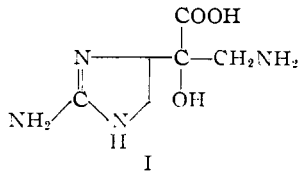
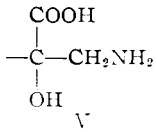
ethyl-2-imidazoline (I).



I

Roseonine, $C_6H_{12}O_3N_4$, has been characterized through the following derivatives: dipicrate (II), m.p. 237° dec. (calcd. for $C_{18}H_{18}O_{17}N_{10}$: C, 33.45; H, 2.81; N, 22.09, mol. wt., 646.40. Found: C, 33.48; H, 3.17; N, 22.09; mol. wt., 654[8]); dihydrochloride (III), m.p. 215°, $[\alpha]^{18}D$ +51.0° (H_2O) (Calcd. for $C_6H_{14}O_3N_4Cl_2$: C, 27.65; H, 5.41; N, 21.46; Cl, 27.16. Found: C, 27.51; H, 5.40; N, 19.88; Cl, 27.97); methyl ester dipicrate (IV), m.p. 202° (Calcd. for $C_{19}H_{20}O_{17}N_{10}$: C, 34.56; H, 3.05; N, 21.21. Found: C, 34.66; H, 3.40; N, 21.39); pK'_a, 2.4, 9.3 and 11.9. The Sakaguchi,[9] biacetyl,[9] Pauly and α-amino acid reactions as well as the Kuhn–Roth and N-methyl determinations on I were negative.

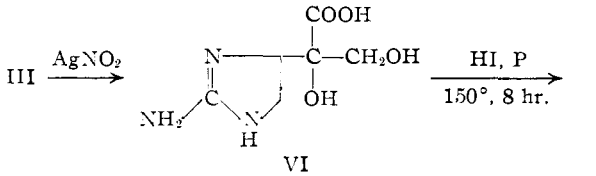
Permanganate oxidation of III at room temperature gave guanidine and a small amount of glycine. When treated with periodate, it consumed one mole of oxygen in 15 minutes with the formation of one mole each of ammonia and formaldehyde, and coupling this with the formation of glycine, it was apparent that the grouping $>C(OH)\cdot CH_2NH_2$ was present. Another mole of periodate was consumed after 20 hours, a behavior identical to that of serine.



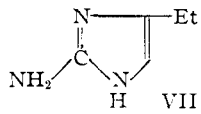
V

These results, together with the pK'_a values[10] suggested the existence of the grouping V.

The Van Slyke amino nitrogen determination gave one mole after seven minutes. A second mole was detected after 30 minutes, the behavior of this second amino group being exactly similar to that of 2-amino-2-imidazoline. III was converted into 2-amino-4(or 5)-ethylimidazole (VI) through the sequences



VI



VII

The action of one mole of silver nitrite gave VI, m.p. 190° dec. (Calcd. for $C_6H_{11}O_4N_3$: C, 38.09: H, 5.86; N, 22.21. Found: C, 37.77; H, 5.62; N, 19.95; negative Van Slyke nitrogen after seven minutes, positive periodate test). Heating VI in a sealed tube with hydroiodic acid and phosphorus gave a substance with one C-methyl group, positive Pauly and bromine test[11] and negative N-methyl (or ethyl); monopicrate, m.p. 182°. (Calcd. for $C_{11}H_{12}O_7N_6$: C, 38.83; H, 3.56; N, 24.70; mol. wt., 340.25. Found: C, 39.05; H, 3.72; N, 23.91; mol. wt., 356[8]). Hence the structure of this compound is apparently that represented by VII. The conversion of VI to VII could be explained by dehydration of the tertiary hydroxyl group, rearrangement to the stable imidazole ring, and decarboxylation at some stage.

No other structure except that represented by I can account for the described facts satisfactorily. Details of the present structural studies will be reported in a Japanese journal shortly.

The authors are greatly indebted to their collaborator, Prof. S. Hosoya, Tokyo University.

(8) Obtained from the extinction coefficient by the method of K. G. Cunningham, *et al.*, *J. Chem. Soc.*, 2305 (1951).

(9) The negativity of these color reactions for structure 1 is in accord with the observations of J. D. Mold, *et al.*, THIS JOURNAL, **75**, 6321 (1953).

(10) Since the value 9.3 (NH_3^+) is considerably lower than the value 10.28 assigned to the ε-amino in hydroxylysine (D. Van Slyke, *et al.*, *J. Biol. Chem.*, **133**, 287 (1940)), the neighboring effect of an additional substituent was anticipated. Placing a carboxyl as shown in V to make an isoserine residue resulted in favorable agreement with existing data: *i.e.*, isoserine (2.78, 9.27), O. H. Emerson, *et al.*, *J. Biol. Chem.*, **92**, 449 (1931), and D,L-α-methylisoserine (2.7, 9.15), E. H. Flynn, *et al.*, THIS JOURNAL, **75**, 5867 (1953). However, the first constant of roseonine (2.4) is slightly lower, and this might be caused by an additional neighboring positive group, which in this case proved to be the β-guanido group.

(11) Imidazoles with a free nuclear methine group decolorize bromine, whereas imidazolines do not.

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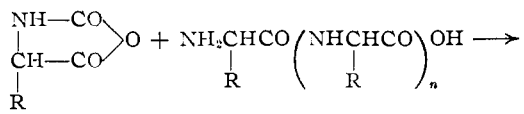
RECEIVED APRIL 17, 1954

A NEW SYNTHETIC METHOD OF PROTEIN ANALOGS HAVING PERIODIC ARRANGEMENT OF AMINO ACIDS

Sir:

The only method for preparing the polypeptides having several ten thousandths molecular weight of natural protein orders is the method of α-amino-N-carboxylic acid anhydride found by H. Leuchs[1] and established by Woodward and Schramm.[2] We recently found a new synthetic method of protein analogs having molecular weight of protein order, by polymerizing ω-amino acids, such as polypeptide consisting of some kinds of amino acids, β-alanine and ε-aminocapronic acid, as well as α-amino acids.[3] N-Carbothiophenyl-α-amino acids[4] expell thiophenol and carbon dioxide to be polymerized in polypeptides having high molecular weight by melting or by heating in the proper solvents such as dioxane only or benzene, having a small quantity of pyridine. The free amino group $-NH_2$ is the initiator of the polymerization in the α-amino acid–N-carboxylic acid anhydride method, and, on the other hand, the free carboxylic acid group –COOH is in this latter reaction.

Leuch's method:

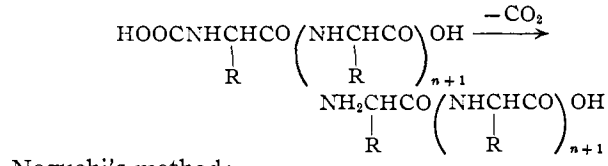


(1) H. Leuchs, *Ber.*, **39**, 857 (1906).

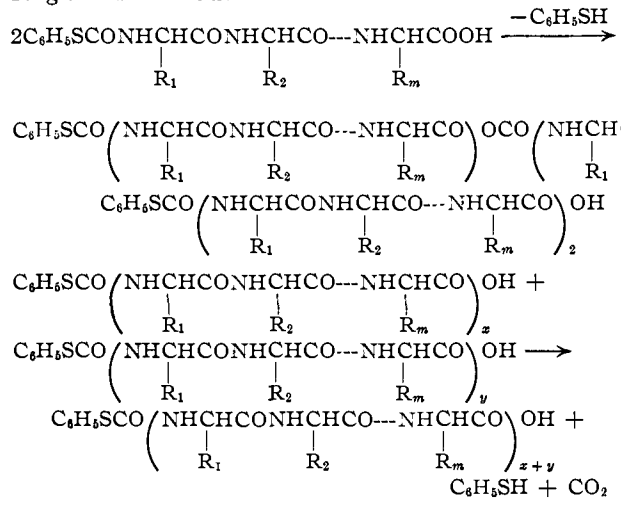
(2) R. B. Woodward and C. H. Schramm, THIS JOURNAL, **69**, 1551 (1947).

(3) J. Noguchi, *J. Chem. Soc. of Japan*, **74**, 961 (1953).

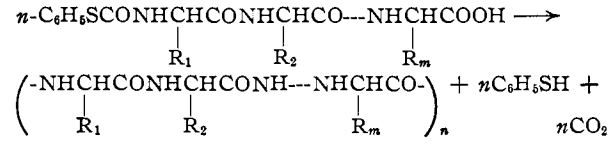
(4) G. C. H. Ehrensvard, *Nature*, **159**, 500 (1947); A. Lindemann, N. H. Khan and K. Hofmann, THIS JOURNAL, **74**, 476 (1952); J. Noguchi, *J. Chem. Soc. of Japan*, **74**, 963 (1953).



Noguchi's method:



In general:



We omitted the end groups of the polymer in the above formula, because the purified polymer corresponded to the theoretical values of its polypeptide in elementary analysis and did not show any trace of thiophenyl group by the chemical test or by the infrared absorption analysis. The infrared test showed a little free $-COOH$ group at one end, but the presence of free $-NH_2$ group at the other end was uncertain. It might be that the hydantoin group showed at this end as shown by Lindemann, etc.[5] The arrangement of each of the amino acids is not clear in the polypeptide prepared by copolymerizing them. But if the oligopeptide, having a clear arrangement of each of the amino acids, which was prepared by bonding with each amino acid in order, would be polymerized, each amino acid should be clearly arranged in regular periodicity.[6] If the method could be obtained, it would be possible to prepare the crystal model of silk fibroin, "poly-glycyl-L-alanine," by Meyer and Mark[7] and the models of "periodicity hypothesis" of amino acids consisting of protein by Bergmann.[8]

N-Carbothiophenyl-polypeptides, having "clear arrangement of each amino acid" polymerized in protein analogs by keeping at 60–80° for 1000 hr. in the saturated solution of dioxane, or in benzene having a small quantity of pyridine. The amino residues will come naturally to periodic arrangements. X-Ray analysis and infrared analysis of these polymers show the periodicity.[9]

By the above method, we have reached a new field, from which we shall be able to go a step farther concerning protein structure, because we could prepare the protein analogs having a clear arrangement of each of the amino acids as well as high molecular weight, a step which by the former method we could not take. Moreover, N-carbothiophenylam

TABLE I

Polymer	Mol. wt. (Osmotic method)[10]	[η]	Solvent
Polyglycine	135,160	0.530	$Cl_2CHCOOH$
Poly-DL-alanine[11]	22,400	.292	$Cl_2CHCOOH$
Poly-L-leucine[11]	26,689	.312	$Cl_2CHCOOH$
Poly-DL-phenylalanine	59,400	.068	$Cl_2CHCOOH$
Poly-β-alanine[11]	43,500	.063	HCOOH
Poly-ε-aminocaproic acid	26,200	.194	CH_3COOH, $ClCH_2COOH$ (1:1)
Polyglycyl-DL-alanine[a]	34,813	.053	H_2O
Polyglycyl-DL-phenylalanine[a]	30,603	.118	$Cl_2CHCOOH$
Polyglycyl-L-leucine[11a]	37,906	.121	$Cl_2CHCOOH$
Poly-β-alanyl-DL-alanine[a]	26,065	.055	H_2O
Poly-ε-aminocapronyl-DL-alanine[a]	65,000	.02	H_2O
Copoly-(glycine, DL-alanine)	11,457	.093	H_2O
Copoly-(L-leucine, DL-phenylalanine)	161,000	2.42	$Cl_2CHCOOH$
Copoly-(glycyl-L-leucine, glycyl-DL-phenylalanine)[b]	13,702	0.108	$Cl_2CHCOOH$

[a] They have alternative periodic arrangement. [b] It has periodic arrangement about glycine.

(5) A. Lindemann, N. H. Khan and K. Hofmann, THIS JOURNAL, **74**, 476 (1952).

(6) E. T. Wilson and E. Pacsu (*J. Org. Chem.*, **7**, 126 (1942)), prepared the polypeptide having periodic arrangement of amino acids by polymerizing tripeptide ester, but they were not high molecular weight of protein order. It is difficult to polymerize dipeptide ester because it is easy to form diketopiperazine.

(7) K. H. Meyer and H. Mark, *Ber.*, **61**, 1932 (1928).

(8) M. Bergmann and C. Niemann, *J. Biol. Chem.*, **110**, 471 (1935); *ibid.*, **115**, 77 (1936).

(9) M. Asai lectured on them at the 7th Meeting of The Japanese Chemical Society, April 1, 1954, and showed the clear differences between our periodic polymer, the copolymer and the polymers consisting of each amino acid itself.

(10) They were measured at dilute solution of about 4–5 g./l. As mentioned above, the presence of free amino ends of the polymers was uncertain, so we could not determine the molecular weights by the end group test. We cannot say whether such association as Katchalski (*Advances in Protein Chem.*, **6**, 166 (1951)), has pointed out, did occur or not in these solvents, but the polymers did not have low molecular weights, because some of them formed films when we evaporated the solvents.

(11) M. Frankel, *Experientia*, **9**, 179 (1953) prepared by another method, but they were lower molecuar weight by the amino end group test.

ino acids remain stable for a long period, but N-carboxylic acid anhydrides are unstable in the presence of moisture and cannot survive storage, so that it is easier to copolymerize several sorts of amino acids. Some protein analogs were prepared by this method (Table I).

A detailed account of this work will be published in *J. Chem. Soc. of Japan*.

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RECEIVED MARCH 19, 1954

ISOLATION, STRUCTURE AND SYNTHESIS OF A LATHYRUS FACTOR FROM *L. ODORATUS*[1]

Sir:

The isolation from *Lathyrus odoratus* seeds of a crystalline substance capable of producing the skeletal abnormalities characteristic of lathyrism has recently been accomplished.[2-4] The substance (I) obtained in this Laboratory, m.p. 193–194° dec.,[5] was water-soluble, ninhydrin-positive, and gave analytical values agreeing with the formula $C_8H_{13}O_3N_3$.[3] It showed only one ninhydrin spot when subjected to paper chromatography in three different solvent systems; however, after hydrolysis in 11.7 *N* hydrochloric acid for 8 hours at 120° this spot disappeared and was replaced by two others. On concentrating and cooling the hydrolysis mixture, L-glutamic acid hydrochloride precipitated. This fragment was identified by m.p., ultimate analysis, infrared spectrum, optical rotation and microbiological assay. When the filtrate was made alkaline and distilled, a volatile base was evolved which was identified as ammonia by conversion to ammonium chloride and demonstrating the absence of carbon by the method of Pepkowitz.[6] This result together with a sharp band at 4.45 μ in the infrared spectrum pointed to the presence of a nitrile function in I. On this basis the remaining hydrolysis product could be only sarcosine, α-alanine or β-alanine. Comparative paper chromatograms clearly pointed to β-alanine as the actual degradation product, and its presence in the hydrolysate was verified by isolation of the β-naphthalene sulfonate of β-alanine,[7] m.p. 134–136°, both alone and mixed with an authentic sample. It was concluded that I is β-(γ-L-glutamyl)-aminopropiononitrile or the α-glutamyl isomer. The γ-glutamyl structure was favored because I showed pK values[3] of 2.2 ± 0.2 and 9.1 ± 0.1. Accordingly a substance of this structure was synthesized by the method of King and Kidd[8] by condensation of β-aminopropiononitrile with N-phthaloyl-L-glutamic anhydride and subsequent removal of the phthaloyl substituent with hydrazine. The synthetic product melted with decomposition at 193.5–194°[5] and showed no depression on admixture with isolated I. The identity of the two products was confirmed by a comparison of their infrared spectra which were alike in all respects. The lathyrus activity of the synthetic compound in rats is being investigated.

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RECEIVED APRIL 9, 1954

(1) Supported in part by grants from the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service.

(2) H. P. Dupuy and J. G. Lee, *J. Am. Pharm. Assoc.*, **43**, 61 (1954).

(3) G. F. McKay, J. J. Lalich, E. D. Schilling and F. M. Strong, *Arch. Biochem. Biophys.*, in press.

(4) E. D. Schilling, *Federation Proc.*, **13**, 290 (1954).

(5) Bath preheated to 180°.

(6) L. P. Pepkowitz, *Anal. Chem.*, **23**, 1716 (1951).

(7) H. H. Weinstock, H. K. Mitchell, E. F. Pratt and R. J. Williams, THIS JOURNAL, **61**, 1421 (1939).

(8) F. E. King and D. A. A. Kidd, *J. Chem. Soc.*, 3315 (1949).

PHENYLACETYLGLUTAMINE AS A CONSTITUENT OF NORMAL HUMAN URINE

Sir:

Phenylacetylglutamine (PAG) was first described by Thierfelder and Sherwin[1] who isolated the compound from the urine of individuals fed phenylacetic acid. The conjugate is frequently referred to as a "detoxication" product. In investigating the source of the amino acids liberated by acid hydrolysis of human urine,[2] it has been found that PAG is excreted under normal conditions by the adult male to the extent of 250 to 500 mg. per day, and accounts for about 50% of the conjugated glutamic acid in urine.

Identification of PAG has been effected by chromatographic analyses of urine employing acidic and basic ion-exchange resins (Dowex 50 and Dowex 2). PAG and other conjugates were detected in the effluent by hydrolysis of 1-ml. effluent fractions with 6 *N* HCl or 2 *N* NaOH prior to the application of the photometric ninhydrin method.[3] Because of the affinity of the resin for aromatic compounds, PAG, despite its acidic nature, is retarded on columns of Dowex 50X8 and emerges at a position between urea and aspartic acid.[2] The conjugate was more readily determined by chromatography of 4-ml. samples of urine on 0.9 × 30 cm. columns of Dowex 2X4 (200–400 mesh) in the acetate form. Elution was begun with 0.2 *M* sodium acetate buffer at *p*H 5.3.[4] After 100 ml., the *p*H and ionic strength were gradually changed by allowing a 2 *M* sodium acetate buffer of *p*H 4.6[5] to flow into 100 ml. of the initial 0.2 *M* buffer stirred magnetically. The compound giving rise to a major peak at about 195 ml. was identified as PAG by the demonstration that the unknown and a sample of synthetic PAG[6] exhibited identical chromatographic behavior on columns of both Dowex 2 and Dowex 50, together with the finding of equimolar amounts of glutamic acid and ammonia in an acid hydrolysate of the unknown.

The same analytical procedure afforded a chro-

(1) H. Thierfelder and C. P. Sherwin, *Ber.*, **47**, 2630 (1914).

(2) W. H. Stein, *J. Biol. Chem.*, **201**, 45 (1953).

(3) S. Moore and W. H. Stein, *ibid.*, **176**, 367 (1948).

(4) 27.2 g. of $NaOAc{\cdot}3H_2O$ + 5.0 ml. of glacial HOAc + 2.5 ml. of 50% BRIJ-35 solution diluted to 1 l. with water. BRIJ-35 is a detergent manufactured by Atlas Powder Co., Wilmington, Delaware.

(5) 54.4 g. of $NaOAc{\cdot}3\ H_2O$ and 10.0 ml. of glacial HOAc are made to a volume of 100 ml. with water. To this solution 30 ml. of glacial HOAc, 70 ml. of water and 0.2 ml. of BRIJ-35 solution are added.

(6) H. Thierfelder and C. P. Sherwin, *Z. physiol. Chem.*, **94**, 1 (1915).