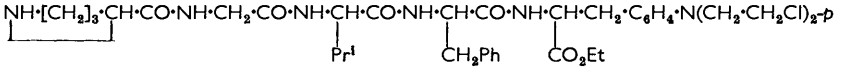
735. *Cyto-active Amino-acids and Peptides. Part X.*[1] *A Pentapeptide and a Basic Dipeptide from Melphalan.*

By J. M. Johnson and J. A. Stock.

The preparation of the ethyl esters of L-prolylglycyl-L-valyl-L-phenylalanyl- and L-arginyl-melphalan is described and some of their biological effects are briefly reported.

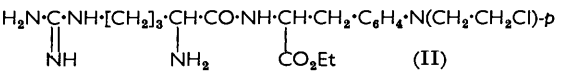
It has been found [1,2] that the hæmatological and anti-tumour activity of peptide derivatives of melphalan [*p*-di-(2-chloroethyl)amino-L-phenylalanine] is influenced by the structure and size of the attached group. A pentapeptide ester (I; Table, No. 8) of the arbitrary sequence prolylglycylvalylphenylalanylmelphalan has now been prepared as the hydrochloride, all the asymmetric centres having the L-configuration. The intermediates (nos. 1—7) in the preparation are set down in the Table in the order in which they were



(I)

made. The peptide chain was extended as far as the protected tetrapeptide ester (no. 5) by stepwise addition of benzyloxycarbonylamino-acids to the free amino-end of the successive precursors. By this procedure, the mixed anhydride method [3] could be used without risk of racemisation.[4] The benzyloxycarbonyl intermediates were hydrogenolysed over palladium–charcoal to give the corresponding peptide ester hydrochlorides (nos. 2, 4, and 8). The melphalan residue, with its reactive " nitrogen mustard " group, was added late in the synthesis to minimise possible decomposition. This addition, in contrast to the previous couplings, was made at the carboxyl end of the peptide intermediate (no. 5), the azide route [5] being chosen to avoid possible racemisation which may occur in the condensation of *N*-benzyloxycarbonylpeptides with amino-acid esters by other methods.[4] The required hydrazide (no. 6) was readily prepared by treatment of the ester (no. 5) with hydrazine in ethanol. Conversion into the azide and reaction of the latter with melphalan ester gave the acylpentapeptide (no. 7), which, although not obtained analytically pure, was subsequently hydrogenolysed in satisfactory yield to the desired pentapeptide ester of melphalan (no. 8; CB **3305**).

All the peptides of melphalan so far described [1,2] have been prepared from neutra amino-acids. It seemed of interest to study the effects of introducing basic or acidic residues, and we have now synthesised L-arginylmelphalan ester (no. 10) as an example of a basic peptide. Tribenzyloxycarbonyl-L-arginine [6] was condensed with melphalan ester, the mixed anhydride procedure again being used. Hydrogenation of the resulting triacylarginyl derivative (no. 9) over palladium–charcoal in ethanolic hydrogen chloride afforded the L-arginylmelphalan ester (II) in the form of its hygroscopic hydrochloride (no. 10; CB 3280).



(II)

Both peptides showed an activity comparable with that of analogues described previously.[2] The pentapeptide, CB **3305**, markedly inhibited the growth of the Walker

[1] Part IX, preceding paper.
[2] Bergel and Stock, *J.*, 1960, 3658; Elson, Haddow, Bergel, and Stock, *Biochem. Pharmacol.*, 1962, in the press.
[3] Boissonnas, *Helv. Chim. Acta*, 1951, **34**, 874; Vaughan, *J. Amer. Chem. Soc.*, 1951, **73**, 3547.
[4] Smart, Young, and Williams, *J.*, 1960, 3902.
[5] Greenstein and Winitz, " Chemistry of the Amino Acids," Wiley, New York, 1961, Vol. II, p. 949.
[6] Zervas, Otani, Winitz, and Greenstein, *J. Amer. Chem. Soc.*, 1959, **81**, 2878; Zervas, Winitz, and Greenstein, *J. Org. Chem.*, 1957, **22**, 1515.

rat carcinoma 256 at a single dose of 5 mg./kg., approximately one-tenth of the toxic dose. It was active against the mouse sarcoma S180 at 10 mg./kg. per day when given for 12 days, and doubled the survival time in a Furth leukæmia test at 2 mg./kg. per day for three weeks. The arginyl-peptide, CB 3280, completely inhibited the Walker tumour at a single dose of 2 mg./kg., approximately one-twelfth of the toxic dose; twelve daily doses of 5 mg./kg. completely suppressed the S180 sarcoma.

Experimental

Benzyloxycarbonylpeptide Esters (Table 1, nos. 1, 3, 5, and 9) (cf. ref. 3).—In general, isobutyl chloroformate (1 equiv.) was added to an ice-cooled solution of the benzyloxycarbonylamino-acid and dry triethylamine (1 equiv.) in dry tetrahydrofuran. The mixture was kept in ice for 20 min., then to it was added a freshly prepared solution of the amino-acid or peptide

No.	Compound [a]	Cryst. from	M. p.	$[\alpha]_D^{22-26}$	(*c* in EtOH)	Yield (%)
1	Z.Val.Phe.OEt	Aq. EtOH	148—149°	−24°	(4·1)	88
2	Val.Phe.OEt,HCl	EtOH–Et_2O	184—186	+21	(1·6)	87
3	Z.Gly.Val.Phe.OEt	Aq. EtOH	153—155	−28	(2·2)	79
4	Gly.Val.Phe.OEt,HCl	EtOH–Et_2O [b]	223—225	−23	(2·1)	82
5	Z.Pro.Gly.Val.Phe.OEt	Aq. EtOH	173—175	−35	(2·0)	74
6	Z.Pro.Gly.Val.Phe.NH·NH_2	— [c]	232—234	— [c]		85
7	Z.Pro.Gly.Val.Phe.Mel.OEt	Aq. EtOH	169—171	— [d]		79
8	Pro.Gly.Val.Phe.Mel.OEt,HCl	EtOH–Et_2O [b]	175—177	−28	(0·9)	51
9	Tri.Z.Arg.Mel.OEt	EtOH–$CHCl_3$	149—152	+16	(2·4) [e]	78
10	Arg.Mel.OEt,2HCl [f]	EtOH–Et_2O [b]	125—130 [g]	+11	(0·8)	95 [h]
11	Arg.Mel.OEt,HO·$C_6H_2(NO_2)_3$	Aq. EtOH [b]	183—185 [i]	+10	(0·5) [d]	—

	Found (%)					Required (%)			
No.	C	H	Cl	N	Formula	C	H	Cl	N
1	67·6	7·2	—	6·5	$C_{24}H_{30}N_2O_5$	67·6	7·1	—	6·6
2	58·1	7·4	10·9	8·4	$C_{16}H_{24}N_2O_3$,HCl	58·4	7·7	10·8	8·5
3	65·0	6·9	—	8·6	$C_{26}H_{33}N_3O_6$	64·6	6·9	—	8·7
4	53·2	7·1	9·3	10·4	$C_{18}H_{27}N_3O_4$,HCl,H_2O	53·5	7·5	8·8	10·4
5	64·6	6·7	—	10·0	$C_{31}H_{40}N_4O_7$	64·1	6·9	—	9·7
6	61·1	6·7	—	14·7	$C_{29}H_{38}N_6O_6$	61·5	6·8	—	14·8
7	61·9	6·8	7·1	9·0	$C_{44}H_{56}Cl_2N_6O_8$ [j]	60·9	6·5	8·2	9·7
8	55·3	6·8	13·6	10·8	$C_{36}H_{50}Cl_2N_6O_6$,HCl	56·1	6·7	13·8	10·9
9	60·5	5·9	8·05	9·8	$C_{45}H_{52}Cl_2N_6O_9$	60·5	5·9	8·0	9·45
11	42·2	4·0	7·3	—	$C_{21}H_{34}Cl_2N_6O_3$,$2C_6H_3N_3O_7$	41·8	4·25	7·5	—

[a] Nomenclature as in ref. 2; Z = Ph·CH_2·O·CO. [b] Amorphous. [c] See Experimental text. [d] Opacity prevented accurate determination (10 cm. tube of *ca.* 1 ml. capacity). [e] In 1 : 1 EtOH–$CHCl_3$. [f] Hygroscopic. [g] Glass; fluid at 145°. [h] Calc. as dihydrochloride. [i] Decomp. [j] Analysis suggests some hydrolysis of the " mustard " group.

ethyl ester hydrochloride (1 equiv.) and triethylamine (1 equiv.) in chloroform. Next day, the mixture was taken to dryness in a vacuum, and the ethyl acetate extract of the residue was washed with 0·1N-hydrochloric acid, sodium hydrogen carbonate solution, and water, dried ($MgSO_4$), and evaporated under reduced pressure. The residual *acyl-peptide* ester was purified by crystallisation (see Table).

*Benzyloxycarbonyl-*L*-prolylglycyl-*L*-valyl-*L*-phenylalanine Hydrazide.*—The corresponding ethyl ester (no. 5; 20·6 g., 18 mmoles) and hydrazine hydrate (5·3 ml., 180 mmoles) were heated for 2 hr. under reflux in ethanol (75 ml.). Evaporation of the solvent gave a colourless solid, sparingly soluble in most solvents. Trituration with hot ethanol, dilution of the cooled mixture with ether, and filtration afforded the hydrazide (8·9 g.; no. 6) which was too insoluble for an accurate rotation measurement.

*Benzyloxycarbonyl-*L*-prolylglycyl-*L*-valyl-*L*-phenylalanylmelphalan Ethyl Ester.*—Conversion of the hydrazide described above (17 mmoles) into the azide, and subsequent coupling with melphalan ester by the general method (*b*) [1] used for the acyldipeptide esters of melphalan, gave the *acylpentapeptide ester* (no. 7).

Peptide Ester Hydrochlorides.—The benzyloxycarbonyl derivatives were hydrogenolysed over 5% palladium–charcoal at room temperature and pressure, in ethanol containing one

equiv. of hydrogen chloride, except for the arginine peptide for which an excess of hydrogen chloride was used. Data for the *ester hydrochlorides* are given in the Table (nos. 2, 4, 8, 10). The arginine dipeptide in the form of its hydrochloride (no. 10) was hygroscopic and was therefore analysed as its *picrate* (no. 11).

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