molar equivalent of triethylamine or other tertiary base. Polymerization proceeded rapidly at room temperature, and the polymer was isolated by precipitation and washing with ethanol or methylene chloride followed by "freeze" drying of the resulting slurry. Preliminary analytical results run a bit low as though a few per cent of water were present. Optical integrity is, however, high. The polymers are slightly soluble in dimethyl sulfoxide, soluble in such solvents as $CHCl_2COOH$, CF_3COOH, and in 60% aqueous lithium bromide, but insoluble in all other solvents which have been tried.

The polymerization can be monitored most conveniently by the n.m.r. spectra in trifluoracetic acid solution.[7] Not only can the methyl ester peak be observed at 3.88, but there is a bonus in that the peak is shifted to 3.93 in such compounds as $HCl \cdot H \cdot Asp\text{-}(OCH_3) \cdot OH$. In low polymers two peaks are observed, and end groups up to a DP of about 15 can be estimated by the size of the spur on the low field side of the main methoxyl peak. These estimates correlate well with measurements of $[\eta]$ (intrinsic viscosity in dichloroacetic acid at 30°). Polymers of DP 15 have an $[\eta]$ of 0.15. Many samples of our polymers with $[\eta]$ 0.20 to 0.24 have been obtained. Preliminary ultracentrifuge results tend to support the mol. wt. assignments.[9]

The successful incorporation of aspartyl residues is particularly noteworthy in view of the well known and extremely facile loss of methanol to give the imide,[10] a reaction which occurs with the peptide intermediates at pH 8 in water or in organic solvents in the presence of an excess of base. Samples of the polyimide $(Asp(imide)\text{-}Gly\text{-}Gly)_n$ of high molecular weight have also been prepared. The n.m.r. shows several differences, particularly the absence of the methoxyl peak and the appearance of two broad glycyl CH_2 peaks at 4.32 and 4.62 rather than a single peak at 4.32.

The polymerization to cyclization ratio is very sensitive to concentration; a 15% solution of tripetide "monomer" gives a low yield of polymer, presumably due to formation of the cyclic hexapeptide.[2] This "cyclic" material has not yet been characterized adequately, but it is in every case very much more soluble in such solvents as ethanol than is the polymer. It also shows very low values of intrinsic viscosity, comparable to the monomer. Where the n.m.r. is useful in estimating end groups, this material shows a relatively small amount of them.

We have applied these new techniques to several other systems with comparable results: $(Asp\text{-}(OCH_3)\text{-}Phe\text{-}Gly)_n$ and $(His\text{-}Gly\text{-}Gly)_n$ from the corresponding tripeptide derivatives and $(Phe\text{-}Gly)_n$ from $HBr \cdot H \cdot Phe\text{-}Gly\text{-}ONP$. This latter is important in showing that dipeptide nitrophenyl esters do not necessarily give cyclic dimers. Related polymers from other "nonfunctional" dipeptides have been described previously.[1]

It is worth noting that it is possible to achieve a wide variety of patterns in these peptide polymers. Tripeptides $(ABC)_n$ have the special property of placing the side groups of A and B in adjacent positions both along the chain direction and also on adjacent turns of an α-helix (if this forms). On the other hand dipeptides $(AB)_n$ and tetrapeptides $(ABCD)_n$ give parallel rows of A's and B's, etc., along the α-helix.

(7) Measurements on Varian A-60, $(CH_3)_4Si$ reference; peaks are given in p.p.m. shift toward lower field.[8]

(8) N. S. Bhacca, L. F. Johnson, and J. N. Shoolery, NMR Spectra Catalog, Varian Associates, 1962.

(9) By way of comparison, P. Doty, J. H. Bradbury, and A. M. Holtzer, *J. Am. Chem. Soc.*, **78**, 947 (1956), and J. C. Mitchell, A. E. Woodward, and P. Doty, *ibid.*, **79**, 3955 (1957), report mol. wt. 8000 for $[\eta] = 0.10$ and 20,000 for $[\eta] = 0.16$ for poly-γ-benzyl glutamate in dicbloroacetic acid. The relationship between $[\eta]$ and molecular weight remains to be established for other peptides. One of the problems under active investigation in our laboratories is that of molecular weight averages and molecular weight distribution. Preliminary studies using both the Archibald technique and sedimentation velocity patterns coupled with end group assays by the DNP method and n.m.r. estimates indicate that the viscosity–molecular weight relationships for the various polymers are at least roughly comparable.

(10) The facile cyclization of ester-amides to five-membered cyclic imides has apparently been discovered and rediscovered several times within the past decade. The first report concerned monoester anilides of methylsuccinic acids: J. E. H. Hancock and R. P. Linstead, *J. Chem. Soc.*, 3490 (1953). Similar closures in aspartic acid derivatives were reported by E. Sondheimer and R. J. Holley, *J. Am. Chem. Soc.*, **76**, 2467 (1954); **79**, 3767 (1957), and by A. R. Battersby and J. C. Robinson, *J. Chem. Soc.* 259 (1955). Recent studies: S. A. Bernhard, A. Berger, J. H. Carter, E. Katchalski, M. Sela, and Y. Shalitin, *J. Am. Chem. Soc.*, **84**, 2421 (1962); A. J. Adler, G. D. Fasman, and E. R. Blout, *ibid.*, **85**, 90 (1963).

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(12) The early work was done at Department of Chemistry, University of South Carolina, Columbia, South Carolina.

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Biosynthesis and Metabolism in Species of Vetch and Lathyrus of γ-Glutamyl-β-cyanoalanine: Relation to the Biosynthesis of Asparagine[1]

Sir:

The metabolism of β-cyanoalanine, recently isolated from the seed of a vetch plant,[2] has been of special interest to us in view of the neurotoxic properties of this amino acid and the widespread distribution of asparagine in animal and plant tissues, since structural considerations suggest the possibility that β-cyanoalanine and asparagine may be metabolically related.[2] A further possible biogenetic relationship involving β-cyanoalanine has been suggested by finding β-cyanoalanine also in *Vicia angustifolia*,[2] a plant in which vicianin, a cyanogenetic glycoside, occurs. That cyanide can be converted *in vivo* to aspartic acid was observed by Bond in studies on the action of fumigants on insects.[3] The present communication reports metabolic evidence, obtained with seedlings of *Vicia sativa* (common vetch) in sterile culture, which establishes that cyanide can serve as an excellent precursor of the cyano-carbon of N-(γ-L-glutamyl)-β-cyano-L-alanine,[4] eq. 1. In addition, N-(γ-L-glutamyl)-β-

(1) Aided by U. S. Public Health Service Grant NB 04316-01 and by Muscular Dystrophy Associations of America, for which appreciation is expressed. We thank Mrs. Jeanne Nelson and Mrs. H. R. Levie for skillful assistance.

(2) C. Ressler, *J. Biol. Chem.*, **237**, 733 (1962).

(3) E. J. Bond, *Can. J. Biochem. Physiol.*, **39**, 1793 (1961).

(4) C. Ressler, S. N. Nigam, and Y.-h. Giza, Abstracts 145th National Meeting of the American Chemical Society, New York, N. Y., September, 1963, American Chemical Society, Washington, D. C., 1963, p. 4A, in which is reported the isolation and identification of N-(γ-L-glutamyl)-β-cyano-L-alanine from the seed of common vetch in connection with the identification of toxic amino acids in lathyrus and vetch peas. These studies are to be presented at the Symposium on Deleterious Compounds of Natural Origin in Foods and Feeds at that meeting. Details are given in a manuscript in preparation.

cyano-L-alanine and β-cyano-L-alanine, like cyanide,[5] have been found in several related species of lathyrus and vetch to serve as excellent precursors of asparagine, the cyano-carbon of β-cyanoalanine providing the amide-carbon of asparagine, eq. 2. γ-Glutamyl-β-cyanoalanine with high specific activity on administration of $KC^{14}N$ has been detected also in a species of lathyrus (*L. sylvestris W.*) which is capable of efficiently utilizing both cyanide and γ-glutamyl-β-cyanoalanine to synthesize asparagine. These data can suggest for certain *leguminosae* a new pathway for the biogenesis of the carbon chain of asparagine (and aspartic acid) from cyanide which involves γ-glutamyl-β-cyanoalanine or β-cyanoalanine, or both, as an intermediate.

$KC^{14}N$ (3.96×10^6 c.p.m.) was administered to the roots of etiolated seedlings of common vetch. Eight days later, the chief radioactive component in aqueous alcohol seedling extracts was separated through chromatography on Dowex 1-X4. Its identity was established as γ-glutamyl-β-cyanoalanine through close comparison with synthetic material,[4] including cochromatography on the amino acid analyzer[6] and crystallization with added carrier to constant specific activity (26.8% incorporated). The position in which the peptide was labeled was established after hydrolysis; the formed aspartic acid contained 99.8% of the recovered activity. This was degraded with *C. welchii*[7] to alanine, which had less than 0.1% of the molar activity of the aspartic acid. When $KC^{14}N$ (1 μmole, 1.05×10^7 c.p.m.) was administered to seedlings of *L. sylvestris W.* in a 10-hr. experiment, a small radioactive fraction corresponding in position to γ-glutamyl-β-cyanoalanine was isolated by chromatography on Dowex 1-X4 and purified by paper chromatography. Its identity as this dipeptide was similarly established by comparison with authentic material,[4] including elution volume (113 ml.) on the amino acid analyzer and radiochemical behavior when chromatographed alone and when cochromatographed on paper in pyridine–acetic acid–water (5:3:2), R_f 0.37, and in *n*-butyl alcohol–pyridine–water (1:1:1), R_f 0.25; and, finally, after dilution with authentic γ-L-glutamyl-β-cyano-L-alanine dicyclohexylammonium salt,[4] by crystallization to constant specific activity.

$$^{-}C^{14}N \longrightarrow \text{N-(}\gamma\text{-L-glutamyl)-}\beta\text{-cyano-L-alanine-4-}C^{14} \quad (1)$$

β-Cyano-L-alanine-4-C^{14} [8] (2.3 to 3.9×10^6 c.p.m.) was administered to etiolated seedlings of *V. villosa*, *L. odoratus*, and *L. sylvestris W.*[9] After 8 days, formed asparagine was isolated from seedling extracts through electrophoresis on paper at pH 5.7 followed by pH 8.6, determined on the amino acid analyzer, and in most cases was mixed with carrier L-asparagine and crystallized to constant specific activity. Activity incorporations into asparagine ranged between 10.2 and 33.5%. In contrast, common vetch seedlings incorporated only 0.36% of the activity into asparagine, whereas the γ-glutamyl-β-cyano-L-alanine became highly labeled (30.1% incorporated). That the β-cyanoalanine had been converted to asparagine by seedlings of *L. sylvestris W.* without randomization of C-4 was established by hydrolysis of the asparagine to aspartic acid followed by degradation[7] to alanine, which showed less than 1% of the specific activity of the aspartic acid.

$$\beta\text{-cyano-L-alanine-4-}C^{14} \longrightarrow \text{L-asparagine-4-}C^{14} \quad (2)$$

γ-L-Glutamyl-β-cyano-L-alanine-4-C^{14}, prepared biosynthetically in 60.5% yield from $KC^{14}N$ by common vetch seedlings, was administered to the roots of *L. odoratus* (5.2×10^6 c.p.m.) and to the stems of *L. sylvestris W.* seedlings (7.8×10^6 c.p.m.). Within 23 and 6.5 hr., respectively, 5.0% and 6.4% of the isotope that had been taken up was incorporated into asparagine, eq. 3.

$$\text{N-(}\gamma\text{-L-glutamyl)-}\beta\text{-cyano-L-alanine-4-}C^{14} \longrightarrow \text{L-asparagine-}C^{14} \quad (3)$$

With the exception of common vetch, which showed 0.9%, incorporations of $KC^{14}N$ into asparagine ranged between 2.6 and 14%. Over-all sequence, eq. 4, can therefore occur in seedlings of *L. sylvestris W.*

$$^{-}C^{14}N \longrightarrow \text{N-(}\gamma\text{-L-glutamyl)-}\beta\text{-cyano-L-alanine-}C^{14} \longrightarrow \text{L-asparagine-}C^{14} \quad (4)$$

It is noted that whereas both cyanide and β-cyanoalanine can serve as effective precursors of asparagine in *V. villosa* and the two lathyrus species, in which glutamyl-β-cyanoalanine is similarly effective, both cyanide and β-cyanoalanine are incorporated much less significantly into asparagine in common vetch, where both compounds accumulate instead to a striking degree as the β-cyanoalanine dipeptide. The formation of β-cyanoalanine, possibly as the dipeptide γ-glutamyl-β-cyanoalanine, may be considered an early step in the conversion of inorganic cyanide to organic-bound carbon, which can appear later as part of the carbon chain of asparagine in a number of *leguminosae*. In seedlings of common vetch it would seem that, in the metabolic sequence between cyanide and asparagine, some step subsequent to the formation of β-cyanoalanine or its peptide is blocked or absent, and that the toxic γ-glutamyl-β-cyanoalanine accumulates as a consequence.

(5) Recently, C^{14}-labeled cyanide was shown in a number of plants to lead to asparagine, labeled in C-4. [S. Blumenthal-Goldschmidt, G. W. Butler, and E. E. Conn, *Nature*, **197**, 718 (1963).] They similarly observed accumulation of radioactivity into unidentified material in *V. sativa* seedlings. We wish to thank Dr. Conn for informing us of their findings prior to publication while some of these studies were in progress.

(6) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).

(7) A. Meister, H. A. Sober, and S. V. Tice, *J. Biol. Chem.*, **189**, 577 (1951).

(8) β-Cyano-L-alanine labeled with C^{14} specifically in C-4 was synthesized by condensation of KCN with diethyl α-acetamido-α-dimethyaminomethylmalonate methiodide [H. Hellmann and E. Folz, *Chem. Ber.*, **88**, 1944 (1955); R. O. Atkinson, *J. Chem. Soc.*, 3317 (1952)] using C^{14}-labeled cyanide. The product was hydrolyzed in alkaline medium to acetyl-β-cyano-DL-alanine-4-C^{14}, which was then deacetylated stereospecifically with amino acid acylase.

(9) Some of these findings were presented at the 47th Annual Meeting of the Federation of American Societies for Experimental Biology, Atlantic City, N. J., April, 1963 [Y.-h. Giza, H. Ratzkin, and C. Ressler, *Federation Proc.*, **22**, 651 (1963)].

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