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Puromycin. Synthetic Studies. VII. Partial Synthesis of Amino Acid Analogs

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Alkaline cleavage of the N-phenylthiourea derivative of puromycin gives 6-dimethylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)-purine (VI). This aminonucleoside loses the bacterial spectrum characteristic of puromycin, but is much more effective against the transplanted mammary adenocarcinoma of the C_3H mouse and *Trypanosoma equiperdum* in mice than is the original antibiotic. A number of α-aminoacyl derivatives of the aminonucleoside VI were synthesized as analogs of puromycin by proper modification of standard peptide synthetic methods. All of these compounds were biologically active.

Puromycin has been shown to have the structure 6-dimethylamino-9-(3′-*p*-methoxy-L-phenylalanylamino-3′-deoxy-D-ribosyl)-purine.[2] Further degradation studies revealed that the 3-amino-3-deoxy-D-ribose was attached to the purine in a β-furanosyl configuration.[3] Since the antibiotic contains three moieties in the sequence purine–aminopentose–amino acid, an interesting type of structural variant would be the exchange of the amino acid for another amino acid or peptide by partial synthesis from the antibiotic. A general method for accomplishing these transformations is described in this paper.

When puromycin (II) dihydrochloride[4] was treated with phenyl isothiocyanate and triethylamine in boiling alcohol, a near quantitative yield of the phenylthiourea III was obtained. In the usual practice of terminal cleavage of a peptide, the action of anhydrous hydrogen chloride on a phenylthiourea in an inert solvent such as nitromethane is employed.[5] With a molecule such as III some difficulties could be anticipated in an acid cleavage. The basic properties imparted by the purine nucleus to the molecule would cause III to precipitate as a hydrochloride before or during cleavage, thus leading to mixtures. If forced conditions were then employed to complete the reaction, the acid-sensitive[2] glycosyl–purine linkage might be cleaved.

These difficulties could be alleviated theoretically if a basic reagent were employed for cleavage of III to the aminonucleoside VI, with concurrent formation of 1-phenyl-4-*p*-methoxybenzyl-2-thiohydantoin. Although no record could be found in the literature of a basic cleavage of this exact type, many properly substituted open chain amides are ring-closed to pyrimidines with a basic catalyst.[6] It follows that III should cleave with base since the driving force for the reaction would be concomitant ring closure of the amino acid moiety to a thiohydantoin. When III was refluxed in methanol with one mole of sodium methoxide, cleavage occurred to the aminonucleoside VI, which crystallized from the reaction mixture in 75% yield and had m.p. 215–216°, $[\alpha]^{25}D$ −24.6° (H_2O). The thiohydantoin could be crystallized from the filtrate in low yield and appeared to have undergone extensive decomposition. This decomposition thus may be the reason why base has not been used heretofore in terminal cleavage of peptides since in peptide work it is necessary to isolate the terminal amino acid derivative for identification.

Methanolic sodium hydroxide also could be used for cleavage, but the yield of VI dropped to 49%. Direct methanolic sodium hydroxide hydrolysis of puromycin base II was slow and after 20 hours a 60% yield of VI was obtained. Since puromycin base is obtainable in 77% yield from its dihydrochloride, the over-all yield is only 47%. Thus, the sodium methoxide cleavage of the phenylthiourea

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

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(4) 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).

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III is the preferred method giving 65–67% over-all yields of VI from puromycin (II) dihydrochloride.

The biological activity of 6-dimethylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)-purine (VI), "the aminonucleoside," proved most interesting. The bacterial spectrum characteristic of puromycin[4] has been completely lost[7] but the activity against *Trypanosoma equiperdum* in mice was inincreased 3–4 fold.[8] Puromycin has a medium order of activity against the transplanted mammary adenocarcinoma of the C_3H mouse; the aminonucleoside VI is much more active, being highly effective against this tumor.[9]

Acetylation of the aminonucleoside VI with acetic anhydride in water gave the N-acetyl derivative IXa, m.p. 190–191°, in 85% yield. Acetylation with acetic anhydride in pyridine afforded a 93% yield of triacetate IXb, m.p. 189–191°. O-Deacetylation of the triacetate IXb with methanolic sodium methoxide gave a 91% yield of IXa. Further hydrolysis of the N-acetyl derivative IXa with hot 0.5 *N* barium hydroxide regenerated the aminonucleoside VI in 80% yield. These last two conversions are useful for a total synthesis of puromycin as is described in the accompanying papers VIII and IX.

In order to establish that the aminonucleoside VI still had the configuration and ring size of the sugar moiety as in the original antibiotic, puromycin (II) was resynthesized from VI. When VI in dilute aqueous sodium carbonate was treated with N-phthalyl-*p*-methoxy-L-phenylalanyl chloride[10] in acetone, coupling to N-phthalylpuromycin (Vb) took place in 80% yield. The identical compound Vb was obtained in 60% yield by fusion of puromycin base II with phthalic anhydride at 160°. The phthalyl group was removed by treatment of II with excess hydrazine[11] in methyl Cellosolve at 100° followed by cleavage of the intermediate with 20% acetic acid in methyl Cellosolve. The yield of puromycin base (II), m.p. 167–168°, was 48% and 12% could be recovered from the filtrate as the N-acetyl derivative.[2] Since it is highly improbable that the configuration and/or ring size could have changed during cleavage, then changed back during resynthesis, this resynthesis serves to prove that the puromycin has the same configuration and ring size as the aminonucleoside VI, namely, β-furanose.[3]

Although the carbobenzoxy blocking group for resynthesis gave about the same over-all yields, it was considered more convenient than the phthalyl blocking group. Reaction of the aminonucleoside VI in dimethylformamide with the carbethoxy mixed anhydride[12] of N-carbobenzoxy-*p*-methoxy-L-phenylalanine gave 64% of pure N-carbobenzoxypuromycin (Va). Hydrogenolysis of Va with a palladium–charcoal catalyst in acetic acid[13] gave a 68% yield of puromycin (II) isolated as the base. The recovery from the acetate salt entailed some crystallization losses since pure puromycin base could be recovered in only 72% yield when dissolved in acetic acid, which indicated that the actual hydrogenolysis was near quantitative.

The above reactions were then applied to glycine with the aminonucleoside. Coupling of phthalylglycyl chloride with the aminonucleoside VI took place in 60% yield in aqueous acetone in the presence of sodium carbonate and in 75% yield with triethylamine in dimethylformamide. The yield with the mixed anhydride of phthalylglycine[12] was poor. Removal of the phthalyl group with hydrazine gave the glycylaminonucleoside (VII, R = H) in 55% yield, but the material was difficult to purify fully. Coupling of carbobenzoxyglycyl chloride with the aminonucleoside in dimethylformamide containing triethylamine gave 63% of product (VIII, R = H). The yields were again better than by the mixed anhydride method.[12] Hydrogenolysis to the glycylaminonucleoside VII in acetic acid proceeded rapidly, but the product was difficult to isolate and the yield of VIII was less than 40%. When the hydrogenolysis was performed in a neutral solvent,[14] methyl Cellosolve, at 60–70°, mere evaporation of the solvent gave a 93% yield of crystalline glycylaminonucleoside (VIII) which was almost analytically pure.

The experience gained with the introduction of glycine and *p*-methoxy-L-phenylalanine onto the aminonucleoside was then applied to a variety of amino acids and one dipeptide. The carbobenzoxy blocking group was used in all cases. The acid chlorides were used for activation of the carboxyl where possible and the couplings were run in dimethylformamide containing triethylamine. This method was successful with N-carbobenzoxy-L-phenylalanine, O,N-dicarbobenzoxy-L-tyrosine, N-carbobenzoxy-L-tryptophan, N-carbobenzoxy-L-leucine, N-carbobenzoxy-*p*-methoxy-L-phenylalanylglycine and N-carbobenzoxy-β-alanine. The latter two also gave good yields of VIII through the azide. For the dipeptide the azide method was more convenient since the dipeptide ester was available from the coupling of N-carbobenzoxy-*p*-methoxy-L-phenylalanine mixed anhydride with glycine ethyl ester. The acid, acid chloride and methyl ester of N,N′-dicarbobenzoxy-L-lysine[15] were oils and no coupling to VIII occurred with the crude acid chloride. However, the hydrazide was obtained crystalline[15] and the corresponding azide[15] coupled satisfactorily with the aminonucleoside. All the intermediate carbobenzoxy derivatives VIII were obtained as crystalline solids. Hydrogenolysis in methyl Cellosolve with a palladium–charcoal catalyst gave the puromycin analogs VII in good yields. All were crystalline except the L-lysyl derivative of the aminonucleoside, which was obtained as a glass.

Dipeptide derivatives of the aminonucleoside VI also could be prepared directly from puromycin. The glycyl and *p*-methoxy-L-phenylalanyl deriva-

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

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tives (IV) of puromycin were obtained as crystalline solids *via* the acid chloride and mixed anhydride,[12] respectively, followed by hydrogenolysis.

All of the amino acid derivatives (IV and VII) of the aminonucleoside were active against *Trypanosoma equiperdum* in mice. The most active was the L-phenylalanyl derivative which had about the same order of activity as the aminonucleoside VI. The other derivatives were $^1/_4$–$^1/_{10}$ as active as VI.[8]

The bacterial spectra of these compounds were more variable.[7] The L-phenylalanine analog had a spectrum almost the same as puromycin. Others were less active. Glycylaminonucleoside, β-alanylaminonucleoside, glycylpuromycin and the aminonucleoside VI were all inactive. Most of these compounds are active against the transplanted mammary adenocarcinoma of the C_3H mouse, but none are as active as VI.[9]

It is interesting to speculate that *in vivo* the amino acids are removed by enzymatic hydrolysis to the aminonucleoside VI which is the active portion of the antibiotic against *Trypanosoma equiperdum*[8] and the mammary adenocarcinoma in mice.[9]

Since the aminonucleoside VI appears to be the fragment of puromycin responsible for the activity against *Trypanosoma equiperdum*, but is inactive against bacteria, this is the first known example where a fragment of an antibiotic is active against one type of organism, but the intact antibiotic is necessary for activity against a different type of organism.

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Experimental

Puromycin Base (II).—A mixture of 20.0 g. of puromycin dihydrochloride,[4] 100 cc. of alcohol and 9.4 cc. of isopropylamine was warmed to complete solution. After 1 hour at 0°, the mixture was filtered and the base washed with water; yield 13.3 g. (77%) in three crops, m.p. 172–173°.[4] Isopropylamine was selected since its hydrochloride is extremely soluble in alcohol,[16] more so than any of the other common aliphatic amines.

N-Phenylthiocarbamyl Derivative of Puromycin (III).—A mixture of 50 g. of puromycin dihydrochloride,[4] 500 cc. of absolute alcohol, 26 cc. of triethylamine and 12 cc. of phenyl isothiocyanate was refluxed for 1 hour, solution taking place at the b.p. The hot solution was poured in a thin stream into 4 l. of ice and water with good manual stirring. The white precipitate was collected, washed with water and air-dried; yield 57 g. (102%) of material suitable for the next step. This material is hydrated and had m.p. 145–150° with shrinking at 110°. In a similar run 6.0 g. of air dried material was heated to boiling with 25 cc. of ethyl acetate. The solid dissolved and the anhydrous material rapidly separated at the b.p. The mixture was cooled and filtered; wt. 4.7 g., m.p. 173–174°. From the filtrate by the addition of heptane was obtained an additional 0.52 g. (87% recovery) with the same m.p. Recrystallization from ethyl acetate afforded white crystals, m.p. 174–175°, $[\alpha]^{25}D$ −46° (2% in acetone). A mixture with puromycin base melted at 155–160°.

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.

6-Dimethylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)-purine. (Aminonucleoside) (IV). (A).—To a stirred solution of 5.9 g. of sodium methoxide (Mathieson) in 390 cc. of methanol was added 57 g. of crude, air-dried III. The mixture was refluxed and stirred until solution was complete, then refluxed 1 hour more. At the b.p. the solid changed crystal form and about 45 minutes was required to complete solution. The hot solution was filtered, then cooled and stirred in an ice-bath for 2 hours. The product was collected and washed with five 10-cc. portions of ice-cold methanol; yield 17.6 g. (66% from puromycin dihydrochloride), m.p. 214–216°. Recrystallization from absolute alcohol gave white crystals, m.p. 215–216°, $[\alpha]^{25}D$ −24.6° (3% in H_2O), $\lambda_{max}^{pH\ 1}$ 269 mμ (ε 18,600); $\lambda_{max}^{H_2O}$ 276 mμ (ε 18,900); $\lambda_{max}^{pH\ 14}$ 275 mμ (ε 17,000).

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.

(B).—A mixture of 1.00 g. of pure III, 5.0 cc. of methanol and 1.7 cc. of 1 *N* methanolic sodium hydroxide was refluxed until solution was complete (1 hour), then for 1 hour longer. Cooling gave 0.32 g. (49%) of product, m.p. 202–204°. Recrystallization from absolute alcohol afforded white crystals, m.p. and mixed m.p. with preparation A, 214–215°.

(C).—A solution of 500 mg. of puromycin base (II) in 5 cc. of 1 *N* methanolic sodium hydroxide was refluxed for 20 hours, then cooled in an ice-bath for 2 hours. The product was collected and washed with methanol; yield 160 mg., m.p. 213–214°, which gave no depression when mixed with preparation A. An additional 30 mg. (total 60%), was obtained from the filtrate.

(D).—A solution of 500 mg. of puromcyin base (II) and 0.14 cc. of phenyl isothiocyanate in 3 cc. of methanol was

(16) A. W. Hofmann, *Ber.*, **15**, 762 (1882).

refluxed for 2 hours. Then 1.15 cc. of 1 *N* methanolic sodium methoxide was added and refluxing continued for 1 hour more. Cooling gave 210 mg. (65%) of product, m.p. and mixed m.p. with preparation A, 214–215°. This is an over-all yield of 47% from puromycin dihydrochloride.

(E).—A solution of 100 mg. of IXa in 5 cc. of 0.5 *N* barium hydroxide was heated on the steam-bath for 1 hour. After addition of 5 cc. of water, the excess baryta was removed with Dry Ice. The filtrate was evaporated to dryness *in vacuo*. A solution of the residue in 3 cc. of water was clarified by filtration and again evaporated *in vacuo*. Trituration of the residue with 3 cc. of ethyl acetate gave 70 mg. (80%) of product, m.p. 209–211°. A mixture with preparation A melted at 212–214°.

6-Dimethylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)-purine Triacetate (IXb).—A solution of 3.0 g. of VI in 17 cc. of reagent pyridine and 9 cc. of acetic anhydride was allowed to stand in a stoppered flask for 21 hours, then poured into 150 cc. of ice-water. The solution was extracted with five 20-cc. portions of chloroform. The combined extracts were evaporated to dryness *in vacuo*. Trituration of the residue with 25 cc. of ether gave 4.0 g. (93%) of product, m.p. 183–188°. Recrystallization from ethyl acetate gave white crystals, m.p. 189–191°, $[\alpha]^{25}D$ +24° (1.7% in $CHCl_3$).

Anal. Calcd. for $C_{18}H_{24}N_6O_6$: C, 51.4; H, 5.77; N, 20.0. Found: C, 51.6; H, 5.84; N, 20.0.

6-Dimethyl-9-(3′-acetamino-3′-deoxy-β-D-ribofuranosyl)-purine (IXa). (A).—To a solution of 500 mg. of VI in 2.5 cc. of water was added 0.24 cc. of acetic anhydride. The mixture was vigorously shaken for 2 minutes, then evaporated to dryness *in vacuo*. Trituration with hot ethyl acetate, then cooling gave 500 mg. (85%) of product, m.p. 186–188°. Recrystallization from alcohol afforded white crystals, m.p. 190–191°, $[\alpha]^{25}D$ −7.4° (4.3% in pyridine).

Anal. Calcd. for $C_{14}H_{20}N_6O_4$: C, 50.3; H, 6.00; N, 25.0. Found: C, 50.0; H, 6.20; N, 24.7.

(B).—A solution of 200 mg. of IXb in 5 cc. of methanol and 0.05 cc. of 1 *N* methanolic sodium methoxide was refluxed for 30 minutes, then evaporated to dryness *in vacuo* leaving 150 mg. (91%) of product, m.p. and mixed with preparation A, 187–189°. A mixture with IXb gave a 30° depression in m.p.

N-Acetyl-*p*-methoxy-L-phenylalanine.—To a stirred mixture of 45 g. of L-tyrosine, 500 cc. of water and a few drops of 60% Aerosol OT heated to 90° on the steam-bath was added dropwise 190 cc. of acetic anhydride over 15 minutes without application of further heat. Near the end of the addition solution took place. After being stirred for 10 minutes longer the solution was evaporated to dryness *in vacuo*, leaving a yellow gum of N-acetyl-L-tyrosine. The gum was dissolved in a warm solution of 30 g. of sodium hydroxide in 140 cc. of water. Then 41 cc. of methyl sulfate was added dropwise with stirring over a period of about 15 minutes. Near the end of the addition it was necessary to add 40 cc. of 10% sodium hydroxide in portions to maintain alkalinity. The solution was heated on the steam-bath for 20 minutes longer, then cooled and acidified. A gum separated which was extracted with chloroform. The product soon began to separate from the chloroform solution. After several hours at 0°, the mixture was filtered and the product washed with chloroform; yield 29 g. (50%), m.p. 144–145°.

This procedure is a modified combination of those described by Karrer[17] and Behr and Clarke.[18] Karrer recorded a m.p. of 147–148° and a yield of 50% from crystalline N-acetyl-L-tyrosine.

***p*-Methoxy-L-phenylalanine Hydrochloride.**—A solution of 14 g. of N-acetyl-*p*-methoxy-L-phenylalanine in 70 cc. of 6 *N* hydrochloric acid was refluxed for 2 hours, then cooled in an ice-bath. The product was collected and washed with cold 6 *N* hydrochloric acid; yield 10.8 g. (79%), m.p. 230–232° dec. No attempt was made to isolate additional product from the filtrate.

Behr and Clarke[18] used a 72-hour sulfuric acid hydrolysis followed by a complicated work-up of the readily soluble sulfate salt. They prepared the hydrochloride by solution of the free amino acid in hot hydrochloric acid, followed by cooling and recorded m.p. 237–238° dec.

***p*-Methoxy-L-phenylalanine.**—To a hot solution of 1.00 g. of the preceding hydrochloride in 10 cc. of water was added 0.3 cc. of 15 *N* ammonium hydroxide. The amino acid rapidly separated as glistening plates. The mixture was cooled, filtered and the product washed with water; yield 0.60 g. (71%), m.p. 250–252° dec. Behr and Clarke[18] record a m.p. of 264–265° dec.

Methyl N-Acetyl-*p*-methoxy-L-phenylalanate.—To a solution of 2.0 g. of N-acetyl-*p*-methoxy-L-phenylalanine in 20 cc. of methanol was added dropwise with swirling 1 cc. of acetyl chloride.[19] After being refluxed for 30 minutes, the solution was evaporated to dryness *in vacuo*. Trituration of the residue with aqueous sodium bicarbonate gave 1.8 g. (86%) of product, m.p. 89–91°. Recrystallization from toluene afforded white crystals, m.p. 104–106°.

Synge[20] recorded m.p. 106–107° for this compound prepared in a different manner.

Methyl *p*-Methoxy-L-phenylalanate Hydrochloride.—To a solution of 1.00 g. of *p*-methoxy-L-phenylalanine hydrochloride in 10 cc. of methanol was added 1 cc. of acetyl chloride dropwise with swirling. The solution was refluxed for 30 minutes, then evaporated to dryness *in vacuo* leaving 1.00 g. (95%) of white solid, m.p. 172–176° with shrinking at 165°. Recrystallization from methanol–chloroform gave white needles, m.p. 180–182° dec. The chloroform clings tenaciously to this compound and is only completely removed in high vacuum at 80° after about 5 hours. This compound was identical with one of the methanolysis products of puromycin.[3]

Anal. Calcd. for $C_{11}H_{15}NO_3 \cdot HCl$: C, 53.7; H, 6.56; N, 5.69. Found: C, 53.5; H, 6.76; N, 5.83.

N-Phthalyl-*p*-methoxy-L-phenylalanyl Chloride and Anilide.—A mixture of 250 mg. of N-phthalyl-*p*-methoxy-L-phenylalanine[21] and 5 cc. of thionyl chloride was refluxed for 1 hour, then evaporated to dryness *in vacuo* (bath 50°). The residual acid chloride, which did not crystallize, was treated with 0.29 cc. of aniline in 5 cc. of acetone. Addition of 5 cc. of water gave 250 mg. (81%) of anilide, m.p. 179–181°. Recrystallization from absolute alcohol afforded white crystals, m.p. 180–181°, $[\alpha]^{25}D$ −107° (1.5% in $CHCl_3$).

Anal. Calcd. for $C_{24}H_{20}N_2O_4$: C, 72.0; H, 5.02; N, 7.00. Found: C, 71.8; H, 5.23; N, 6.87.

Balenovic, Thaller and Filipovic[10] have recorded a m.p. of 81–82° for the acid chloride.

N-Phthalylpuromycin (Vb) (A).—A solution of the acid chloride from 250 mg. of N-phthalyl-*p*-phenylalanine, prepared as in the preceding experiment, in 5 cc. of acetone was added dropwise with swirling to a solution of 250 mg. of the aminonucleoside VI and 250 mg. of anhydrous sodium carbonate in 5 cc. of water cooled in an ice-bath. After 30 minutes at 0° the mixture was diluted with 20 cc. of water, filtered and the product washed with water; yield 370 mg. (80%), m.p. 228–230°. Recrystallization from methyl Cellosolve gave white crystals, m.p. 230–231°, $[\alpha]^{24}D$ −140° (0.8% in pyridine).

Anal. Calcd. for $C_{30}H_{31}N_7O_7$: C, 59.9; H, 5.22; N, 16.3. Found: C, 59.5; H, 5.44; N, 16.5.

(B).—A mixture of 1.00 g. of puromycin base (II), 0.35 g. of phthalic anhydride and 5 cc. of diethyl Carbitol was heated in a bath at 160–165° until the solid dissolved and another separated (about 2 minutes), then for an additional 5 minutes. The mixture was cooled to 0°, filtered and the product washed with alcohol; yield 0.77 g. (60%) of a tan solid, m.p. and mixed m.p. with preparation A 228–230°.

In a pilot run the yield was 66% (170 mg.), m.p. 228–230°. When only one-fifth of the above quantity of solvent was employed, no reaction took place at 160°. With no solvent a temperature of 180° was required for fusion and the product, obtained in 59% yield, was of poor quality, m.p. 215–225°.

N-Carbobenzoxy-*p*-methoxy-L-phenylalanine.—To a stirred solution of 10 g. of *p*-methoxy-L-phenylalanine in 45 cc. of 10% sodium hydroxide and 19 cc. of water cooled in an ice-bath was added 17 cc. of 70% carbobenzoxy chlo-

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(18) L. D. Behr and H. T. Clarke, THIS JOURNAL, **54**, 1630 (1932).

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TABLE I

CARBOXYBENZOXYAMINOACYL ANALOGS (VIII) OF PUROMYCIN

RCHNH$_2$-CO-[a]	Method	Yield, %	$[\alpha]^{24}D$[b]	M.p., °C.	Analyses, % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
Glycyl[13]	A	63	− 7.5	170–172[c]	54.4	5.63	20.2	54.6	5.70	19.9
L-Phenylalanyl[24]	A	71	−28	213–214[c]	60.6	5.79	17.1	60.7	6.04	17.0
O-Cbz-L-tyrosyl[25]	A	62	−20	199–201[c]	61.3	5.42	13.5	61.3	5.55	13.2
L-Tryptophyl[26]	A	63	− 7.2	233–234[c]	60.5	5.58	18.3	60.6	5.86	18.0
L-Leucyl[d]	A	59	−34	213–215[c]	57.7	6.53	18.2	57.5	6.60	18.6
p-MeO-L-phenylalanylglycyl[d]	B[e]	56	−10	160–161[k]	58.1	5.80	16.9	57.8	5.83	16.8
N′-Cbz-L-lysyl[h]	B[f]	54	−27	213–215[c]	59.2	6.13	16.3	59.5	6.15	16.5
β-Alanyl[27]	A[g]	69	−16	197–199[c]	55.3	5.89	19.6	55.5	5.78	19.5
Glycyl-*p*-MeO-L-phenylalanyl	[d]	50	−23	193–195[i]	58.1	5.80	17.0	57.7	5.85	17.3
Bis-*p*-MeO-L-phenylalanyl	[d]	56	−23	193–196[j]	59.3[j]	6.05	13.9	59.6	5.79	13.8

[a] Reference to acid chloride or azide used for coupling. [b] In pyridine (2–3%). [c] Recrystallized from alcohol. [d] See Experimental. [e] Method A gave a 52% yield. See experimental for acid chloride. [f] Method A gave no crystalline product. [g] Method B (see ref. 28 for azide) gave a 66% yield. [h] The azide was prepared from the hydrazide[24] according to ref.[27] since the described procedure[24] gave only unchanged hydrazide. [i] Recrystallized from methanol. [j] This compound crystallizes as the sesquihydrate. Calcd.: $1^1/_2H_2O$, 3.33. Found: H_2O, 3.33 (Fischer). [k] Recrystallized from chloroform.

ride[22] dropwise at such a rate that the temperature was 5–8° (15 minutes). After being stirred in the ice-bath for an additional hour, the mixture was acidified and extracted with 50 cc., then 20 cc. of chloroform. The combined chloroform extracts were back-extracted with excess aqueous sodium bicarbonate. The alkaline extracts were acidified and again extracted with 50 cc., then 20 cc. of chloroform. The extracts, dried with magnesium sulfate, were evaporated to dryness *in vacuo* leaving 14.6 g. (87%) of white solid, m.p. 104–109°, suitable for the next step. Similar yields were obtained starting with amino acid hydrochloride.

Rivers and Lerman[23] record a m.p. of 112° for this compound prepared by O-methylation of N-carbobenzoxy-L-tyrosine methyl ester followed by hydrolysis.

N-Carbobenzoxypuromycin (Va). (A).—To a stirred mixture of 2.0 g. of puromycin dihydrochloride,[4] 1.5 g. of sodium carbonate, 5 cc. of water and 5 cc. of acetone cooled in an ice-bath was added dropwise 0.95 cc. of 70% carbobenzoxy chloride.[22] After 30 minutes in an ice-bath, the solid was collected, and washed successively with two 5-cc. portions of 50% acetone and finally 10 cc. of water. The crude material was recrystallized from alcohol to give 1.2 g. (50%) of product, m.p. 199–200°. Further recrystallization from alcohol gave white crystals, 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.

(B).—To a solution of 300 mg. of N-carbobenzoxy-*p*-methoxy-L-phenylalanine in 2 cc. of dimethylformamide containing 0.13 cc. of triethylamine cooled in an ice-bath was added 0.09 cc. of ethyl chlorocarbonate. After 10 minutes the mixed anhydride formation[12] was complete. A solution of 190 mg. of aminonucleoside VI in 3 cc. of dimethylformamide and 0.19 cc. of triethylamine was warmed to complete solution, then quickly cooled to 0° and treated with the mixed anhydride solution. After 20 hours at 3° protected from moisture, the mixture was concentrated to about one-third volume *in vacuo* then diluted with several volumes of water. The product was collected and washed with water; yield 320 mg. (82%), m.p. 193–196°. Recrystallization from 10 cc. of alcohol gave 250 mg. (64%) of white crystals, m.p. 208–210°. Admixture with preparation A gave no depression in m.p. When the reaction was run for 2 hours at 3°, the yield was only 18%.

Puromycin (II). (A).—A mixture of 230 mg. of Vb, 0.022 cc. of 100% hydrazine hydrate and 1.2 cc. of methyl Cellosolve was heated on the steam-bath until solution was complete (4 minutes), then for an additional 3 minutes when some solid separated. The mixture was evaporated to dryness *in vacuo*. The residue was heated on the steam-bath with 1.15 cc. of methyl Cellosolve and 0.23 cc. of acetic acid until solution was complete (5 minutes), then for an additional 5 minutes. The solution was evaporated to dryness *in vacuo*. The residue was triturated with 5 cc. of water and filtered. The insoluble precipitate consisted of 62 mg. (98%) of alkali soluble phthalhydrazide and 7 mg. (3%) of alkali-insoluble starting material, m.p. 218–225° dec.

The aqueous filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in 2 cc. of alcohol and neutralized with 0.12 cc. of triethylamine. After about 18 hours at 3°, the mixture was filtered and the product washed with 0.5 cc. of alcohol; yield 86 mg. (48%) of puromycin, m.p. and mixed m.p. 166–168°. This compound and authentic puromycin base[4] had identical infrared spectra and gave no depression in m.p. when mixed.

Addition of 0.1 cc. of acetic anhydride to the filtrate gave 24 mg. (12%) of N-acetylpuromycin, m.p. 229–231°. A mixture with an authentic sample (m.p. 233–235°[3]) gave no depression in m.p.

(B).—A solution of 500 mg. of Va in 50 cc. of acetic acid was shaken with hydrogen at atmospheric pressure in the presence of 250 mg. of 10% palladium–charcoal catalyst for 5 minutes when hydrogen uptake (65 mole %) ceased. Clarified by filtration through Celite, the solution was evaporated to dryness *in vacuo*. The residue was dissolved in 3 cc. of warm alcohol, then neutralized with 0.2 cc. of isopropylamine. After several hours at 0°, the product was collected and washed with 0.5 cc. of alcohol; yield 0.26 g. (68%), m.p. 167–169°. Admixture with an authentic sample of puromycin gave no depression in m.p.

6-Dimethylamino-9-(3′-phthalylglycylamino-3′-deoxy-β-D-ribofuranosyl)-purine.—To a warm solution of 300 mg. of the aminonucleoside VI and 0.22 cc. of triethylamine in 5 cc. of dimethylformamide cooled rapidly to 15–20° was added 0.25 g. of phthalylglycyl chloride. After 2 minutes the mixture was poured into 50 cc. of ice-water. The solid was collected and washed with water; yield 370 mg. (76%), m.p. 271–272° dec. Recrystallization from Methyl Cellosolve gave white crystals, m.p. 282–284° dec., $[\alpha]^{24}D$ +43° (1% in 9:1 pyridine:H_2O).

Anal. Calcd. for $C_{22}H_{23}N_7O_6$: C, 55.0; H, 4.83; N, 20.3. Found: C, 54.9; H, 4.96; N, 20.3.

When the reaction was run in 50% acetone containing sodium carbonate as described for Vb, the yield was 60% (0.32 g.), m.p. 274–275°. The mixed anhydride method[12] in dimethylformamide as described for Vb gave variable results. The best yield obtained was 53%, m.p. 255–260° dec.

6-Dimethylamino-9-(3′-carbobenzoxyglycylamino-3′-deoxy-β-D-ribofuranosyl)-purine (VIII, R = H).—To a warm solution of 500 mg. of aminonucleoside VI and 0.99 cc. of triethylamine in 20 cc. of dimethylformamide cooled rapidly to 20° was added an ice-cold solution of 450 mg. of freshly prepared carbobenzoxyglycyl chloride[12] in 2 cc. of dimethyl-

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TABLE II

AMINOACYL ANALOGS (DERIVATIVES OF VI) OF PUROMYCIN

R	Yield, %	$[\alpha]^{24}D$[a]	M.p., °C.	Analyses, % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
L-Phenylalanyl	72	−49°	176–178[b]	57.3	6.18	22.3	57.1	6.47	22.0
Glycyl	93	− 8.4°[c]	209–211[d] dec.	47.8	6.02	27.9	47.9	6.19	27.2
L-Tyrosyl	90	−54	203–205[d]	55.1	5.97	21.4	55.0	6.31	21.4
L-Tryptophyl	83	−49	219–221[d] dec.	57.5	5.89	23.3	57.6	5.91	23.5
L-Leucyl	58	− 8.4	173–175[d]	53.1	7.20	24.1	53.4	7.49	23.7
p-MeO-L-phenylalanylglycyl	74	−34	170–172[d]	54.5	6.12	21.2	54.4	6.01	21.3
β-Alanyl	95	−10.7	110–115[e]	48.2	6.48	26.2	48.2	6.77	26.1
L-Lysyl	82[f]	−15	Glass[f]	51.2	7.19	26.4	50.7	7.19	26.1
Glycyl-*p*-MeO-L-phenylalanyl	88	−26	186–188[g] dec.	54.4[g]	6.41	20.3	53.8	6.78	20.3
p-MeO-L-phenylalanyl-*p*-MeO-L-phenylalanyl	81	−37	157–159[h]	56.9	6.41	16.6	57.1	6.63	16.8

[a] All rotations measured in pyridine (2–3%) unless otherwise specified. [b] Recrystallized from chloroform–heptane. [c] In H_2O (3%). [d] Recrystallized from absolute alcohol. [e] Recrystallized from wet ethyl acetate as a hemihydrate. Calcd.: $^1/_2H_2O$, 2.40. Found: H_2O, 2.98 (Fischer). [f] By solution of the crude product in water, clarification with Norit and evaporation. [g] Crystallized from absolute alcohol as gelatinous crystals which cling tenaciously to solvent. The analysis indicated a hemi-alcoholate after drying in high vacuum at 110° for 6 hours. [h] Crystallized from methanol as a sesquihydrate, m.p. 113–115°, resolidifies and remelts at 157–159°. Calcd.: $1^1/_2H_2O$, 4.00. Found: H_2O, 4.12 (Fischer).

formamide. After 10 minutes the mixture was diluted with 150 cc. of water. To the turbid solution was added 5 cc. of chloroform. The mixture was stirred overnight. The solid was collected and washed with 4 cc. of alcohol; yield 520 mg. (63%), m.p. 169–171°. Recrystallization from absolute alcohol gave white crystals, m.p. 170–172°. See Table I for additional data.

Other compounds prepared in the same way, except that the coupling reaction was allowed to proceed 15–20 hours and the chloroform was omitted for crystallization, are listed in Table I under method A.

6-Dimethylamino-9-(3′-glycylamino-3′-deoxy-β-D-ribofuranosyl)-purine (VII, R = H). (A).—A solution of 4.0 g. of the corresponding pure carbobenzoxy derivative (VIII, R = H) in 30 cc. of methyl Cellosolve was stirred on the steam-bath with 0.3 g. of Norit for 20 minutes, then filtered. To the filtrate was added 0.8 g. of 10% palladium–charcoal catalyst. A stream of hydrogen was passed through the solution heated in a bath at 60–70° until the evolution of carbon dioxide, as tested with barium hydroxide on the exit gases, was complete (1.5 hours). The catalyst was removed by filtration through Celite. Evaporation of the combined filtrate and washings to dryness *in vacuo* left 2.69 g. (93%) of product, m.p. 209–213° dec. Recrystallization of a similar preparation from absolute alcohol gave white crystals, m.p. 209–211° dec., $[\alpha]^{23}D$ −8.4° (3% in H_2O).

Similar reductions for the preparation of other VIII derivatives are listed in Table II.

(B).—Treatment of 6-dimethylamino-9-(3′-phthalylglycylamino-3′-deoxy-β-D-ribofuranosyl)-purine with hydrazine as described for Vb gave 55% of product, m.p. 201–202° dec. Admixture with preparation A gave no depression in m.p.

N-Carbobenzoxy-L-leucyl Chloride.—To a magnetically stirred solution of 1.00 g. of N-carbobenzoxy-L-leucine[29] in 10 cc. of reagent ether cooled in an ice-bath was added 0.87 g. of phosphorus pentachloride. The mixture was stirred for two hours at 0° protected from moisture. The ether was removed *in vacuo* (bath 0°). To the remaining oil was added 25 cc. of cold petroleum ether (20–40°) and the evaporation repeated. The residual oily acid chloride was washed with cold petroleum ether (20–40°) by decantation, then immediately dissolved in dimethylformamide and coupled with the aminonucleoside VI (0.92 g.) in the usual fashion.

Ethyl N-Carbobenzoxy-*p*-methoxy-L-phenylalanylglycinate.—To a solution of 3.6 g. of N-carbobenzoxy-*p*-methoxy-L-phenylalanine in 10 cc. of chloroform and 1.5 cc. of triethylamine cooled to 5° in an ice-bath was added 1.1 cc. of ethyl chlorocarbonate.[12] After 15 minutes at 0° a solution of 1.54 g. of ethyl glycinate hydrochloride in 10 cc. of dimethylformamide containing 1.5 cc. of triethylamine was added. An immediate brisk evolution of carbon dioxide took place which was complete in 15 minutes. The mixture was then diluted with 50 cc. of water. The separated aqueous layer was extracted with two 20-cc. portions of chloroform. The combined chloroform solutions, washed with excess aqueous sodium bicarbonate and dried with magnesium sulfate, were evaporated to dryness *in vacuo*. Crystallization of the semi-solid residue (5.0 g.) from absolute alcohol gave 3.3 g. (73%) of product, m.p. 131–133°. Further recrystallization from the same solvent afforded white crystals, m.p. 132–134°, $[\alpha]^{24}D$ 0.0° (2% in $CHCl_3$).

Anal. Calcd. for $C_{22}H_{26}N_2O_6$: C, 63.7; H, 6.34; N, 6.75. Found: C, 63.6; H, 6.20; N, 6.71.

N-Carbobenzoxy-*p*-methoxy-L-phenylalanylglycine.—To a solution of 200 mg. of the corresponding ester in 6 cc. of alcohol was added 0.24 cc. of 10% aqueous sodium hydroxide. After 15 minutes the solution was acidified with acetic acid, concentrated to a thin syrup *in vacuo*, then diluted with water. The product was collected and washed with water; yield 130 mg. (73%), m.p. 155–157°. Recrystallization from chloroform gave white crystals, m.p. 157–159°, $[\alpha]^{24}D$ −17° (1.5% in pyridine).

Anal. Calcd. for $C_{20}H_{22}N_2O_6$: C, 62.1; H, 5.75; N, 7.25. Found: C, 61.8; H, 5.83; N, 7.11.

N-Carbobenzoxy-*p*-methoxy-L-phenylalanylglycyl Chloride.—A mixture of 1.6 g. of the corresponding acid, 15 cc. of anhydrous ether and 1.0 g. of phosphorus pentachloride was magnetically stirred in an ice-bath protected from moisture for 1 hour. During this time the solid changed over from the acid to the acid chloride and was collected, then washed with petroleum ether; yield 1.6 g. (95%), m.p. 88–92° dec. This material was not further purified, but was immediately coupled with the aminonucleoside VI in the usual fashion.

N-Carbobenzoxy-*p*-methoxy-L-phenylalanylglycyl Hydrazide.—A solution of 5.5 g. of the corresponding ester and 2.1 cc. of 100% hydrazine hydrate in 15 cc. of absolute alcohol was refluxed for 2 hours. Evaporation to dryness *in vacuo* and trituration with heptane gave 4.8 g. (91%) of product, m.p. 151–153°, suitable for the next step. Recrystallization of a sample from 50% alcohol afforded white crystals, m.p. 158–160°, $[\alpha]^{24}D$ −10.9° (2% in pyridine).

Anal. Calcd. for $C_{20}H_{24}N_4O_5$: C, 60.0; H, 6.08; N, 14.1. Found: C, 59.8; H, 5.94; N, 14.0.

6-Dimethylamino-9-(3′-N-carbobenzoxy-*p*-methoxy-L-phenylalanylglycylamino-3′-deoxy-β-D-ribofuranosyl)-purine.—To a stirred mixture of 1.18 g. of N-carbobenzoxy-*p*-methoxy-L-phenylalanylglycyl hydrazide, 11.8 cc. of water, 2.4 cc. of acetic acid, 5 cc. of chloroform and 2.4 cc. of 5 *N* hydrochloric acid cooled to 5° in an ice-bath was added dropwise a solution of 0.24 g. of sodium nitrite in 5 cc. of water over a period of about 10 minutes. The hydrazide gradually dissolved and stirring was continued at 0° for an additional 15 minutes after solution was complete. The chloroform layer was separated and washed several times

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with ice-water until the *p*H of the washings was 4. Dried with magnesium sulfate, the chloroform solution of azide was added to a solution of 0.87 g. of aminonucleoside VI in 20 cc. of dimethylformamide. After 18 hours at room temperature protected from moisture the mixture was poured into 150 cc. of water and stirred for 90 minutes during when the chloroform evaporated and the product crystallized. The solid was collected and washed with ethyl acetate; yield 1.10 g. (56%), m.p. 153–155°. See Table I for additional data. Other compounds prepared by this method are listed in Table I under method B.

N-Carbobenzoxyglycylpuromycin (I, R = H). (A).—To a solution of 4.0 g. of puromycin (II) dihydrochloride[4] in 20 cc. of dimethylformamide and 3.1 cc. of triethylamine cooled in an ice-bath was added the mixed anhydride from 1.84 g. of carbobenzoxyglycine, 1.24 cc. of triethylamine and 0.87 cc. of ethyl chlorocarbonate in 10 cc. of dimethylformamide prepared in the usual manner.[12] After 18 hours at room temperature protected from moisture, the mixture was poured into 150 cc. of water and the oil collected by two 25-cc. extractions with chloroform. The combined extracts were washed with 90 cc. of 0.1 *N* hydrochloric acid in portions to remove unchanged puromycin, then with excess aqueous sodium bicarbonate. Dried with magnesium sulfate, the chloroform was evaporated to dryness *in vacuo*. The oily residue was crystallized from 20 cc. of methanol; yield 2.2 g. (45%), m.p. 183–186°. Additional data are listed in Table I.

(B).—To a solution of 5.8 g. of puromycin dihydrochloride[4] in 25 cc. of dimethylformamide and 7.2 cc. of triethylamine was added a solution of carbobenzoxyglycyl chloride (freshly prepared from 2.68 g. of acid)[13] in 5 cc. of dimethylformamide. After 18 hours the reaction mixture was processed as in procedure A; yield 3.5 g. (50%), m.p. 189–191°. When the coupling reaction was run for 2 hours, the yield was only 35%.

6-Dimethylamino-9-(3′-N-carbobenzoxy-*p*-methoxy-L-phenylalanyl-*p*-methoxy-L-phenylalanylamino-3′-deoxy-β-*d*-ribofuranosyl)-purine (I, R = *p*-$MeOC_6H_4CH_2$-).—To a solution of the mixed anhydride from 5.04 g. of N-carbobenzoxy-*p*-methoxy-L-phenylalanine, 2.2 cc. of triethylamine and 1.52 cc. of ethyl chlorocarbonate in 25 cc. of dimethylformamide (see preparation of Va) was added a solution of 7.0 g. of puromycin (II) dihydrochloride[4] in 35 cc. of dimethylformamide and 5.4 cc. of triethylamine. After 18 hours at room temperature protected from moisture, the mixture was poured into 250 cc. of water and allowed to stand for 1 hour. The product was collected and washed with water, then 25 cc. of cold absolute alcohol; yield 7.8 g. (78%), m.p. 189–192°. See Table I for additional data.

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[CONTRIBUTION FROM THE CHEMICAL AND BIOLOGICAL RESEARCH SECTION, LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID COMPANY]

Puromycin. Synthetic Studies. VIII. Synthesis of 3-Amino-3-deoxy-D-ribofuranoside Derivatives. A Second Synthesis of 3-Amino-3-deoxy-D-ribose

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A synthesis of methyl 2,5-di-*O*-acetyl-3-acetamido-3-deoxy-D-ribofuranoside (XX), useful for the total synthesis of puromycin, is described starting with D-xylose and proceeding through the key intermediates, methyl D-xylofuranoside, methyl 2,3-anhydro-D-lyxofuranoside and methyl 3-acetamido-3-deoxy-D-arabinofuranoside. Acid hydrolysis of methyl 2,5-di-*O*-acetyl-3-acetamido-3-deoxy-D-ribofuranoside (XX) completes a second synthesis of 3-amino-3-deoxy-D-ribose. The synthesis of another 3-aminopentose, namely, 3-amino-3-deoxy-D-arabinose, is described also.

The identity of the aminopentose moiety from puromycin with 3-amino-3-deoxy-D-ribose, synthesized from methyl β-L-arabinopyranoside, has been the subject of a previous paper in this series.[2] Since degradation studies have revealed that the 3-amino-3-deoxy-D-ribose is attached in the β-furanose form to 6-dimethylaminopurine,[3] it would be necessary to obtain 3-amino-3-deoxy-D-ribose in a blocked furanose form as an intermediate for the total synthesis of the antibiotic. The necessary furanose derivative, methyl 2,5-di-*O*-acetyl-3-acetamido-3-deoxy-D-ribofuranoside (XX), has now been obtained during a second synthesis of 3-amino-3-deoxy-D-ribose *via* methyl D-xylofuranoside. The conversion of XX to puromycin is the subject of the accompanying paper IX of this series.

Starting with 3-amino-3-deoxy-D-ribose[2] it should be possible to obtain a blocked furanose derivative in an additional five to seven steps.[4] The same key transformations described in the first synthesis of 3-amino-3-deoxy-D-ribose[2] were applied to a known sugar furanoside, methyl D-xylofuranoside (II),[10] and the number of steps in the total synthesis were considerably reduced.

Levene, Raymond and Dillon[10] have made an extended study of the proportion of furanosides and pyranosides formed when a number of sugars were treated with methanol containing 0.5% hydrogen chloride at 25°. Of particular interest was the data obtained with D-xylose. They found that in 5 hours an 87% yield of α- and β-methyl D-xylofuranosides were formed mixed with about 5% free D-xylose and 6% of the pyranose ethers. Percival and Zobrist[11] treated this mixture with acetone, anhydrous copper sulfate, sulfuric acid and acetalde-

(1) To whom inquiries concerning this paper should be directed.

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(4) Direct acylation of sugars generally leads predominately to acyl derivatives of the pyanose form. For example, benzoylation of D-ribose in pyridine gives 35% of tetra-*O*-benzoyl-β-D-ribopyranose and an unspecified amount of the corresponding α-pyranose.[5] Benzoylation of D-xylose under conditions considered most favorable for furanoside derivatives gave 62% of β-D-xylopyranoside tetrabenzoate, 3% of α-furanose and traces of β-furanose.[6] Preparation of tetra-*O*-acetyl-D-ribofuranose by an unequivocal synthesis required four steps and gave an over-all yield of 24%.[6] Later Zinner[7] found conditions for increasing the proportion of β-D-ribofuranose tetraacetate to 35% on direct acetylation of D-ribose. The synthesis of the furanose tetraacetate of D-xylose requires seven steps[8] and has recently been accomplished in 5 steps.[9]

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