the reaction was stopped. The solution was filtered, acidified with methanolic hydrogen chloride and evaporated to dryness. The crystalline residue was recrystallized from an isopropyl alcohol–ether mixture and melted at 216–218° (X).

Anal. Calcd. for $C_{15}H_{23}N\cdot HCl$: C, 71.0; H, 9.5; N, 5.5. Found: C, 70.7; H, 9.6; N, 5.5.

N-Cyclohexylmethyl-N-methylbenzylamine (X).—A solution of 7.1 g. (50 mmoles) of N-methylcyclohexanecarboxamide[4] (XI) in 80 ml. of toluene was refluxed with 2 g. (50 mmoles) of sodium amide for 3 hours. After cooling, 8.5 g. (50 mmoles) of benzyl bromide was added and refluxing continued for 3 hours. The reaction mixture was cooled, filtered, concentrated and the residue was distilled *in vacuo;* yield 8.5 g. (74%) of N-benzyl-N-methylcyclohexanecarboxamide (XII), b.p. 146–149° (1 mm.).

Anal. Calcd. for $C_{15}H_{21}NO$: C, 77.9; H, 9.2. Found: C, 77.6; H, 9.1.

To a suspension of 1.7 g. (45 mmoles) of lithium aluminum hydride in 120 ml. of anhydrous ether, 6.95 g. (30 mmoles) of the above amide was added. The reaction mixture was stirred at 25° for 18 hours, then decomposed by adding 5.1 ml. of ethyl acetate, 1.7 ml. of water, 3.4 ml. of 15% aqueous sodium hydroxide and 5.1 ml. of water, filtered and concentrated. The residue was distilled *in vacuo* and yielded 5.2 g. (79%) of N-cyclohexylmethyl-N-methylbenzylamine, b.p. 106–108° at 1 mm. The hydrochloride prepared from this base, after recrystallization from isopropyl alcohol–ether melted at 216–218°.

Anal. Calcd. for $C_{15}H_{23}N\cdot HCl$: C, 71.0; H, 9.5. Found: C, 70.8; H, 9.4.

4-Acetamino-3-ethylacetophenone (XIV).—To a mixture of 50 g. (0.3 mole) of 2-ethylacetanilide,[8] 225 g. (1.69 moles) of aluminum chloride and 125 g. of carbon disulfide, 80 g. (0.78 mole) of acetyl chloride was added gradually. The reaction is exothermic and was completed by warming on a steam-bath for 5 minutes. After distilling off the carbon disulfide and excess acetyl chloride, the red mass was poured on to ice and the reaction product extracted with benzene. After recrystallization from an ethyl acetate–ether mixture the product melted at 113–115°, yield 36 g. (57%).

Anal. Calcd. for $C_{12}H_{15}NO_2$: C, 70.2; H, 7.4. Found: C, 70.2; H, 7.3.

(8) H. Paucksch, *Ber.*, **17**, 767 (1884); J. v. Braun, O. Bayer and G. Blessing, *ibid.*, **57**, 392 (1924).

4-Acetamino-3-ethylbenzoic Acid.—To a cooled solution of 24 g. (0.15 mole) of bromine in 130 ml. of water containing 17.4 g. (0.43 mole) of sodium hydroxide, 10 g. (0.049 mole) of 4-acetamino-3-ethylacetophenone dissolved in 25 ml. of dioxane was added dropwise with stirring at 0°. After 3 hours the reaction mixture was partially neutralized and extracted with chloroform. The aqueous solution was acidified to *p*H 2 which precipitated the acid. After cooling, the acid was filtered off and recrystallized from isopropyl alcohol; yield 4.0 g. (40%), m.p. 277–280°.

Anal. Calcd. for $C_{11}H_{13}NO_3$: C, 63.7; H, 6.3. Found: C, 63.5; H, 6.3.

4-Amino-3-ethylbenzoic Acid (XV).—A suspension of 15 g. of 4-acetamino-3-ethylbenzoic acid in 50 ml. of concentrated hydrochloric acid was refluxed for 4 hours. After cooling, the amino acid hydrochloride was filtered off and dissolved in alcohol. The solution was adjusted to *p*H 5 by adding alcoholic sodium hydroxide. After filtering off the sodium chloride the solution was concentrated *in vacuo* and the residue recrystallized from an alcohol–water mixture; yield 6 g. (50%), m.p. 152–154°.

Anal. Calcd. for $C_9H_{11}NO_2$: C, 65.4; H, 6.7. Found: C, 65.7; H, 6.6.

***cis*-4-Amino-3-ethylcyclohexanecarboxylic Acid (XVI).**—Hydrogenation of 8.3 g. (50 mmoles) of 4-amino-3-ethylbenzoic acid in 100 ml. of water with 1.0 g. of platinum oxide (hydrogen uptake 2.71 l. in 24 hours, calcd. 3.36 l.), after removal of the water, gave a sirupy residue. On addition of alcohol, crystals separated. After recrystallization from water–alcohol 4.0 g. (46%) of a *cis*-4-amino-3-ethylcyclohexanecarboxylic acid, m.p. 253–255°, was obtained.

Anal. Calcd. for $C_9H_{17}NO_2$: C, 63.1; H, 10.0. Found: C, 63.2; H, 9.8.

6-Ethyl-3-isoquinuclidone (XVII).—The *cis*-4-amino-3-ethylcyclohexanecarboxylic acid (2.5 g.) was cyclized to 6-ethyl-3-isoquinuclidone by heating to 250° for 3 minutes. After cooling, the melt was dissolved in benzene, filtered and evaporated to dryness. The residue was recrystallized from hexane; yield 2.0 g., m.p. 77–80°; infrared absorption band of –CO–NH– group 1707 cm.$^{-1}$.

Anal. Calcd. for $C_9H_{15}NO$: C, 70.6; H, 9.9. Found: C, 70.4; H, 9.6.

SUMMIT, NEW JERSEY

[CONTRIBUTION FROM THE BIOCHEMICAL AND ORGANIC CHEMICAL RESEARCH SECTIONS, RESEARCH DIVISION, AMERICAN CYANAMID CO.]

The Structure of the Antibiotic Puromycin[1,2]

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The oxidation of puromycin with alkaline permanganate yielded anisic acid. The cleavage of puromycin with alcoholic hydrogen chloride gave 6-dimethylaminopurine dihydrochloride, the ester of *p*-methoxy-L-phenylalanine hydrochloride and 3-amino-3-deoxy-D-ribose hydrochloride. From the chemical and physical data on the antibiotic and its fragments, puromycin was shown to be 6-dimethylamino-9-[3-deoxy-3-(*p*-methoxy-L-phenylalanylamino)-β-D-ribofuranosyl]-β-purine.

Porter and co-workers[4,5] of these laboratories reported the isolation of a new antibiotic from the substrate of a new species of actinomycete, *Streptomyces albo-niger*.[6] Since the partial structure of this antibiotic[7] showed it to be a purine derivative, puromycin was assigned as its generic name. Porter, *et al.*,[4,5] found that puromycin inhibited the growth of both gram positive and gram negative bacteria *in vitro*. Hewitt and co-workers[8] reported that the antibiotic had curative properties in mice and rabbits infected with *Trypanosoma*

(1) Puromycin is the generic name for Stylomycin, which is the American Cyanamid Co. trademark for puromycin. The trademark Achromycin, associated in some earlier publications with puromycin, has been reassigned as the trademark for the non-related antibiotic tetracycline.

(2) Presented at the Meeting-in-Miniature of the New York Section of the American Chemical Society, New York, February, 1954, and at the 126th Meeting of the American Chemical Society, New York, September, 1954.

(3) To whom inquiries should be addressed.

(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 & Chemotherapy*, **2**, 409 (1952).

(5) J. N. Porter, G. C. Krupka and N. Bohonos, U. S. Patent 2,763,642.

(6) C. W. Hesseltine, J. N. Porter, N. Deduck, M. Hauck, N. Bohonos and J. H. Williams, *Mycologia*, **46**, 16 (1954).

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

(8) R. I. Hewitt, W. S. Wallace, A. R. Gumble, E. R. Gill and J. H. Williams, *Am. J. Trop. Med. and Hyg.*, **2**, 254 (1953).

equiperdum. The effects of puromycin on certain experimental tumors were demonstrated by Troy and associates.[9]

General Considerations

Puromycin (I) is a stable, optically active, white, crystalline substance melting at 175.5–177°. It is sparingly soluble in water as well as organic solvents. The empirical formula, $C_{22}H_{29}N_7O_5$, was established from analyses and molecular weight determination. Group analyses showed the presence of one amino nitrogen by Van Slyke's procedure, one O-methyl and two N-methyl groups and five active hydrogens. A Kuhn–Roth determination indicated the absence of terminal methyl groups. An aqueous solution of the compound was basic (pH 8.35), and titration showed it to be a diacidic base with pK_a values of 6.8 and 7.2. The infrared absorption spectrum suggested OH or NH groups, an aromatic nucleus and a substituted carboxamide group. The ultraviolet absorption spectra exhibited maxima in 0.1 N NaOH at 275 mμ (ϵ 20,300) and in 0.1 N HCl at 268 mμ (ϵ 19,500).

The antibiotic readily formed salts with acids and was crystallized routinely as a dihydrochloride, monosulfate or picrate. While puromycin was resistant to methylation with diazomethane, it reacted with acetic anhydride to form a derivative which analyzed for two O-acetyl and one N-acetyl groups. Triacetylpuromycin was partially deacetylated with methanolic ammonia[10] to form N-acetylpuromycin.

Oxidation

When puromycin was dissolved in sodium hydroxide and oxidized with potassium permanganate, a crystalline acid was produced. This product melted at 180.5–182°, gave a neutral equivalent of 157.1 and analyzed for a $C_8H_8O_3$ compound containing one methoxy group. The infrared absorption spectrum showed aromatic bands and also confirmed the presence of a carboxyl group and an ether linkage. The compound was identified as anisic acid (II) by comparison of its chemical and physical properties with those of an authentic sample.

Although the presence of a p-methoxyphenyl moiety in puromycin was thus established, the question arose as to whether an anisoyl (A) or anisylidene (B) type structure was present in the antibiotic. It was noted that the ultraviolet absorption spectrum of puromycin after refluxing with hydrobromic acid showed only a 5 to 7 millimicron bathochromic shift. The ultraviolet absorption spectra of a series of representative models were examined (Table I).

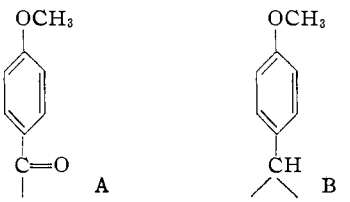


TABLE I

ULTRAVIOLET ABSORPTION CHARACTERISTICS OF PUROMYCIN AND MODEL COMPOUNDS

Compound	0.1 N NaOH Max., mμ	$E \times 10^{-4}$
Puromycin	275	2.03
Prod. of HBr treatment of puromycin	280	2.60
Anisic acid	248	1.37
p-Hydroxybenzoic acid	280	1.63
p-Methoxyacetophenone	275	1.30
p-Hydroxyacetophenone	325	2.31
Anisaldehyde	285	0.20
p-Hydroxybenzaldehyde	330	2.79
Anisalcohol	273	0.10
p-Hydroxybenzyl alcohol	275	.01
Methyl p-tolyl ether	282	.09
p-Cresol	286	.20

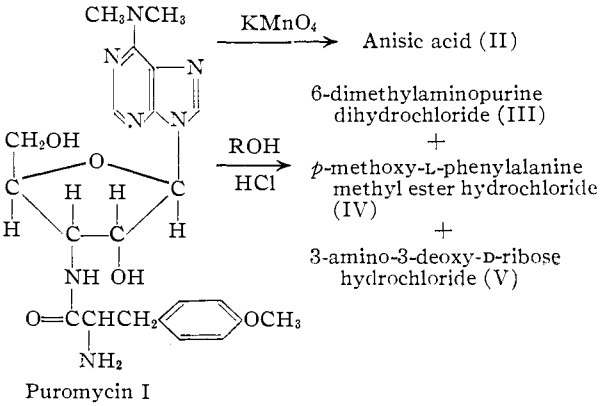
It was found that only those compounds which carried no chromophoric group on the carbon *para* to the methoxy group displayed a similar spectral change upon demethylation. A bathochromic shift of nearly tenfold magnitude was exhibited by the other models. Thus, it was concluded that the anisic acid arose from an anisylidene type (B) structure in the molecule. An examination of the extinction coefficients of the model compounds further indicated that the anisylidene group did not contribute appreciably to the ultraviolet absorption of puromycin.

Hydrolysis, Alcoholysis

Hydrolytic cleavage with concomitant biological inactivation of the antibiotic occurred in dilute acid or on heating in alkali. In contrast to the antibiotic, the hydrolysates consumed periodic acid and gave positive tests with Brady, Tollens and Fehling reagents.

Since aqueous acids caused extensive decomposition, alcoholic hydrogen chloride was used for the further degradation of puromycin (see Chart I).

CHART I



a. 6-Dimethylaminopurine Dihydrochloride (III).—When puromycin was refluxed for 45 minutes in an alcoholic solution of hydrogen chloride, a white precipitate (III) formed. The substance analyzed for $C_7H_9N_5 \cdot 2HCl$ and melted at 225°; it contained two N-methyl groups and one active

(9) W. Troy, S. Smith, G. Personeus, L. Moser, E. James, S. J. Sparks, M. Stevens, S. Halliday, D. McKenzie and J. J. Oleson, "Antibiotics Annual 1953–1954," Medical Encyclopedia, Inc., New York, N. Y., 1953, pp. 186–190.

(10) J. Davoll and B. A. Lowy, THIS JOURNAL, 73, 1654 (1951).

hydrogen. Compound III was stable and appeared amphoteric in nature by being soluble in both acid and alkali. It precipitated as a dihydrochloride which was converted to the monohydrochloride by recrystallization from alcohol. The ease of this conversion indicated a striking difference in the basicity of the two salt-forming groups. The ultraviolet absorption spectra (Table III), the infrared absorption spectrum and the similarity of these absorption spectra to those of benzimidazole and theophylline[11] suggested a dimethylated aminopurine structure for compound III. The ultraviolet absorption spectrum for compound III (Table III) specifically indicated a 6-dimethylaminopurine. For example, aniline absorbed at 230 and 280 mμ[12] while dimethylaniline had maxima at 252 and 296 mμ.[13] The substitution of two alkyl groups for the two hydrogens of the aromatic amine caused a bathochromic shift of about 20 mμ. In the purines the ultraviolet absorption has been shown to be due mainly to the pyrimidine moiety.[14] The spectra of 4-(6)-aminopyrimidine with maxima at 233 and 269 mμ and 4-(6)-dimethylaminopyrimidine with maxima at 250 and 286 mμ[15] showed a similar shift of about 18 mμ on alkylation. The dialkylation of adenine at the 6-position should shift its absorption from 260 mμ to about 280 mμ which would then compare favorably with the absorption of compound III. A synthetic sample of 6-dimethylaminopurine[16] was obtained and found to be identical with the natural $C_7H_9N_5$ fragment (III).

TABLE II

MELTING POINTS OF 6-DIMETHYLAMINOPURINE AND ITS SALTS

6-Dimethyl-aminopurine	Ref.	Base	Picrate	HCl	2HCl
Natural		257.5–258.5	245	253	225
Synthetic	17	257–258	244–245	253[a]	225

[a] G. E. Elion, E. Burgi and G. H. Hitchings, THIS JOURNAL, **74**, 412 (1952).

In order to ascertain the point of linkage between the dimethylaminopurine and the remainder of the molecule, the ultraviolet absorption spectra of 7- and 9-substituted purine[17] were investigated (Table III). It was noted that puromycin absorbed at a somewhat shorter wave length than the dimethylaminopurine. Analogously it was found that 9-alkyl substitution of the purine caused a hypsochromic shift while 7-substitution produced a bathochromic shift. Therefore, the 9-position of 6-dimethylaminopurine was postulated as the point of attachment to the remainder of the molecule.

TABLE III

ULTRAVIOLET ABSORPTION CHARACTERISTICS OF 6-DIMETHYLAMINOPURINE AND ITS 7- AND 9-ETHYL DERIVATIVES

Compound	0.1 *N* NaOH Max., mμ	0.1 *N* NaOH $E \times 10^{-4}$	0.1 *N* HCl Max., mμ	0.1 *N* HCl $E \times 10^{-4}$
Puromycin	275	2.03	268	1.95
6-Dimethylaminopurine (natural)	280–282	1.70	275–277	1.51
6-Dimethylaminopurine (synth.)	281	1.78	277	1.59
6-Dimethylamino-9-ethyl-β-purine	277.5	1.83	270	1.75
6-Dimethylamino-7-ethyl-purine	295	1.94	290	2.33

b. O-Methyl-L-tyrosine Methyl Ester Hydrochloride (IV).—If the solution from which the 6-dimethylaminopurine had been obtained was refluxed for a further period of about 18 hours, complete alcoholysis was effected. The solution reacted with 2,4-dinitrophenylhydrazine, Tollens and Fehling reagents. The Molisch test showed a questionably slight development of a purple ring while the Fuchsin aldehyde test remained negative.

From this alcoholysate a white crystalline solid (IV) was obtained which melted at 183–185°. The empirical formula, $C_{11}H_{15}NO_3 \cdot HCl$, was established from analytical data. Group analyses showed the presence of one amino nitrogen, one O-methyl group and an ester group. Compound IV gave a positive ninhydrin test and was optically active. The infrared absorption spectrum showed the presence of ester and O-methyl groups, an aromatic nucleus and an amine hydrochloride. The ultraviolet absorption spectra of IV were similar to those of anisole which implied a lack of conjugation of the aromatic portion with the ester carbonyl. The compound was identified as O-methyl-L-tyrosine methyl ester hydrochloride by comparison with an authentic sample.[18] To substantiate the identity of IV, a carbanilide was prepared and found to be identical with N-carbanilido-O-methyl-L-tyrosine[19] obtained from an authentic sample.

c. 3-Amino-3-deoxy-D-ribose Hydrochloride (V).—Another white crystalline solid (V) was isolated from the 24-hour hydrogen chloride alcoholysate which analyzed for $C_5H_{11}NO_4 \cdot HCl$ and melted at 158–158.5°. The substance was optically active ($[\alpha]^{25}D$ −24.6°, 4% in water) and gave positive reactions with Brady and Fehling reagents. The presence of one amino nitrogen was established by Van Slyke's method. The infrared absorption spectrum showed hydroxyl and amine hydrochloride bands but no absorption from 5 to 6 μ. Compound V did not absorb in the ultraviolet region of the spectrum.

Deamination of the compound with nitrous acid and steam distillation after the addition of phosphoric acid yielded furfural which was identified by comparison of its ultraviolet absorption spectrum and its phenylhydrazine derivative with those

(11) In the imidazole series, amphoteric compounds result when neither nitrogen is substituted, also a broad absorption band usually occurs in the region from 2500 to 2850 cm.$^{-1}$. For example, 2-benzimidazole and some other benzimidazoles absorb in this region (H. M. Randall, R. G. Fowler, N. Fuson and J. R. Dangl, "Infrared Determination of Organic Structures," D. Van Nostrand Co., Inc., New York, N. Y., 1949, p. 216). Theophylline has absorption at 2620 cm.$^{-1}$ which is not present in caffeine or theobromine (E. K. Blout and M. Fields, THIS JOURNAL, **72**, 483 (1950)). 6-Dimethylaminopurine has absorption at 2600 cm.$^{-1}$ which was absent in puromycin.

(12) L. Doub and J. M. Vandenbelt, *ibid.*, **69**, 271 (1947).

(13) H. B. Klevens and J. R. Platt, *ibid.*, **71**, 1714 (1949).

(14) L. F. Cavalieri and A. Bendich, *ibid.*, **72**, 2587 (1950).

(15) D. J. Brown and L. N. Short, *J. Chem. Soc.*, 331 (1953).

(16) B. R. Baker, J. P. Joseph and R. E. Schaub, *J. Org. Chem.*, **19**, 631 (1954).

(17) B. R. Baker, R. E. Schaub aud J. P. Joseph, *ibid.*, **19**, 638 (1954).

(18) B. R. Baker, J. P. Joseph and J. H. Williams, THIS JOURNAL, **77**, 1 (1955).

(19) L. D. Behr and H. T. Clarke, *ibid.*, **54**, 1630 (1932).

of an authentic sample. The original fragment was, therefore, postulated to be a straight chain aminopentose occurring in the hemiacetal form.

Compound V consumed 4 moles of periodate which established the presence of an oxygen or an amine substituent on each of its carbons. The isolation of formaldehyde (0.4 mole) and ammonia (0.4 mole) indicated the oxidation of a primary alcohol group and a carbon containing a primary amino group, respectively. No carbon dioxide was produced, further demonstrating (along with the positive Fehling test) that the potential carbonyl was an aldehyde. The absence of carbon dioxide in the oxidation mixture also showed that the C_5-compound was a straight chain sugar, thus eliminating the possibility of rearrangement during the deamination and dehydration to produce furfural.

The location of the amino group on the aminopentose was next determined. The aminopentose gave a negative ninhydrin test while the same test was positive with glucosamine, thus indicating that the amino group was not on the second carbon. The acetylation of V in aqueous solution yielded an N-acetyl derivative which consumed only two moles of periodate establishing that the acetamido group was not terminal, *i.e.*, on the first or fifth carbons. Since the amino group of V is fully substituted in puromycin as evidenced by the mono-N-acetyl derivative obtained on acetylation of **I** and since the antibiotic did not consume periodate, the amino group was not on the fourth carbon of V. Therefore, compound V must be a straight chain 3-aminopentose.

The 3-aminopentose on catalytic reduction with Raney nickel at 100° and 1700 lb. pressure consumed 1 mole of hydrogen. The presumed reduction of the carbonyl group was further substantiated by a negative Fehling test. The aminopentitol resulting from the reduction was found to be optically inactive, a fact which was attributed to internal compensation of the molecule. Thus, the arabinose and lyxose structures were eliminated, since only ribose and xylose allow a *meso*-configuration for the pentitol.

The natural 3-aminopentose was compared with a sample of 3-amino-3-deoxy-D-ribose prepared by an unequivocal synthesis.[20] The two compounds were found to be identical on the basis of infrared absorption spectra, optical rotations and mixed melting point determinations.

Linkage of Fragments

The isolation and identification of 6-dimethylaminopurine, O-methyl-L-tyrosine and 3-amino-3-deoxy-D-ribose permitted the postulation of the partial structure of puromycin. It had been shown that the 9-position of the purine formed the site of attachment to the remainder of the molecule. It had also been established from spectrophotometric evidence that the 9-position of III contained a nonconjugated substituent. The ease with which III was cleaved from the remainder of the molecule suggested a glycosidic link. The formation of a triacetate of puromycin and its subsequent deacetylation with alcoholic ammonia to yield N-acetylpuromycin showed the presence of two free hydroxyl groups in I. The free amino group was placed in the O-methyl tyrosine moiety, since compound I failed to consume periodic acid. This latter fact and the presence of bands in the infrared spectrum of I typical of an amide indicated that the amino acid was attached through its carboxyl to the amino group on the 3-position of the sugar.

To establish whether the glycosidic linkage was of the α- or β-configuration and to determine whether the sugar contained a furanoside or pyranoside ring system, O-methyl-L-tyrosine was cleaved from puromycin by sodium methoxide hydrolysis of the thiourea derivative resulting from the interaction of puromycin and phenyl isothiocyanate. Periodate oxidation of the resulting product, 6-dimethylamino-9-[3-amino-3-deoxyribosyl]-β-purine,[18] "the aminonucleoside," showed the uptake of only one mole of oxidant establishing the furanose nature of the pentose portion. The molecular rotation of the dialdehyde resulting from the periodate oxidation was found to be −4,827. Identical periodate oxidations were carried out on 6-dimethylamino-9-β-D-glucopyranosyl-β-purine.[21] As expected two moles of oxidant were consumed; carbon 3 of the glucose unit was eliminated, and the resulting dialdehyde had a molecular rotation of −5,107. Thus, the two dialdehydes were considered identical,[22] and the β-configuration between the carbohydrate and the purine was suggested.

The complete structure of puromycin was thus postulated to be 6-dimethylamino-9-[3-deoxy-3-(*p*-methoxy-L-phenylalanylamino)-β-D-ribofuranosyl]-β-purine which was subsequently confirmed by total synthesis.[23]

Acknowledgment.—The authors wish to thank L. Brancone and staff for the microanalyses and W. Fulmor and staff for the rotations and spectral data.

Experimental[24]

Puromycin, 6-Dimethylamino-9-[3-deoxy-3-(*p*-methoxy-L-phenylalanylamino)-β-D-ribofuranosyl]-β-purine (I).—Puromycin and its salts used in these experiments were produced by Porter, *et al.*[4] Puromycin base was recrystallized from water, m.p. 175.5–177°, $[\alpha]^{25}D$ −11° (ethanol). *Anal.* Calcd. for $C_{22}H_{29}N_7O_5$: C, 56.0; H, 6.20; N, 20.8; amino nitrogen, 2.79; N-CH_3, 6.36; mol. wt., 471.5. Found: C, 56.1; H, 6.48; N, 21.1; amino nitrogen, 3.13; N-CH_3, 7.97; mol. wt. (Rast), 464; neut. equiv., 470.5.

Puromycin Dihydrochloride.—When puromycin was dissolved in warm normal hydrochloric acid solution and cooled quickly, the dihydrochloride was obtained. It was recrystallized several times by dissolving in warm dilute hydrochloric acid and cooling to bring about crystallization; m.p. 174° dec. The infrared absorption has been reported.[5] *Anal.* Calcd. for $C_{22}H_{29}N_7O_5 \cdot 2HCl \cdot 2H_2O$: C, 45.6; H, 6.08; N, 16.9; Cl, 12.2. Found: C, 45.8; H, 6.04; N, 17.2; Cl, 12.1.

Puromycin Sulfate.—The sulfate of I was prepared by dissolving 3 g. of I in 100 ml. of 50% aqueous methanol containing 3 ml. of concentrated sulfuric acid. The white crystalline product was isolated and recrystallized from water, m.p. 180–187° dec. *Anal.* Calcd. for $C_{22}H_{29}N_7O_5 \cdot H_2SO_4$: C, 46.4; H, 5.49; N, 17.2; S, 5.63. Found: C, 46.1; H, 5.90; N, 16.5; S, 5.90.

(20) B. R. Baker and R. E. Schaub, THIS JOURNAL, **75**, 3864 (1953); *J. Org. Chem.*, **19**, 646 (1954); B. R. Baker, R. E. Schaub and J. H. Williams, THIS JOURNAL, **77**, 7 (1955).

(21) B. R. Baker, J. P. Joseph, R. E. Schaub and J. H. Williams, *J. Org. Chem.*, **19**, 1780 (1954).

(22) R. Chang and V. B. Lythgoe, *J. Chem. Soc.*, 1992 (1950), and references cited therein have shown several such comparisons.

(23) B. R. Baker, R. E. Schaub, J. P. Joseph and J. H. Williams, THIS JOURNAL, **77**, 12 (1955).

(24) Melting points reported herein are uncorrected.

Puromycin Picrate.—The picrate of I was prepared by adding an aqueous solution of picric acid to a solution of puromycin or its salts, m.p. 146–149°. *Anal.* Calcd. for $C_{22}H_{29}N_7O_5 \cdot 2C_6H_3N_3O_7 \cdot H_2O$: C, 43.1; H, 3.91; N, 19.3. Found: C, 42.9; H, 3.99; N, 19.2.

Triacetylpuromycin.—The base of puromycin (100 mg.) in 10 ml. of acetic anhydride containing 3 drops of pyridine was heated on a steam-bath for a half-hour. The product crystallized after concentrating and cooling. It was recrystallized from acetone; yield 80 mg., m.p. 217.5–218°. *Anal.* Calcd. for $C_{28}H_{35}N_7O_8$: C, 56.3; H, 5.90; N, 16.4; 3 acetyl, 21.6. Found: C, 56.2; H, 6.01; N, 16.4; acetyl, 22.6.

N-Acetylpuromycin.—The monoacetyl derivative was prepared by partial deacetylation of 1 g. of the triacetyl derivative of I in 75 ml. of methanol at room temperature with 150 ml. of methanol saturated wih ammonia at 0°. The mixture was shaken and cooled for several hours. The white crystalline product was isolated and recrystallized from ethanol; yield 700 mg., m.p. 236–237°. *Anal.* Calcd. for $C_{22}H_{31}N_7O_6$: C, 56.1; H, 6.09; N, 19.1; Ac, 8.38. Found: C, 56.3; H, 6.24; N, 19.4; Ac, 7.84.

Anisic Acid (II).—The hydrochloride of I (1.5 g.) was dissolved in 50 ml. of 10 *N* sodium hydroxide and 50 ml. of water. Solid potassium permanganate was added slowly to maintain an excess. When the oxidation was complete, the excess permanganate was destroyed with methanol. The reaction mixture was filtered, acidified to *p*H 1 and extracted with ethyl acetate. The ethyl acetate was concentrated to dryness; yield 210 mg. This solid was recrystallized from aqueous methanol and dried, m.p. 180.5–182°. *Anal.* Calcd. for $C_8H_8O_3$: C, 63.1; H, 5.30; O–Me, 20.4. Found: C, 62.9; H, 5.68; O–Me, 20.3.

6-Dimethylaminopurine (III).—Twelve grams of I was dissolved in 250 cc. of 2 *N* NaOH and refluxed for 4 hours. After standing overnight, the solution was adjusted to *p*H 4.5 and extracted 3 times with an equal volume of amyl acetate. The white crystalline product was obtained by concentrating the amyl acetate and recrystallizing from chloroform, m.p. 257.5–258.5°. *Anal.* Calcd. for $C_7H_9N_5$: C, 51.5; H, 5.56; N, 42.9. Found: C, 51.6; H, 5.76; N, 43.0.

Dihydrochloride of III.—Three grams of I was dissolved in 150 cc. of ethanol saturated with hydrogen chloride. A white solid precipitated after 30 minutes of refluxing and cleavage appeared complete after 1 hour. The product was isolated and dried; yield 1.5 g., m.p. 225° dec. *Anal.* Calcd. for $C_7H_9N_5 \cdot 2HCl$: C, 35.6; H, 4.69; N, 29.7; Cl, 30.0. Found: C, 36.1; H, 4.92; N, 29.5; Cl, 29.9.

Monohydrochloride of III.—Recrystallization of the dihydrochloride of III from 80% ethanol gave a monohydrochloride of III, m.p. 253° dec.

6-Dimethylaminopurine Picrate.—The free base of III and the dihydrochloride of III were characterized by preparing the picrate. A saturated solution of picric acid was added to an aqueous solution of III or its dihydrochloride. A picrate precipitated as heavy yellow needles in either case and was filtered off and dried. The picrate prepared from the free base of III melted at 235–236.5°. The picrate prepared from the dihydrochloride melted at 245°.

***p*-Methoxy-L-phenylalanine Methyl Ester Hydrochloride** (IV).—Three grams of I was refluxed in 150 ml. of methanol saturated with hydrogen chloride. Compound III was removed after six hours and refluxing continued for 18 hours. After the solution was reduced to a sirup *in vacuo* and 500 cc. of chloroform was added, a white solid was isolated; yield 1 g. It was recrystallized from ethanol using Norite for decolorization, m.p. 183–185° dec. *Anal.* Calcd. for $C_{11}H_{15}O_3N \cdot HCl$: C, 53.7; H, 6.56; N, 5.70; Cl, 14.3; OCH_3(2), 25.3; NH_2, 5.70. Found: C, 53.1; H, 6.72; N, 5.77; Cl, 13.9; OCH_3, 23.2; NH_2, 6.03.

Carbanilide of IV.—A solution of 100 mg. of IV in 1.5 cc. of water and 0.5 cc. of 10% NaOH was shaken with 0.15 cc. of phenyl isocyanate for about one hour. After separating the diphenylurea, the alkaline solution was acidified with hydrochloric acid and the crude product was isolated, washed and recrystallized from 50% alcohol obtaining white leaflets, m.p. 176.5–177.5°.[19]

3-Amino-3-deoxy-D-ribose Hydrochloride (V).—Three grams of I was refluxed in 120 cc. of ethanol saturated with hydrogen chloride. The precipitate of III was filtered off after two hours and refluxing was continued for a total of 24 hours. The product (400 mg.) was obtained by concentrating to sirup and triturating with 80% ethanol and subsequently with absolute ethanol. It was recrystallized from a water–ethanol mixture and dried, m.p. 158–158.5°. *Anal.* Calcd. for $C_5H_{11}O_4N \cdot HCl$: C, 32.4; H, 6.52; N, 7.55; Cl, 19.1; NH_2, 7.55. Found: C, 32.6; H, 6.72; N, 7.62; Cl, 19.4; NH_2, 7.70.

N-Acetyl Derivative of V.—A mixture of 1 g. of V, 0.5 g. of sodium acetate and 1 ml. of acetic anhydride in 25 ml. of water was allowed to react for 10 minutes at room temperature and then concentrated to dryness. The product was isolated from the residue by dissolving in ethanol and precipitating with 5 volumes of ether, m.p. 74–76°. *Anal.* Calcd. for N-acetyl, 22.5. Found: N-acetyl, 22.1.

Catalytic Reduction of V.—Five hundred and fifty mg. of V was dissolved in 7 ml. of water containing 3 ml. of 1 *N* sodium hydroxide. The solution was placed in a hydrogenation bomb, the volume was made up to 13 ml. and 0.25 teaspoonful of Raney nickel catalyst was added. The hydrogenation was run at 100° and 1700 lb. pressure for 4 hours. One mole of hydrogen was taken up. The catalyst was filtered off; the solution was reduced to dryness. This residue was taken up in 45 ml. of water to yield a theoretical concentration of 1 g. per 100 ml. The solution gave a negative Fehling test indicating reduction of the carbonyl groups. Polarimetric examination of the solution showed it to be optically inactive.

TABLE IV

MOLES PERIODATE PER MOLE OF COMPOUND

Time	V	N-Ac of V	"Amino-nucleoside"
10 min.	1.7	0.7	0.99
1 hr.	3.2	1.17	1.09
3 hr.	3.8	1.4	
20 hr.			1.18
3 days	3.8	2.7	

Deamination of V and Furfural Formation.—Fifty mg. of V and 20 mg. of sodium nitrite were dissolved in 50 ml. of water, and the solution was acidified with 1 ml. of concentrated hydrochloric acid. When the reaction had subsided, 250 ml. of sirupy phosphoric acid was added and the solution was steam distilled at 170°.[25] The steam distillate was collected and diluted to a total volume of 500 ml. The ultraviolet spectrum of the solution showed a maximum at 278 mμ which was considered typical for furfural. To characterize the furfural further, an aliquot of the solution was treated with 2,4-dinitrophenylhydrazine to form the hydrazone which compared favorably with a known sample, the 2,4-dinitrophenylhydrazone of furfural.

Periodate Oxidation.—Samples of the substance to be oxidized were weighed, *ca.* 0.5 millimole, and dissolved in 10 ml. of water; 30 ml. of 0.15 *N* periodic acid was added and allowed to react. At specified time intervals 1-ml. aliquots were withdrawn and the consumption of periodic acid determined by the arsenite method.[26]

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(25) For further details of this procedure see S. Dunstan and A. E. Gillam, *J. Chem. Soc.*, S 140 (1949).

(26) E. L. Jackson, "Organic Reactions," John Wiley and Sons, Inc., New York, N. Y., 1944, Vol. II, p. 361.