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# Amino Acid Composition of Crystalline Pancreatic Amylase from Swine[1]

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The amino acids present in three times crystallized electrophoretically and enzymically homogeneous pancreatic amylase from swine have been determined quantitatively. The results are presented and discussed briefly.

## Introduction

Pancreatic amylase is a protein that, as far as is known, contains no non-protein prosthetic groups. It appears to exert its distinctive action, at least in part, by virtue of the active groupings of some of its amino acids[3,4] and probably also because of the arrangement of the amino acids in the protein molecule.[3] Free primary amino groups, presumably from lysine, are essential to the action of pancreatic amylase.[3,4] There is also some indication that lysine may be lost early in the hydrolysis of this protein with an accompanying loss of amylase activity.[3,5]

It is evident that quantitative information about the amino acid make-up of the amylase is essential to an understanding of its action. This report gives results of analyses to determine the amino acid content of crystalline electrophoretically and enzymatically homogeneous, highly active swine pancreatic amylase.

## Experimental

**Crystalline Amylase.**—Several batches of three times crystallized pancreatic amylase were prepared from swine pancreatin as described previously.[6] They all gave the same high amylase activity of 16,000.[6-8] After activity and other measurements had been made, the crystalline amylase was lyophilized[9] and the dry composite sample used for most of the analyses. Holding the lyophilized protein in a vacuum oven at 100° for 24 hours[10] caused a loss of weight of 1.8%. No further loss of weight occurred in 24 hours at 110° in the vacuum oven. Previous work had shown[6] the three times crystallized pancreatic amylase to be homogeneous by electrophoresis and by sedimentation measurements. In addition, evidence that the crystalline protein is enzymically homogeneous had been obtained by selective inactivation studies[6] and by comparisons of the solubility of the protein and of the active amylase.[6]

Hydrolyses.—For most of the work, and unless otherwise stated, the protein was hydrolyzed according to the method suggested by Block[11] and by Rees[12] by refluxing the protein at 120° and at ordinary pressure for 20 hours with 6 *N* hydrochloric acid. The hydrochloric acid was removed by repeated distillation under reduced pressure and the residue dissolved in 10% isopropyl alcohol. The completeness of the hydrolysis was confirmed by measurements of amino nitrogen using the micromanometric Van Slyke apparatus.[13-15] Other hydrolysis procedures[13-16] gave the same results as judged by distribution of nitrogen measurements.[13-15]

**Methods of Analysis for Individual Amino Acids.**—Although the yields of crystalline pancreatic amylase are considered good,[6] nevertheless, the amounts of the crystalline protein available for the analyses were limited. Therefore, the selection of methods for the analyses also was limited. Chromatographic techniques were chosen for most of the work because they promised reliable information with relatively small expenditure of protein. Several colorimetric methods also were employed and in addition the amylase was analyzed for its distribution of nitrogen.[13-15]

Many chromatographic procedures were investigated. Those finally adopted were patterned, in general, upon methods described by Block[11] and by Block and Bolling[17a] but with variations and adaptations proposed by other workers[18,19] and resulting from experience gained during the course of this investigation.[20] Before being adopted for use with the crystalline amylase, the methods of analysis were applied to crystalline egg albumin.[21] The methods finally selected gave values for the amino acid content of crystalline egg albumin[20] that, except for alanine, agreed well with values considered satisfactory for this protein as reported by other workers. However, the values for alanine were consistently higher than those reported by other workers for this protein.

In addition to the usual control of each chromatographic analysis by several concentrations of the pure amino acid[22]

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under consideration, the accuracy of the procedures frequently was verified by the simultaneous analysis of known mixtures of pure amino acids[22] and by recovery values for amino acids added to hydrolyzates of the crystalline egg albumin.

The individual amino acids were determined, in general, as follows: arginine, by a modification of the Sakaguchi method,[23,24] by chromatographic techniques[19,20] and by Van Slyke distribution of nitrogen with decomposition of the arginine by alkali[13c-15]; histidine by Van Slyke distribution of nitrogen[13-15]; lysine, by Van Slyke distribution of nitrogen[13-15] and by a chromatographic procedure[19,20]; hydroxyproline by chromatographic[11] and by colorimetric[25] procedures; methionine, by a colorimetric method[26] and by a chromatographic procedure[11]; phenylalanine, by colorimetric procedure.[17b,27,28] Tyrosine and tryptophan were determined in the intact protein by ultraviolet spectrophotometry.[29] So far, erratic values have been obtained for tryptophan after alkaline hydrolyses of crystalline egg albumin or of the crystalline amylase. Therefore, such results are omitted from this report. Tyrosine was determined also by a colorimetric[30] and by a chromatographic[11] procedure. The remaining amino acids were determined by chromatographic techniques: alanine,[11,18,20] aspartic acid,[18,19] cysteine,[11] cystine,[11,17a,20] glutamic acid,[18,19]; glycine,[11,17a,18] the leucines as a unit,[11,17a,31] proline,[11,17a] serine,[11,17a] threonine[11,17a] and valine.[11,17a] The chromatographic work was carried out in an air conditioned room at 27°. The general procedure selected involved the use of sheets of filter paper[11] approximately 18″ by 22″. Tightly covered rectangular fish tanks with glass covers were found satisfactory. After the spots formed by the amino acids had been sprayed with suitable reagents, they were analyzed quantitatively by the use of a photoelectric densitometer[32] and in some cases of a compensating polar planimeter.[33]

## Results and Discussion

**Distribution of Nitrogen in Crystalline Pancreatic Amylase from Swine.**—Average values for two or more determinations of the distribution of nitrogen[13-15] in three times crystallized pancreatic amylase from swine are given in Table I.

Free amino nitrogen was determined in a micromanometric Van Slyke apparatus[13-15] and total nitrogen by micro Kjeldahl procedures.[34] The three times crystallized protein contains 15.92% nitrogen on a moisture-free,[10] ash-free[6] basis. This value is the average of a number of determinations made in this Laboratory and also by Dr. Elek[35] who carried out ultimate analyses on several different samples of the crystalline amylase.[6] The average value for arginine given in Table I is based on the evolution of ammonia when aliquots of solutions containing the basic amino acids were boiled with sodium hydroxide[13c,15] in a specially designed all glass apparatus.

Table I

Distribution of Nitrogen in Crystalline Pancreatic Amylase by Van Slyke Method

| Distribution | Total nitrogen,[a] % |
|---|---|
| Amide N | 8.8 |
| Melanin N | 5.1 |
| Total N of filtrate | 56.8 |
| Arginine[b] N | 14.2 |
| Cystine[c] N | 1.7 |
| Histidine N | 6.6 |
| Lysine N | 4.6 |
| Total distribution | 97.8 |
| Amino N before hydrolysis | 3.2 |
| Amino N after hydrolysis | 90.8 |

[a] Three times crystallized maltase-free, protease-free pancreatic amylase[6]; total nitrogen, 15.92% on moisture-free,[10] ash-free[6] basis. [b] Based on evolution of ammonia upon boiling an aliquot of basic amino acids with alkali.[13c,15] [c] Average value for two chromatographic methods.[11,17a,20]

**Determination of Individual Amino Acids in Crystalline Pancreatic Amylase from Swine.**—Average values for the percentages of different amino acids found in three times crystallized maltase-free, protease-free pancreatic amylase from swine[6] are given in Table II. These data represent a number of independent determinations for each amino acid, sometimes carried out by more than one method. Several entirely separate hydrolyses of the protein are represented. In many cases, the chromatographic procedure for a given amino acid carried out with different hydrolyzates involved the use of different solvent systems and of different developing agents. The results thus obtained were in good agreement and are averaged. In general, the methods adopted appear satisfactory. The range of values for any given amino acid by any of the methods adopted was within ±5% with the exception of proline and cystine where the range was within ±10%.

So far, the values obtained by chromatographic procedures for histidine and for arginine in crystalline egg albumin and in crystalline pancreatic amylase have been erratic. Therefore, such values are omitted from this report. Failure to detect hydroxyproline and cysteine in pancreatic amylase should be mentioned. Hydroxyproline could not be detected by chromatographic procedures even when relatively high concentrations of the hydrolyzates were used. The small amount found by colorimetric analyses,[25] Table II, probably resulted from interference by another amino acid or amino acids. The possibility that cysteine, present in the original protein, was oxidized during the hydrolysis of the protein cannot be excluded entirely. However, previous work in this Laboratory has given considerable evidence that sulfhydryl groups are not present in the intact active amylase molecule.[3,4] The sulfur recovered in cystine and methionine accounts for 80% of the total sulfur, 1.36%, present in the protein. It is possible that sulfur

agreed well with those reported in the literature.[11,17a,18,19] These amino acids were purchased from Eastman Kodak Company, Rochester, N. Y.; Merck and Company, Rahway, N. J.; Nutritional Biochemical Company, Cleveland, Ohio; and Pfanstiehl Company, Waukegan, Ill. Each of the amino acids used as a control was in its natural optically active form except for aspartic acid, leucine, norleucine, isoleucine, methionine, serine, threonine and tryptophan. These amino acids were mixtures of the D- and L-forms.

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TABLE II

ANALYSES OF CRYSTALLINE PANCREATIC AMYLASE FROM SWINE FOR AMINO ACIDS

| Amino acid | Grams of amino acid per 100 g. protein[a,b,c] | Number of separate detns.[d] | Duplicate values in each detns.[d] | Type of method | Literature references to method[e] |
|---|---|---|---|---|---|
| Alanine | 6.9 | 4 | 5 | Chromatographic | 11, 18, 20 |
| Arginine | 7.1 | 2 | 1 | Van Slyke | 13–15 |
| | 5.8 | 3 | 2 | Colorimetric | 23, 24 |
| Aspartic acid | 14.5 | 5 | 7 | Chromatographic | 18, 19 |
| Cysteine | 0 | 5 | 4 | Chromatographic | 11 |
| Cystine | 2.3 | 7 | 4 | Chromatographic | 11, 17a, 20 |
| Glutamic acid | 10.5 | 4 | 8 | Chromatographic | 18, 19 |
| Glycine | 6.7 | 4 | 6 | Chromatographic | 11, 17a, 18 |
| Histidine | 3.9 | 2 | 1 | Van Slyke | 13–15 |
| Hydroxyproline | 0 | 6 | 5 | Chromatographic | 11 |
| | 0.13 | 2 | 2 | Colorimetric | 25 |
| Leucines[f] | 11.5 | 4 | 4 | Chromatographic | 11, 17a, 31 |
| Lysine | 3.8 | 2 | 1 | Van Slyke | 13–15 |
| | 4.9 | 3 | 4 | Chromatographic | 19, 20 |
| Methionine | 2.1 | 5 | 2 | Chromatographic | 11 |
| Phenylalanine | 10.1 | 2 | 2 | Colorimetric | 17b, 27, 28 |
| Proline | 3.6 | 6 | 5 | Chromatographic | 11, 17a |
| Serine[g] | 4.1 | 3 | 6 | Chromatographic | 11, 17a |
| Threonine[g] | 3.9 | 5 | 6 | Chromatographic | 11, 17a |
| Tryptophan | 6.7 | 4 | 2 | Ultraviolet analysis | 29 |
| Tyrosine | 5.3 | 4 | 2 | Ultraviolet analysis | 29 |
| | 4.9 | 5 | 6 | Chromatographic | 11 |
| | 6.4 | 2 | 1 | Colorimetric | 30 |
| Valine | 7.8 | 5 | 5 | Chromatographic | 11, 17a |

[a] Three times crystallized maltase-free, protease-free pancreatic amylase from swine,[6] lyophilized. [b] Average values on moisture-free basis,[10] corrected for ash.[6] [c] Include data from different hydrolyzates. The range of values was within ±5%. [d] Different concentrations of an amino acid compared by the use of different volumes of the hydrolyzate are reported as *one* determination. [e] Numbers indicate references in text. [f] These values are for total leucines. [g] Corrected according to Rees.[12]

may be present in the protein also as inorganic or as organic sulfate. So far, the protein has not been analyzed for these forms of sulfur. It also is possible that some of the cystine or methionine was destroyed during the hydrolysis of the protein. However, no indication of the presence of cysteic acid or of methionine sulfoxide was found in the chromatographic procedures with the hydrolyzates of the protein. In fact, no unaccounted for spots were encountered in any of the chromatographic work with crystalline pancreatic amylase.

The data given in Table III have been selected as representing the most convincing and the most reliable values so far obtained for the amino acid composition of crystalline pancreatic amylase from swine. The analyses account for 102.5% of the

TABLE III

AMINO ACID COMPOSITION OF CRYSTALLINE PANCREATIC AMYLASE FROM SWINE

| Amino acid | Grams of amino acid per 100 g. of protein[a,b] | Grams of amino acid residue per 100 g. of protein[a,b] | Nitrogen as per cent. of total[a] nitrogen | Carbon as per cent. of total[a] carbon | Moles of amino acid per $10^5$ g. protein |
|---|---|---|---|---|---|
| Alanine | 6.9 | 5.51 | 6.8 | 5.5 | 77.4 |
| Arginine[e] | 5.8 | 5.20 | 11.7 | 4.7 | 33.3 |
| Aspartic acid | 14.5 | | 9.6 | 10.4 | |
| Amide $NH_3$[c] | 1.6 | | 8.3 | | |
| Asparagine[d] | | 10.74 | | | 94.1 |
| Free aspartic acid[d] | | 1.70 | | | 14.8 |
| Cystine | 2.3 | 2.13 | 1.7 | 1.4 | 9.6 |
| Glutamic acid | 10.5 | 9.22 | 6.3 | 8.5 | 71.4 |
| Glycine | 6.7 | 5.09 | 7.9 | 4.2 | 89.2 |
| Histidine[e] | 3.9 | 3.45 | 6.6 | 3.6 | 25.1 |
| Leucines[e,f] | 11.5 | 9.92 | 7.7 | 12.5 | 87.7 |
| Lysine | 4.9 | 4.30 | 5.9 | 4.8 | 33.5 |
| Methionine | 2.1 | 1.85 | 1.2 | 1.7 | 14.1 |
| Phenylalanine | 10.1 | 9.00 | 5.4 | 13.1 | 61.2 |
| Proline | 3.6 | 3.04 | 2.8 | 3.7 | 31.3 |
| Serine[g] | 4.1 | 3.40 | 3.4 | 2.8 | 39.0 |
| Threonine[g] | 3.9 | 3.31 | 2.9 | 3.1 | 32.7 |
| Tryptophan | 6.7 | 6.11 | 5.8 | 8.6 | 32.8 |
| Tyrosine | 5.3 | 4.77 | 2.6 | 6.3 | 29.3 |
| Valine | 7.8 | 6.60 | 5.9 | 7.9 | 66.6 |
| "Terminal" $H_2O$[h] | | 0.05 | | | |
| Totals | 112.2 | 95.4 | 102.5 | 102.8 | 843.1 |
| Water of hydrolysis of asparagine | | | | | 94.1 |
| Total | | | | | 937.2 |
| Water taken up during hydrolysis[i] | | 16.8 | | | 933.3 |

[a] Three times crystallized, maltase-free, protease-free pancreatic amylase.[6] C, 50.57; H, 7.37; N, 15.92; S, 1.36; P, 0.00; ash, 0.73. [b] Values corrected for ash[6] and moisture.[10] [c] Amide nitrogen corrected as recommended by Rees[12] for destruction of serine and threonine during acid hydrolysis of protein. [d] All of the amide nitrogen was assigned arbitrarily to asparagine. [e] Tentative value. [f] Values are for total leucines. [g] Corrected for destruction during acid hydrolysis of protein.[12] [h] Calculated according to Brand.[36] [i] By difference between columns 2 and 3[36] includes water taken up in hydrolysis of acid amide groups.

TABLE IV

VALUES FOR THE MINIMUM MOLECULAR WEIGHT OF SWINE PANCREATIC AMYLASE CALCULATED FROM ITS AMINO ACID COMPOSITION

| Amino acid | Grams per 100 g. of protein | Moles per $10^5$ g. of protein | Molar ratio[a] tyrosine as standard Tyrosine residues 1 | 5 | 10 | 14 | 15 | Minimum molecular weight[b] |
|---|---|---|---|---|---|---|---|---|
| Cystine | 2.3 | 9.58 | 0.327 | 1.64 | 3.27 | 4.58 | 5 | 52,000 |
| Methionine | 2.1 | 14.1 | .481 | 2.41 | 4.81 | 6.73 | 7 | 49,700 |
| Histidine | 3.9 | 25.1 | .857 | 4.29 | 8.57 | 12 | 13 | 51,700 |
| Tyrosine | 5.3 | 29.3 | 1 | 5 | 10 | 14 | 15 | 51,200 |
| Proline | 3.6 | 31.3 | 1.07 | 5.35 | 11 | 15 | 16 | 51,000 |
| Threonine | 3.9 | 32.7 | 1.12 | 5.60 | 11 | 16 | 17 | 51,900 |
| Tryptophan | 6.7 | 32.8 | 1.12 | 5.60 | 11 | 16 | 17 | 51,900 |
| Arginine | 5.8 | 33.3 | 1.14 | 5.70 | 11.4 | 16 | 17 | 51,000 |
| Lysine | 4.9 | 33.5 | 1.14 | 5.70 | 11.4 | 16 | 17 | 50,600 |
| Serine | 4.1 | 39.0 | 1.33 | 6.65 | 13 | 19 | 20 | 51,200 |
| Phenylalanine | 10.1 | 61.2 | 2.09 | 10.45 | 21 | 29 | 31 | 50,800 |
| Valine | 7.8 | 66.6 | 2.27 | 11.35 | 23 | 32 | 34 | 51,000 |
| Glutamic acid | 10.5 | 71.4 | 2.44 | 12 | 24 | 34 | 37 | 51,800 |
| Alanine | 6.9 | 77.4 | 2.64 | 13 | 26 | 37 | 40 | 51,600 |
| Leucines | 11.5 | 87.7 | 2.99 | 15 | 30 | 42 | 45 | 51,300 |
| Glycine | 6.7 | 89.2 | 3.04 | 15 | 30 | 43 | 46 | 51,500 |
| Asparagine | 12.4 | 94.1 | 3.21 | 16 | 32 | 45 | 48 | 51,400 |
| Free aspartic acid[c] | 2.0 | 14.8 | 0.505 | 2.53 | 5 | 7 | 7.58 | |
| Average | | | | | | | | 51,300 ± 450 |

[a] When value was within 3% of an integer, it was rounded off to that integer. [b] Calculated as follows:[36] $M.W._{min} = M.W._{AA} \times R_{AA} \times 100$/grams AA per 100 g. protein. [c] These values were omitted in calculating minimum molecular weight.

nitrogen and for 102.8% of the carbon but for only 95.4% of the protein in terms of its amino acid residues. Taken as a whole, the recoveries given in Table III are good. Although some of the values undoubtedly include compensating errors, it does not appear probable that additional amino acids are present in the protein in any considerable concentrations. On the other hand, the possibility of the presence of traces of additional amino acids or of somewhat different concentrations of some of the amino acids reported here cannot be excluded entirely. The reproducibility of the data given in Table III was within ±5%.

Further study of the data given in Table III shows a number of interesting correlations that indicate that the analytical data are reasonably satisfactory. Calculations, according to Brand, *et al.*,[36] give 310.5 atoms of "non-α-nitrogen" and 826.6 atoms of "α-nitrogen" per $10^5$ grams of the protein. The value of 826.6 atoms of α-nitrogen corresponds reasonably well with 843.1, the value for the sum of the moles of amino acids per $10^5$ g. of the protein, as determined directly from the analytical data, and shown in Table III. The water taken up during the hydrolysis of the protein, 16.8 g. per 100 g. of the protein, corresponds to 933.3 moles of water per $10^5$ g. of protein. This value agrees well with 937.2, the value for the sum of the moles of amino acids and of the moles of acid amide linkages per $10^5$ g. of protein obtained from the analytical data and given in Table III. This close agreement between the moles of water taken up in the hydrolysis of the protein and the moles of amino acids obtained shows that only peptide linkages, including acid amide linkages have been hydrolyzed in the protein.

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TABLE V

NATURE AND NUMBER OF SIDE CHAINS IN CRYSTALLINE PANCREATIC AMYLASE FROM SWINE, MOLECULAR WEIGHT, 51,300

| Type of group | Number of groups per mole[a] | | |
|---|---|---|---|
| Cationic groups | | | |
| Ammonium[b] (ε-amino from lysine[c] + one free terminal α-amino group) | 18 | | |
| Guanidino (arginine)[c] | 17 | | |
| Imidazole (histidine)[c] | 13 | | |
| Total cationic groups | 48 | | |
| Anionic groups | | | |
| Carboxyl groups (free aspartic acid[c] + glutamic acid[c] + one free terminal carboxyl group[b,c]) | — | | |
| Total anionic groups | 46 | | |
| Total ionic groups | | 94 | |
| Non-ionic polar groups[c] | | | |
| Methionyl (methionine) | 7 | | |
| Indole (tryptophan) | 17 | | |
| Phenolic hydroxyl (tyrosine) | 15 | | |
| Aliphatic hydroxyl (serine, threonine) | 37 | | |
| Amide groups[b] | 48 | | |
| Total non-ionic polar groups | 124 | 124 | |
| Total polar groups | | | 218 |
| Non-polar groups[c] | | | |
| Hydrogen (glycine) | 46 | | |
| Paraffins (alanine, valine, leucines) | 119 | | |
| Benzene (phenylalanine) | 31 | | |
| Pyrrolidine (proline) | 16 | | |
| Total non-polar groups | 212 | | 212 |

[a] Calculated according to Brand[36] and to Gordon[42] and their co-workers. [b] From data in Table I. [c] From data in Tables III and IV.

**Minimum Molecular Weight of Crystalline Pancreatic Amylase from Swine.**—The minimum molecular weights for swine pancreatic amylase given in Table IV were calculated according to Brand, *et al.*,[36] from the data given in Table III for the best values so far available for the amino acid make-up of the protein. Although the molar ratio for tyrosine was not the lowest found in the protein, this amino acid was chosen as the standard for these calculations because the determinations of tyrosine in the protein appear to be particularly satisfactory. Tyrosine had been determined in the amylase by three independent methods. The value used as the standard and considered as probably the most accurate was obtained by spectrophotometric analysis of the intact protein.[29] However, it also was close to the average of the values obtained for tyrosine by the other two methods of analysis, Table II. As shown in Table IV, the minimum molecular weight values for crystalline pancreatic amylase from swine, calculated from its amino acid make-up, averaged 51,300 ± 450. This value corresponds reasonably well with the value of 45,000 reported by Danielsson[37] for the molecular weight of crystalline pancreatic amylase from swine[38] as calculated from sedimentation and diffusion measurements.[37] The correspondence between the value obtained here for the minimum molecular weight of the protein from its amino acid composition and the value derived from sedimentation and diffusion data leads to the conclusion that pancreatic amylase from swine exists as a single molecular unit in solution rather than as a polymer of several units as has been reported for insulin.[39-41] This conclusion appears justified. However, at present, values for minimum molecular weights of a protein, calculated from analytical data for amino acids, must be interpreted with caution. Relatively small differences in the values for the amino acids, especially those present in low concentrations, materially change the values calculated for the average minimum molecular weight of the protein.

**Side Chain Groups.**—The data given in Table V for the side chain groups of crystalline pancreatic amylase were obtained from the data given in Tables I, III and IV by calculations according to Brand[36] and to Gordon[42] and their co-workers. These data must be considered tentative. Their satisfactory evaluation and interpretation must await the results of titration and of other studies of the properties of the protein. However, it is interesting to note that the presence of one terminal $\alpha$-amino group and of one terminal carboxyl group suggests the presence of only one polypeptide chain. The relatively high number of glycine residues would permit flexibility of the polypeptide chain. On the other hand, the part played by five residues of cystine in a protein consisting of a single polypeptide chain is not clear. Information about the arrangement of the amino acids in the protein molecule is being sought.

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[CONTRIBUTION FROM THE LABORATORY OF ORGANIC CHEMISTRY OF THE UNIVERSITY OF WISCONSIN]

# The Synthesis of N-(2-Benzyl-4-$\Delta^2$-oxazolinoyl)-valine[1]

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Methyl N-[2-benzyl-4(L)-($\Delta^2$-oxazolinoyl)]-D-valinate and its D,D-(Ib)-isomer were prepared in good over-all yields starting with carbobenzyloxyserine. The key step in this synthesis was the condensation of methyl serylvalinate with methyl phenyliminoacetate hydrochloride to give the desired oxazoline. N-(2-Benzyl-4(L)-$\Delta^2$-oxazolinoyl)-D-valine (Ia) was obtained in 14% yield when L-seryl-D-valine was condensed with the iminoester, but D-seryl-D-valine underwent negligible reaction. The structures of these compounds were confirmed by elemental analysis, infrared spectra and identification of hydrolysis products.

The synthesis of the $\Delta^2$-oxazoline with a peptide linkage at the 4-position (Ia or b) has been carried out as an extension of the recently reported preparation of the corresponding oxazole II.[7] Starting with carbobenzyloxyserine (III), the preparative route necessitated the synthesis of the hitherto unknown methyl serylvalinate (VI) or serylvaline (VIII) as key intermediates. This dipeptide (or the corresponding ester) was condensed with methyl (or ethyl) phenyliminoacetate to yield the desired oxazoline I (a or b).

Yields of the intermediates IV and V were excellent. The conversion of V to the oxazoline could best be done through methyl serylvalinate by hydrogenolysis of the carbobenzyloxy derivative V and allowing the latter material to condense with methyl phenyliminoacetate hydrochloride (Route B). In this way, both methyl N-[2-benzyl-4(L)-

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(7) H. Adkins, R. M. Ross and D. C. Schroeder, THIS JOURNAL, **72**, 5401 (1950).