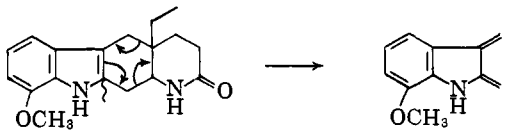
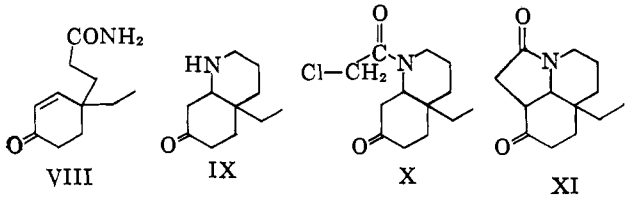
system VI, as was easily determined by the mass spectral fragmentation,[7] which in both cases showed a major peak at *m/e* 173, corresponding to the expected formation of the dimethylene indole VII. The rest of the fragmentation patterns was almost identical for the two substances.



VI VII

Steps were now taken to favor cyclization in the non-linear sense.

Ketalization of the cyclohexenone IV, followed by reaction with aqueous ammonia, gave the crystalline ketal amide VIII, m.p. 64–66°, which upon reduction with lithium aluminum hydride, followed by successive treatment with aqueous acid and base, gave the desired 10-ethyl-7-ketodecahydroquinoline (IX), m.p. 47–50°. Advantage was then taken of the relative position of the amino group in order to introduce the third ring on the proper side of the carbonyl group. Acylation with chloroacetyl chloride led to the chloroacetamide X, m.p. 75–77°, which was smoothly cyclized to the tricyclic ketolactam XI, m.p. 116–118°, by means of potassium *t*-butoxide in benzene.

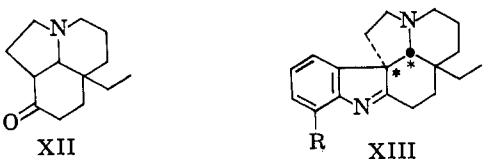


VIII IX X XI

The Fischer indole cyclization with the *o*-methoxyphenylhydrazone of XI[8] gave only neutral (indole) substances after heating with acetic acid, showing that the additional substitution next to the carbonyl group was not sufficient to lead to angular cyclization. The situation was expected to be considerably more favorable in the related tricyclic ketoamine XII: two of the three trigonal atoms present in the five-membered ring in the transition state for the desirable cyclization become tetrahedral with the reduction of the amide link.

The keto-amine XII was easily prepared by ketalization, lithium aluminum hydride reduction, and regeneration of the ketonic function. It was obtained as an oil, b.p. 110 ± 5° (0.1 mm.), and was characterized as its picrate, m.p. 157–160°.

We were now ready to perform the crucial last stages of the total synthesis.[9] Cyclization of the *o*-methoxyphenylhydrazone of XII with hot acetic acid, followed by lithium aluminum hydride (to convert the indolenine, XIII, R = OCH_3, into the indoline), and acetylation with acetic anhydride gave a crude material from which



XII XIII

crystalline *dl*-aspidospermine, m.p. 195–195.5°, was readily obtained.

The identity of the synthetic material as *dl*-aspidospermine was rigorously established by the superposability of the infrared and mass spectra[10] with those of the natural alkaloid.

Cyclization of the phenylhydrazone of XII to a mixture containing XIII, R = H, followed by reductive cleavage with potassium borohydride[11] gave *dl*-quebrachamine, m.p. 113–116° (reported[12] m.p. 112–115°), identical by infrared analysis, thin layer chromatography, and mass spectroscopy[10] with authentic material.[13]

(7) We wish to thank Prof. C. Djerassi for determining and interpreting these mass spectra.

(8) The Fischer indole synthesis toward the junction between two rings was first reported by V. Georgian, *Chem. Ind.* (London), 1124 (1957).

(9) The stereochemistry of the various bicyclic and tricyclic intermediates which are described in this communication is left open at this point. This stereochemical ambiguity is not operationally significant here: the indolenine XIII is formed under conditions which would lead to equilibration at the two centers marked by asterisks *via* reverse Mannich reaction (*cf.* G. F. Smith and J. T. Wróbel, *J. Chem. Soc.*, 792 (1960)). The most stable relative arrangement of the three asymmetric centers of XIII woud thus be expected to result whatever the stereochemistry of the intermediates or the detailed course of the indolenine cyclization process. There are good *conformational* arguments that this most stable arrangement should coincide with that of dehydroaspidospermine (XIII, R = OCH_3) and there is some *experimental* evidence in support of this conclusion (private communication from Dr. G. F. Smith).

(10) We are very grateful to Professor K. Biemann for this comparison.

(11) *Cf.* K. Biemann and G. Spiteller, ref. 3.

(12) F. Walls, O. Collera, and A. Sandoval, *Tetrahedron*, **2**, 173 (1958).

(13) This work was supported by grants from the National Institutes of Health and the National Science Foundation.

THE CHANDLER LABORATORIES
COLUMBIA UNIVERSITY
NEW YORK 27, N. Y.
GILBERT STORK
JOSEPH E. DOLFINI

RECEIVED AUGUST 9, 1963

Synthesis of Peptide Polymers with Repeating Sequences

Sir:

We have now succeeded in developing a general route to an important new class of peptide polymers having known repeating sequences in chains of random length. These polymers should prove of great value in working out relationships between structure and physical, chemical, and biological properties. There is also the not entirely remote prospect that appropriately constituted polyfunctional polymers may exhibit catalytic activity.

Polymers with repeating sequences have, of course, been investigated before.[1] The pronounced ease of ring closure to cyclic di- and hexapeptides as well as to other ring sizes[2] has, however, raised questions about practical routes to such polymers. Furthermore, the efficient incorporation of amino acids having functional side chains has received little attention.

We have found that the active ester method[3] is suitable for preparing (L-Asp-(OCH_3)-Gly-Gly)$_n$[4] with average degrees of polymerization which we estimate to be 25 (75 amino acid residues, wt. av. mol. wt. 12,000) or more. A concentrated solution of HBr·H·Asp-(OCH_3)Gly-Gly-ONP of high analytical purity (C, H, N, Br, $-OCH_3$, –ONP all agree closely with theory[6]) in dimethyl sulfoxide, dimethylformamide, or N-methylpyrrolidone was treated with the exact

(1) Previous work has been summarized in the admirable review by E. Katchalski and M. Sela, *Advan. Protein Chem.*, **13**, 449 456, (1958). *Cf.* also C. H. Bamford, H. Elliott, and W. E. Hanby, "Synthetic Polypeptides," Academic Press, New York, N. Y., 1956, p. 26.

(2) These syntheses have been elegantly pursued by R. Schwyzer and others: R. Schwyzer and P. Sieber, *Helv. Chim. Acta*, **41**, 2186, 2190 (1958), and references therein.

(3) M. Bodanszky, *Nature*, **175**, 685 (1955); B. Iselin, W. Rittel, P. Sieber, and R. Schwyzer, *Helv. Chim. Acta*, **40**, 373 (1957).

(4) The following abbreviations are used; Asp, Gly, Phe are the standard amino acid abbreviations[5]; Z = benzyloxycarbonyl, NP = *p*-nitrophenyl; Asp(OCH_3) is the β-methyl ester of aspartic acid; Asp(imide) is the α-aminosuccinimide group; DP = degree of polymerization, *i.e.*, the number of tripeptide units.

(5) *Cf.*, *e.g.*, M. Goodman and G. W. Kenner, *Advan. Protein Chem.*, **12**, 465 (1957).

(6) Microanalyses by Dr. F. Pascher, Bonn, and by Mrs. L. Ross, FSU. Techanical assistance by Mr. E. Heimer.

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 dichloroacetic 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).

(11) This work is the culmination of several years of research effort. The early stages were made possible by an unrestricted grant from Research Corporation and by an unrestricted grant PRF213c from the Petroleum Research Fund. The work has subsequently received generous support from the National Science Foundation, NSF G 4179, from the National Institutes of Health, NIH RG 5695 and RG 7828, and from the Air Force Office of Scientific Research, AF-AFOSR-62-279. It has also received support within the Institute of Molecular Biophysics under a contract with the Division of Biology and Medicine, U. S. Atomic Energy Commission. Grateful acknowledgment is made to the donors of the Petroleum Research Fund, to the Research Corporation, and to the granting agencies for their generous support and faith in the ultimately successful outcome of this line of work.

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

DEPARTMENT OF CHEMISTRY AND
INSTITUTE OF MOLECULAR BIOPHYSICS
THE FLORIDA STATE UNIVERSITY
TALLAHASSEE, FLORIDA

D. F. DETAR
W. HONSBERG
U. HONSBERG
A. WIELAND
M. GOUGE
H. BACH
A. TAHARA
W. S. BRINIGAR
F. F. ROGERS, JR.[11,12]

RECEIVED JUNE 19, 1963

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.