COMMUNICATIONS TO THE EDITOR

PUROMYCIN. SYNTHETIC STUDIES. VII. PARTIAL SYNTHESIS OF AMINO ACID ANALOGS

Sir:

Puromycin has been shown to have the structure 6-dimethylamino-9-(3′-*p*-methoxy-L-phenylalanylamino-D-ribosyl)-purine.[1] An interesting type of structural variant would be the exchange of the amino acid moiety for a different amino acid or peptide.

Treatment of puromycin dihydrochloride[2] with phenyl isothiocyanate and triethylamine in boiling alcohol gave a nearly quantitative yield of phenylthiocarbamyl derivative (I), m.p. 174–175°, $[\alpha]^{25}D$ −45.6° (acetone). *Anal.* Calcd. for $C_{29}H_{34}N_8O_6S$: C, 57.4; H, 5.66; N, 18.5; S, 5.29. Found: C, 57.3; H, 5.73; N, 18.3; S, 5.50. Cleavage of I with boiling methanolic sodium methoxide[3] was complete in 1 hour. The solution deposited 65–70% of a crystalline "aminonucleoside," 6-dimethylamino-9-(3′-amino-β-D-ribofuranosyl)-purine[5] (II), m.p. 215–216°, $[\alpha]^{25}D$ −24.6° (H_2O). *Anal.* Calcd. for $C_{12}H_{18}N_6O_3$: C, 49.0; H, 6.16; N, 28.6. Found: C, 49.4; H, 6.39; N, 28.4.

The "aminonucleoside" (II) did not exhibit the bacterial spectrum[6] characteristic of puromycin,[2] but the activity against *Trypanosoma equiperdum* was increased 3–4 fold.[7] Puromycin has a medium order of activity against the transplanted mammary adenocarcinoma of the C3H mouse; the "aminonucleoside" is much more active, being highly effective against this tumor.[8]

In order to establish that the "aminonucleoside" still had the configuration and ring size of the sugar moiety as in the original antibiotic, puromycin was resynthesized from II. Treatment of II in dimethylformamide with the mixed anhydride[9] of N-carbobenzoxy-*p*-methoxy-L-phenylalanine[10] gave 64% of pure N-carbobenzoxy puromycin (III), m.p. 208–210°. *Anal.* Calcd. for $C_{30}H_{35}N_7O_7$: C, 59.5; H, 5.83; N, 16.4. Found: C, 59.4; H, 5.92; N, 16.5. Hydrogenolysis of III in Methyl Cellosolve at 60–70° in the presence of 10% palladium–charcoal gave puromycin base identical with an authentic sample.[2]

A variety of amino acids such as L-phenylalanine, L-tyrosine, L-lysine, L-tryptophan, L-leucine, β-alanine, glycine and *p*-methoxy-L-phenylalanylglycine, by activation of their N-carbobenzoxy derivatives as the mixed anhydride, acid chloride or azide, were coupled with the "aminonucleoside" to give analogs of puromycin. These compounds were active against *Trypanosoma equiperdum*[7] and the mammary adenocarcinoma[8] in mice. Some of these analogs had anti-bacterial activity, the most effective being the L-phenylalanyl analog.[6]

It is interesting to speculate that *in vivo* the amino acids are enzymatically removed to give the "aminonucleoside" which is the active portion of the antibiotic against *Trypanosoma equiperdum* and the mammary adenocarcinoma in mice.

LEDERLE LABORATORIES DIVISION
AMERICAN CYANAMID COMPANY
PEARL RIVER, NEW YORK

B. R. BAKER
JOSEPH P. JOSEPH
JAMES H. WILLIAMS

RECEIVED APRIL 14, 1954

(1) C. W. Waller, P. W. Fryth, B. L. Hutchings and J. H. Williams, THIS JOURNAL, **75**, 2025 (1953).

(2) J. N. Porter, R. I. Hewitt, C. W. Hesseltine, G. Krupka, J. A. Lowery, W. S. Wallace, N. Bohonos and J. H. Williams, *Antibiotics and Chemotherapy*, **2**, 409 (1952).

(3) In the usual practice of terminal cleavage of a peptide,[4] the action of anhydrous hydrogen chloride on the phenylthiourea in an inert solvent such as nitromethane is employed. These conditions were considered incompatible with I.

(4) P. Edman, *Acta Chem. Scand.*, **4**, 283 (1950).

(5) The details of the determination of the configuration of the sugar moiety as β-furanose will be published by C. W. Waller, *et al.*

(6) Private communication from Dr. J. N. Porter of these laboratories.

(7) R. I. Hewitt, A. Gumble, W. S. Wallace and J. H. Williams, *Am. J. Trop. Med.*, in press.

(8) J. J. Oleson, *et al.*, to be published.

(9) R. A. Boissonas, *Helv. Chim. Acta*, **34**, 874 (1951).

(10) R. P. Rivers and J. Lerman, *J. Endocrinol.*, **5**, 223 (1948).

TYROSINE-O-SULFATE IN A PEPTIDE FROM FIBRINOGEN

Sir:

One of the two peptides released from bovine fibrinogen when it is clotted by thrombin, peptide B, was found to give rise to tyrosine on acid hydrolysis.[1] The phenolic hydroxyl group of this tyrosine appears not to be free in the intact peptide, however, for it does not react with fluorodinitrobenzene (FDNB), and the ultraviolet absorption spectrum of the peptide shows no trace of the peaks at 275 and 293 mμ characteristic of tyrosine in neutral and alkaline solution, respectively. There is now good reason to believe that the tyrosine is, in fact, present as the O-sulfate derivative. The evidence for this is as follows.

1. Mild hydrolysis with acid, but not with alkali, liberates the phenolic group, making it reactive to FDNB, and causing the ultraviolet absorption characteristic of tyrosine to appear. The reaction is complete in 4 minutes in *N* HCl at 93°. Such marked lability to acid and stability to alkali is characteristic of aryl sulfates. The amount of tyrosine present, estimated spectrophotometrically after acid hydrolysis, corresponds to one mole per 2800 g. of peptide, in good agreement with the approximate minimum molecular weight of 3000 based on the lysine content.[1]

2. Tyrosine-O-sulfate was prepared by the action of concentrated sulfuric acid on tyrosine in the cold[2] and crystallized as the K salt. Its ultraviolet absorption spectrum differs markedly from that of tyrosine, showing a much weaker absorption with a maximum near 263 mμ. It is hydrolyzed by acid at a rate close to that at which the phenolic hydroxyl group of the tyrosine in peptide B is liberated.

3. The formation of inorganic sulfate on mild

(1) F. R. Bettelheim and K. Bailey, *Biochim. Biophys. Acta*, **9**, 578 (1952).

(2) H. C. Reitz, R. E. Ferrel, H. Fraenkel-Conrat and H. S. Olcott, THIS JOURNAL, **68**, 1024 (1946).