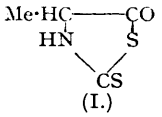
126. *Studies in the Azole Series. Part XXVIII. A New Method of Peptide Synthesis: Alanyl-peptides.*

By A. H. Cook and A. L. Levy.

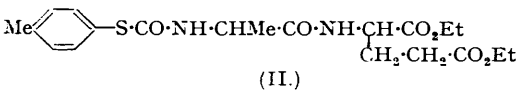
2-Thio-4-methyl-5-thiazolidone (I) has been used to provide alanyl-peptides in three different ways: (*a*) by the " ester " method described in the preceding paper, which gives lower yields than in the analogous glycine series; (*b*) by reaction with amino-acids in alkaline solution, followed by acidification with weak acids, preferably at an elevated temperature; and (*c*) by direct combination with amino-acids in hot acetic acid. Mixtures of the required peptide and its component amino-acids which result from (*b*) and (*c*) are conveniently separated on a column of cation-exchange resin.

When attempts were made to apply the general synthetic method described in the preceding paper for glycyl-peptides to the corresponding alanine derivative (I), the greater stability of (I) foreshadowed by earlier studies with model bases (Cook, Heilbron, and Levy, Part XXVI, *J.*, 1950, 642) precluded complete success. Thus, with alanine ester, the formation of alanylalanine ester was shown by the isolation of alanine anhydride but the yield was small. As with 2-thio-5-thiazolidone, a better result was obtained by coupling with a dipeptide ester, and alanylglycylglycine methyl ester hydrochloride was obtained in 40% yield by reaction with glycylglycine methyl ester and triethylamine in chloroform and then adding dry hydrogen chloride. This was later improved to 66% by acidifying with ethanolic acetic acid, followed by hydrogen chloride. As a model for the reaction with simple α-amino-esters, the condensation

of (I) with ethyl L-glutamate was studied; DL-alanyl-L-glutamic ester so obtained was isolated as its crystalline derivative, *ethyl* DL-N-[(p-*tolylthio*)*formyl*]*alanyl*-L-*glutamate* (II), in a yield of

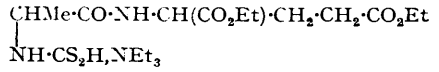


(I.)



(II.)

18%. The same overall yield also resulted from the four-stage synthesis: alanine methyl ester hydrochloride ⟶ *N*-[(*p*-tolylthio)formyl]alanine methyl ester ⟶ *N*-[(*p*-tolylthio)-formyl]alanine ⟶ *N*-[(*p*-tolylthio)formyl]alanyl chloride ⟶ (II) (cf. Ehrensvärd, *Nature*, **1947, 157**, 500; Levy, *J.*, in the press).



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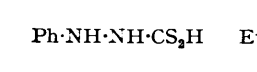

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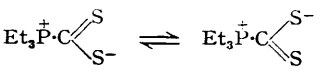
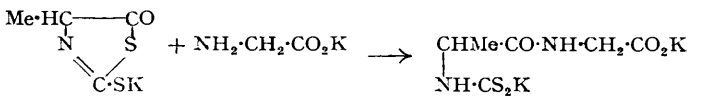


(V.)

It appeared from the work described in Part XXVI (*loc. cit.*) that opening of the ring in (I) by amines proceeded with excellent yield, but that acidification tended to cause reversion to (I) in substantial amount, even under anhydrous conditions. Some experiments were therefore carried out to see if carbon disulphide could be eliminated from (III) without the use of acids. It was shown in Part XXVI (*loc. cit.*) that the morpholine analogue of (III) readily lost its carbon disulphide as the highly insoluble morpholinium salt of 4-dithiocarboxymorpholine, but attempts to obtain this compound from (III) by treatment with two equivalents of morpholine were not successful. It is known that phenylhydrazine forms the stable compound (IV) with carbon disulphide (Fischer, *Annalen*, 1877, **190**, 114), but preliminary experiments with 2-thio-5-thiazolidone indicated the consumption of four equivalents of phenylhydrazine and did not show promise. Triethylphosphine and carbon disulphide form a red addition compound (Hofmann, *Ber.*, 1880, **13**, 1732), which may be formulated as the hybrid (V). It was hoped that the basic character of triethylphosphine would allow it to replace triethylamine in (III), when it might then detach carbon disulphide to give (V) and the required dipeptide ester. Indeed, when (I) was treated with equivalent amounts of ethyl glutamate and triethylphosphine in ethyl acetate, (V) was deposited in 50% of the theoretically possible amount during two hours, but the filtrate failed to yield (II) on treatment with tolyl chloro(thiolformate). However, replacing the triethylamine by triethylphosphine in the synthesis of glycylglycine ester discussed in the preceding paper resulted in a slight increase in yield, though the yield of glycyl-L-tyrosine ester was unaffected by a similar change. Some further properties of (V) are mentioned in the Experimental section.

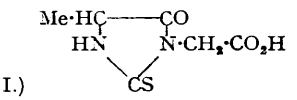
As basic methods for removing carbon disulphide were unpromising, further study was given to acidification procedures. Following the action of boron trifluoride–ether on (III), (II) was obtained in a yield of 14% but, from the action of oxalic acid, only triethylamine oxalate could be isolated. Very slow acidification of (III) with hydrogen chloride in chloroform or in water (cf. Part XXVI, *loc. cit.*) did not yield any (II) on suitable treatment with *p*-tolyl chloro-(thiolformate).

(VI.)  (VII.)

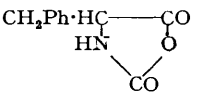
In view of these difficulties the possibility of effecting a useful interaction between 2-thio-5-thiazolidones and free amino-acids or peptides in aqueous solution was explored. The thio-thiazolidone (I) was dissolved in one equivalent of N-potassium hydroxide to give a solution of the monopotassium salt (VI) (pH 8·7), and a solution of glycine in one equivalent of N-potassium hydroxide added. The pH at once rose to 10·52 and then fell rapidly as the ring opened to give (VII), no further change appearing to take place after 10 minutes (pH 6·0). This fall in pH was to be expected during ring-fission, for not only is a strong base thereby consumed, but an acid ($^-NH{\cdot}CS_2H$) is liberated which is considerably stronger than the original mercaptothiazolone. As in the procedure developed for the model ammonium dithiocarbamate in Part XXVI (*loc. cit.*), the solution was titrated with N-hydrochloric acid during two hours, allowing time for loss of carbon disulphide to occur between each addition of acid. The " steps," however, were far less pronounced than in the model case, and (I) began to separate at pH 4·9 after only 15% of

the theoretical quantity of acid had been added, and continued till the end (pH 2·9), a yield of 68% being recovered. Paper chromatography of the filtrate showed that a little alanylglycine had been produced, together with much glycine and a moderate quantity of alanine. In an otherwise similar experiment, where an equivalent of boric acid was present, a 77% recovery of (I) was obtained and no alanylglycine could be detected in the filtrate. The absence of any unchanged (VI) in the solutions before acidification was shown by their stability to carbon dioxide, which liberates (I) from (VI), but not from (VII). In a slow titration of the crystalline barium salt of *N*-dithiocarboxyglycine with N-hydrochloric acid, carbon disulphide was liberated but the " steps " of pH drift were much less definite than with the ammonium salt of *N*-dithiocarboxyalanine amide; clearly the electrometric method is not so indicative of dithiocarbamic acid rupture in the case of peptides. A more promising result was obtained by acidifying (VII) with two equivalents of acetic acid, whereby the yield of (I) fell to 31% (45% on another occasion), paper chromatography showing that alanylglycine had been synthesised in fair quantity, though it was contaminated with glycine and alanine. Propionic acid gave a 28% recovery of (I), and very slow acidification with acetic acid yielded only a trace, but in each case the alanylglycine produced was mixed with its component amino-acids. The isolation of alanylglycine from such solutions by crystallisation proved difficult, owing to its tendency to form mixed crystals with the alanine and the glycine also present. This analytical problem was overcome, however, by " chromatography " on a column of the sulphonic acid-containing exchange-resin " Zeokarb 215 " (see Partridge and Westall, *Biochem. J.*, 1949, **44**, 418); the amino-acids were preferentially eluted by 0·1N-ammonia, followed immediately by the pure peptide. An attempt to use the column of resin to acidify (VII) and separate the products at the same time was not successful, for copious crystallisation of (I) took place and interfered with the flow of liquid.

Even milder conditions of acidification were achieved by opening the barium salt of (I) with barium glycine and decomposing the resulting barium salt of (VII) with carbon dioxide at 100°. Barium carbonate was precipitated, though incompletely even after 20 minutes, but the alanylglycine was once more contaminated with alanine and glycine, chromatography on " Zeokarb 215 " giving the crystalline peptide. When (VII) was treated with aqueous lead acetate, a yellow lead salt was clearly precipitated, which however with hydrogen sulphide yielded lead sulphide and the usual mixture of alanine, glycine, and peptide. Boiling the lead salt with water gave black lead sulphide, but the product was soluble in ethanol and was probably the 2-thiohydantoin (VIII) formed from an intermediate *iso*thiocyanate.



(VIII.)



(IX.)

A variation on the above techniques consisted in shaking a mixture of (I) and glycine with two equivalents of aqueous sodium hydrogen carbonate [whereupon the solids dissolved with evolution of carbon dioxide (neither, separately, gave carbon dioxide with the hydrogen carbonate)] and acidifying with two equivalents of acetic acid at 70°. Carbon disulphide was freely liberated, and no (I) separated on cooling. The method was also applicable to 2-thio-5-thiazolidone, which was in this way coupled with valine to give crystalline glycylvaline.

A third approach to the synthesis of alanyl-piptides from (I) was initiated by the observation that boiling equivalent quantities of (I) and glycine in water for a few moments led to substantial synthesis of alanylglycine. It was, of course, mixed with alanine and glycine, but no higher peptides were present, and the yield as judged from the relative intensity of the ninhydrin spots after chromatography of a sample of the product on paper was of the same order as that produced in the more elaborate experiments already described. When (I) was heated alone with water, it was hydrolysed completely to alanine without formation of any polymeric material. The effect of other solvents, and of different proportions of the reactants on the peptide synthesis was examined; the best procedure was to boil equimolecular amounts of (I) and glycine in acetic acid, which gave a mixture of alanylglycine and glycine (free from alanine). On a preparative scale, alanylglycine resulted in 48% yield from this reaction after " chromatography " on " Zeokarb 215." The analogous condensation with amino-acids other than glycine was less ready, though the synthesis of alanyl-glycylglycine, -alanine, -valine, and -arginine was demonstrated by paper chromatography. The direct method can also be used for α-amino-esters, and the syntheses of alanylglycine ester and alanylvaline ester by heating the components in acetic acid was established.

It was interesting to note that when anhydrocarboxyphenylalanine (IX) (Leuchs and Geiger, *Ber.*, 1908, **41**, 1721; Levy, *Nature*, in the press) was heated with glycine, tetraglycine, alanine, or valine in acetic acid, only the lower two or three polyphenylalanyl peptides were produced without any further polymerisation.

A list of R_F values of alanyl- and phenylalanyl-peptides is given at the end of the Experimental section.

Experimental.

Reaction of (I) *with Alanine Ester.*—2-Thio-4-methyl-5-thiazolidone (0·74 g.), DL-alanine methyl ester hydrochloride (0·70 g., 1 equiv.), and triethylamine (1·0 g., 2 equivs.) were dissolved in ethanol (10 c.c.) and kept at room temperature for 45 minutes. The solution was acidified with ethanolic hydrogen chloride and cooled in ice, and a crop of triethylamine hydrochloride removed; the filtrate was evaporated to dryness and a further crop precipitated by adding acetone. The filtrate was evaporated, and the residue rubbed with ethyl acetate to remove any thiothiazolidone. The remaining material was treated with saturated sodium hydrogen carbonate and extracted with chloroform, whereafter evaporation gave a basic-smelling oil which was kept at 100° in a vacuum to afford crystals of alanine anhydride, m. p. 275°.

Reaction of (I) *with Glycylglycine Methyl Ester.*—2-Thio-4-methyl-5-thiazolidone (0·73 g.), glycylglycine methyl ester hydrochloride (0·91 g., 1 equiv.), and triethylamine (1·0 g., 2 equivs.) were kept for 1·5 hours in chloroform (10 c.c.), and the solution was saturated with dry hydrogen chloride. A little ether was added. A semi-crystalline sludge obtained after 2 days was treated with ethanol–acetone to yield crude alanylglycylglycine methyl ester hydrochloride (0·5 g., 40%), m. p. 127—148°, which recrystallised from ethanol to give a product m. p. 153°, which was not depressed on admixture with material made according to Pacsu and Wilson (*J. Org. Chem.*, 1942, **7**, 126) and gave a single spot (R_F 0·25) on a paper chromatogram.

The same starting materials were kept for 50 minutes in ethanol (10 c.c.), acetic acid (0·65 c.c.) was added, and after 1·25 hours dry hydrogen chloride (2 equivs.) was passed in. Sufficient ethanol was added to re-dissolve a little triethylamine hydrochloride which separated, and the solution seeded with L-alanylglycylglycine methyl ester hydrochloride and kept overnight. The product which crystallised (0·84 g., 66%) had m. p. 158°, after contracting at 146°, and was essentially the same as the alanylglycylglycine methyl ester hydrochloride described above.

Ethyl DL-N-[(p-*Tolylthio*)*formyl*]*alanyl*-L-*glutamate.*—(*a*) 2-Thio-4-methyl-5-thiazolidone (0·73 g.) in chloroform (20 c.c.) was kept with ethyl L-glutamate (1·0 g., 1 equiv.) and triethylamine (0·5 g., 1 equiv.) for 1 hour. Dry hydrogen chloride was passed in, the solution concentrated in a vacuum, and the syrup dissolved in ethyl acetate, to which a little ether was then added. The solution was extracted with water (5 × 1—2 c.c.), and the aqueous phase treated with solid potassium carbonate at 0° and extracted with ether (3 times), some insoluble oil being rejected. The ethereal solution was dried ($MgSO_4$) and treated with *p*-tolyl chloro(thiolformate) (Levy, *J.*, in the press) until precipitation of triethylamine hydrochloride was complete. After 30 minutes, this was washed with water and with 2N-hydrochloric acid, dried, and evaporated to yield *ethyl* DL-N-[(p-*tolylthio*)*formyl*]*alanyl*-L-*glutamate* (0·4 g., 18%), m. p. 94°, which was recrystallised from ether without change of m. p. (Found: C, 56·8; H, 6·4; N, 6·4. $C_{20}H_{28}O_6S$ requires C, 56·6; H, 6·6; N, 6·6%).

(*b*) Alanine nitrile (Part XXVI) (23 g.) was added cautiously at 0° to a saturated solution of hydrogen chloride in methanol (120 c.c.), and kept overnight at room temperature. Ammonium chloride was filtered off, and the DL-alanine methyl ester hydrochloride (20·3 g.) crystallised [m. p. 155° (decomp.)] by adding ether to the filtrate. A further crop (10·2 g.; total yield, 66%), m. p. 152° (decomp.), was obtained by dilution with ethyl acetate and storage overnight at 0°. The ester hydrochloride (1·4 g.) in dry pyridine (3 c.c.) was treated, with cooling, with a solution of *p*-tolyl chloro(thiolformate) (1·87 g.) in pyridine (2 c.c.), and the whole stored overnight at 0°. The mixture was diluted with chloroform and extracted with 2N-hydrochloric acid, and the chloroform layer dried and evaporated, to yield *N*-[(*p*-tolylthio)formyl]alanine methyl ester (2·0 g., 80%), m. p. 83°. This ester (7·0 g.) was heated under reflux with acetic acid (25 c.c.) and concentrated hydrochloric acid (25 c.c.) for 15 minutes, water (25 c.c.) was added, and the mixture cooled to room temperature, to give *N*-[(*p*-tolylthio)formyl]-alanine (5·5 g., 83%), as flakes of m. p. 163°. This acid (1·2 g.) was boiled with benzene (30 g.), and phosphorus pentachloride (1·2 g.) added to the warm suspension, exothermic dissolution occurring. The benzene solution was concentrated under diminished pressure, and light petroleum added so long as the *N*-[(*p*-tolylthio)formyl]alanyl chloride (0·56 g., 44%) which crystallised [compact rosettes of spears, m. p. 102° (decomp.)] remained free from gum. The chloride (0·26 g.) in dry ether (15 c.c.) was cooled to 0°, and ethyl L-glutamate (0·40 g., 2 equivs.) added; after 1 hour at room temperature, the mixture was extracted with 2N-hydrochloric acid, dried, and evaporated to yield ethyl DL-*N*-[(*p*-tolylthio)formyl]alanyl-L-glutamate (0·31 g., 72%), m. p. 88°.

Experiments with Triethylphosphine.—Triethylphosphine (Hibbert, *Ber.*, 1906, **39**, 161) (0·59 g.) and ethyl glutamate (1·0 g.) in ethyl acetate (5 c.c.) were added to 2-thio-4-methyl-5-thiazolidone (0·73 g.) in ethyl acetate (5 c.c.). After 2 hours at room temperature, the deep-red prisms of triethylphosphine–carbon disulphide (0·5 g.) were removed, and the filtrate concentrated to small bulk. Treatment with *p*-tolyl chloro(thiolformate) (0·9 g.) and pyridine (2 c.c.), and working up in the usual way, yielded only *p*-tolyl dithiocarbonate, m. p. 95° (Found: C, 65·7; H, 5·4. Calc. for $C_{15}H_{14}OS_2$: C, 65·6; H, 5·14%).

2-Thio-5-thiazolidone (0·67 g.) in chloroform (10 c.c.) was treated with glycine ester (0·51 g., 1 equiv.) and triethylphosphine (0·6 g., 1 equiv.) in chloroform (3 c.c.). After 20 hours at room temperature, addition of ethanolic hydrogen chloride caused glycylglycine ethyl ester hydrochloride (0·72 g., 73%; m. p. 174—176°) to crystallise.

Triethylphosphine–carbon disulphide could be generated from its components in acetic acid, and was decomposed on contact with carbon tetrachloride. When warmed with glycine amide in ethanol, the

carbon disulphide was transferred and the amino-amide precipitated as its *N*-dithiocarboxy-derivative. Keeping α-aminobenzyl cyanide with triethylphosphine–carbon disulphide in chloroform for 4—5 days caused separation of yellow 2 : 4-dithio-5-phenylhydantoin, m. p. 264—265° (decomp.) (negative glyoxal test; cf. Cook, Heilbron, and Levy, *J.*, 1947, 1598).

Use of Boron Trifluoride.—2-Thio-4-methyl-5-thiazolidone (0·73 g.) in chloroform (10 c.c.) was treated with ethyl L-glutamate (1·0 g., 1 equiv.) and triethylamine (0·5 g., 1 equiv.), and boron trifluoride–ether (1·1 g., 2 equivs.) was added after 1 hour (heat evolved). After a further hour, ethanolic hydrogen chloride was added, and the mixture evaporated to a syrup and mixed with *p*-tolyl chloro(thiolformate) (1·5 c.c.) in pyridine (10 c.c.). After 2·5 hours the solution was concentrated under diminished pressure, dissolved in ethyl acetate, and washed with 2N-sulphuric acid; drying and evaporating then yielded ethyl DL-*N*-[(*p*-tolylthio)formyl]alanyl-L-glutamate (0·3 g., 14%), m. p. 89—91°, after rubbing with a little ether.

Reaction of (I) *with Glycine in Alkaline Solution.*—Powdered 2-thio-4-methyl-5-thiazolidone (0·588 g.) was dissolved in N-potassium hydroxide (4·0 c.c., 1 equiv.) (pH then 8·7), and a solution of glycine (0·3 g., 1 equiv.) in N-potassium hydroxide (4·0 c.c.) added. The resulting pH changes were as follows:

pH	10·52	10·26	9·88	9·48	8·82	7·54	6·96	6·70
Time (mins.)	0	0·5	1	1·5	2	2·5	3	3·5
pH	6·55	6·44	6·38	6·27	6·18	6·14	6·05	6·03
Time (mins.)	4	4·5	5	6	7	8	10	11

The solution was then titrated slowly with N-hydrochloric acid (8·0 c.c.) during about 2 hours, the pH changes being followed for an average of 3 minutes after each addition. Initially, the rise was 0·2 pH unit during this period. (I) began to crystallise after 1·2 c.c. of acid had been added, the pH drift at this point being 0·15 unit in 3 minutes but becoming less as the titration proceeded. Finally, the mixture was extracted with ether, and the dried extracts were evaporated to yield (I) (0·40 g., 68%).

Experiment with Barium Glycine-N-dithiocarboxylate.—Glycine (1·5 g.) and barium hydroxide octahydrate (6·3 g.) were dissolved in water (15 c.c.) (warming), and shaken overnight with carbon disulphide (1·5 c.c.). On filtration of the solution from a little insoluble material, the required salt crystallised in dense, colourless prisms, m. p. above 300°. When titrated with N-hydrochloric acid (2 equivs.) during 1 hour, carbon disulphide was liberated, and the pH fell from 4·35 to 1·70. However, the rise in pH following each addition of acid was not marked, being about 0·2 unit in 3 minutes.

Use of Acetic Acid and a Cation-exchange Column.—2-Thio-4-methyl-5-thiazolidone (2·95 g.) was added to a solution of glycine (1·5 g., 1 equiv.) in N-potassium hydroxide (40 c.c., 2 equivs.), and the whole shaken. After 2 hours, acetic acid (2·4 g.) was added, and the 2-thio-4-methyl-5-thiazolidone which slowly separated (1·17 g.) was collected after an additional 2 hours. Extraction of the filtrate with ethyl acetate gave a further 0·15 g. of (I) (total recovery, 45%). The aqueous layer was evaporated to dryness in a vacuum, and the residue digested with several portions of hot ethanol to remove potassium acetate (the extract yielded a little glycine on cooling). The remaining material was dissolved in water (15 c.c.) (it contained glycine, alanylglycine, and alanine—paper chromatography) and run on to a column (25 cm. high in a 50-c.c. burette) of "Zeokarb 215" (~10 g., sieved to be retained between 40 and 60 B.S.S.). The "Zeokarb" was prepared for use by running through it (*a*) sodium chloride solution, (*b*) 4N-hydrochloric acid, and (*c*) distilled water. The zone of adsorption of the amino-acids and peptide was clearly visible, as the resin had changed from orange to yellow-brown, and the column was run with distilled water until this zone had grown to its full length. Development was then continued with 0·1N-ammonia, and the effluent collected in 5-c.c. fractions when a positive ninhydrin test was first given. The pH changes in the effluent were not a reliable guide to its composition, so 3 μl. of each fraction were run on a paper chromatogram for analysis. The first 7 fractions contained glycine and alanine, the eighth glycine, alanylglycine, and alanine, and the ninth to eleventh the peptide, with rapidly diminishing amounts of its component amino-acids, the last being pure alanylglycine. Fractions 10 and 11 were evaporated to dryness, and the residue crystallised from 50% ethanol-acetone to give pure alanylglycine (about 0·2 g.).

Use of Barium and Lead Salts.—Glycine (1·5 g.) in 0·34N-barium hydroxide (120 c.c.) was shaken for 45 minutes with 2-thio-4-methyl-5-thiazolidone (2·95 g.), and the solution (pH 8) treated with a stream of carbon dioxide (pH then 7), raising the temperature slowly. Precipitation of barium carbonate did not begin until the solution was almost boiling; this temperature was maintained for 45 minutes. The carbonate was removed (1·95 g.), and the filtrate (which still contained Ba^{++}) run on to a column of "Zeokarb 215" similar to that used above, except that resin retained between 100 and 120 B.S.S. was used. The overlap of amino-acids and peptide was less in this case, and 5 fractions containing pure alanylglycine were obtained on elution with ammonia, which yielded the solid peptide on evaporation.

(I) (0·735 g.) was shaken with glycine (0·375 g.) in N-potassium hydroxide (10 c.c.), and the resulting solution treated with aqueous lead acetate. The derivative was precipitated cleanly, yellow at first, but becoming almost white when the lead acetate was in excess. A portion of this lead derivative was suspended in water and treated with hydrogen sulphide. It dissolved to give a deep-brown solution, from which lead sulphide was precipitated after addition of a little acetic acid and boiling. The colourless filtrate contained glycine, alanylglycine, and alanine, as shown by paper chromatography. Another portion of the lead derivative was boiled with water for 15 minutes, being completely decomposed to give lead sulphide. The filtrate, however, contained only traces of ninhydrin-reactive materials, and on evaporation the solid residue was completely soluble in hot ethanol.

Use of Sodium Hydrogen Carbonate.—2-Thio-4-methyl-5-thiazolidone (0·73 g.), glycine (0·37 g.), and sodium hydrogen carbonate (0·85 g.) were shaken with water (10 c.c.) for 50 minutes, dissolving with evolution of carbon dioxide. The solution was heated to 70°, acetic acid (0·6 c.c.) was added, and the whole allowed to cool to room temperature. Carbon disulphide separated in small globules, no (I) was

recovered, and the solution contained a considerable proportion of alanylglycine, as shown by paper chromatography.

2-Thio-5-thiazolidone (0·67 g.), DL-valine (0·59 g.), and sodium hydrogen carbonate (0·84 g.) were shaken with water (10 c.c.) until dissolved, and no more carbon dioxide was evolved (~20 minutes). The pale pink solution was heated to 80°, and acetic acid (1·5 c.c.) added, after which the solution was evaporated to dryness in a vacuum. The residue was completely soluble in hot ethanol, though on storage overnight with ether a little glycine and valine separated. The filtrate was evaporated, dissolved in water, and run on to a column of "Zeokarb 215" (~15 g.; 100—120 B.S.S.) as in the previous examples. Development with 0·1N-ammonia gave 5 fractions (5 c.c.) containing glycine and valine, followed by 8 of pure glycylvaline. These eight were evaporated to give the solid peptide (0·4 g.), which was rubbed with methanol to remove a little colour and thereafter had m. p. 239° (decomp.).

Reactions with (I) *in Acetic Acid.*—2-Thio-4-methyl-5-thiazolidone (3·0 g.) and glycine (1·5 g.) were refluxed in acetic acid (85 c.c.) for 5 minutes, and the solvent was evaporated off in a vacuum. The syrupy residue was dissolved in water (10 c.c.), and filtered on to a column (23 × 1·5 cm.) of "Zeokarb 215" (~15 g.; 100—120 B.S.S.), which was washed through with water until acetic acid ceased to emerge and the yellow zone of adsorbed material extended for 18 cm. The column was then washed with 0·1N-ammonia, and the effluent collected in 10-c.c. fractions until the yellow zone reached the foot of the column. Fractions 1—5 contained glycine, 7—9 alanylglycine with diminishing quantities of glycine, and 10—25 alanylglycine, though with nos. 21—25 the ninhydrin spots on the paper chromatogram were slightly elongated, suggesting the presence of an impurity (perhaps alanylalanylglycine). Fractions 10—25 were evaporated to dryness and rubbed with ethanol, to give a slightly deliquescent sample of alanylglycine (1·4 g., 48%). Recrystallisation from 50% ethanol-acetone gave pure material (0·77 g.), m. p. 215° (decomp.).

Syntheses of alanylglycylglycine, etc. (theoretical section), were performed only on a micro-scale (1—2 mg.), with heating for about 1 minute and analysis of a little of the resulting acetic acid solution directly on a paper chromatogram. The new spot, additional to the amino-acid or peptide used, was assumed to be the alanyl derivative. The procedure was similar for the experiments with anhydro-*N*-carboxyphenylalanine, the first spot below the amino-acid being assumed to be the monophenylalanyl derivative, the next the diphenylalanyl, and so on.

R_F *Values of peptides.*

(The paper chromatograms were run with butanol–acetic acid as the mobile phase, as described in Part XXVII, and the R_F values are corrected for glycine = 0·1, unless otherwise stated.)

Alanylglycine	0·15	Phenylalanylglycine	0·34	(G = 0·07)
Alanylglycylglycine	0·107	Diphenylalanylglycine	0·55	,,
Alanylalanine	0·26	Triphenylalanylglycine	0·75	,,
Alanylvaline	0·43	Phenylalanyltetraglycine	0·155	,,
Alanylarginine	0·29	Diphenylalanyltetraglycine	0·37	,,
Alanylglycylglycine methyl ester HCl	0·25	Triphenylalanyltetraglycine	0·54	,,
Alanylvaline methyl ester	0·58	Phenylalanylalanine	0·53	,,
		Phenylalanylvaline	0·575	,,
		Diphenylalanylvaline	0·75	,,

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