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 $C_{29}H_{34}N_8O_5S$: 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.

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(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).

acid hydrolysis of peptide B can be shown by the addition of Ba^{++}. Estimation of the amount of sulfate liberated, by a turbidimetric method using Ba^{++}, gave a value of about 0.9 mole per mole of tyrosine. The rate of liberation of inorganic sulfate parallels that of the appearance of the tyrosine absorption peak at 275 mμ.

4. Tyrosine-O-sulfate has been detected in hydrolysates of peptide B prepared by heating in 0.2 *M* $Ba(OH)_2$ for 24 hours at 125°. It was freed of other amino acids and Ba^{++} by passage through a column of Dowex 50 in the H^+ form, and shown to be identical with synthetic tyrosine-O-sulfate by paper chromatography with 3 different solvent systems, and by paper electrophoresis at *p*H 2.4. The amount found, estimated as tyrosine after acid hydrolysis, was 75% of that expected from the tyrosine content of the peptide.

5. The new peptide formed on mild acid hydrolysis of peptide B behaves as a less acidic molecule than the original on paper electrophoresis at *p*H 6.8, 4.1 and 2.4. This is consistent with the loss of a strongly acid group.

The modification in the properties of the phenolic group by conjugation with sulfuric acid may help to account for the assertions of Lorand[3] and Lorand and Middlebrook[4] that the peptide material released from fibrinogen by thrombin is free of tyrosine.

(3) L. Lorand, *Nature*, **167**, 992 (1951).
(4) L. Lorand and W. R. Middlebrook, *Biochim. Biophys. Acta*, **9**, 581 (1952).

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ALKALI METAL–AMMONIA SOLUTIONS: GROSS CHEMICAL DIFFERENCES

Sir:

Reactions in liquid ammonia have been reported in which the alkali metals differ from calcium solutions[1]; in which the rates of reaction of the alkalies differ[2]; or in which the alkalies differ in the efficiency with which they bring about reduction of organic compounds.[3] But in the case of liquid ammonia solutions of $BF_3 \cdot NH_3$ our experimental results show a straight-forward difference in the *stoichiometry* of the reactions with different alkali metals. Such inherent differences in the chemical reactivity of these metal solutions have not been demonstrated previously.

Solutions of $BF_3 \cdot NH_3$ were titrated with solutions of the alkali metals in an apparatus[4] which provided for the quantitative recovery of all products. Also, reactions of $BF_3 \cdot NH_3$ solutions with an excess of alkali metal were carried out, followed by back-titration with ammonium iodide solutions to determine the excess metal present.

Similar titrations were carried out with potassium amide, using triphenylmethane as indicator, to investigate the extent of total solvolysis. Results are reported in Table I.

(1) W. M. Burgess and J. W. Eastes, THIS JOURNAL, **63**, 2674 (1941).
(2) G. W. Watt and P. I. Mayfield, *ibid.*, **75**, 1760 (1953).
(3) A. L. Wilds and N. A. Nelson, *ibid.*, **75**, 5360 (1953).
(4) G. W. Watt and C. W. Keenan, *ibid.*, **71**, 3833 (1949).

TABLE I

TITRATION OF $BF_3 \cdot NH_3$–LIQUID NH_3 SOLUTIONS

Titrant	Moles titrant per mole $BF_3 \cdot NH_3$ By forward-titration	By back-titration
Li	2.91	2.97
Na	2.50	2.62
K	1.00	1.02[a]
Cs		1.03
KNH_2	2.6	3.00

[a] Determined by analysis of hydrogen evolved in presence of excess potassium, which was added in form of solid pieces to $BF_3 \cdot NH_3$ solution.

All final end-points were one-drop excesses stable for at least 30 minutes. Of particular interest were the observations of transient end-points in the direct titrations with both sodium and lithium. In the case of sodium there was found a transient end-point at about one equivalent,[5] and in the case of lithium at both one and two and one-half equivalents. These transient end-points were approached sharply and persisted for as long as 40 minutes, at which time the blue color due to excess metal faded suddenly. The subsequent titration reactions were as rapid as normal ionic titrations.

The following over-all equations are in agreement with our findings. Supporting analytical data will be reported in a later paper.

$$6Li + 2BF_3 \cdot NH_3 + 2NH_3 \longrightarrow 6LiF + (NH_2)_2BNHBNH + 3H_2$$

$$5Na + 2BF_3 \cdot NH_3 + 2NH_3 \longrightarrow 5NaF + (NH_2)_2BNHB(F)NH_2 + 5/2H_2$$

$$K + BF_3 \cdot NH_3 \longrightarrow KF + BF_2NH_2 + 1/2H_2[6]$$

$$3KNH_2 + BF_3 \cdot NH_3 \longrightarrow 3KF + B(NH_2)_3 + NH_3$$

These experimental data suggest a mechanistic explanation based on differences in the alkali metal–oxidant interaction. Such an approach has been defended recently in another case.[3]

(5) For an earlier description of the reaction with sodium see C. A. Kraus and E. H. Brown, *ibid.*, **51**, 2690 (1929).
(6) C. W. Keenan and W. J. McDowell, *ibid.*, **75**, 6348 (1953).

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STRUCTURAL STUDIES WITH BACITRACIN A

Sir:

In partial hydrolysis studies with bacitracin A using hydrochloric acid a considerable number of peptides have been isolated as DNP (dinitrophenyl) derivatives which appear to be of satisfactory purity as judged by C.C.D. (countercurrent distribution), two dimensional P.C. (paper chromatography) and P.E. (paper electrophoresis). Each peptide has been completely hydrolyzed and the hydrolysate studied by P.C. and P.E. The DNP amino acid has been extracted from the hydrolysate and identified by a combination of P.C., P.E. and C.C.D. A summary of part of the work is given in Table I. Over-all analytical data, supporting the composition indicated by the results in Table I for many of the peptides, are given in Table II.