crystallized by the addition of 10 cc. of heptane, followed by chilling; yield 2.6 g. (58%), m.p. 105–107°. Recrystallization of a similar preparation from the same solvents gave white crystals, m.p. 107–108°, $[\alpha]^{25}D$ +86° (3% in $CHCl_3$).

Anal. Calcd. for $C_{15}H_{19}NO_6 \cdot {}^1/_2H_2O$: C, 56.6; H, 6.34; N, 4.40; H_2O, 2.83. Found: C, 56.6; H, 6.56; N, 4.54; H_2O, 3.93 (Fischer).

Methyl 2-*O*-Mesyl-5-*O*-benzoyl-3-acetamido-3-deoxy-α-D-arabinofuranoside (XVIIIa).—To a solution of 2.6 g. of XVa in 26 cc. of reagent pyridine was added 1.3 cc. of methanesulfonyl chloride with cooling. After 43 hours in a stoppered flask, the mixture was diluted with 3 volumes of water and extracted with chloroform. The combined extracts, washed with aqueous sodium bicarbonate and dried with magnesium sulfate, were evaporated to dryness *in vacuo* leaving 3.0 g. (92%) of product as a colorless gum which could not be crystallized.

Anal. Calcd. for $C_{16}H_{21}NO_8S$: N, 3.62. Found: N, 3.33.

Methyl 2,5-Di-*O*-mesyl-3-acetamido-3-deoxy-α-D-arabinofuranoside (XVIa).—To a solution of 15 g. of XIVa in 254 cc. of reagent pyridine was added 15 cc. of methanesulfonyl chloride in portions with ice-cooling at such a rate that the temperature was 10–20°. After 48 hours in a stoppered flask, just sufficient water to dissolve the pyridine hydrochloride was added. The solution was concentrated to about one-third *in vacuo* (bath 50°). Upon dilution with 200 cc. of water crystals began to separate. After 2 hours at 0°, the mixture was filtered and the product washed with water; yield 22.1 g. (84%), m.p. 122–124°. Recrystallization of a similar preparation from absolute alcohol gave white crystals, m.p. 125–126°, $[\alpha]^{28}D$ +104° (2% in pyr.).

Anal. Calcd. for $C_{10}H_{19}NO_9S_2$: C, 33.2; H, 5.30; N, 3.88. Found: C, 33.6; H, 5.36; N, 3.93.

Methyl 2,5-Di-*O*-mesyl-3-acetamido-3-deoxy-β-D-arabinofuranoside (XVIb).—Mesylation of 14.8 g. of XIVb as described for XVIa gave 21.7 g. (84%) of product, m.p. 165–166°. Recrystallization from alcohol afforded white crystals, m.p. 169–170°, $[\alpha]^{24}D$ −88° (2% in pyridine).

Anal. Calcd. for $C_{10}H_{19}NO_9S_2$: C, 33.2; H, 5.30; N, 3.88. Found: C, 33.5; H, 5.48; N, 3.89.

Methyl 2,5-Di-*O*-acetyl-3-acetamido-3-deoxy-α-D-ribofuranoside (XXa).—A mixture of 3.0 g. of XVIa, 3.4 g. of anhydrous sodium acetate and 36 cc. of methyl cellosolve containing 5% water was refluxed for 21 hours. The cooled mixture was filtered from sodium methanesulfonate (1.4 g.), then evaporated to dryness *in vacuo*. The residue was heated on the steam-bath with 30 cc. of reagent pyridine and 30 cc. of acetic anhydride for 1 hour. Dilution with 150 cc. of iced water gave a clear solution which was extracted with three 50-cc. portions of chloroform. The combined extracts, dried with magnesium sulfate and clarified with Norit, were evaporated to dryness *in vacuo*. The residue, twice dissolved in 20 cc. of toluene and evaporated *in vacuo* to remove pyridine, readily solidified; yield 2.25 g. (94%), m.p. 85–87°. In a larger run the yield was 11.8 g. (98%), m.p. 85–87°. Recrystallization of a pilot run (360 mg. (90%), m.p. 85–87°) from 1:1 benzene–heptane gave white crystals, m.p. 90–91°, $[\alpha]^{23}D$ +135° (2% in $CHCl_3$).

Anal. Calcd. for $C_{12}H_{19}NO_7$: C, 49.8; H, 6.62; N, 4.85. Found: C, 49.5; H, 6.80; N, 5.07.

Methyl 2,5-Di-*O*-acetyl-3-acetamido-3-deoxy-β-D-ribofuranoside (XXb).—From 10 g. of XVIb as described for XXa was obtained 7.5 g. (94%), m.p. 92–93°. Recrystallization from benzene–heptane gave white crystals, m.p. 98–99°, $[\alpha]^{24}D$ +34.6° (2% in $CHCl_3$).

Anal. Calcd. for $C_{12}H_{19}NO_7$: C, 49.8; H, 6.62; N, 4.85. Found: C, 49.7; H, 6.56; N, 4.69.

3-Amino-3-deoxy-D-ribose Hydrochloride (XIX).—Hydrolysis of 330 mg. of XXa in 7 cc. of 1% and 0.1 cc. of concentrated hydrochloric acid for 19 hours as described for methyl 2,4-di-*O*-acetyl-3-acetamido-3-deoxy-α-D-ribopyranoside[2] gave 120 mg. (57%) of product, m.p. 150–151° dec. Recrystallization from 0.2 cc. of water by addition of 0.3 cc. of concentrated hydrochloric acid afforded white crystals, m.p. 159° dec., which had an infrared spectrum identical with authentic XIX.[2]

Similarly hydrolysis of 330 mg. of XXb gave 150 mg. (71%) of crude XIX, m.p. 147° dec. Recrystallization afforded white crystals, m.p. 159° dec., identical with authentic XIX.[2]

PEARL RIVER, NEW YORK

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

Puromycin. Synthetic Studies. IX. Total Synthesis

BY B. R. BAKER,[1] ROBERT E. SCHAUB, JOSEPH P. JOSEPH AND JAMES H. WILLIAMS

RECEIVED JULY 19, 1954

The conversion of methyl 3-amino-3-deoxy-D-ribofuranoside triacetate to 1-*O*-acetyl-2,5-di-*O*-benzoyl-3-acetamido-3-deoxy-D-ribofuranoside is described. Condensation of the titanium chloride complex of 1-chloro-2,5-di-*O*-benzoyl-3-acetamido-3-deoxy-D-ribofuranoside with chloromercury 2-methylmercapto-6-dimethylaminopurine followed by desulfurization and *O*-debenzoylation afforded 6-dimethylamino-9-(3′-acetamino-3′-deoxy-β-D-ribofuranosyl)-purine (VII) identical with that obtained from puromycin. Since VII previously has been converted to puromycin (XI), a total synthesis of the antibiotic has been completed starting with D-xylose.

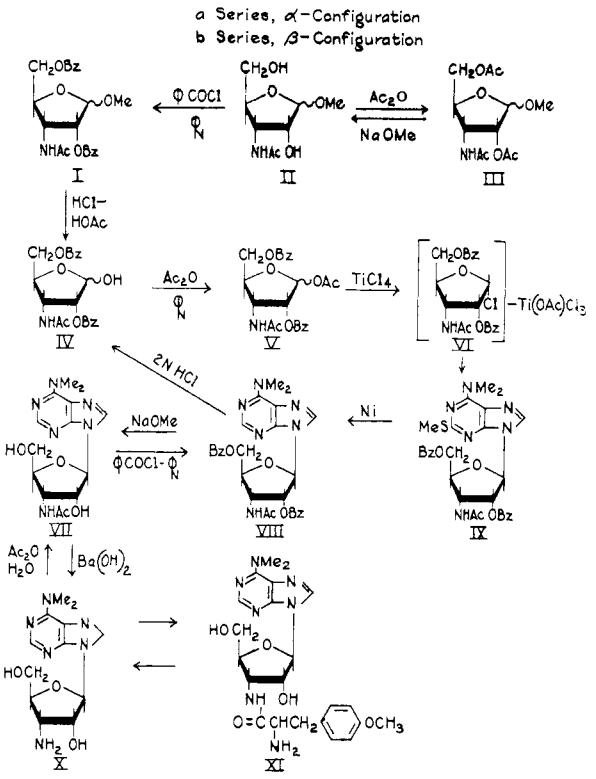
The synthesis of methyl 3-amino-3-deoxy-D-ribofuranoside triacetate (III) from D-xylose has been described in the previous paper of this series.[2] The conversion of this compound to the nucleoside, 6-dimethylamino-9-(3′-acetamido-3′-deoxy-β-D-ribofuranosyl)-purine (VII) is now presented. Since VII has been converted to puromycin (XI) *via* the aminonucleoside (X),[3] this sequence completes the steps for a total synthesis of the antibiotic from D-xylose.

O-Deacetylation of crystalline methyl 3-amino-3-deoxy-β-D-ribofuranoside triacetate (IIIb) with methanolic sodium methoxide gave, in quantitative yield, IIb as a glass which afforded 64–72% of a crystalline dibenzoate Ib when treated with benzoyl chloride in pyridine at 3°. A study of acid hydrolysis conditions for cleavage of the glycosidic linkage revealed that the optimum conditions were 3:10 concentrated hydrochloric acid:acetic acid at 50° for 25 minutes. 2,5-Di-*O*-benzoyl-3-acetamido-3-deoxy-D-ribose (IV), m.p. 154°, was obtained in 52% yield. Similarly, IV was prepared from methyl 3-amino-3-deoxy-α-D-ribofuranoside triace-

(1) Papers VII, VIII and IX of this series were presented at the Sixth Summer Seminar in Natural Products at the University of New Brunswick, Canada, during the week of August 17, 1954, and at the A.C.S. Meeting in New York, September, 1954.

(2) B. R. Baker, R. E. Schaub and J. H. Williams, THIS JOURNAL, **77**, 7 (1955), paper VIII of this series.

(3) B. R. Baker, J. P. Joseph and J. H. Williams, *ibid.*, **77**, 1 (1955), paper VII of this series.



tate (IIIa) in 23% over-all yield, although the dibenzoate Ia did not crystallize. The over-all yield was more than doubled when crude Ia was cleaved with 30% hydrogen bromide in acetic acid,[4] forming 2,5-di-*O*-benzoyl-3-acetamido-3-deoxy-D-ribofuranosyl bromide. Hydrolysis with aqueous acetone–silver carbonate formed crystalline IV in 58% over-all yield from IIIa. Similar over-all yields of IV from IIIb were obtained by this process.

This same key intermediate, IV, also can be obtained from puromycin (XI) *via* the aminonucleoside X and its N-acetyl derivative VII. Benzoylation of VII in pyridine gave the 2′,5′-di-*O*-benzoate VIII in quantitative yield as an analytically pure glass. Hydrolysis with the refluxing two-phase system, 2 *N* hydrochloric acid–ethylene dichloride, gave a maximum yield of IV of 41% after 4 hours. The identity of the 3-acetamido-3-deoxy-2,5-di-*O*-benzoyl-D-ribose (IV) prepared by both methods serves as an additional chemical proof that the aminonucleoside X and puromycin XI have the D-ribose moiety attached to the purine in the furanose form.[5] Acetylation of IV with acetic anhydride in pyridine at 100° gave a quantitative yield of acetate V as a mixture of α- and β-forms from which one anomer, probably the β-form, could be crystallized.

The standard method for preparing a 1-haloaceto sugar, namely, hydrogen chloride or bromide in acetic acid, is not suitable for the preparation of haloacylated sugar furanoses because of their great ease of hydrolysis during the cold aqueous sodium bicarbonate treatment for work-up. Davoll, Lythgoe and Todd[6] have circumvented this difficulty with D-ribofuranose tetraacetate by use of ethereal hydrogen chloride, then simple removal of the excess solvent and reagent by evaporation *in vacuo*. This type of procedure, unfortunately, was unsuccessful with 1-*O*-acetyl-2,5-di-*O*-benzoyl-3-acetamido-3-deoxy-D-ribofuranoside (V) due to the rapid formation of an ether-insoluble hydrochloride salt of the weakly basic amide group which stopped further reaction.

Pacsu[7] has observed that D-glucopyranose pentaacetate will react with titanium tetrachloride in chloroform to give a titanium complex of 1-chloro-D-glucopyranose tetraacetate. Cold water washings break the complex and remove the titanium salts leaving the chloro sugar, which can be isolated in good yield, in the chloroform layer. When titanium tetrachloride reacted with V, the time of contact with cold water necessary to decompose the titanium complex VI was sufficient to hydrolyze the chloro sugar back to IV. At this point it was realized that perhaps the titanium complex VI could be treated directly with chloromercury 2-methylmercapto-6-dimethylaminopurine[8] to give the nucleoside IX. When the condensation was run in boiling ethylene dichloride for 18 hours, a crude nucleoside was isolated as a gum. The ultraviolet analysis of the gum showed the yield of IX to be 94%. It is probable that the titanium is transferred to the purine as the insoluble chloromercury salt of the purine rapidly dissolves on addition of VI, then another precipitate separates. If the reaction mixture is worked up at this point no appreciable nucleoside IX is formed.[9]

Raney nickel desulfurization[10] of X afforded VIII as a glass in 54% yield (determined by ultraviolet analysis). Removal of the *O*-benzoyl groups by alcoholysis in methanolic sodium methoxide gave 6-dimethylamino-9-(3′-acetamido-3′-deoxy-β-D-ribofuranosyl)-purine (VII), m.p. 188°, in 41% yield. This material, as well as its *O*-diacetate, were identical in all respects with the natural VII and its *O*-diacetate obtained from puromycin. Thus a total synthesis of puromycin from D-xylose has been completed since VII has been previously converted to puromycin.[3]

Acknowledgment.—The authors wish to thank W. McEwen and J. Poletto for large-scale preparation of some of the intermediates, L. Brancone and staff for the microanalyses and W. Fulmor and staff for the rotations and spectra.

Experimental

Methyl 2,5-Di-*O*-benzoyl-3-acetamido-3-deoxy-β-D-ribofuranoside (Ib).—To a solution of 5.1 g. of crude IIIb[2] in 100 cc. of methanol was added 1.8 cc. of 1 *N* methanolic sodium methoxide. After being refluxed for 30 minutes, the solution was evaporated to dryness *in vacuo* leaving a glass. The

(4) H. G. Fletcher, THIS JOURNAL, **75**, 2624 (1953), has used this procedure to convert methyl 2,3,5-tri-*O*-benzoyl-D-ribofuranoside to 2,3,5-tri-*O*-benzoyl-D-ribose.

(5) C. W. Waller, P. W. Fryth, B. L. Hutchings and J. H. Williams, N. Y. Meeting-in-miniature, Feb., 1954.

(6) J. Davoll, B. Lythgoe and A. R. Todd, *J. Chem. Soc.*, 967 (1948).

(7) E. Pacsu, *Ber.*, **61**, 1508 (1928).

(8) B. R. Baker, J. P. Joseph, R. E. Schaub and J. H. Williams, *J. Org. Chem.*, **19**, 1780 (1954), paper IV of this series.

(9) The use of titanium complexes of chlorofuranose sugars for the synthesis of nucleosides does not appear to be general and is probably limited to 2- and 3-aminopentofuranose derivatives as will be reported in future papers.

(10) R. Mozingo, D. E. Wolf, S. A. Harris and K. Folkers, THIS JOURNAL, **65**, 1013 (1943).

residue was dissolved in 50 cc. of reagent pyridine and treated dropwise with shaking and ice-cooling with 5.1 cc. of benzoyl chloride at such a rate that the temperature was 5–7°. After 68 hours at 3° in a stoppered flask, the mixture was diluted with 200 cc. of water and extracted with three 50-cc. portions of chloroform. The combined extracts, washed with aqueous sodium bicarbonate, dried with magnesium sulfate and clarified with Norit, were evaporated to dryness *in vacuo*. Crystallization of the residue (8.2 g.) from 14 cc. of benzene by the addition of heptane to turbidity gave 4.7 g. (64%) of product, m.p. 139–141°. In other runs the yields were 64–72%. Recrystallization from the same solvents gave white crystals, m.p. 139–141°, $[\alpha]^{25}D$ +83° (1.5% in $CHCl_3$).

Anal. Calcd. for $C_{22}H_{23}NO_7$: C, 64.0; H, 5.57; N, 3.39. Found: C, 63.9; H, 5.57; N, 3.52.

Similarly from 29.1 g. of IIIa was obtained 47.4 g. (114%) of crude **methyl 2,5-di-*O*-benzoyl-3-acetamido-3-deoxy-α-D-ribofuranoside** (Ia) as a glass which could not be crystallized.

6-Dimethylamino-9-(2′,5′-di-*O*-benzoyl-3′-acetamido-3′-deoxy-β-D-ribofuranosyl)-purine (VIII) from Puromycin.—To a solution of 11.2 g. of VII[3] in 100 cc. of reagent pyridine was added 9.6 cc. of benzoyl chloride with ice-cooling at such a rate that the temperature was 7–9° (7 minutes). After 17 hours at room temperature in a stoppered flask, the mixture was poured into 400 cc. of iced water and extracted with three 100-cc. portions of methylene chloride. The combined extracts were washed with aqueous sodium bicarbonate, dried with magnesium sulfate and evaporated to dryness *in vacuo* leaving 18.3 g. (101%) of a glass which could not be crystallized, but was nearly pure.

Anal. Calcd. for $C_{28}H_{28}N_6O_6$: C, 61.8; H, 5.18; N, 15.4. Found: C, 62.0; H, 5.34; N, 14.7.

2,5-Di-*O*-benzoyl-3-acetamido-3-deoxy-D-ribose (IV). (A).—To a solution of 5.0 g. of Ib in 50 cc. of acetic acid was added 15 cc. of concentrated hydrochloric acid. The solution was stirred in a 50° bath for 25 minutes, then diluted with 175 cc. of ice-water. The gum which separated was collected by extraction with 75 cc., then two 50-cc. portions of chloroform. Washed with aqueous sodium bicarbonate and dried with magnesium sulfate, the combined extracts were evaporated to dryness *in vacuo*. Crystallization of the residue (3.6 g.) from 10 cc. of ethyl acetate by the addition of heptane to turbidity followed by standing at room temperature overnight gave 2.5 g. (52%) of product, m.p. 144–147°. Several recrystallizations of a similar preparation from the same solvents gave white crystals, m.p. 153–154°, $[\alpha]^{24}D$ +108° (2% in pyridine). The compound gave a positive Benedict test in dilute alcohol.

Anal. Calcd. for $C_{21}H_{21}NO_7$: C, 63.1; H, 5.26; N, 3.51; MeO, 0.0. Found: C, 63.2; H, 5.89; N, 3.41; MeO, 0.0.

When the above reaction time was increased to 1 hour or decreased, the yield was lowered. Hydrolysis at room temperature for 30 minutes gave 71% recovery of starting material. The use of 48% aqueous hydrobromic acid–acetic acid mixture at room temperature, boiling 1 *N* hydrochloric acid and chloroform for 2 hours or boiling 0.1 *N* hydrochloric acid in 50% alcohol for two hours gave crude products which at best gave only a poor Benedict test and from which no IV could be isolated.

(B).—Hydrolysis of 1.27 g. of crude Ia (obtained in 108% yield) as in procedure A gave 0.255 g. (23% from IIIa) of product, m.p. and mixed m.p. 146–149°.

(C).—To a solution of 46.6 g. of crude Ia (obtained in 114% yield) in 93 cc. of methylene chloride was added 233 cc. of 30% hydrogen bromide in acetic acid.[4] After 2 hours the solution was diluted with 116 cc. of methylene chloride and washed twice with 930-cc. portions of ice-water, then excess aqueous sodium bicarbonate. The methylene chloride solution was treated successively with 460 cc. of acetone, 4.6 cc. of water and 46 g. of silver carbonate. After being vigorously stirred for 30 minutes, the solution was filtered through Celite, then evaporated to dryness *in vacuo*. Crystallization of the residue from 120 cc. of ethyl acetate by addition of 100 cc. of heptane gave 19.1 g. (48% from IIIa) of product in three crops, m.p. between 142 and 150°. Retreatment of the residue from the mother liquors with hydrogen bromide, then silver carbonate gave an additional 3.4 g. (total 58%) of product. Acetolysis of Ib gave similar over-all yields of IV from IIIb.

(D).—A solution of 6.0 g. of VIII (prepared from puromycin) in 60 cc. of ethylene dichloride and 60 cc. of 2 *N* hydrochloric acid was refluxed for 4 hours. The separated aqueous layer was extracted with ethylene dichloride. The combined organic solutions were further processed as in preparation A; yield 1.8 g. (41%), m.p. and mixed m.p. with preparation A, 150–152°. A reflux period of 2 hours gave a 31% yield whereas increase in reflux time to 19 hours gave 24% yield. Hydrolysis procedure A gave only 18% yield.

1-*O*-Acetyl-2,5-di-*O*-benzoyl-3-acetamido-3-deoxy-α(and β)-D-ribofuranoside (V).—A solution of 2.5 g. of IV in 5 cc. of reagent pyridine and 5 cc. of acetic anhydride was heated on the steam-bath for 1 hour, then diluted with 25 cc. of iced water and extracted with one 25 cc. and two 15-cc. portions of chloroform. The combined extracts, dried with magnesium sulfate, were evaporated to dryness *in vacuo* leaving 2.7 g. (98%) of a slightly gummy solid, m.p. 127–131°. Recrystallization from 8 cc. of ethyl acetate by addition of 8 cc. of heptane and followed by standing at room temperature overnight gave 1.5 g. (54%) of one anomer, m.p. 149–151°. Recrystallization from the same solvent afforded white crystals, m.p. 152–154°, $[\alpha]^{24}D$ +63° (1.5% in pyridine).

Anal. Calcd. for $C_{23}H_{23}NO_8$: C, 62.6; H, 5.21; N, 3.18. Found: C, 62.2; H, 5.31; N, 3.46.

The filtrate from the 1.5 g. gave on evaporation 1.1 g. (40%) of a gum which could not be crystallized and had $[\alpha]^{24}D$ +84° (2% in pyridine).

2-Methylmercapto-6-dimethylamino-9-(2′,5′-di-*O*-benzoyl-3′-acetamido-3′-deoxy-β-D-ribofuranosyl)-purine (IX).—To a solution of 990 mg. of V (α,β-mixture, m.p. 127–131°) in 8.5 cc. of ethylene dichloride was added a solution of 0.30 cc. of titanium tetrachloride in 4.4 cc. of ethylene dichloride. A yellow precipitate separated which quickly redissolved. After being refluxed for 1 hour, the yellowish-brown solution was added to a stirred mixture of 1.25 g. of chloromercury 2-methylmercapto-6-dimethylaminopurine,[8] 1.35 g. of Celite and 90 cc. of ethylene dichloride which had been dried previously by distilling off 20 cc. of solvent. The chloromercury purine rapidly dissolved and in a few minutes a different precipitate separated. The mixture was refluxed and stirred for 18 hours, then treated with 45 cc. of water and stirred without heating for 15 minutes. The mixture was filtered and the solids washed with hot chloroform. The separated organic layer from the combined filtrate and washings was evaporated *in vacuo* leaving a glass. This was dissolved in 25 cc. of chloroform and washed with 25 cc. of 30% aqueous potassium iodide, then water. Dried with magnesium sulfate and clarified with Norit, the chloroform solution was evaporated to dryness *in vacuo* leaving 1.33 g. (100%) of a glass which had λ_{max} 282.5 mμ (ε 17,000) in methyl cellosolve, corresponding to a maximum purity of 94%. The lower peak[8] was masked by benzoate adsorption at 230 mμ.

Anal. Calcd. for $C_{29}H_{30}N_6O_6S$: C, 59.0; H, 5.12; N, 14.2. Found: C, 58.1; H, 5.50; N, 12.6.

6-Dimethylamino-9-(2′,5′-di-*O*-benzoyl-3′-acetamido-3′-deoxy-β-D-ribofuranosyl)-purine (VIII) (Synthetic).—A solution of 1.28 g. of the preceding IX in 75 cc. of methyl cellosolve was stirred with 2 spoonsful of desulfurizing Raney nickel[10] on the steam-bath for 40 minutes. The hot solution was filtered through Celite and the catalyst washed several times with hot methyl cellosolve. Evaporation of the combined filtrate and washings to dryness *in vacuo* left 0.705 g. (60%) of a glass which had λ_{max} 275 mμ (ε 16,900) in methyl cellosolve or a maximum purity of 90%.

Anal. Calcd. for $C_{28}H_{28}N_6O_6$: C, 61.8; H, 5.18; N, 15.5. Found: C, 59.4; H, 5.80; N, 12.1.

6-Dimethylamino-9-(3′-acetamido-3′-deoxy-β-D-ribofuranosyl)-purine (VII) (Synthetic).—A solution of 690 mg. of the preceding VIII in 15 cc. of dry methanol and 0.14 cc. of 1 *N* methanolic sodium methoxide was refluxed for 30 minutes, then evaporated to dryness *in vacuo*. The residue was dissolved in 3 cc. of hot alcohol and the solution was clarified with Norit. Cooling afforded 130 mg. (30%) of white crystals, m.p. 184–186°. Recrystallization from alcohol raised the m.p. to 187–188°. A mixture with VII obtained from puromycin[3] gave no depression in m.p. and both had identical infrared spectra and specific rotations.

Anal. Calcd. for $C_{14}H_{20}N_6O_4$: C, 50.0; H, 6.00; N, 25.0. Found: C, 49.8; H, 5.93; N, 25.2.

The filtrate from the 130 mg. was evaporated to dryness *in vacuo*. Acetylation of the residue with acetic anhydride in pyridine as described for X[3] gave a glass. Crystallization from ethyl acetate afforded 60 mg. (11%) of white crystals, m.p. 185–187°. A mixture with 6-dimethylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)-purine triacetate obtained from puromycin[3] gave no depression in m.p. and both compounds had the same infrared spectra.

PEARL RIVER, NEW YORK

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

Puromycin. Synthetic Studies. X. A Novel Breakdown of the Purine Ring System

BY B. R. BAKER AND JOSEPH P. JOSEPH

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6-Dimethylamino-9-(5′-methanesulfonyl-3′-amino-3′-deoxy-β-D-ribofuranosyl)-purine 2′,3′-carbonate (VI) has been found to quaternerize easily to V. The latter is very labile to 0.1 *N* base at 25°, hydrolyzing rapidly to 5′,N[4]-cyclo-3-(2′,3′-carbonyl-3′-amino-3′-deoxy-β-D-ribofuranosyl)-4-formamidoimidazole-5-(N,N-dimethyl)-carboxamidine methanesulfonate (IV) which in turn hydrolyzes more slowly to 5′,N[4]-cyclo-3-(2′,3′-carbonyl-3′-amino-3′-deoxy-β-D-ribofuranosyl)-4-aminoimidazole-5-carboxamide (VII). Biological implications are suggested for this sequence.

The total synthesis of the aminonucleoside, 6-dimethylamino-9-(3′-amino-3′-deoxy-β-D-ribofuranosyl)-purine (I), from D-xylose[1] and its partial synthesis from the antibiotic, puromycin,[2] have been described recently. Since the aminonucleoside I is highly active against the transplanted adenocarcinoma of the C_3H mouse[3] as well as *Trypanosoma equiperdum*[4] in mice, it was considered desirable to prepare some functional analogs of the aminonucleoside I by replacement of the 5′-hydroxyl with other groups (IX) *via* a 5′-mesylate. Although the general method of approach failed, a novel breakdown of the pyrimidine portion of the purine ring system was observed and is the subject of this paper.

The conversion of adenosine to its 5′-methylmercapto derivative *via* 2′,3′-isopropylidene-5′-tosyladenosine has been described by Satoh and Makino[5] as well as Baddiley, Trauth and Weygand.[6] A similar sequence with the aminonucleoside I was investigated. Reaction of I with carbobenzoxy chloride and triethylamine in dimethylformamide afforded the carbobenzoxy derivative II in 78% yield. Ring closure of II to the cyclic urethan III with elimination of benzyl alcohol proceeded smoothly in 93% yield with a catalytic amount of sodium methoxide in dimethylformamide.[7] The formation of this cyclic urethan III was substantiated by the hypsochromic shift of the carbonyl absorption of the carbobenzoxy group from 5.87 to 5.62 μ in the infrared as would be expected for a five-membered ring bearing a carbonyl. With the 2′- and 3′-groups now effectively blocked in III, the remaining 5′-hydroxyl was mesylated smoothly in pyridine at 3° to give VI in 80% yield as a crude gum insoluble in water and readily soluble in chloroform. However, after standing at room temperature for several days or, more efficiently, by refluxing in chloroform for two hours, the gum changed to an isomeric crystalline product, m.p. 283° dec., in 91% yield which was now insoluble in chloroform, but readily soluble in water. The salt-like character of this compound could be explained most readily by reaction of the 5′-mesylate with the 3-nitrogen of the purine ring to form a 3,5′-cyclonucleoside V. That the purine ring system had been further alkylated readily could be seen by the shift in the ultraviolet maximum in water from 275 mμ for an ordinary 9-nucleoside derivative of 6-dimethylaminopurine[8] such as I to 288 mμ.

Clark, Todd and Zussman[9] have described the formation of a similar quaternary salt when 2′,3′-isopropylidene-5′-tosyladenosine was heated at 100° in acetone or dioxane. Their proposed structure was beautifully substantiated by X-ray crystallographic analysis. Their results also serve to explain why only about 2% yield of 5′-methylmercaptoadenosine can be obtained from the 5′-tosylate.[5,6] The rate of monomolecular quaternization is obviously much more rapid than the bimolecular displacement reaction with mercaptide ion. Since the 6-dimethylaminopurine nucleoside (VI) appears to quaternerize even more rapidly than in the adenine series, the use of VI as an intermediate in displacement reactions could not possibly lead to useful yields of functional analogs of the aminonucleoside IX.

The usual routine measurement of the ultraviolet absorption spectra at *p*H 1, 7 and 14 on the quaternary salt V, showed an instability at *p*H 14. Within five minutes the initial peak at 230 mμ began to shift and in one hour was at 235 mμ. Then a slower shift to 272 mμ over 27 hours (Graph 1) gradually took place, thus indicating at least two break-down products. The quaternary salt V absorbed at 288 mμ at *p*H 1 and 7 showing that it could not protonate further which contrasts to an ordinary nucleoside such as I which will protonate in acid giving a hypsochromic shift of 7–8 mμ.[8]

(1) B. R. Baker, R. E. Schaub, J. P. Joseph and J. H. Williams, paper IX of this series, THIS JOURNAL, **77**, 12 (1955).

(2) B. R. Baker, J. P. Joseph and J. H. Williams, paper VII of this series, *ibid.*, **77**, 1 (1955).

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