TABLE I

STOICHIOMETRY OF THE CONDENSATION OF OROTIC ACID AND PRPP

The reaction mixture (24 ml.) contained 0.48 ml. of tris-(hydroxymethyl)-aminomethane (TRIS) buffer (1 *M*, *p*H 8.0), 0.48 ml. of $MgCl_2$ (0.1 *M*), 1.20 ml. of PP (0.01 *M*, *p*H 8.5), 1.44 ml. of NaF (0.5 *M*) 0.48 ml. of 4,7-C^{14}-sodium orotate (0.01 *M*, 186,000 c.p.m./μmole), 0.40 ml. of PRPP (2.85×10^{-3} *M*, 112,000 c.p.m./μmole) and 4.8 ml. of enzyme fraction I (containing 20.6 mg. of protein). One-half of the reaction mixture was immediately placed in a boiling water-bath for 0.5 min.; the remainder was incubated at 30° for 40 minutes and then heated in a boiling water-bath for 0.5 min.

	μmoles		
	0 min.	40 min.	Δ
Orotic acid[a]	2.40 (2.38)[e]	1.84 (1.87)	−0.56 (−0.51)
PRPP[b]	0.57 (0.52)	0.03 (0.00)	−0.54 (−0.52)
U5P[c]	.00 (0.00)	.55 (0.55)	+ .55 (+0.55)
CO_2[d]		.60	+ .60
PP	(0.09)	(0.48)	(+0.39)[f]

[a] Determined spectrophotometrically at 295 mμ. [b] Determined spectrophotometrically by the removal of orotic acid (see equation 1). [c] Determined spectrophotometrically at 260 mμ. [d] Estimated by trapping in NaOH the $C^{14}O_2$ released enzymatically from orotic acid and measuring the radioactivity. [e] Values in parentheses were determined after chromatography on Dowex 1 anion-exchange resin; orotic acid and U5P were estimated by optical density measurements at 280 and 262 mμ, respectively; PRPP and PP were estimated by radioactivity measurements. [f] This value is low because of inorganic PPase activity; 0.22 μmole was recovered as 0.44 μmole of inorganic orthophosphate.

phate by 5'-nucleotidase.[6] The specific activity of the U5P (24,700 c.p.m./μmole) was one-half that of the orotic acid.

The accumulation of O5P was demonstrated with a more purified enzyme preparation (II) which was free of decarboxylase and relatively poor in PPase. In an experiment in which 0.27 μmole of 4,7-C^{14}-orotic acid was consumed, 0.26 μmole of O5P was isolated by ion-exchange chromatography. The O5P was identified by its absorption spectrum (identical with that of orotidine[7]: peak at 266 mμ, $\lambda_{280}/\lambda_{260} = 0.66$ at *p*H 7), and its enzymatic decarboxylation (0.26 μmole $C^{14}O_2$ liberated; equation 2). The synthesis of larger amounts of O5P is made difficult by the rapid decline in reaction rate due to the accumulation of O5P and PP.

The stoichiometry of the O5P pyrophosphorylase reaction (equation 1) with O5P (prepared by phosphatase transfer[8]) and PP as substrates was observed with the results shown in Table II. In the presence of larger amounts of PP the reaction proceeds rapidly to completion. Thus, in a 1-ml. incubation mixture containing partially purified O5P pyrophosphorylase (0.02 mg. of protein), 0.036 μmole of O5P and 0.30 μmole of PP, 0.037 μmole of orotic acid was formed in 17 minutes. No reaction occurred in the absence of added PP.

(6) L. A. Heppel and R. J. Hilmoe, *J. Biol. Chem.*, **188**, 665 (1951).

(7) A. M. Michelson, W. Drell and H. K. Mitchell, *Proc. Natl. Acad. Sci.*, **37**, 396 (1951).

(8) A compound having an absorption spectrum identical to O5P synthesized by the enzymatic condensation of orotic acid and PRPP has been prepared from orotidine by phosphate transfer from phenylphosphate with a malt phosphatase (G. Brawerman and E. Chargaff, THIS JOURNAL, **75**, 2020 (1953)). It was further identified as O5P by its properties on ion-exchange chromatography, by its enzymatic decarboxylation to U5P (0.061 μmole yielded 0.061 μmole of U5P estimated spectrophotometrically), and by the molar ratios of orotic acid:pentose:phosphate of 1.08:1.04:1.06.

TABLE II

STOICHIOMETRY OF THE PYROPHOSPHORLYSIS OF O5P

The reaction mixture (21.6 ml.) contained 0.36 ml. of TRIS buffer (1 *M*, *p*H 8.0), 0.36 ml. of $MgCl_2$ (0.1 *M*), 1.8 ml. of NaF (0.05 *M*), 1.8 ml. of PP (1.2×10^{-3} *M*, 750,000 c.p.m./μmole), 13.2 ml. of O5P (9.8×10^{-5} *M*) and 3.6 ml. of the enzyme fraction II (0.42 mg. of protein). One half of the reaction mixture was immediately placed in a boiling water-bath for 0.5 min.; the other half was incubated at 30° for 22 min. and then placed in a boiling water-bath for 0.5 min.

	μmoles		
	0 min.	22 min.	Δ
O5P[a]	0.65 (0.58)[d]	0.39 (0.29)	−0.26 (−0.29)
PP	(1.04)	(0.84)	(−0.20)
Orotic acid[b]	.00 (0.00)	.32 (0.30)	+ .32 (+0.30)
PRPP[c]	.00 (0.00)	.22 (0.16)	+ .22 (+0.16)

[a] Estimated spectrophotometrically at 266 mμ. [b] Determined by the decrease in optical density at 280 mμ in the presence of dihydroörotic dehydrogenase and reduced diphosphopyridine nucleotide (I. Lieberman and A. Kornberg, *Biochim. Biophys. Acta*, 12, 223 (1953)). [c] See footnote *b*, Table I. [d] See footnote *e*, Table I. The relatively low value at 22 min. for PRPP and the high value for PP at 22 min. suggest the destruction of PRPP to yield PP.[5]

O5P pyrophosphorylase did not convert adenine or uracil to their respective nucleotides in detectable amounts (8-C^{14}-adenine and 2-C^{14}-uracil of high specific activities were used in these experiments). It is noteworthy that another yeast enzyme has been purified which catalyzes the condensation of adenine with PRPP to form A5P[9] and which is inactive in forming O5P from orotic acid. O5P decarboxylase also is specific for O5P, attacking neither orotidine nor orotic acid.

(9) A. Kornberg, I. Lieberman and E. S. Simms, unpublished results.

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RECEIVED APRIL 12, 1954

STRUCTURE OF A NEW AMINO ACID OBTAINED FROM ROSEOTHRICIN[1]

Sir:

Roseothricin (H-277) is a streptothricin-like antibiotic obtained from *Streptomyces roseochromogenus*.[2] Though fairly toxic, it possesses high antibacterial activity. The hydrolysis of roseothricin hydrochloride with 20% hydrochloric acid in a sealed tube for 48 hours at 100° gave two ninhydrin-positive products, which were separated by solubility differences of their picrates. One was β,ε-diaminohexanoic acid, an amino acid also present in streptothricin,[3] streptolin,[3,4] and viomycin,[3,5] and characterized by American workers.[6,7] The other substance, for which the trivial name of "roseonine" is designated, has now been shown to be 2-amino-4(or 5)-(1-carboxy-1-hydroxy-2-amino)-

(1) An imidazoline structure was postulated at the 6th Annual Meeting, Chemical Society of Japan, Kyoto, April, 1953.

(2) S. Hosoya, *et al.*, *Jap. J. Exptl. Med.*, **20**, 121 (1949); **20**, 481, 683, 771 (1950).

(3) H. E. Carter, *et al.*, Abstracts of Papers 120th Meeting, American Chemical Society, New York, N. Y., September, 1951, p. 3L.

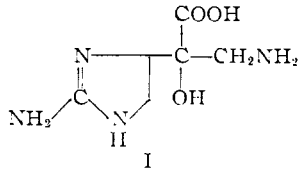
(4) E. E. Smissman, *et al.*, Abstracts of Papers 121st Meeting, American Chemical Society, Milwaukee, Wisconsin, April, 1952, p. 80.

(5) T. H. Haskell, *et al.*, THIS JOURNAL, **74**, 599 (1952).

(6) H. E. Carter, *et al.*, *ibid.*, **74**, 3704 (1952).

(7) E. E. van Tamelen and E. E. Smissman, *ibid.*, **74**, 3714 (1952).

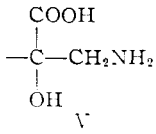
ethyl-2-imidazoline (I).



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°, $[\alpha]^{13}D$ +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.

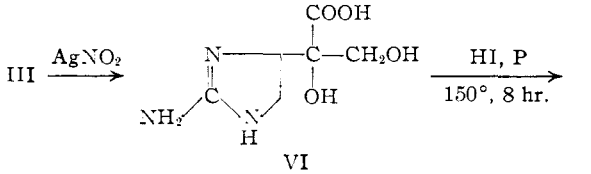
Permanganate oxidation of III at room temperature gave guanidine and a small amount of glycine. When treated with periodate, it consumed one mole of oxygen in 15 minutes with the formation of one mole each of ammonia and formaldehyde, and coupling this with the formation of glycine, it was apparent that 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.



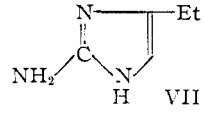
V

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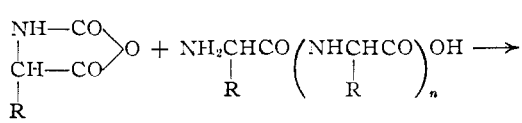
A NEW SYNTHETIC METHOD OF PROTEIN ANALOGS HAVING PERIODIC ARRANGEMENT OF AMINO ACIDS

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).