydride *in vacuo* gave a semi-solid residue which was extracted with ether. The extract was filtered and the solvent removed. Distillation of the residual oil gave the *acetamido-ester* (Table 2). During the preparation of N-*acetyl*-D-*phenylalanine ethyl ester* (Table 2, No. 3) removal of the excess of acetic anhydride gave a colourless solid which recrystallised from water.

N-*Formyl*-L-*phenylalanine Methyl Ester.*—L-Phenylalanine methyl ester hydrochloride (Table 1, No. 5) (4·3 g., 0·02 mole), suspended in ethanol (20 ml.), was treated with triethylamine (2·8 ml., 0·02 mole). Addition of an excess of ether precipitated the triethylamine hydrochloride which was filtered off, and the filtrate was evaporated to a residual pale-yellow oil. Fractional distillation *in vacuo* gave 2·85 g. (80%) of free ester, b. p. 87—88°/0·45 mm. The ester was immediately dissolved in 98—100% formic acid (25 ml.) and treated dropwise with acetic anhydride (9 ml.) at $<15°$. Then the mixture was stirred at room temperature for 1 hr. after which ice–water (20 ml.) was introduced and the mixture concentrated *in vacuo*. Fractional distillation of the oily residue gave 2·8 g. (68% calc. on L-phenylalanine methyl ester hydrochloride) of the *formyl ester*, b. p. 145—150°/0·5 mm., n_D^{23} 1·5300, $[\alpha]_D^{23}$ 28·95° (*c* 2·5 in EtOH) (Found: C, 62·7; H, 6·2; N, 6·7. $C_{11}H_{13}NO_3$ requires C, 63·8; H, 6·3; N, 6·8%).

Acetamido-hydrazides.—A solution of the acetamido-ester (0·016 mole) and hydrazine hydrate (0·096 mole) in ethanol (20 ml.) was heated under reflux for 1 hr. Evaporation *in vacuo* followed by recrystallisation of the residue gave the colourless *hydrazides* (Table 3).

N-*Formyl*-L-*phenylalanine Hydrazide.*—A solution of *N*-formyl-L-phenylalanine methyl ester (1·4 g., 6·75 mmoles) and hydrazine hydrate (2 ml., 41 mmoles) in methanol (5 ml.) was set aside at room temperature for 20 hr. Evaporation *in vacuo* at 40° and recrystallisation of the colourless residue from ethanol–ether gave the *hydrazide* (0·66 g., 47%), m. p. 138—143° (Found: C, 57·9; H, 6·7; N, 20·8. $C_{10}H_{13}N_3O_2$ requires C, 58·0; H, 6·3; N, 20·3%).

Acyl-dipeptide Ethyl Esters of Melphalan (Table 4).—(*a*) *Mixed carbonic–carboxylic anhydride method.* The procedure described in Part VIII [1] was used.

(*b*) *Azide method.* An acylamino-hydrazide (20 mmoles) was dissolved in glacial acetic acid (4 ml.), and concentrated hydrochloric acid (4 ml., 40 mmoles) and water (20 ml.) were added. The solution was cooled to 5° and a solution of sodium nitrite (2·8 g., 40 mmoles) in the minimum of cold water added portionwise. The resulting solution or suspension was extracted several times with ice-cold ethyl acetate, and the organic layer washed with ice-cold saturated sodium hydrogen carbonate solution, then with ice-cold water, dried ($MgSO_4$), and filtered. During the extraction and washing, finely crushed ice was always present in the separatory funnel. Meanwhile triethylamine (2·8 ml., 20 mmoles) was added to melphalan ethyl ester hydrochloride (7·4 g., 20 mmoles) in ice-cold ethyl acetate (50 ml.), the mixture stirred to assist dissolution of the ester, and the whole added to the previously prepared azide solution. After 24 hr. at room temperature the mixture was washed with 0·1N-hydrochloric acid, sodium hydrogen carbonate solution, and water, dried ($MgSO_4$), and evaporated to dryness *in vacuo*. The residual *acyl-dipeptide esters* were purified by recrystallisation.

(*c*) *Carbodi-imide method.* A mixture of the acylamino-acid (10 mmoles), melphalan ethyl ester hydrochloride (10 mmoles), and triethylamine (10 mmoles) in tetrahydrofuran (50 ml.) was treated with dicyclohexylcarbodi-imide (11 mmoles) and set aside for 12 hr. A few drops of glacial acetic acid were added, the precipitate was removed, and the filtrate evaporated to dryness *in vacuo*. A chloroform solution of the residue was washed with 0·1N-hydrochloric acid, water, sodium hydrogen carbonate solution, and water, and dried ($MgSO_4$) and the chloroform removed *in vacuo*. The residue was purified by recrystallisation.

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