19. *Cyto-active Amino-acids and Peptides. Part V.*[1] *Derivatives of* p-*Amino- and* p-*Mercapto-phenylalanine.*

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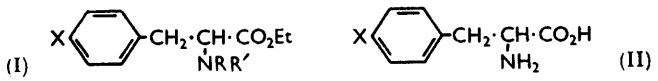
A number of optically active *p*-alkyl- and *p*-aralkyl-aminophenylalanines has been prepared, three of which carry also the 2-chloroethyl group. *p*-2-Chloroethylamino-L-phenylalanine was obtained as its ethyl ester. *p*-2-Chloroethylthio-L-phenylalanine has been synthesised *via* *p*-mercapto-L-phenylalanine (L-thiotyrosine) and the *S*-2-hydroxyethyl derivative. The results of some biological tests are given.

The relatively high activity of difunctional " nitrogen mustard " derivatives of phenylalanine [2,3,4] and higher homologues [5] against the Walker rat carcinoma 256 and their effects on circulating leucocytes [6,7] prompted the synthesis of some monochloro- (" monofunctional ") and halogen-free (" non-functional ") analogues. It was hoped that such compounds might show more pronounced effects than other " monofunctional " agents not having an amino-acid structure.

The starting material for the compounds listed in the Table was *p*-nitro-*N*-phthaloyl-L- or -D- or *N*-acetyl-*p*-nitro-L-phenylalanine ethyl ester (I; X = NO_2, RR′ = phthaloyl; or R = H, R′ = Ac) described previously.[2,3] The secondary amines (I; X = NHEt, $NHPr^n$, and $Ph{\cdot}CH_2{\cdot}NH$, RR′ = phthaloyl) required for the synthesis of the three chloroethylamino-compounds (Nos. 1, 2, and 3) were prepared from the primary amine (I;



(I) (II)

X = NH_2, RR′-phthaloyl) which has now been obtained crystalline in both its L- and its D-form, without preliminary purification as the hydrochloride.[2] When this amine was heated with ethanol and propan-1-ol in the presence of Raney nickel,[8,9] the amines (I; X = NHEt and $NHPr^n$, RR′ = phthaloyl) were formed. While a heating time of 2 hr., as given by Ainsworth,[9] was satisfactory for the higher alcohol, this was insufficient for the ethylamino-compound, which had to be heated with Raney nickel for at least 4 hr. to ensure complete absence of the initial primary amine. Boiling for only 2 hr. led to an apparently pure material which was converted into a biologically active chloroethyl compound. However, the observed anti-tumour effect was found to be due to contamination by the di-(2-chloroethyl) derivative [II; X = $(Cl{\cdot}CH_2{\cdot}CH_2)_2N$; CB 3025] formed from small amounts of primary amine (I; X = NH_2, RR′ = phthaloyl) whose presence was revealed by a positive diazotisation and coupling test of the intermediate (I; X = NHEt, RR′ = phthaloyl). This impurity could not be removed satisfactorily by recrystallisation. On the other hand prolonged heating had the disadvantage of lowering the yield and causing slight contamination with the tertiary amine (I; X = NEt_2, RR′ = phthaloyl) which was, however, eliminated during the subsequent stages.

The ethylamine (I; X = NHEt, RR′ = phthaloyl) was also prepared from the nitro-compound (I; X = NO_2, RR′ = phthaloyl) by catalytic reductive ethylation.[10] Alkylation by this method was, however, not always satisfactory. Moreover, a diazotisation

[1] Part IV, Bergel and Stock, *J.*, 1957, 4563.
[2] *Idem*, *J.*, 1954, 2409.
[3] Bergel, Burnop, and Stock, *J.*, 1955, 1223.
[4] Bergel and Stock, *Brit. Emp. Cancer Camp. Ann. Rep.*, 1953, **31**, 6.
[5] Davis, Roberts, and Ross, *J.*, 1955, 890.
[6] Elson, *Ann. New York Acad. Sci.*, 1958, **68**, 826.
[7] Elson, Talbot, Cardinali, and Lotz, *Brit. Emp. Cancer Camp. Ann. Rep.*, 1957, **35**, 58.
[8] Rice and Kohn, *J. Amer. Chem. Soc.*, 1955, **77**, 4052.
[9] Ainsworth, *ibid.*, 1956, **78**, 1635.
[10] Emerson, " Organic Reactions," Wiley, New York, 1948, Vol. IV, p. 174.

and coupling test indicated that the crystalline product was again contaminated with traces of primary amine (I; X = NH_2, RR′ = phthaloyl). Attempts to prepare the derivative (I; X = NHEt, RR′ = phthaloyl) by catalytic reductive alkylation of the amine (I; X = NH_2, RR′ = phthaloyl) were unsuccessful. The intermediate benzylamine (I; X = Ph·CH_2·NH, RR′ = phthaloyl) was prepared by hydrogenation over Raney nickel of the Schiff's base (I; X = Ph·CH:N, RR′ = phthaloyl). The secondary bases were submitted to hydroxyethylation, chlorination, and hydrolysis in the usual

No.	Compound	CB no.	Isomer	Cryst. from	M. p.*	$[\alpha]_D$	Yield (%)
1	II; X = Cl·CH_2·CH_2·NEt	3135	L	—[a]	186—187°	−33°[b] +6·5[c]	37[d]
2	II; X = Cl·CH_2·CH_2·NPr^n	3155	L	—[a]	175—176	+3·5[f]	58[d]
3	II; X = Cl·CH_2·CH_2·N·CH_2Ph	3153	L	—[a]	173—175	+16[g]	34[d]
4	I; X = Cl·CH_2·CH_2·NH, R = R′ = H (dihydrochloride)	3198	L	E[j]	159—162	+26[e]	75
5	II; X = NMe_2	3167	L	A[h]	206 208	−40[i]	61
6	II; X = NMe_2	3192	D	A[h]	206—207	+41[i]	51
7	II; X = NHEt	3165	L	B[j]	215—217	−38[k]	53
8	II; X = NEt_2	3193	L	B[j]	183—184	+9[l]	33
9	II; X = Ph·CH_2·NH	3194	L	C[n]	214—216	+14[p]	75
10	II; X = HS (hydrochloride)	3195	L	D[j]	231—233	−4[q]	37[r]
11	II; X = HO·CH_2·CH_2·S (hydrochloride)	3196	L	—[s]	193—196	−6[t]	85
12	II; X = Cl·CH_2·CH_2·S (hydrochloride)	3175	L	D[j]	197—200	+16[u]	94

No.	Formula	Found (%) C	H	N	S	Cl	Required (%) C	H	N	S	Cl
1	$C_{13}H_{19}O_2N_2Cl$[e]	57·7	7·3	10·0	—	13·0	57·6	7·1	10·35	—	13·1
2	$C_{14}H_{21}O_2N_2Cl$[e]	59·0	7·3	9·7	—	12·2	59·0	7·4	9·8	—	12·5
3	$C_{13}H_{21}O_2N_2Cl,H_2O$	62·0	6·7	8·0	—	10·3	61·6	6·6	8·0	—	10·1
	$C_{13}H_{21}O_2N_2Cl$[e]	—	—	8·2	—	10·7	—	—	8·4	—	10·7
4	$C_{13}H_{19}O_2N_2Cl,2HCl$[w]	46·0	6·3	8·1	—	—	45·4	6·2	8·15	—	—
5	$C_{11}H_{16}O_2N_2$	63·5	7·95	13·4	—	—	63·4	7·7	13·45	—	—
6	,,	,,	7·9	13·2	—	—	,,	,,	,,	—	—
7	,,[e]	63·25	,,	13·45	—	—	,,	,,	,,	—	—
8	$C_{13}H_{20}O_2N_2,\frac{1}{3}H_2O$[em]	64·5	8·6	11·6	—	—	64·4	8·6	11·6	—	—
		64·2	8·4	11·6	—	—					
9	$C_{16}H_{18}O_2N_2$[e]	71·3	6·8	10·6	—	—	71·1	6·7	10·4	—	—
10	$C_9H_{11}O_2NS,HCl$	45·9	5·5	5·9	13·7	—	46·2	5·2	6·0	13·7	—
11	$C_{11}H_{15}O_3NS,HCl$	46·8	5·7	4·9	12·0	—	47·5	5·8	5·0	11·5	—
12	$C_{11}H_{14}O_2NSCl,HCl$	44·4	5·2	4·65	—	—	44·6	5·1	4·7	—	—

* All the compounds melted with decomposition.

A, Aq. MeOH. B, MeOH–EtOH. C, Aq. EtOH. D, Conc. HCl. E, EtOH–Et_2O.

[a] Purified by repptn. (Expt. section). [b] *c* 1·25 in MeOH at 23°. [c] *c* 2·2 in N-HCl at 21°. [d] Calc. on the intermediate *p*-monoalkylamino-*N*$^\alpha$-phthaloylphenylalanine ester. [e] Compound dried 3—4 hr. at 110°/0·5 mm. [f] *c* 1·95 in N-HCl at 25°. [g] *c* 2·36 in N-HCl at 25°. [h] Slightly tinted plates. [i] *c* 1·0 in 0·1M-phosphate buffer (pH 7) at 22°. [j] Colourless or slightly tinted prisms [k] *c* 3·29 in H_2O at 24°. [l] *c* 2·20 in N-HCl at 25°. [m] Analytical figures given for two separate preparations. [n] Colourless needles. [p] *c* 2·77 in N-HCl at 24°. [q] *c* 2·35 in N-HCl at 25°. [r] Calc. on the intermediate *N*-acetyl-*p*-nitrophenylalanine ester. [s] Precipitated from solution in MeOH or EtOH by addition of Et_2O. [t] *c* 3·0 in H_2O at 24°. [u] *c* 3·7 in EtOH at 22°. [v] *c* 0·35 in MeOH at 25°. [w] Dried 30 min. at 90°/1 mm.

manner [2] and gave the three *N*-chloroethyl-*N*-alkyl- or -benzyl-amino-L-phenylalanines (Nos. 1, 2, 3).

When the crude non-crystalline intermediate (I; X = HO·CH_2·CH_2·NH·CH_2Ph, RR′ = phthaloyl) was hydrogenolysed over palladium–charcoal, the 2-hydroxyethylamino-compound (I; X = HO·CH_2·CH_2·NH, RR′ = phthaloyl) was formed. A similar reaction with the free amino-acid (II; X = Cl·CH_2·CH_2·NH·CH_2Ph) led to a crystalline deliquescent hydrochloride which was characterised as the analytically pure hydrochloride of the ester (I; X = Cl·CH_2·CH_2·NH, R = R′ = H).

Several " non-functional " analogues were synthesised for comparative biological purposes (Nos. 5, 6, 7, 8, and 9). The preparation of *p*-dimethylamino-DL-phenylalanine

by an azlactone synthesis has been reported by others.[11] *p*-Dimethyl- and *p*-diethylamino-phenylalanine have been claimed by Harper *et al.*[12] but these authors do not indicate the method of preparation or the configuration of the compounds, nor do they give analytical or physical data. We prepared the *p*-dimethylamino-L- and -D-phenylalanine (II; X = NMe_2) by catalytic reductive methylation of the nitro-compound (I; X = NO_2, RR′ = phthaloyl) and subsequent hydrolysis. An attempted dimethylation of the amine (I; X = NH_2, RR′ = phthaloyl) by the Eschweiler–Clarke modification of the Leuckart reaction,[13] using formaldehyde and formic acid, gave an intractable gum. *p*-Ethylamino-L-phenylalanine (II; X = NHEt) was prepared by hydrolysis of the intermediate (I; X = NHEt, RR′ = phthaloyl) discussed above, and was purified by chromatography on powdered cellulose. Catalytic reductive diethylation [10] of the nitro-compound (I; X = NO_2, RR′ = phthaloyl) with acetaldehyde gave a non-crystalline product which yielded, on hydrolysis, a crude granular hydrochloride. Paper chromatography revealed the presence of a substantial quantity of a fast-moving impurity, together with the required diethylamine II; X = NEt_2) and a trace of the primary amine (II; X = NH_2). The major impurity was possibly a higher alkyl derivative of *p*-aminophenylalanine formed from a polymer of acetaldehyde, but no attempt has been made to isolate this substance. Pure *p*-diethylamino-L-phenylalanine was obtained by chromatography on cellulose and subsequent crystallisation. *p*-Benzylamino-L-phenylalanine (II; X = $Ph{\cdot}CH_2{\cdot}NH$) was readily prepared by hydrolysis of the corresponding phthaloyl ester.

The synthesis of the " hemi-sulphur mustard " L-derivative (II; X = $Cl{\cdot}CH_2{\cdot}CH_2{\cdot}S$) involved the initial preparation of *p*-mercapto-L-phenylalanine. For this, we employed the diazonium xanthate method used recently by Colescott, Herr, and Dailey [14] in the preparation of the DL-form from a malonic ester intermediate. Our first experiments started with the L-phthaloyl derivative (I; X = NH_2, RR′ = phthaloyl) but hydrolysis of the product was unsatisfactory. We therefore employed the L-acetyl compound [3] (I; X = NH_2, R = H, R′ = Ac) which was diazotised and then converted into the xanthate (I; X = $EtO{\cdot}CS_2$, R = H, R′ = Ac) and thence into the hydrochloride of the *p*-mercapto-L-phenylalanine (II; X = SH), best purified through the mercuric chloride complex. Reaction of this thiol with ethylene oxide at room temperature in the presence of excess of sodium hydrogen carbonate proceeded rapidly without involving the α-amino-group significantly, conversion into the hydroxyethyl derivative (II; X = $HO{\cdot}CH_2{\cdot}CH_2{\cdot}S$) being practically complete in about 2 min. Brief heating of this compound with concentrated hydrochloric acid [15] gave the hydrochloride of the " hemi-sulphur mustard " (II; X = $Cl{\cdot}CH_2{\cdot}CH_2{\cdot}S$).

In tests on the Walker rat carcinoma 256, the " monofunctional " derivatives, CB 3135, 3153, 3155, and 3175 (see Table) appeared much less effective than the corresponding difunctional compound, *p*-di-(2-chloroethyl)amino-L-phenylalanine [II; X = $(Cl{\cdot}CH_2{\cdot}CH_2)_2N$] (CB 3025, Melphalan),[4] without showing on the whole a corresponding decrease in toxicity in the animal not bearing a tumour. The first sample of CB 3135, which has one chlorine atom less than Melphalan, produced anti-timour effects at ten times the dose of the latter (1 mg./kg.) but, as outlined above, this was due to contamination by the di-(2-chloroethyl) analogue. Pure CB 3135 was inactive below the toxic dose (75 mg./kg.), even though its chemical reactivity as measured by its rate of hydrolysis at pH 7 and 37° (determination by Dr. W. Davis) was about four times that of CB 3025. A mixture of the latter and CB 3135 (0·25 mg./kg.; 25 mg./kg.) proved equally ineffective. This suggested that contamination of the first sample of CB 3135 by CB 3025 exceeded 1%, indicating an enrichment of the " difunctional " impurity during the final stages of the synthesis. While the benzyl compound CB 3153 exerted some activity at 150 mg./kg.,

[11] Elliott, Fuller, and Harington, *J.*, 1948, 85.
[12] Harper, Furst, and Morris, *Wasmann J. Biol.*, 1950, **8**, 299.
[13] Moore, " Organic Reactions," Wiley, New York, 1949, Vol. V, p. 301.
[14] Colescott, Herr, and Dailey, *J. Amer. Chem. Soc.*, 1957, **79**, 4232.
[15] Connors and Ross, *Chem. and Ind.*, 1958, 366.

the propyl derivative CB 3155 at 150 mg./kg. and the " half-sulphur mustard " CB 3175 at 10 mg./kg. (toxic at 25 mg./kg.) were inactive. The last result is the more interesting as the " half-sulphur mustard " $HO{\cdot}CH_2{\cdot}CH_2{\cdot}S{\cdot}CH_2{\cdot}CH_2Cl$ showed some promise in clinical trials.[16] However, in general, difunctionality seems necessary for substantial activity, as with other " mustard derivatives." [17] Of the " non-functional " phenylalanine derivatives, CB 3167 was tested with negative results.

Experimental

p-*Amino*-N$^{\alpha}$-*phthaloyl*-L- *and* D-*phenylalanine Ethyl Ester* (I; X = NH_2, RR′ = phthaloyl). —These compounds were originally characterised as the hydrochlorides.[2] Each has now been obtained crystalline as the free base directly from the (palladium–charcoal) hydrogenation product of the corresponding nitro-compound. The L-*amine* crystallised from aqueous methanol, in yellow prisms (91%), m. p. 102—103°, $[\alpha]_D^{21}$ −213° (*c* 1·0 in EtOH) (Found: C, 67·4; H, 5·3; N, 8·2. $C_{19}H_{18}O_4N_2$ requires C, 67·4; H, 5·4; N, 8·3%). The D-*amine* (90%) had m. p. 102—104°, $[\alpha]_D^{23}$ +212° (*c* 1·0 in EtOH) (Found: N, 8·2%).

p-*Ethylamino*-N$^{\alpha}$-*phthaloyl*-L-*phenylalanine Ethyl Ester* (I; X = NHEt, RR′ = phthaloyl).—(*a*) *p*-Nitro-*N*-phthaloyl-L-phenylalanine ethyl ester [2] (7·4 g., 0·02 mol.) and acetaldehyde (1·4 ml., 0·025 mol.) were hydrogenated in ethanol (200 ml.) in the presence of anhydrous sodium acetate (0·4 g., 0·005 mol.) over Raney nickel (2 g.) and Adams platinum catalyst (0·2 g.) (cf. Emerson [10]). Hydrogen uptake was complete in a few hours. Evaporation of the filtrate to a smaller volume gave the *ester* as yellow prisms (5·95 g., 81%), m. p. 124—126° (unchanged on recrystallisation from ethanol), $[\alpha]_D^{22}$ −226° (*c* 0·47 in dioxan) (Found: C, 68·9; H, 6·05; N, 7·65. $C_{21}H_{22}O_4N_2$ requires C, 68·8; H, 6·05; N, 7·65%). A diazotisation and coupling test, carried out as in (*b*), indicated very slight contamination by the primary amine (I; X = NH_2, RR′ = phthaloyl).

(*b*) In one experiment, *p*-amino-*N*$^{\alpha}$-phthaloyl-L-phenylalanine ethyl ester (5·0 g.), ethanol-moist Raney nickel (17 g.), and ethanol (45 ml.) were heated for 2 hr. under reflux with stirring (cf. Rice and Kohn [8] and Ainsworth [9]). The hot mixture was filtered through " Hyflo " and evaporated to a smaller volume. The cooled solution deposited yellow prisms (4·8 g., 89%) of the ester, m. p. 126—128°, mixed m. p. of 124—126° with the product described in (*a*) (Found: C, 68·9; H, 6·25; N, 7·65%). The material (m. p. 126—128°) gave a pink colour when treated with nitrite in acid solution, then with an alkaline solution of R-acid (2-naphthol-3 : 6-disulphonic acid). A control experiment, in which the amine was omitted, gave no colour. The intensity of the colour indicated contamination by not more than *ca.* 0·5% of primary amine. Recrystallisation from ethanol did not remove the colour-producing material.

Heating the primary amine with the Raney-nickel for a longer period (4—6 hr.) gave a somewhat lower yield (*ca.* 70%), and the recrystallised product had a lower m. p. (123—126°). It was, however, free from primary amine (diazotisation and R-acid coupling test), though the experiments in which it was hydrolysed to the free amino-acid (II; X = NHEt) (see below) indicated slight contamination by the tertiary amine (I; X = Et_2N, RR′ = phthaloyl).

p-n-*Propylamino*-N$^{\alpha}$-*phthaloyl*-L-*phenylalanine Ethyl Ester* (I; X = NHPr^n, RR′ = phthaloyl).—The method of preparation was as above, except that propan-1-ol was used instead of ethanol. The heating time was 4·5 hr. The gummy product crystallised from methanol in yellow prisms (0·44 g., 78%) of the *ester*, m. p. 94—97°. Recrystallisation from methanol raised the m. p. to 97—98° and the pure compound had $[\alpha]_D^{22}$ −202° (*c* 1·62 in EtOH) (Found: C, 69·1; H, 6·6; N, 7·3. $C_{22}H_{24}O_4N_2$ requires C, 69·45; H, 6·4; N, 7·4%).

p-*Benzylideneamino*-N$^{\alpha}$-*phthaloyl*-L- *and* -D-*phenylalanine Ethyl Ester* (I; X = Ph·CH:N, RR′ = phthaloyl).—*p*-Amino-*N*$^{\alpha}$-phthaloyl-L-phenylalanine ethyl ester (6·0 g.), benzaldehyde (2·4 ml., 1·3 mol. equiv.), anhydrous sodium acetate (0·5 g.), and methanol (35 ml.) were heated 1 hr. under reflux. The *azomethine* crystallised, on cooling, as pale yellow prisms, m. p. 110—111°, unchanged on recrystallisation from methanol (6·55 g., 86%; the yield tended to be a little lower in the absence of sodium acetate), $[\alpha]_D^{24}$ −239° (*c* 0·90 in EtOH) (Found: C, 73·2; H, 5·3; N, 6·4. $C_{26}H_{22}O_4N_2$ requires C, 73·2; H, 5·2; N, 6·6%).

The D-*isomer* was prepared similarly in 75% yield from the corresponding primary amine.[2]

[16] Seligman and Rutenburg, *Ann. New York Acad. Sci.*, 1958, **68**, 1181.
[17] Haddow, Kon, and Ross *Nature*, 1948, **162**, 824.

The product had m. p. 110—111°, $[\alpha]_D^{21}$ +246° (*c* 1·2 in EtOH) (Found: C, 73·05; H, 5·3; N, 6·6%).

p-*Benzylamino*-N$^{\alpha}$-*phthaloyl*-L- *and* -D-*phenylalanine Ester* (I; X = Ph·CH$_2$·NH, RR′ = phthaloyl).—The L-azomethine (6·53 g.) was hydrogenated at room temperature and pressure over Raney nickel in ethanol–ethyl acetate (1 : 1 by vol.; 100 ml.). The filtrate was evaporated, and the residual gum crystallised from methanol. The *benzylamine* formed colourless needles, m. p. 118—120° (5·87 g.; 90%), $[\alpha]_D^{21}$ −185° (*c* 1·58 in EtOH) (Found: C, 72·4; H, 5·5; N, 6·6. $C_{26}H_{24}O_4N_2$ requires C, 72·9; H, 5·65; N, 6·5%). The D-*benzylamine* was obtained similarly and had m. p. 118—120°, $[\alpha]_D^{21}$ +188° (*c* 0·98 in EtOH) (Found: N, 6·5%).

p-*Benzylamino*-L-*phenylalanine* (II; X = Ph·CH$_2$·NH).—*p*-Benzylamino-N$^{\alpha}$-phthaloyl-L-phenylalanine ethyl ester (1·6 g.) was heated for 4 hr. under reflux with 6N-hydrochloric acid (15 ml.). The solution became homogeneous within 45 min. The cooled solution was filtered from the phthalic acid and evaporated to dryness under vacuum, and the residue dissolved in water (5 ml.). Neutralisation with concentrated sodium acetate solution precipitated the crude *amino-acid* which was collected, washed with water, dried, and crystallised from aqueous ethanol (Table, No. 9).

p-(N-2-*Chloroethyl*-N-*ethyl*)-, p-(N-2-*Chloroethyl*-N-n-*propyl*)- *and* p-(N-*Benzyl*-N-2-*chloroethyl*)-*amino*-L-*phenylalanine* (II).—The hydroxyethylation and subsequent chlorination of *p*-ethyl-, *p*-*n*-propyl-, and *p*-benzyl-amino-N$^{\alpha}$-phthaloyl-L-phenylalanine ethyl ester were carried out with ethylene oxide and freshly distilled phosphorus oxychloride by the method used earlier for the "nitrogen-mustard" derivative of phenylalanine.[2] All the intermediate hydroxyethyl derivatives were gums. The chlorinated products were severally heated under reflux for 4 hr. with 6N-hydrochloric acid. The isolation, using sodium acetate, followed the published method.[2] Each of the products resisted crystallisation but was purified for analysis and biological testing by dissolution in excess of N-hydrochloric acid, precipitation from the filtered solution by the addition of N-sodium hydroxide and thorough washing of the precipitate with water (Nos. 1, 2, and 3).

The rates of hydrolysis of CB 3135 (II; X = Cl·CH$_2$·CH$_2$·NEt) and CB 3025 [II; X = (Cl·CH$_2$·CH$_2$)$_2$N] [2] at pH 7 and 37°, determined by Dr. W. Davis using a Pye automatic titrator, were: CB 3135, $k = 6{\cdot}66 \times 10^{-4}$ sec.$^{-1}$ (half-life 17·4 min.); CB 3025, $k = 1{\cdot}45 \times 10^{-4}$ sec.$^{-1}$ (half-life 79·7 min.).

p-2-*Chloroethylamino*-L-*phenylalanine Ethyl Ester* (I; X = Cl·CH$_2$·CH$_2$, R = R′ = H).—*p*-(*N*-Benzyl-*N*-2-chloroethyl)amino-L-phenylalanine (1·35 g.) was debenzylated by hydrogenation, at room temperature and pressure, in ethanol (10 ml.) containing 10N-ethanolic hydrogen chloride (0·4 ml., 1 equiv.). The granular product (0·85 g.), m. p. 129—131° (decomp.), obtained by addition of ether to the filtered mixture, was converted into colourless plates, m. p. 136—137°, by reprecipitation from ethanol. The compound became sticky on storage in a stoppered tube and gave an unsatisfactory analysis. The tetraphenylboron derivative was also hygroscopic. The compound was therefore converted into the *ester hydrochloride* with cold 10N-ethanolic hydrogen chloride (2 days at room temperature) (Table, No. 4).

p-2-*Hydroxyethylamino*-N$^{\alpha}$-*phthaloyl*-L-*phenylalanine Ethyl Ester* (I; X = HO·CH$_2$·CH$_2$·NH, RR′ = phthaloyl).—*p*-Benzyl-(2-hydroxyethyl)amino-N$^{\alpha}$-phthaloyl-L-phenylalanine ethyl ester was prepared, as mentioned above, by the usual procedure,[2] from the corresponding benzylamine (I; X = Ph·CH$_2$·NH, RR′ = phthaloyl) (2·2 g.). It did not crystallise but was successfully hydrogenolysed at ordinary temperature and pressure over 5% palladium–charcoal (3 g.) in ethanol (150 ml.). The *hydroxyethylamine* crystallised from aqueous methanol in pale yellow plates (1·32 g., 68%, calc. on the intermediate *p*-benzylamino-N$^{\alpha}$-phthaloyl-L-phenylalanine ethyl ester), m. p. 108—111°, $[\alpha]_D^{24}$ −194° (*c* 2·07 in EtOH). Recrystallisation from aqueous methanol raised the m. p. to 110—112° (Found: C, 65·85; H, 6·1; N, 7·2. $C_{21}H_{22}O_5N_2$ requires C, 65·95; H, 5·8; N, 7·3%).

p-*Dimethylamino*-N-*phthaloyl*-L- *and* -D-*phenylalanine Ethyl Ester* (I; X = Me$_2$N, RR′ = phthaloyl).—The conditions of Bowman and Stroud [18] were used. *p*-Nitro-*N*-phthaloyl-L-phenylalanine ethyl ester [2] (32 g.) and 40% aqueous formaldehyde (50 ml., 8 mol. equiv.) were hydrogenated in ethanol (480 ml.) over 5% palladium–charcoal (16 g.) in the presence of anhydrous sodium acetate (16 g., 2 equiv.). Approximately the theoretical volume (5 mol.) of hydrogen was absorbed. The filtrate and ethanol washings were evaporated until sodium acetate began to crystallise. Hot water was added to a faint turbidity, the sodium acetate

[18] Bowman and Stroud, *J.*, 1950, 1342.

redissolving. The cooled solution deposited yellow plates of the *dimethylamino-compound* (27 g., 85%), m. p. 120—122°, $[\alpha]_D^{21}$ −214° (*c* 1·0 in MeOH). Recrystallisation from a small volume of ethanol raised the m. p. to 123—124° (Found: C, 68·8; H, 6·15; N, 7·7. $C_{21}H_{22}O_4N_2$ requires C, 68·8; H, 6·05; N, 7·65%). The D-*isomer* was prepared in 84% yield in the same way from *p*-nitro-*N*-phthaloyl-D-phenylalanine ethyl ester.[2] It formed yellow plates, m. p. 123—124°, $[\alpha]_D^{21}$ +221° (*c* 1·0 in MeOH) (Found: C, 69·0; H, 6·05; N, 7·65%).

p-*Dimethylamino*-L- *and* -D-*phenylalanine* (II; X = NMe_2).—*p*-Dimethylamino-*N*-phthaloyl-L-phenylalanine ethyl ester (27·0 g.) was heated under reflux for 5 hr. with 6N-hydrochloric acid (200 ml.). Evaporation of the solution to smaller bulk, cooling, removal of phthalic acid, neutralisation with 4N-lithium hydroxide, and dilution with ethanol gave a crude product (13·2 g.) from which the pure L-*amino-acid* (9·3 g.) was obtained by crystallisation. The D-*amino-acid* was prepared similarly (Nos. 5 and 6).

p-*Ethylamino*-L-*phenylalanine* (II; X = NHEt).—In a preliminary experiment, a crystalline substance was obtained by the lithium hydroxide treatment of an ethanol solution of the hydrolysis product of *p*-ethylamino-N^{α}-phthaloyl-L-phenylalanine ethyl ester. The substance gave two ninhydrin-positive components when run on Whatman No. 1 paper with butan-1-ol–propan-1-ol–0·1N-aqueous ammonia (2 : 1 : 1 by vol.), the main spot having R_F 0·33, and the minor R_F 0·62 (= R_F of *p*-diethylaminophenylalanine). The second component was not removed by crystallisation from aqueous ethanol. The compound was therefore prepared and purified as follows.

p-Ethylamino-N^{α}-phthaloyl-L-phenylalanine ethyl ester (1·0 g.) was heated for 4 hr. under reflux with 6N-hydrochloric acid (15 ml.). The solution was cooled, filtered from the phthalic acid, and evaporated to dryness under a vacuum. The residue was dissolved in sufficient (1·5 ml.) 2N-aqueous ammonia to give a neutral solution, and diluted with butan-1-ol–propan-1-ol (2 : 1; 4·5 ml.). The mixture was placed on a powdered cellulose (80 g. dry wt.) column (37 × 3·5 cm.) made up in butan-1-ol–propan-1-ol–0·1N-aqueous ammonia (2 : 1 : 1). The column was eluted with the same solvent at *ca.* 2 ml. per min. The first 130 ml. of eluate were discarded and 7 ml. fractions were then collected. Fractions 31—52 inclusive contained ninhydrin-positive material of R_F 0·32 only (run on Whatman No. 1 in the same solvent), and were combined and evaporated. The crystalline white residual *amino-acid* was recrystallised (No. 7).

p-*Diethylamino*-L-*phenylalanine* (II; X = NEt_2).—*p*-Nitro-*N*-phthaloyl-L-phenylalanine ethyl ester [2] (10·0 g.) and acetaldehyde (10·5 ml., 7 mol. equiv.) were hydrogenated at room temperature and pressure in the presence of sodium acetate (0·5 g. anhydrous; 2 equiv.) over 5% palladium–charcoal (5 g.) in ethanol (250 ml.) containing water (15 ml.) (which appeared to assist the alkylation). Approximately the calculated volume (5 mol.) of hydrogen was absorbed. Evaporation of the filtrate and separation from sodium acetate by extraction into ether gave a gum.

Most (8 g.) of the gum was hydrolysed in the same way as the ethylamine analogue. The crude amino-acid hydrochloride (5·5 g.) was isolated in granular form by addition of ether to a solution in methanol, and subsequent trituration of the semisolid precipitate with ethyl acetate. Chromatography on Whatman No. 1 paper with butanol–propanol–0·1N-aqueous ammonia (2 : 1 : 1 by vol.) revealed three ninhydrin-positive components having R_F values 0·93, 0·63 (main component) and 0·35 (very faint; = R_F of *p*-aminophenylalanine) respectively. Part (1·45 g.) of the crude hydrochloride was run on a column similar to that used in the purification of *p*-ethylaminophenylalanine but containing a larger amount (175 g.) of cellulose. The first 300 ml. eluate were rejected, and 10 ml. fractions were then collected. Substantial overlap of the three components occurred but evaporation of combined fractions 24—36 (which contained the bulk of the required material of R_F 0·62) and crystallisation of the residue gave chromatographically pure p-*diethylamino*-L-*phenylalanine* (No. 8). Analysis of two preparations indicated that one-third of a mol. of water was strongly held. An attempted purification by ion-exchange chromatography on Amberlite IR 120 was unsatisfactory.

N-*Acetyl*-S-(*ethoxythiocarbonyl*)-p-*mercapto*-L-*phenylalanine Ethyl Ester* (I; X = Et·O·CS_2, R = H, R′ = Ac).—The method resembled that of Colescott *et al.*[14] *p*-Amino-N^{α}-acetyl-L-phenylalanine ethyl ester [3] (4·1 g. of gum) was dissolved in 6N-hydrochloric acid (15 ml.) and diazotised at 0—5° by the dropwise addition of a solution of sodium nitrite (1·5 g., 1·3 equiv.) in water (3 ml.). The slight excess of nitrous acid was destroyed by the addition of a crystal of sulphamic acid. The solution was carefully neutralised (pH 7) by the addition, with ice-cooling

and stirring, of powdered, anhydrous sodium carbonate. Potassium ethylxanthate (3·42 g., 1·3 mol.), dissolved in water (5 ml.), was added dropwise during about 5 min. to the cooled, stirred solution. The diazonium ethylxanthate was precipitated as a thick yellow gum. The mixture was heated for 50 min. with stirring at 60—70°, gas evolution occurring. The mixture was cooled, the gummy product extracted into ethyl acetate, the solution dried ($MgSO_4$), and the solvent removed under a vacuum. The residue (A; 5·2 g.) did not crystallise. However, in another similar experiment (not recorded in detail here because of complications due to substantial hydrolysis of the ethyl ester group, caused, it is believed, by having the solution too alkaline during the heating of the diazonium ethylxanthate solution) a crystalline neutral product (3·4 g., 30% calc. on the intermediate *p*-nitro-compound) was isolated by ether extraction. Recrystallisation from aqueous Cellosolve gave almost colourless needles of N-*acetyl*-S-(*ethoxythiocarbonyl*)-p-*mercapto*-L-*phenylanine ethyl ester*, m. p. 69—72°, $[\alpha]_D^{24}$ +25° *c* 0·99 in EtOH) (Found: C, 54·2; H, 6·1; N, 3·9. $C_{16}H_{21}O_4NS_2$ requires C, 54·1; H, 6·0; N, 3·9%).

p-*Mercapto*-L-*phenylalanine* (II; X = SH).—Most (4·6 g.) of the gummy ester (A above) was heated for 4 hr. under reflux with 6N-hydrochloric acid (30 ml.). The solution was evaporated to a smaller volume, then cooled. The colourless product (B) was collected, washed with a little concentrated hydrochloric acid, and dried (NaOH). It weighed 2·52 g., and an iodine titration indicated that it contained 45% of the required compound. The material was taken up in boiling 6N-hydrochloric acid (15 ml.), and zinc dust (1 g.) was added to the boiling solution in small quantities during 15 min. The solution was filtered, and concentrated hydrochloric acid (15 ml.) was added. The cooled solution yielded a crystalline substance (C; 1·95 g.) of improved iodine titre (67% of calc. SH); further treatment with zinc and acid did not improve this titre. The mother-liquors from (B) above gave, on further evaporation, a second crop of almost colourless crystals (D; 0·5 g.) having an iodine titre of 82% of theory. Materials (C) and (D) were sufficiently pure for conversion into the *S*-chloroethyl derivative described below. Analytically pure material was obtained by treatment of the mercuric chloride complex with hydrogen sulphide and final isolation as p-*mercapto*-L-*phenylalanine hydrochloride* (iodine titre *ca.* 99%) (No. 10).

p-2-*Hydroxyethylthio*-L-*phenylalanine* (II; X = $HO{\cdot}CH_2{\cdot}CH_2{\cdot}S$).—*p*-Mercapto-L-phenylalanine hydrochloride (0·50 g.), purified through the mercuric chloride complex, was added to air-free water (10 ml.) through which carbon dioxide was passing. Sodium hydrogen carbonate (1·0 g.) was added with shaking, then ethylene oxide (1 ml.). The solution was set aside for 10 min. (In a preliminary experiment it was found that the iodine titre of aliquot parts fell to zero quite rapidly, the reaction with ethylene oxide being practically complete in 1—2 min.) Concentrated hydrochloric acid (2·5 ml.) was added to the filtered solution which was evaporated to dryness under vacuum. The residue was heated with a little water on a steam-bath for 2 min. (to decompose any chloroethyl derivative which may have been present), the solution evaporated, the residue extracted with boiling ethanol, and the ethanol solution evaporated. The residue was taken up in methanol and precipitated by addition of ether, to give the *amino-acid hydrochloride* (0·50 g.) (No. 11). When the product was run on Whatman No. 1 paper, with butanol–propanol–propionic acid–water (10 : 5 : 2 : 5), only one ninhydrin-positive component was revealed (R_F 0·5).

p-2-*Chloroethylthio*-L-*phenylalanine* (II; X = $Cl{\cdot}CH_2{\cdot}CH_2{\cdot}S$).—*p*-2-Hydroxyethylthio-L-phenylalanine hydrochloride (0·5 g.) was suspended in concentrated hydrochloric acid (10 ml.) and heated to the b. p. Complete dissolution occurred. After 1 minute's further boiling, crystals began to separate, and the mixture set to a solid mass of colourless crystals within a further 0·5 min. The mixture was cooled at once (a longer heating period, with addition of acid to redissolve the product results in a lower yield), and the product was collected and washed with a little concentrated hydrochloric acid.

The *amino-acid hydrochloride* (0·5 g.) (No. 12) was analytically pure, and had R_F 0·7 in the system used with the parent hydroxy-compound. Paper chromatography also showed that complete reconversion into the hydroxy-compound occurred when an aqueous solution of the chloro-compound was boiled for 3 min. In ethanol, no hydrolysis was detected when the solution was heated at the b. p. for 1 min.

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