158. *Polypeptides. Part VIII.*[1] *Synthesis of Oxazoline Peptides.*

By Leo Benoiton, R. W. Hanson, and H. N. Rydon.

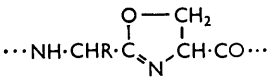
Although 2-benzamidomethyl-Δ^2-oxazoline hydrochloride (V) is formed by the action of thionyl chloride on 2-hippuramidoethanol at 0°, the analogous reaction with hippuryl- and aceturyl-serine leads only to the replacement of hydroxyl by chlorine. The action of bases on the *O*-toluene-*p*-sulphonyl derivatives of hippuryl- and benzyloxycarbonylglycyl-serine esters yields the corresponding dehydroalanine peptides, but 2-benzamidomethyl-4-carbamoyl-Δ^2-oxazoline (XIX) is produced by the action of potassium acetate on *O*-toluene-*p*-sulphonyl-*N*-hippurylserine amide. The benzamido-group makes the two new oxazolines, (V) and (XIX), weaker bases than the corresponding 2-methyl compound and greatly increases their rates of decomposition in aqueous solution.

On the basis of their finding that certain Δ^2-oxazolines, unlike serine, reacted with di-isopropyl phosphorofluoridate (DFP) under "physiological" conditions, Porter, Rydon, and Schofield [2] suggested that the "reactive" serine residue in DFP-sensitive enzymes, such as chymotrypsin and cholinesterase, was, in fact, a Δ^2-oxazoline residue, as (I), and owed its unique reactivity to this chemical modification. It was clearly desirable to study the reactivity towards DFP of Δ^2-oxazolines more closely analogous to (I) than were the simple compounds studied in the earlier work and the present paper is concerned with the synthesis of such compounds.

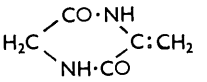
The only previous attempt to cyclise a serine peptide to a Δ^2-oxazoline is that of Bergmann and Miekeley,[3] who treated *N*-glycyl-DL-serine methyl ester with thionyl chloride

[1] Part VII, Jarvis, Rydon, and Schofield, *J.*, 1961, 1752.
[2] Porter, Rydon, and Schofield, *Nature*, 1958, **182**, 927.
[3] Bergmann and Miekeley, *Z. physiol. Chem.*, 1924, **140**, 927.

and obtained a chlorine-containing product of intermediate structure, which reacted with ammonia to give a product subsequently shown [4] to be the dioxopiperazine (II).

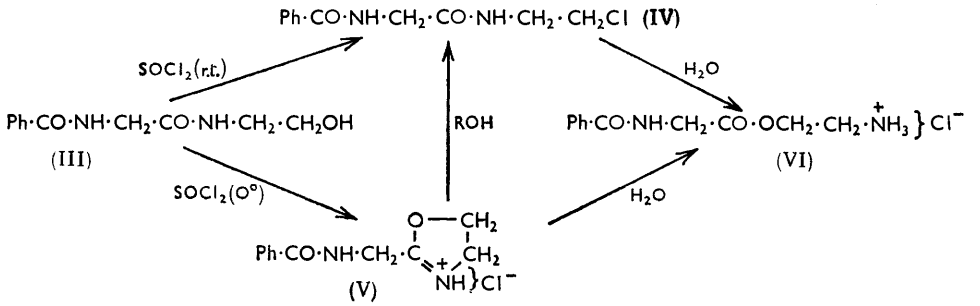


(I)



(II)

In view of difficulties in exploratory experiments on the cyclisation of some *N*-protected serine peptides, we decided to study the simpler case of the cyclisation of 2-hippuramidoethanol (III) to 2-benzamidomethyl-Δ^2-oxazoline (V). Treatment of 2-hippuramidoethanol



with thionyl chloride at room temperature gave a high yield of the chloro-compound (IV), but at 0°, in chloroform solution, a water-soluble, crystalline hydrochloride was obtained; the analytical, spectroscopic, and chemical properties of this salt showed it to be 2-benzamidomethyl-Δ^2-oxazoline hydrochloride (V). It was rather unstable and could not be recrystallised, since in hot aqueous solvents it was converted into the *O*-peptide hydrochloride (VI), also formed by boiling the chloro-compound (IV) with water,[5] while attempted recrystallisation from anhydrous isobutyl alcohol yielded the chloro-compound (IV). The latter isomerisation, which was shown to be complete in 24 hours in anhydrous ethanol at room temperature, is analogous to reactions described by Gabriel and Heyman [6] and others [7] and clearly involves attack at C-5 by chloride ion, thus:

in a manner analogous to that proposed by Porter, Rydon, and Schofield [2] for the second stage of the attack of DFP on Δ^2-oxazolines.

The free base was liberated from the hydrochloride (V) by treatment with ethanolic sodium ethoxide, as a thick oil which resisted purification; the same compound was also obtained by the condensation of ethanolamine with hippuronitrile [8] and ethyl benzamidoacetimidate.[9] The reactions of this base with water are discussed below (p. 828).

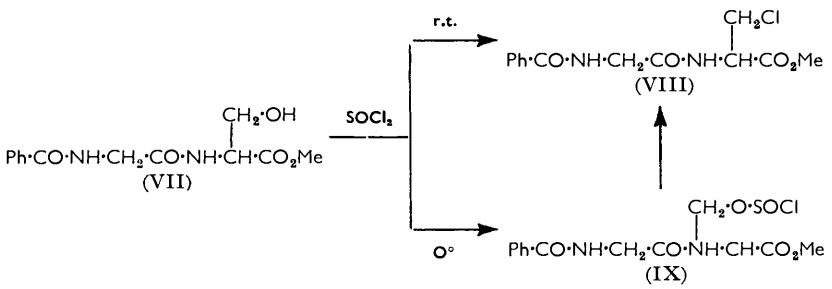
Boyd and Hansen [10] prepared a number of Δ^2-oxazolines from the corresponding ethanolamine derivatives by the action of toluene-*p*-sulphonyl chloride in pyridine. When this

[4] Bergmann, Miekeley, and Kann, *Z. physiol. Chem.*, 1925, **146**, 247.
[5] Botvinik, Aveava, and Mistryuka, *Doklady Akad. Nauk S.S.S.R.*, 1952, **82**, 727.
[6] Gabriel and Heyman, *Ber.*, 1890, **23**, 2493.
[7] Gabriel and Stelzner, *Ber.*, 1896, **29**, 2381; Wislicenus and Körber, *Ber.*, 1902, **35**, 164; Takeda, *J. Pharm. Soc. Japan*, 1917, **426**, 691; Wenker, *J. Amer. Chem. Soc.*, 1938, **60**, 2152.
[8] Cf. Loder, U.S.P. 2,402,196; Yoyama, U.S.P. 2,846,439.
[9] Cf. Elliott, *J.*, 1949, 589; McCasland and Horswill, *J. Amer. Chem. Soc.*, 1951, **73**, 3744.
[10] Boyd and Hansen, *J. Amer. Chem. Soc.*, 1953, **75**, 5896; cf. Boyd and Rittner, *ibid.*, 1960, **82**, 2032.

procedure was applied to 2-hippuramidoethanol, the product, isolated by pouring the reaction mixture into water, was not the expected oxazoline salt but the toluene-*p*-sulphonate of 2-aminoethyl hippurate, no doubt resulting from an isomerisation analogous to that of (V) to (VI) above. An attempt to cyclise 2-hippuramidoethanol by the action of phosgene at 0° gave a high yield of the chloroformate,

$$Ph{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CH_2{\cdot}O{\cdot}COCl.$$

The reaction of thionyl chloride with *N*-hippurylserine methyl ester (VII) resembled that with 2-hippuramidoethanol in that at room temperature the product was the chloro-compound (VIII); disappointingly, however, the product of this reaction at 0° was the chlorosulphinate (IX), which readily lost sulphur dioxide to give the chloro-compound.



N-Aceturylserine ethyl ester behaved similarly.

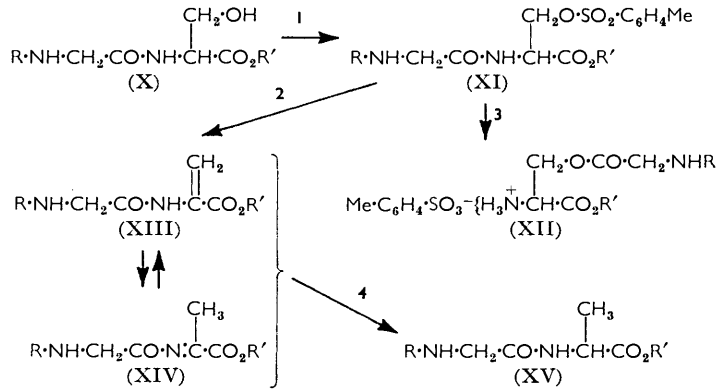
Treatment of *N*-hippurylserine methyl ester (X; R = Ph·CO·, R′ = Me) with toluene-*p*-sulphonyl chloride in pyridine [10] gave the *O*-toluene-*p*-sulphonyl derivative (XI; R = Ph·CO, R′ = Me), which was transformed into the *O*-peptide (XII; R = Ph·CO; R′ = Me) when heated in ethyl acetate. A parallel series of transformations was observed with *N*-benzyloxycarbonylglycylserine benzyl ester (X; R = $Ph{\cdot}CH_2{\cdot}O{\cdot}CO$, R′ = CH_2Ph), the last stage in this case being best carried out in acetonitrile. In the hope of " freezing " the isomerisation reaction at an intermediate stage, *N*-hippuryl-*O*-toluene-*p*-sulphonyl-DL-serine methyl ester (XI; R = Ph·CO, R′ = Me) was heated in acetonitrile containing an excess of triethylamine. The crystalline product, obtained in good yield, was at first thought to be the desired oxazoline peptide, since its infrared spectrum contained a band at 1658 $cm.^{-1}$ (C=N stretching), similar to that found at 1666 $cm.^{-1}$ for the oxazoline (V) and at 1674 $cm.^{-1}$ for 2-methyl-Δ^2-oxazoline and other Δ^2-oxazolines.[11] A similar compound, with a band at 1672 $cm.^{-1}$, was formed in the same way from *N*-benzyloxycarbonylglycyl-*O*-toluene-*p*-sulphonylserine benzyl ester (XI; R = $Ph{\cdot}CH_2{\cdot}O{\cdot}CO$, R′ = $Ph{\cdot}CH_2$).

However, the compound from *N*-hippurylserine methyl ester did not behave like a Δ^2-oxazoline, yielding the carboxylic acid with aqueous sodium hydroxide and the amide with methanolic ammonia, and further investigation showed both products to be the dehydroalanine peptides (XIII) formed by β-elimination [12] of the *O*-toluene-*p*-sulphonyl group from (XI). Thus, acid hydrolysis of the product from hippurylserine methyl ester gave glycine and pyruvic acid, while catalytic hydrogenation yielded hippurylalanine methyl ester (XV; R = Ph·CO, R′ = Me); similarly, catalytic hydrogenation of the product from benzyloxycarbonylglycylserine benzyl ester gave glycylalanine (XV; R = R′ = H). Repetition of the reaction sequence with *N*-hippuryl-L-serine methyl ester in place of the DL-compound gave the same, optically inactive, product, asymmetry having

[11] Thompson, Brattain, Randall, and Rasmussen, " The Chemistry of Penicillin," Princeton Univ. Press, 1949, p. 382.

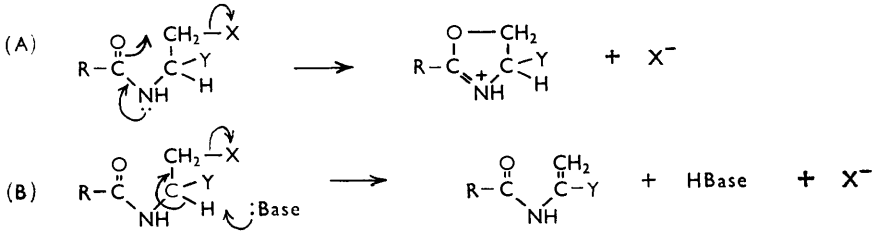
[12] Cf. Linstead, Owen, and Webb, *J.*, 1953, 1211; Riley, Turnbull, and Wilson, *J.*, 1957, 1373; Cohen, Oosterbaan, Jansz, and Berends, *J. Comp. Cell. Physiol.*, 1959, **54**, Suppl. 1, 231; Samuel and Silver, *J.*, 1963, 289.

been destroyed at the β-elimination stage. The presence of the C=N stretching frequency in the infrared absorption spectra of these dehydroalanine peptides suggests that they have the tautomeric structure (XIV), in which the conjugated system is more extended than in (XIII).



Reagents: 1, *p*-$C_6H_4Me{\cdot}SO_2Cl$–C_5H_5N. 2, NEt_3–MeCN. 3, EtOAc or MeCN. 4, H_2–Pd.

Comparison of the probable mechanism for oxazoline formation (A) with that for β-elimination (B) shows that an electron-attracting substituent Y (*e.g.*, CO_2Me) will hinder



the former, by opposing the required electron shifts, and assist the latter, by facilitating removal of the α-hydrogen atom. It is also clear that elimination, rather than cyclisation, is likely to occur if too strong a base is used. No success attended the use, with the ester (XI; R = Ph·CO, R′ = Me), of the weaker bases, ammonia, pyridine, and potassium acetate, but replacement of the methoxycarbonyl by the amide group led to a successful preparation of a Δ^2-oxazoline peptide.

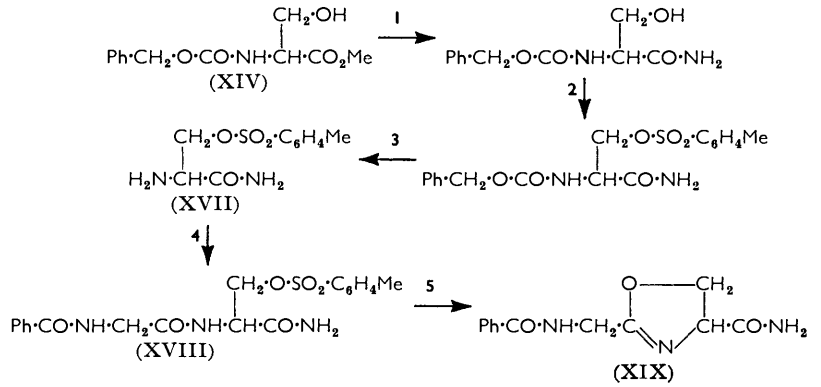
The peptide amide (XVIII) required for this purpose could not be satisfactorily prepared by the action of toluene-*p*-sulphonyl chloride on *N*-hippurylserine amide and was synthesised from *N*-benzyloxycarbonylserine methyl ester (XVI) by the annexed reaction sequence, the final coupling, (XVII) ⟶ (XVIII), being brought about by means of dicyclohexylcarbodi-imide.

The action of pyridine on the peptide amide (XVIII) did not give a dehydroalanine derivative, but the product was a discoloured gum, which could not be purified; refluxing the amide (XVIII) with fused potassium acetate in methanol, however, gave a solid product which, after several recrystallisations, afforded the required Δ^2-oxazoline peptide (XIX); this compound is much lower-melting than the isomeric unsaturated amide from (XIII; R = Ph·CO, R′ = Me) and its structure follows from its conversion, in aqueous solution, into *N*-hippurylserine amide.

The behaviour of partially neutralised aqueous solutions of the two Δ^2-oxazolines, (V) and (XIX), resembled that of Δ^2-methyl-2-oxazoline,[13] the pH of the solutions rising

[13] Porter, Rydon, and Schofield, *J.*, 1960, 2686.

rapidly to a maximum, owing to the formation of the strongly basic *O*-acylethanolamines, and then falling again as these are transformed into the isomeric, non-basic, *N*-acylethanolamines. As in the earlier work, the basic strengths of the oxazolines were evaluated by extrapolation of the pH–time curves to zero-time and the first-order velocity constants,



Reagents: 1, NH_3–MeOH. 2, *p*-$C_6H_4Me{\cdot}SO_2Cl$–C_5H_5N. 3, HBr–AcOH. 4, $Ph{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CO_2H$. 5, KOAc–MeOH.

k_1, for the disappearance of the protonated oxazolines from the slope of the early, linear, portions of these curves. The results are collected in the following Table, in which the results for some other Δ^2-oxazolines are included for comparison.

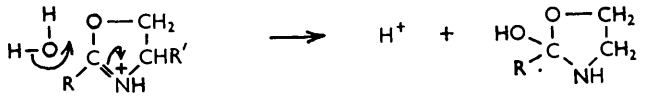
Substituted Δ^2-oxazolines.

Substituents	pK_a	10^3k_1 (min.$^{-1}$)	Reference
2-Methyl	5·51	20·5 (room temp.)	13
2-Benzamidomethyl	4·35	{ 805 (17°) 1035 (21°) }	Present paper
2-Benzamidomethyl-4-carbamoyl-	2·53	857 (room temp.)	Present paper
2-Phenyl-	4·4	—	2
2-Phenyl-4-carbamoyl-	2·9	—	2

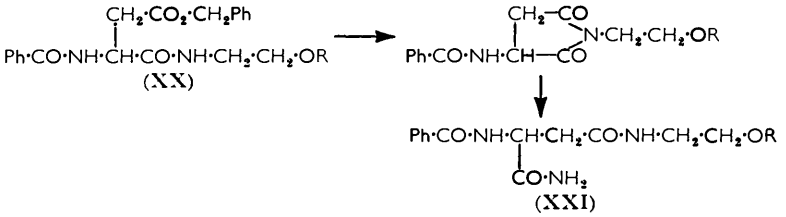
The effect of the various substituents on the basic strengths of the oxazolines is as expected, the electron-withdrawing 2-benzamidomethyl, 2-phenyl, and 4-carbamoyl groups all weakening the bases. The effect of introducing the electron-withdrawing benzamido-group into the 2-methyl group in greatly increasing the initial rate of disappearance of the protonated oxazoline is consistent with the view [13,14] that the first, and rate-determining,* stage in the reaction is the hydration to the hydroxyoxazolidine by nucleophilic attack at C-2 by water as illustrated, and supports Martin and Parcell's overall interpretation [14] of the reactions.

* Although this is not explicitly stated by Martin and Parcell,[14] it is a matter of simple arithmetic to show, from their results, that, in the case of 2-methyl-Δ^2-oxazoline the rate-determining stage in the initial conversion into *O*-acetylethanolamine is the hydration to the hydroxyoxazolidine and not the ring-opening of the latter compound. It is experimentally established [13,15] that the end-products of the whole series of reactions are *N*-acetylethanolamine and *O*-acetylethanolamine hydrochloride and that the equilibrium system contains very little 2-hydroxy-2-methyloxazolidine. Even if the equilibrium constant (k_3/k_4 on Martin and Parcell's notation) for the interconversion of the latter compound and *N*-acetylethanolamine is set as low as 1·0, the first-order velocity constant for the ring-opening of the hydroxy-oxazolidine to *O*-acetylethanolamine is about 2×10^4 times that for the hydration of the oxazoline and it is clear that this hydration must be the rate-determining process in the initial disappearance of the oxazoline. Although these considerations apply strictly only to the 2-methyl compounds, it seems likely that the conclusion will also be valid for more complicated compounds.

[14] Martin and Parcell, *J. Amer. Chem. Soc.*, 1961, **83**, 4835.
[15] Greenhalgh, Heggie, and Weinberger, *Canad. J. Chem.*, 1963, **41**, 1662.



2-(β-Benzyl *N*-benzoylaspartyl)aminoethanol (XX; R = H) was required for the preparation of the aspartic acid analogue of (V). Condensation of β-benzyl *N*-benzoylaspartate and ethanolamine by the carbodi-imide and the cyanomethyl ester method gave only poor yields; the *O*-acetyl derivative (XX; R = Ac) was prepared in good yield by the carbodi-imide coupling of β-benzyl *N*-benzoylaspartate and *O*-acetylethanolamine, but attempted removal of the *O*-acetyl group by the action of aqueous ammonia [16] gave the isoasparagine derivative (XXI; R = Ac), no doubt by way of the imide,[17] as shown.



N-Hippuryl-*S*-methylcysteine methyl ester was prepared by coupling hippuric acid and *S*-methylcysteine methyl ester and converted into the amide by ammonolysis; lack of time prevented the carrying out of a projected study of the action of bases on the methiodides of these compounds as a possible route to oxazolines.

Experimental

The purity of most compounds was checked by descending chromatography on Whatman No. 1 paper with butan-1-ol–acetic acid–water (100 : 17 : 37 v/v) (R_{FA}), butan-1-ol–pyridine–water (39 : 21 : 39) (R_{FB}), or phenol saturated with 10% aqueous sodium citrate (R_{FC}) for development; spots were revealed with ninhydrin or by the chlorine–starch–iodide method.[18] Infrared spectra were measured, for potassium bromide discs, on a Hilger H800 instrument with a sodium chloride prism; the principal bands down to about 1360 cm.$^{-1}$, with suggested assignments, are listed below, intensities being designated in the conventional manner. Evaporations were carried out under reduced pressure.

Derivatives of 2-Hippuramidoethanol.

2-Hippuramidoethanol.—Hippuryl chloride [19] (10 g.) and sodium hydroxide (30 ml.) were added alternately, in small portions, during 1 hr. to a stirred, ice-cooled solution of ethanolamine (3·1 g.) in N-sodium hydroxide (30 ml.). After being stirred for a further 45 min., the solution was acidified to Congo Red with 5N-hydrochloric acid, filtered, neutralised to litmus with 2N-sodium hydroxide, and evaporated to dryness, ethanol being added and sodium chloride removed by filtration from time to time. Recrystallisation of the residue from a small volume of water gave the required compound (6·9 g.; 62%), m. p. 125—126° (lit.,[20] m. p. 126·5°), R_{FA} 0·72 (Found: C, 59·5; H, 6·25; N, 12·6. Calc. for $C_{11}H_{14}N_2O_3$: C, 59·4; H, 6·35; N, 12·6%).

Action of Thionyl Chloride on 2-Hippuramidoethanol.—(*a*) *At room temperature.* 2-Hippuramidoethanol (5·56 g.) was treated with thionyl chloride (18 ml.); after 2·5 hr., ether was added and the precipitate twice recrystallised from isobutyl alcohol; the resulting *N*-2-*chloroethylhippuramide* (4·2 g., 70%) had m. p. 144°, R_{FA} 0·88 (Found: C, 54·4; H, 5·2; Cl, 15·2; N, 11·5. $C_{11}H_{13}ClN_2O_2$ requires C, 54·9; H, 5·4; Cl, 14·7; N, 11·6%). Boiling this in water for 10 min., followed by evaporation and recrystallisation of the residue from isobutyl alcohol, afforded 2-aminoethyl hippurate hydrochloride, m. p. 141° (lit.,[5] m. p. 139·5°), R_{FA} 0·42 [*picrate*,

[16] Cf. Botvinik and Aveava, *Zhur. obshchei Khim.*, 1957, **27**, 1910.
[17] Cf. Battersby and Robinson, *J.*, 1955, 259; Swallow and Abraham, *Biochem. J.*, 1958, **70**, 364.
[18] Rydon and Smith, *Nature*, 1952, **69**, 922.
[19] Fischer, *Ber.*, 1905, **38**, 613.
[20] Cf. Hanby and Rydon, *J.*, 1947, 513.

m. p. 158° (from isobutyl alcohol (Found: C, 45·5; H, 3·8; N, 15·3. $C_{17}H_{17}N_5O_{10}$ requires C, 45·2; H, 3·8; N, 15·5%)]; extrapolation to zero time of the pH–time curve for a half-neutralised solution gave pK_a 8·5.

(*b*) *At* 0°. 2-Hippuramidoethanol (5·56 g.) was added in small portions, in 1·5 hr., to a stirred, ice-cooled mixture of thionyl chloride (50 ml.) and chloroform (100 ml.). After 16 hr. at 4°, the solution was concentrated. *2-Benzamidomethyl-Δ²-oxazoline hydrochloride* (3·4 g., 57%) crystallised and was collected and washed with anhydrous chloroform; it had m. p. 136—139°, R_{FA} 0·44 (Found: C, 53·9; H, 5·4; Cl, 15·4; N, 11·8. $C_{11}H_{13}ClN_2O_2$ requires C, 54·9; H, 5·4; Cl, 14·7; N, 11·6%), $\nu_{max.}$ 3341s (NH stretching). 3100m (aromatic CH stretching), 2981 (methylene CH stretching), 1666s (C=N), 1635s (amide C=O), 1603m and 1590s (aromatic CC), 1546s (amide NH), 1488m (aromatic CC), 1444m (aromatic CC or methylene CH deformation), 1419m (not assigned) and 1385m (not assigned) cm.$^{-1}$. This hydrochloride (2·41 g.) was added to sodium ethoxide (0·68 g.) in anhydrous ethanol (10 ml.) at 0°. Ether was added and sodium chloride removed by filtration; evaporation gave the free base (1·96 g., 96%) as a thick oil, R_{FA} 0·43, which could be neither crystallised nor distilled.

The hydrochloride was dissolved in anhydrous ethanol and kept at room temperature; chromatography from time to time showed that isomerisation to *N*-2-chloroethylhippuramide was complete after 24 hr. Addition of 0·25M-calcium picrate [20] to the hydrochloride precipitated an oil which crystallised; recrystallisation from isobutyl alcohol gave 2-aminoethyl hippurate picrate, m. p. and mixed m. p. 158°.

Condensation of Hippuronitrile and Ethanolamine.—The nitrile [21] (8·0 g.) and ethanolamine (3·05 g.) were refluxed in absolute ethanolic sodium ethoxide (from sodium, 10 mg., and ethanol, 60 ml.) for 6 hr. Evaporation gave a thick oil, R_{FA} 0·44, which was clearly (infrared spectrum) mainly 2-benzamidomethyl-Δ²-oxazoline.

Condensation of Ethyl Benzamidoacetimidate and Ethanolamine.—The acetimidate hydrochloride [21] (12·1 g.) was suspended in cold ether (200 ml.) and treated, with shaking, with potassium carbonate (6·9 g.) in ice-cold water (30 ml.). The ether layer was separated and the aqueous layer extracted with more ether (2 × 100 ml.). Evaporation of the combined ethereal solutions gave *ethyl benzamidoacetimidate* (9·3 g., 90%) as fine needles, m. p. 73—75°, raised to 76—77° by recrystallisation from ether (Found: C, 63·7; H, 7·3; N, 14·1. $C_{11}H_{14}N_2O_2$ requires C, 64·1; H, 6·8; N, 13·6%); this compound was previously described [22] as a yellow oil. The ester (8·25 g.) and ethanolamine (2·44 g.) were kept at 50° in anhydrous chloroform (60 ml.) for 15 hr. Evaporation of the product gave a thick red-brown oil, the main constituent of which was 2-benzamidomethyl-Δ²-oxazoline (R_{FA} 0·44 and infrared spectrum).

*Action of Toluene-*p*-sulphonyl Chloride on 2-Hippuramidoethanol.*—The chloride (10·48 g.) was added in small portions in 15 min. to a stirred solution of the amido-alcohol (5·56 g.) in anhydrous pyridine (20 ml.) at −10°. After 3 hr. at 4°, the solution was poured into water (50 ml.) and ice (50 g.). The precipitated *2-hippuryloxyethylammonium toluene-*p*-sulphonate* (7·02 g., 68%) crystallised from isobutyl alcohol as the *monohydrate*, m. p. 112—113°, R_{FA} 0·44 (Found: C, 52·8; H, 5·7; N, 6·5. $C_{18}H_{22}N_2O_6S,H_2O$ requires C, 52·5; H, 5·9; N, 6·5%).

Action of Phosgene on 2-Hippuramidoethanol.—A brisk stream of phosgene was passed through a suspension of 2-hippuramidoethanol (3·0 g.) in chloroform (300 ml.), cooled in a freezing mixture, until dissolution was complete (45 min.). After a further 6 hr. in the freezing mixture, the solution was kept overnight at room temperature while a stream of dry air was drawn through it. Evaporation gave *2-hippurylamidoethyl chloroformate* (3·16 g., 82%), m. p. 107° (decomp.), unchanged on recrystallisation from ethyl acetate or chloroform, R_{FA} 0·72 (Found: C, 51·1; H, 4·8; Cl, 12·3; N, 10·0. $C_{12}H_{13}ClN_2O_4$ requires C, 50·6; H, 4·6; Cl, 12·5; N, 9·8%).

Derivatives of Hippurylserine.

N-*Hippurylserine Methyl Ester.*—*NN'*-Dicyclohexylcarbodi-imide (22·7 g.), in anhydrous pyridine (22 ml.), was added to hippuric acid (17·9 g.) and DL-serine methyl ester hydrochloride [23] (15·6 g.), dissolved in the same solvent (60 ml.). Next day, the precipitated dicyclohexylurea was removed by filtration and the filtrate evaporated to dryness. The residue was dissolved in chloroform (200 ml.) and shaken with 2N-hydrochloric acid (200 ml.); the

[21] Goldberg and Kelly, *J.*, 1947, 1372.
[22] Freudenberg, *Ber.*, 1932, **65**, 1183.
[23] Prepared, in 96% yield, by the procedure used by Guttmann and Boissonnas (*Helv. Chim. Acta*, 1958, **41**, 1852) for the preparation of the L-isomer.

product which crystallised was collected and triturated with 2N-sodium hydroxide (20 ml.) and water (20 ml.). Two recrystallisations from aqueous ethanol (charcoal) gave the DL-ester as the monohydrate (17·9 g., 64%), m. p. 96—98° (lit.,[24] m. p. 89—90°), R_{FA} 0·80 (Found: C, 52·5; H, 6·3; N, 9·6. Calc. for $C_{13}H_{16}N_2O_5,H_2O$: C, 52·3; H, 6·1; N, 9·4%). The L-ester, prepared similarly in 62% yield, had m. p. 112—113°, $[\alpha]_D^{21}$ +10·8° (*c* 3·0 in pyridine), R_{FA} 0·79, and was also a *monohydrate* (Found: C, 52·7; H, 6·3; N, 9·3%).

N-*Hippuryl*-DL-*serine Amide*.—The methyl ester monohydrate (1·5 g.) was kept at room temperature overnight in a closed flask with methanol (90 ml.), saturated at 0° with ammonia. Evaporation and recrystallisation from aqueous ethanol gave the *amide* (1·07 g., 80%), m. p. 176—177°, R_{FA} 0·49, R_{FB} 0·74 (Found: C, 55·0; H, 5·9; N, 15·9. $C_{12}H_{15}N_3O_4$ requires C, 54·3; H, 5·7; N, 15·8%).

N-*Hippuryl*-DL-*serine*.—The methyl ester monohydrate (2·98 g.), in acetone (20 ml.), was kept at room temperature for 15 min. with N-sodium hydroxide (10 ml.). The solution was then acidified to Congo Red with concentrated hydrochloric acid and evaporated until crystallisation occurred. The product (2·14 g.) was collected, a further quantity (0·47 g.) being obtained from the filtrate by evaporation to dryness, extraction with ethanol, re-evaporation, and trituration of the residue with ethyl acetate. Recrystallisation from acetonitrile gave the *peptide* (2·16 g., 81%), m. p. 174—176°, R_{FA} 0·57, R_{FB} 0·41 (Found: C, 54·6; H, 5·5; N, 10·75. $C_{12}H_{14}N_2O_5$ requires C, 54·1; H, 5·3; N, 10·5%).

Methyl β-*Chloro*-α-*hippuramidopropionate*.—*N*-Hippuryl-DL-serine methyl ester monohydrate (2·98 g.) was treated dropwise with thionyl chloride (10 ml.) and kept at room temperature for 2·5 hr. Ether (60 ml.) was then added and the precipitate (1·96 g.) collected, a further quantity (0·71 g.) being obtained from the mother-liquors by evaporation. Two recrystallisations from water (charcoal) gave the *chloro-ester* (1·82 g., 61%), m. p. 152—155°, R_{FA} 0·93 (Found: Cl, 11·4; N, 9·2. $C_{13}H_{15}ClN_2O_4$ requires Cl, 11·9; N, 9·4%).

N-*Hippuryl*-O-*toluene*-p-*sulphonyl*-DL-*serine Methyl Ester*.—Toluene-*p*-sulphonyl chloride (4·19 g.) was added, in small portions, in 30 min. to a stirred solution of the methyl ester monohydrate (2·98 g.), in pyridine (20 ml.), at −5°. After 3·5 hr. at 0°, the solution was poured on ice (80 g.) and water (40 ml.). The solidified oil, collected and well washed with water, was the required *derivative* (3·01 g., 69%); a sample, recrystallised from ethyl acetate and then from acetone–ether, had m. p. 104—105°, R_{FA} 0·91 (Found: C, 56·1; H, 5·2; N, 6·4. $C_{20}H_{22}N_2O_7S$ requires C, 55·3; H, 5·1; N, 6·45%).

This compound (0·5 g.) was heated for 2 hr. in refluxing ethyl acetate (15 ml.). The oil, which separated on cooling, was triturated with anhydrous ethyl acetate and twice precipitated from chloroform with ether, affording O-*hippuryl*-DL-*serine methyl ester toluene*-p-*sulphonate monohydrate*, m. p. 70° (decomp.), R_{FA} 0·54 (Found: C, 51·5; H, 5·4; N, 6·0. $C_{20}H_{24}N_2O_8S,H_2O$ requires C, 51·5; H, 5·6; N, 5·9%).

N-*Hippuryldehydroalanine Methyl Ester*.—(i) *N*-Hippuryl-*O*-toluene-*p*-sulphonyl-DL-serine methyl ester (12·3 g.) was heated for 1 hr. in refluxing anhydrous acetonitrile (300 ml.), containing triethylamine (5·8 g.). The cooled product was filtered and the filtrate concentrated until crystallisation began. The crystalline product was collected and washed with anhydrous ethyl acetate and ether. The washings and filtrate were combined and evaporated to give an oily residue, to which ethyl acetate (80 ml.) was added; washing with water (2 × 20 ml.), drying, and evaporation gave a further quantity of solid. The combined solid products (5·95 g.) were recrystallised from methanol–ether, yielding the *ester* (5·06 g., 68%) as fine needles, m. p. 146—148°, R_{FA} 0·89, R_{FB} 0·75 (Found: C, 59·8; H, 5·3; N, 10·6. $C_{13}H_{14}N_2O_4$ requires C, 59·5; H, 5·4; N, 10·7%), $\nu_{max.}$ 3354s (NH stretching), 3072w, 2976w and 2870w (CH stretching), 1737s (ester C=O), 1674s (C=N), 1639s (amide C=O), 1607m and 1576m (aromatic CC), 1535s (amide NH), 1490s (aromatic CC), 1437s and 1376m (methyl CH deformation) cm.$^{-1}$.

This ester (150 mg.) was heated for 15 hr. at 90° with 5N-hydrochloric acid (15 ml.). Benzoic acid (40 mg., 57%), m. p. 121°, was isolated from the cooled filtrate by filtration. Chromatography of the de-salted (Zeo-Karb 225) filtrate showed the presence of glycine (R_{FA} 0·02; R_{FC} 0·43), but no serine could be detected. Pyruvic acid was identified in the hydrolysate as its 2,4-dinitrophenylhydrazone, m. p. and mixed m. p. 215° (decomp.) with an infrared absorption spectrum identical with that of an authentic specimen. Determination of pyruvic acid [25] in another hydrolysate showed the yield to be 98% (mean of two determinations).

[24] Clayton, Farrington, Kenner, and Turner, *J.*, 1957, 1398.
[25] Rindi and Ferrari, *Experientia*, 1956, **12**, 398.

Hydrogenation of the ester (750 mg.) over 5% palladised charcoal in methanol (50 ml.) and recrystallisation of the product from aqueous methanol gave *N*-hippuryl-DL-alanine methyl ester (610 mg., 81%), m. p. 136° (lit.,[26] m. p. 136°), which yielded glycine (R_{FA} 0·03; R_{FC} 0·42) and alanine (R_{FA} 0·06; R_{FC} 0·58) on acid hydrolysis.

(ii) *N*-Hippuryl-*O*-toluene-*p*-sulphonyl-DL-serine methyl ester (4·34 g.) and anhydrous potassium acetate were heated for 2 hr., with rigorous exclusion of moisture, in refluxing anhydrous ethanol (50 ml.). The cooled product was filtered, concentrated to 5 ml., and allowed to crystallise at 4°. The solid product (1·98 g.) was collected and washed with ether; the filtrate and washings were freed from ether by evaporation, poured into water, and kept for some time at 4°; further solid (0·49 g.) crystallised. Recrystallisation of the combined solids from aqueous methanol gave *N*-hippuryldehydroalanine methyl ester (1·86 g., 71%), m. p. 140—142°, raised by further recrystallisation to 146—148°, not depressed on admixture with an authentic specimen.

(iii) *N*-Hippuryl-L-serine methyl ester monohydrate (1·49 g.) and toluene-*p*-sulphonyl chloride (1·91 g.) were heated for 1 hr. in refluxing anhydrous acetonitrile (40 ml.), containing triethylamine (2·54 g.). The solution was then evaporated to dryness and the residue taken up in ethyl acetate (50 ml.) and washed with water (2 × 20 ml.). Evaporation, trituration with ether, and two recrystallisations from methanol–ether gave *N*-hippuryldehydroalanine methyl ester (0·94 g., 71%), m. p. and mixed m. p. 147—148°, $[\alpha]_D^{20}$ 0·0° (*c* 3 in dimethylformamide), identical (chromatography, infrared spectrum) with material from the DL-ester.

N-*Hippuryldehydroalanine Amide*.—(i) The methyl ester (4·0 g.) was kept overnight at room temperature in a closed flask with methanol (200 ml.) saturated at 0° with ammonia. Evaporation to dryness and recrystallisation from aqueous acetonitrile gave the *amide* (1·05 g., 28%), m. p. 202°, R_{FA} 0·72, R_{FB} 0·70 (Found: C, 57·9; H, 5·4; N, 17·5. $C_{12}H_{13}N_3O_3$ requires C, 58·3; H, 5·3; N, 17·0%), $\nu_{max.}$ at 3393s and 3309s (NH stretching), 3059w and 2936w (CH stretching), 1679s (C=N), 1639s (amide CO), 1614s and 1584s (aromatic CC), 1538s (amide NH), 1495s (aromatic CC), 1434s and 1385s (methyl CH deformation) cm.$^{-1}$. Evaporation of the recrystallisation mother-liquors gave a white, hygroscopic, ninhydrin-positive solid, R_{FA} 0·20, R_{FB} 0·57, which resisted attempts at purification.

(ii) *N*-Hippuryl-*O*-toluene-*p*-sulphonyl-DL-serine methyl ester (3·0 g.) was dissolved in anhydrous methanol (30 ml.), and the solution was saturated at 0° with ammonia. Next day, the solution was concentrated to 5 ml. and poured into half-saturated aqueous potassium hydrogen carbonate (10 ml.). The solid (0·38 g., 22%), recrystallised from aqueous methanol, had m. p. and mixed m. p. 206—208° and was identical (chromatography; infrared spectrum) with amide prepared by method (i); the mother-liquors contained a similar by-product to that encountered in method (i).

N-*Hippuryldehydroalanine*.—The methyl ester (270 mg.) was suspended in 0·8N-sodium hydroxide (2·5 ml.), and the mixture warmed gently until a clear solution was obtained. After 5 min. the solution was acidified to Congo Red with 2N-hydrochloric acid. The precipitate (220 mg., 86%) was collected, washed with water, and recrystallised from acetic acid, affording the *peptide*, m. p. 206° (decomp.), R_{FA} 0·83, R_{FB} 0·60 (Found: C, 58·4; H, 4·9; N, 11·4. $C_{12}H_{12}N_2O_4$ requires C, 58·1; H, 4·9; N, 11·3%), $\nu_{max.}$ 3325s (NH stretching), 3009m and 2905s (CH stretching), 2681m and 2567m (carboxyl OH stretching), 1658s (C=N), 1645s (amide C=O), 1581m (aromatic CC), 1538s (amide NH), 1492s (aromatic CC), 1449m and 1392m (methyl CH deformation) cm.$^{-1}$.

O-*Toluene*-p-*sulphonyl*-DL-*serine Amide*.—*N*-Benzyloxycarbonyl-DL-serine methyl ester (57 g.), b. p. 207—214°/2 mm., prepared in 77% yield by the method of Riley, Turnbull, and Wilson,[27] was kept overnight at room temperature in methanol (1 l.) saturated at 0° with ammonia. Evaporation and two recrystallisations of the residue from ethyl acetate containing *ca.* 2% of ethanol gave N-*benzyloxycarbonyl*-DL-*serine amide* (44·6 g., 84%), m. p. 115° (Found: N, 11·9. $C_{11}H_{14}N_2O_4$ requires N, 11·8%). A stirred solution of this compound (37 g.) in anhydrous pyridine (160 ml.) was treated at 0° with toluene-*p*-sulphonyl chloride (35·7 g.), added in small portions in 30 min. After 4 hr. at 4°, the solution was poured into ice (500 g.) and water (500 ml.). The oil which separated crystallised on refrigeration and rubbing; two recrystallisations from ethanol gave N-*benzyloxycarbonyl*-O-*toluene*-p-*sulphonyl*-DL-*serine amide* (37·0 g., 61%), m. p. 112° (Found: C, 55·8; H, 5·6; N, 7·25. $C_{18}H_{20}N_2O_6S$ requires

[26] Curtius and Lambotte, *J. prakt. Chem.*, 1896, **70**, 117.
[27] Riley, Turnbull, and Wilson, *Chem. and Ind.*, 1953, 1181.

C, 55·1; H, 5·1; N, 7·1%). This amide (21·5 g.) was treated at room temperature, with occasional shaking, with 5N-hydrogen bromide in glacial acetic acid (55 ml.). After 1 hr., ether (1 l.) was added and the mixture kept at 4° until crystallisation was complete. The solid was collected, washed with ether, and recrystallised from a little water, affording *O-toluene-p-sulphonyl-DL-serine amide hydrobromide* (13·3 g., 71%), m. p. 140—143°, R_{FA} 0·27, R_{FB} 0·56 (Found: N, 8·5. $C_{10}H_{15}BrN_2O_4S$ requires N, 8·3%).

N-*Hippuryl-O-toluene-p-sulphonyl-DL-serine Amide.*—The above hydrobromide (3·39 g.) was added to a solution of hippuric acid (1·79 g.) and triethylamine (1·01 g.) in anhydrous chloroform (12·5 ml.). *NN'*-Dicyclohexylcarbodi-imide (2·26 g.) was then added and the mixture kept at room temperature for 6 hr. The solid which separated (4·85 g.) was collected, washed with chloroform, and extracted with boiling acetone (2 × 40 ml.). The *amide* (1·43 g., 33%) crystallised from the extract and, after two recrystallisations from ethanol, had m. p. 126—128°, R_{FA} 0·24, R_{FB} 0·65 (Found: C, 54·3; H, 5·5; N, 9·9. $C_{19}H_{21}N_3O_6S$ requires C, 54·4; H, 5·05; N, 10·0%).

2-*Benzamidomethyl-4-carbamoyl-*Δ^2*-oxazoline.*—The above dipeptide amide (1·92 g.) was refluxed for 2 hr. with freshly fused and powdered potassium acetate (0·48 g.) in anhydrous methanol (20 ml.). After cooling to 4°, the mixture was filtered and the filtrate concentrated to 3 ml. and poured into half-saturated sodium hydrogen carbonate solution (10 ml.). The filtered solution was immediately extracted with chloroform (3 × 25 ml.), and the extract dried (Na_2SO_4) and evaporated. The residue (0·34 g.) solidified after some days in a vacuum-desiccator; recrystallisation from ethyl acetate containing *ca.* 5% of methanol, followed by one recrystallisation from acetonitrile and two more from ethyl acetate–methanol, gave the *oxazoline peptide* (86 mg., 8%), m. p. 130° (Kofler block), R_{FA} 0·18, R_{FB} 0·69 (Found: C, 58·5; H, 5·4; N, 16·9. $C_{12}H_{13}N_3O_3$ requires C, 58·3; H, 5·3; N, 17·0%), $\nu_{max.}$ 3395s, 3316s, and 3193m (NH stretching), 3062w and 2910w (CH stretching), 1671s (composite, resolved in Nujol into 1679s, C=N, and 1663s, amide C=O), 1608s and 1579m (aromatic CC), 1544s (amide NH), 1492m (aromatic CC) and 1415m (not assigned) cm.$^{-1}$.

In another experiment the filtered hydrogen carbonate solution was kept at room temperature for several days, during which it deposited *N*-hippuryl-DL-serine amide (51% yield), m. p. and mixed m. p. 174° after recrystallisation from aqueous ethanol.

Derivatives of Aceturylserine.

Aceturic acid [28] (8·2 g.), dissolved in 1 : 1 chloroform–acetonitrile (30 ml.) containing triethylamine (7·07 g.) was treated with DL-serine ethyl ester hydrochloride [29] (11·9 g.), followed by *NN'*-dicyclohexylcarbodi-imide (15·9 g.) in more solvent (20 ml.). After 18 hr. at room temperature, acetic acid (0·5 ml.) was added; dicyclohexylurea was removed by filtration 2 hr. later and the filtrate evaporated to dryness. Extraction of the residue with acetone, re-evaporation, and three re-crystallisations of the residue from acetonitrile containing a little water gave N-*aceturyl-DL-serine ethyl ester* (8·3 g., 51%), m. p. 95—97°, R_{FA} 0·58 (Found: C, 46·7; H, 6·8; N, 11·95. $C_9H_{16}O_5N_2$ requires C, 46·5; H, 6·95; N, 12·05%).

This peptide (2·9 g.) was added in small portions, at 0°, in 1 hr., to a stirred mixture of thionyl chloride (30 ml.) and chloroform (50 ml.); after 2 hr. at 0°, the mixture was kept overnight at 4°. The deposited solid (3·4 g., m. p. 77—78°) was collected, washed with chloroform, and dried in a vacuum-desiccator; it contained sulphur and chlorine, reacted vigorously with water and ethanol, and was clearly the *O*-chlorosulphinate. Heating it for 3 hr. in refluxing anhydrous acetonitrile or alone *in vacuo* converted it quantitatively into *ethyl α-aceturylamino-β-chloropropionate*, m. p. 134° (from isobutyl alcohol), R_{FA} 0·82 (Found: C, 43·1; H, 6·0; N, 11·2; Cl, 13·8. $C_9H_{15}ClN_2O_4$ requires C, 43·1; H, 6·0; N, 11·2; Cl, 14·2%).

Derivatives of Benzyloxycarbonylglycylserine.

N-*Benzyloxycarbonylglycyl-O-toluene-p-sulphonyl-DL-serine Benzyl Ester.*—Toluene-*p*-sulphonyl chloride (3·60 g.) was added in small portions in 30 min. at 0° to a stirred solution of *N*-benzyloxycarbonylglycyl-DL-serine benzyl ester [30] (6·65 g.) in anhydrous pyridine. After 3·5 hr. at 4°, the mixture was poured into ice (80 g.) and water (80 ml.) and acidified to litmus

[28] Herbst and Shemin, *Org. Synth.*, 1959, Coll. Vol. II, p. 11.
[29] Attenburrow, Elliott, and Penny, *J.*, 1948, 310.
[30] Fölsch, *Acta Chim. Scand.*, 1958, **12**, 561.

with concentrated hydrochloric acid. The oil which separated crystallised when rubbed and was then collected, washed with water, dried in a vacuum-desiccator, and dissolved in warm ethyl acetate (100 ml.). Unchanged starting material (2·15 g.) crystallised when this solution was cooled to 4° and was removed. Evaporation of the filtrate gave the required *ester* (4·97 g., 54%), m. p. 98—100°; a sample, recrystallised from methanol–ether, had m. p. 101—103°, R_{FA} 0·94, R_{FB} 0·95 (Found: C, 60·2; H, 5·4; N, 5·8. $C_{27}H_{28}N_2O_8S$ requires C, 60·0; H, 5·2; N, 5·2%).

O-*Benzyloxycarbonylglycyl*-DL-*serine Benzyl Ester*.—The above ester (1·00 g.) was heated for 3·5 hr. in refluxing, slightly aqueous, acetonitrile (50 ml.). The solution was evaporated to dryness, and the residue dissolved in a little ethanol and re-evaporated. Treatment with ether and repeated recrystallisation of the solid product (0·85 g.) from ethanol–ether gave the O-*peptide toluene*-p-*sulphonate* (0·54 g., 52%), m. p. 137°, R_{FA} 0·72, R_{FB} 0·94 (Found: C, 58·3; H, 5·5; N, 5·2. $C_{27}H_{30}N_2O_9S$ requires C, 58·1; H, 5·4; N, 5·0%).

N-*Benzyloxycarbonylglycyldehydroalanine Benzyl Ester*.—*N*-Benzyloxycarbonylglycyl-*O*-toluene-*p*-sulphonyl-DL-serine benzyl ester (5·95 g.) was refluxed for 90 min. with triethylamine (3·08 ml.) in anhydrous acetonitrile (150 ml.). The cooled product was filtered and evaporated to dryness and the residue dissolved in ethyl acetate (50 ml.) and washed with water (2 × 20 ml.). Evaporation of the dried solution and treatment of the residue with ether gave a solid (3·25 g.) which was collected by filtration, a further crop (0·38 g.) being obtained by concentration of the mother-liquors. The combined solids were extracted with warm benzene (50 ml.); evaporation of the extract and two recrystallisations of the residue from benzene–light petroleum ether (b. p. 40—60°) gave the *peptide ester* (2·44 g., 60%). m. p. 94—95°, R_{FA} 0·91, R_{FB} 0·95 (Found: C, 65·6; H, 5·6; N, 6·9. $C_{20}H_{20}N_2O_5$ requires C, 65·2; H, 5·5; N, 7·6%), $\nu_{max.}$ 3313s (NH stretching), 3045w and 2955w (CH stretching), 1724s (ester C=O), 1691s (C=N), 1658m and 1630m (amide C=O), 1542s (amide NH), 1501m (aromatic CC), 1455m and 1400m (methyl CH deformation) cm.$^{-1}$.

Catalytic hydrogenation in aqueous t-butyl alcohol over 5% palladised charcoal resulted in the uptake of 3 mol. of hydrogen with the production of glycyl-DL-alanine, m. p. 224—226°, after recrystallisation from aqueous ethanol (lit.,[31] m. p. 231—233°), hydrolysed by 6N-hydrochloric acid to glycine (R_{FA} 0·02, R_{FC} 0·43) and alanine (R_{FA} 0·06, R_{FC} 0·58).

Behaviour of Δ^2-*Oxazolines in Aqueous Solution.*

2-*Benzamidomethyl*-Δ^2-*oxazoline*.—The hydrochloride, dissolved in water, was treated with aqueous sodium hydroxide of such strength as to give a 0·1M-solution of the degree of neutralisation required. The pH of the solution was determined with a Cambridge pH meter (glass and calomel electrodes) at intervals, zero time being taken as the time of " half-addition " of the alkali. The following Table summarises the results of the individual experiments, pK_a being calculated from the extrapolated initial pH of the solution and k_1 from the slope of the initial, linear part of the pH–time curve:

				10^3k_1 (min.$^{-1}$)		
Expt. no.	NaOH (mol.)	Temp.	pK_a	17°	21°	Max. pH
1	0·75	17°	4·58	824		6·55
2	0·75	21	4·46		1010	6·60
3	0·50	21	4·12		1074	6·78
4	0·50	21	4·23		1048	6·76
5	0·25	17	4·15	785		7·13
6	0·25	21	4·52		1007	7·08
		Mean	4·34	805	1035	
		S.E.	±0·08	±20	±51	

2-*Benzamidomethyl*-4-*carbamoyl*-Δ^2-*oxazoline*.—The base was dissolved in water and half-neutralised with hydrochloric acid to give a 0·01M-solution; the single experiment performed gave pK_a 2·53, 10^3k_1 857 min.$^{-1}$, and max. pH 3·0.

Derivatives of 2-*Aspartylaminoethanol.*

Dibenzyl N-*Benzoylaspartate*.—*N*-Benzoyl-DL-aspartic acid[32] (90 g.), toluene-*p*-sulphonic acid monohydrate (10 g.), benzyl alcohol (500 ml.), and benzene (500 ml.) were refluxed for 48

[31] Bergmann and Zervas, *Ber.*, 1932, **65**, 1192.
[32] Fischer, *Ber.*, 1899, **32**, 2459.

hr. in an apparatus fitted with a Dean and Stark tube. The cooled solution was shaken for 15 min. with magnesium oxide (10 g.), filtered, and evaporated, affording an oil which crystallised on trituration with 1 : 1 ether–light petroleum (b. p. 40—60°). Recrystallisation from ether gave the DL-*ester* (135 g., 85%), m. p. 72—74° (Found: C, 71·9; H, 5·7; N, 3·6. $C_{25}H_{23}NO_5$ requires C, 71·9; H, 5·6; N, 3·4%).

The L-*ester*, prepared similarly in 78% yield and recrystallised from aqueous methanol, had m. p. 88°, $[\alpha]_D^{19}$ −11·3° (*c* 2·1 in MeOH) (Found: C, 72·3; H, 5·5; N, 3·9%).

β-*Benzyl* N-*Benzoyl*-DL-*aspartate*.—N-Sodium hydroxide (132 ml.) was added dropwise in 90 min. to a stirred solution of the dibenzyl DL-ester (50·2 g.) in 25% aqueous acetone (1 l.). After a further 30 min. at room temperature the acetone was removed under reduced pressure and the residual solution extracted with ether (3 × 200 ml.), acidified with concentrated hydrochloric acid, and kept at 4° for several hours. Recrystallisation of the precipitated solid from ethyl acetate gave the *monoester* (22·7 g., 58%), m. p. 145—146°, raised to 148° by recrystallisation from 50% aqueous ethanol (Found: C, 66·0; H, 5·4; N, 4·3. $C_{18}H_{17}NO_5$ requires C, 66·0; H, 5·2; N, 4·3%); this compound is formulated as the β-benzyl ester by analogy with the products of partial alkaline hydrolysis of dimethyl *N*-benzoylaspartate [33] and dibenzyl *N*-benzyloxycarbonylaspartate.[34]

2-(β-*Benzyl* N-*Benzoylaspartyl*)*aminoethanol*.—(i) β-Benzyl *N*-benzoyl-DL-aspartate (3·27 g.), ethanolamine (0·61 g.), and *NN'*-dicyclohexylcarbodi-imide (2·27 g.) were kept overnight in chloroform (40 ml.); two drops of acetic acid were then added and the mixture kept at room temperature for a further 2 hr. The solution was then filtered and the filtrate was diluted with more chloroform to 100 ml., washed successively with a saturated aqueous sodium hydrogen carbonate (10 ml.), 2N-hydrochloric acid (10 ml.), and water (10 ml.), dried, and evaporated. The residue was extracted with hot acetone (40 ml.) and the extract evaporated. Two recrystallisations from ethyl acetate containing a little ethanol gave the required *peptide* (1·21 g., 32%), m. p. 122°, R_{FA} 0·92. R_{FB} 0·86 (Found: C, 65·4; H, 6·3; N, 7·7. $C_{20}H_{22}N_2O_5$ requires C, 64·9; H, 6·0; N, 7·6%).

(ii) β-Benzyl *N*-benzoyl-DL-aspartate (3·27 g.), chloroacetonitrile (2 ml.), and triethylamine (1·11 g.) were refluxed for 1·5 hr. in anhydrous ethyl acetate (25 ml.). The product was cooled to 4°, filtered, diluted to 100 ml. with more ethyl acetate, and washed as in (i). Evaporation of the dried solution and trituration with ether afforded β-*benzyl α-cyanomethyl* N-*benzoyl*-DL-*aspartate* (3·00 g., 82%), m. p. 98—100°, raised to 101° by recrystallisation from ether (Found: C, 65·3; H, 4·9; N, 7·7. $C_{20}H_{18}N_2O_5$ requires C, 65·6; H, 5·0; N, 7·7%). This ester (1·83 g.) and ethanolamine (0·335 g.) were refluxed for 6 hr. in anhydrous ethyl acetate (25 ml.) containing one drop of acetic acid. The cooled solution was diluted to 75 ml. with ethyl acetate, washed as above, dried and evaporated. Three recrystallisations of the residue from ethyl acetate containing a little ethanol afforded the required peptide (0·53 g., 28%), m. p. and mixed m. p. 120—122°.

2-(N-*Benzoyl*-DL-*aspartyl*)*aminoethanol*.—The above peptide benzyl ester (750 mg.) was hydrogenated over 5% palladised charcoal in 95% ethanol (100 ml.). The mixture was heated to the b. p., filtered from catalyst, and evaporated. The resulting *peptide* (530 mg., 93%) was chromatographically homogeneous (R_{FA} 0·81; R_{FB} 0·80) and had m. p. 166°, unchanged on recrystallisation from methanol (Found: N, 10·0, 10·2. $C_{13}H_{16}N_2O_5$ requires N, 10·0%).

2-(β-*Benzyl* N-*Benzoylaspartyl*)*aminoethyl Acetate*.—β-Benzyl *N*-benzoyl-DL-aspartate (3·27 g.) and 2-aminoethyl acetate hydrochloride [35] (1·39 g.), in anhydrous *NN*-dimethylformamide (30 ml.), were treated with triethylamine (1·01 g.), followed by *NN'*-dicyclohexylcarbodi-imide (2·26 g.). After 16 hr. at room temperature, the mixture was filtered and evaporated to dryness. The residual oil was dissolved in ethyl acetate (50 ml.), and the filtered solution washed with 2N-hydrochloric acid (10 ml.), saturated aqueous sodium hydrogen carbonate (10 ml.), and water (10 ml.) and evaporated. Trituration of the residue with light petroleum (b. p. 40—60°) and crystallisation from carbon tetrachloride gave the required *peptide* (3·36 g., 81%), m. p. 115°, raised to 116° by recrystallisation from ethyl acetate, R_{FA} 0·88, R_{FB} 0·90 (Found: C, 63·8; H, 6·0; N, 6·8. $C_{22}H_{24}N_2O_6$ requires C, 64·1; H, 5·9; N, 6·8%).

This peptide (1·02 g.), in ethanol (12 ml.), was kept for 30 min. with aqueous ammonia

[33] Pauly and Weir, *Ber.*, 1910, **43**, 661.
[34] Berger and Katchalski, *J. Amer. Chem. Soc.*, 1951, **73**, 4084.
[35] Crane and Rydon, *J.*, 1947, 527.

(*d* 0·880; 12 ml.). Evaporation of the filtered product, followed by two recrystallisations from methanol containing a little water, gave 2-(*N-benzoyl*-DL-*isoasparaginyl*)*aminoethyl acetate* (0·49 g., 62%), m. p. 138°, R_{FA} 0·81 (Found: C, 56·2; H, 6·2; N, 13·0. $C_{15}H_{19}N_3O_5$ requires C, 56·1; H, 6·0; N, 13·1%).

Derivatives of S-*Methylcysteine.*

Methanol (20 ml.) was cooled to −10° and purified thionyl chloride (5·2 ml.) added dropwise in 10 min., followed by *S*-methyl-L-cysteine [36] (2·70 g.). The mixture was stirred overnight at room temperature and kept for a further 24 hr. Evaporation, followed by recrystallisation of the thoroughly dried residue from acetonitrile, gave S-*methyl*-L-*cysteine methyl ester hydrochloride* (3·30 g., 89%), m. p. 150—151°, $[\alpha]_D^{17}$ +27·1° (*c* 3·05 in dimethylformamide) (Found: Cl, 18·9; N, 7·3. $C_5H_{12}ClNO_2S$ requires Cl, 19·1; N, 7·5%).

This hydrochloride (2·60 g.) and hippuric acid (2·51 g.) were dissolved in anhydrous chloroform (12 ml.), containing triethylamine (1·44 g.). The solution was cooled to 0°, *NN'*-dicyclohexylcarbodi-imide (3·18 g.) added, and the mixture kept overnight at room temperature. The filtered product was diluted to 100 ml. with chloroform, washed as usual, dried, and evaporated to dryness. The residue was extracted with hot acetone (18 ml.), and the cooled extract filtered and evaporated to dryness. Trituration with ether gave N-*hippuryl*-S-*methyl*-L-*cysteine methyl ester* (3·56 g., 82%), m. p. 96° after three recrystallisations from acetone–ether, $[\alpha]_D^{17}$ −22·2° (*c* 3·06 in dimethylformamide) (Found: C, 54·0; H, 5·6; N, 8·6. $C_{14}H_{18}N_2O_4S$ requires C, 54·2; H, 5·8; N, 9·0%). Treatment with methanol saturated with ammonia gave the *amide*, m. p. 126—128° (from aqueous acetonitrile), $[\alpha]_D^{20}$ −38·6° (*c* 3·0 in dimethylformamide) (Found: N, 14·4. $C_{13}H_{17}N_3O_3S$ requires N, 14·2%).

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