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$_6$H$_4$CH$_2$-).—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.

(2) B. R. Baker and R. E. Schaub, *J. Org. Chem.*, **19**, 646 (1954), paper III of this series.

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

(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%.[7] 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]

(5) R. Jeanloz, H. G. Fletcher and C. S. Hudson, THIS JOURNAL, **70**, 4052 (1948).

(6) G. A. Howard, B. Lythgoe and A. R. Todd, *J. Chem. Soc.*, 1052 (1947).

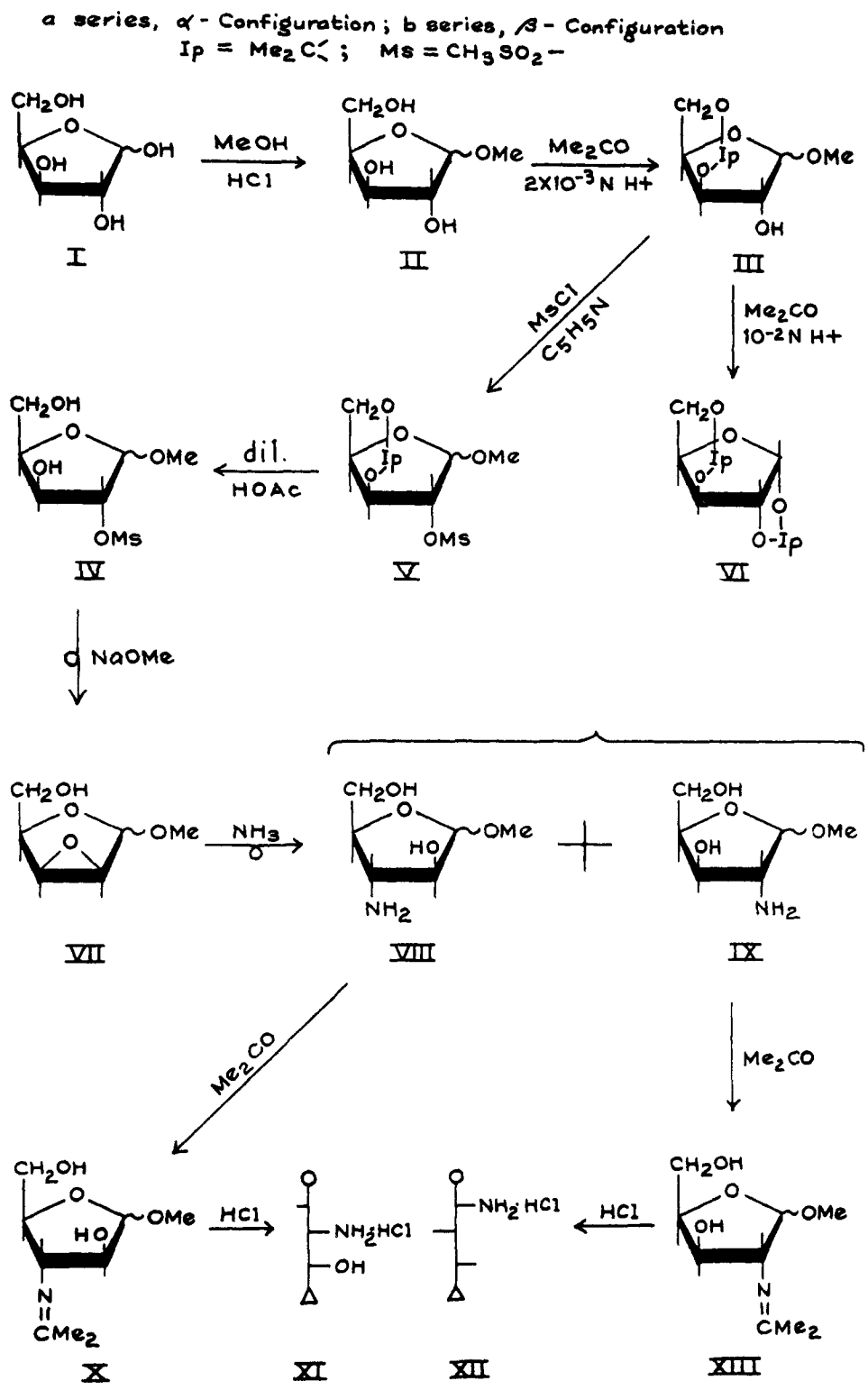
(7) H. Zinner, *Chem. Ber.*, **83**, 153 (1950).

(8) P. Chang and B. Lythgoe, *J. Chem. Soc.*, 1992 (1950).

(9) H. G. Fletcher, THIS JOURNAL, **75**, 2624 (1953).

(10) P. A. Levene, A. L. Raymond and R. T. Dillon, *J. Biol. Chem.*, **95**, 699 (1932).

(11) E. E. Percival and R. Zobrist, *J. Chem. Soc.*, 4306 (1952).



hyde (as an accelerator) to obtain the isopropylidene derivative III. Their procedure was only vaguely described, but repetition in this Laboratory as closely as possible gave 1,2:3,5-di-*O*-isopropylidene-D-xylofuranoside (VI). As a result of over twenty runs with varying conditions, it has now been established how to obtain the monoisopropylidene derivative III with certainty. The concentration of sulfuric acid is quite critical. With no sulfuric acid reaction was slow and incomplete with either anhydrous copper sulfate or copper sulfate monohydrate[12] with or without acetaldehyde. The usual 0.2% sulfuric acid[13] as catalyst (5×10^{-2} *N*) gave almost exclusively the diisopropylidene derivative VI. When the sulfuric acid concentration was reduced by $^1/_{25}$, that is, to 2×10^{-3} *N*, reaction was selective and gave consistent yields of the desired III of 53–57% based on D-xylose (I). When the concentration was only 5 times this amount (1×10^{-2} *N*), then the amount of diisopropylidene derivative VI became appreciable. Other variants found were (a) the acetaldehyde recommended by Percival and Zobrist[11] was without effect; (b) *p*-toluenesulfonic acid (2×10^{-3} *N*) could be used to give the same selective results obtained with the same normality of sulfuric acid; (c) acetic acid gave no reaction; (d) potassium bisulfate, an insoluble acid catalyst, caused considerable decomposition and great lowering of the yield.

Percival and Zobrist[11] described the product III as an oil with b.p. 110° (0.1 mm.) and $[\alpha]_D$ −26° (H_2O). It has now been observed that III obtained by use of 2×10^{-3} *N* sulfuric acid could readily be separated into two fractions, (a) 33% yield, b.p. 85°

(12) P. Brigl, K. Gronemeier and A. Schulz, *Ber.*, **72**, 1052 (1939).

(13) (a) P. A. Levene and A. L. Raymond, *J. Biol. Chem.*, **102**, 317 (1933); (b) *Ber.*, **56**, 863 (1923); (c) P. A. Levene and E. T. Stiller, *J. Biol. Chem.*, **104**, 299 (1934).

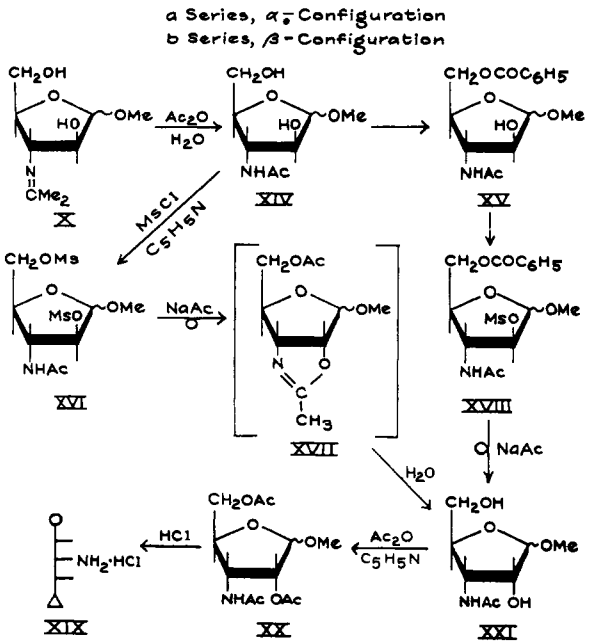
(0.1 mm.), $[\alpha]_D$ +17.6° (H_2O) and (b) 21% yield, b.p. 108° (0.1 mm.), $[\alpha]_D$ −64° (H_2O). Both fractions had the proper combustion values and infrared spectra expected for III. It is clear from the higher positive rotation of fraction (a) that it is the α-anomer and that fraction (b) is the β-anomer. When the reaction time was increased from the 17 hours used in the runs to standardize the acid concentration to 60 hours, the combined yield was 72% (41% α and 31% β).

The rotation of −26° (H_2O) recorded by Percival and Zobrist[11] indicates that they obtained a mixture consisting of 54% α-anomer and 46% β-anomer. The large difference in boiling points observed for the two isomers is probably due to the facts that the α-methoxyl is in a *cis* position favorable for intramolecular hydrogen bonding with the 2-hydroxyl, which would cause lowering of the b.p. The β-methoxyl is *trans* to the 2-hydroxyl and the latter can only bond intermolecularly in the normal fashion of alcohols.

Percival and Zobrist[11] tosylated their III mixture and isolated one crystalline isomer with $[\alpha]_D$ −45° (MeOH) in 50% yield which has the β-configuration (see Experimental). It has now been found that methyl 3,5-*O*-isopropylidene-α-D-xylofuranoside (IIIa) can be mesylated to Va as an analytically pure gum with $[\alpha]_D$ +66° (MeOH) in 92–96% yield. When Va was hydrolyzed to IVa with boiling methanol containing 1% hydrogen chloride, as suggested by Percival and Zobrist,[11] then converted to methyl 2,3-anhydro-α-D-lyxofuranoside (VIIa) with methanolic sodium methoxide at 0° for 17 hours, the over-all yield was 40% of distilled product which partially solidified and gave only fair combustion values. The use of 70% acetic acid at 50° for hydrolysis of the isopropylidene group[14] followed by the same formation of the oxide with cold sodium methoxide gave an over-all yield of 52% of VIIa which completely solidified, melted at 55–60°, and gave good combustion values. When the time of oxide formation was increased to 3 days at 0°, the over-all yield for the two steps was increased to 76%. The crude distilled product had m.p. 72–75°, $[\alpha]^{26}_D$ +44° (H_2O). Recrystallization from benzene gave the pure oxide, m.p. 80–82°, $[\alpha]^{26}_D$ +67° (H_2O). Oxide formation with boiling methanolic sodium methoxide was complete in 5 minutes, but the yield of distilled product was considerably lowered and the quality inferior.

This series of reactions was also run with methyl 3,5-*O*-isopropylidene-β-D-xylofuranoside (IIIb) in the same manner as in the α-series. The mesyl derivative Vb was obtained as a gum, $[\alpha]_D$ −35° (MeOH), in quantitative yield. Hydrolysis of the isopropylidene group with 70% acetic acid at 50° and oxide formation to methyl 2,3-anhydro-β-D-lyxofuranoside (VIIb) with methanolic sodium methoxide at 0° for 3 days proceeded in 71% yield, isolated as a distilled, hygroscopic semi-solid, which was analytically pure and had $[\alpha]_D$ −77° (H_2O). Recrystallization from ether afforded the pure oxide, m.p. 74–75°, $[\alpha]^{25}_D$ −102° (H_2O). A mixture with the isomeric VIIa melted below room temperature.

During the time the above experiments were in progress, Percival and Zobrist published a second paper[15] describing the conversion of methyl 2-*O*-tosyl-D-xylofuranoside to methyl 2,3-anhydro-D-lyxofuranoside (VII) with sodium hydroxide in alcohol at 75°. A 23% yield of one crystalline anomer, m.p. 81°, $[\alpha]_D$ +57° (H_2O), was isolated, which they stated was the α-anomer. A 71% yield of a non-crystalline undistilled fraction of doubtful purity believed to be a mixture of VIIa and VIIb also was obtained with $[\alpha]_D$ +4° (H_2O). This material probably also contained some methyl 2,3-anhydro-D-lyxopyranoside. Since Percival and Zobrist[15] started with crystalline methyl 2-*O*-tosyl 3,5-*O*-isopropylidene-β-D-xylofuranoside and obtained a mixture of approximately 70% of the α- and 30% of the β-anomer of anhydro-D-lyxofuranoside, the anomerization of β to α and any ring expansion must have taken place during the methanolic hydrogen chloride treatment for removal of the isopropylidene group, a result which could be anticipated in view of the data obtained by Levene, Raymond and Dillon.[10] The dilute acetic acid procedure for removal of the isopropylidene group, described in the Experimental, causes much less, about 15%, anomerization.



Treatment of methyl 2,3-anhydro-α-D-lyxofuranoside (VIIa) with ammonium hydroxide might be expected to give a mixture of methyl 3-amino-3-deoxy-α-D-arabinofuranoside (VIIIa) and methyl 2-amino-2-deoxy-α-xylofuranoside (IXa). The product was a gum which could only be crystallized from acetone, in which case rapid formation of a crystalline N-isopropylidene derivative (Xa or XIIIa) took place in 52–54% yield from crude, distilled VIIa. That acetone had condensed with the amine group was shown clearly by the combustion data and by C=N absorption at 6.0 μ in the

(14) This reagent has been used for the selective hydrolysis of 1,2-5,6-di-*O*-isopropylidene-3-*O*-mesyl-D-glucofuranoside to 1,2-*O*-isopropylidene-3-*O*-mesyl-D-glucofuranoside by B. Helferich, H. Dressler and R. Grieber. *J. prakt. Chem.*, **153**, 286 (1939).

(15) E. E. Percival and R. Zobrist, *J. Chem. Soc.*, 564 (1953).

infrared. That the crystalline N-isopropylidene derivative had the desired structure Xa was proven by hydrolysis to a crystalline aminopentose hydrochloride in 80% yield which gave a negative ninhydrin test and was therefore 3-amino-3-deoxy-D-arabinose hydrochloride (XI).[16,17]

When the oxide ring of VIIb was opened with ammonia, a gum was again obtained which could only be crystallized from acetone as an N-isopropylidene derivative Xb in 47% yield. That this was also a 3-aminopentose derivative was proven by hydrolysis to 3-amino-3-deoxy-D-arabinose hydrochloride (XI), identical with that obtained from Xa.

Treatment of methyl N-isopropylidene-3-amino-3-deoxy-α-D-arabinofuranoside (Xa) with acetic anhydride in water caused hydrolysis of the isopropylidene group and N-acetylation to XIVa, m.p. 116°, in 90% yield. Although a crystalline monobenzoyl derivative, presumably XVa, could be prepared in 58% yield from XIVa in order to mesylate selectively the 2-hydroxyl to XVIIIa, this extra step to block the 5-hydroxyl is unnecessary. Dimesylation of XIVa proceeded in 84% yield to XVIa, m.p. 126°, Treatment with sodium acetate in boiling 95% methyl cellosolve caused displacement of both mesylate groups, the 5-mesylate by acetate and the 2-mesylate by the neighboring 3-acetamino group[2,18] with inversion to the oxazoline acetate XVIIa, though not necessarily concurrently. The oxazoline ring was opened and the acetate hydrolyzed by the water present to give XXIa, isolated as its crystalline acetyl derivative XXa, m.p. 91°, in 94–98% over-all yield from XVIa. Hydrolysis with 1% hydrochloric acid gave 3-amino-3-deoxy-D-ribose hydrochloride (XIX) identical with that described in an earlier paper in this series.[2]

Similarly, methyl N-isopropylidene-3-amino-3-deoxy-β-D-arabinofuranoside (Xb), gave a crystalline N-acetyl derivative (XIVb) in 98% yield, which was converted to the dimesylate XVIb, m.p. 170°, in 84% yield. Reaction with sodium acetate and acetylation afforded crystalline XXb in 91–94% yield which, in turn, was hydrolyzed to the same 3-amino-3-deoxy-D-ribose hydrochloride (XIX) obtained from the α-series.

It is interesting to note that this synthesis proceeds through all four D-pentose configurations in the order: *xylo*, *lyxo*, *arabino* and *ribo*.

(16) The amino sugar gave a negative ninhydrin test in 1.5% sodium hydroxide solution, conditions under which 2-amino-2-deoxy-D-glucose gave a blue color. 2-Amino-2-deoxy-D-xylose (XII) should similarly give a blue ninhydrin test under these conditions, thus proving that the compound in question was a 3-amino sugar. The ninhydrin test should be done under specified conditions since in 3% aqueous sodium bicarbonate, 2-amino-2-deoxy-D-glucose, 3-amino-3-deoxy-D-ribose[2] and 3-amino-3-deoxy-D-arabinose all gave a purple ninhydrin test. M. Wolfrom and K. Anno (THIS JOURNAL, **75**, 1038 (1953)) stated that 2-amino-2-deoxy-D-xylose hydrochloride rotated +80° → +40° (H_2O) and gave a positive ninhydrin test, but the conditions were not specified. Since the sugar in question rotates −110° (H_2O), it is clearly isomeric to 2-amino-2-deoxy-D-xylose hydrochloride and serves as an alternate structure proof that this compound was indeed 3-amino-3-deoxy-D-arabinose hydrochloride.

(17) When analytically pure oxide VIIa was treated with ammonia, the yield of Xa was over 90% and no 2-amino isomer could be detected. From a preparative stand-point the over-all yield is superior if crude distilled oxide is employed.

(18) G. E. McCasland, R. K. Clark and H. E. Carter, THIS JOURNAL, **71**, 637 (1949).

Acknowledgment.—The authors are indebted to W. McEwen and J. Poletto for large scale preparation of some of the intermediates, to L. Brancone and staff for the microanalyses and to W. Fulmor and staff for the infrared spectra and optical rotations.

Experimental

Methyl 3,5-*O*-Isopropylidene-α(and β)-D-xylofuranoside (III).—A mixture of 11 g. of D-xylose and 250 cc. of methanol containing 0.5% hydrogen chloride was stirred for 5 hours, solution being complete in about 2 hours. After the addition of 6 g. of silver carbonate, the mixture was stirred for 0.5 hour, then filtered through Celite. The neutral filtrate was concentrated to a sirup *in vacuo* (bath 40°). The crude II was readily soluble in acetone and was used without further purification. If bath temperatures much above 40° were employed the compound was transformed into material insoluble in acetone.

To a solution of the crude xyloside (II) in 125 cc. of acetone was added 0.25 cc. of 1 *N* sulfuric acid and 25 g. of anhydrous copper sulfate. The mixture was shaken for 17 hours, then filtered. The filtrate, neutralized with 0.2 cc. of concentrated ammonium hydroxide, was evaporated to dryness *in vacuo* (bath to 70°). The residue (11.4 g.) was dissolved in 12 cc. of water and extracted with 24 cc., then 12 cc. of chloroform. Dried with magnesium sulfate, the combined extracts were evaporated to dryness *in vacuo*. The residue (9.3 g.) was distilled through a semi-micro Vigreux column to give the α-anomer IIIa as a colorless liquid, b.p. 85–88° (0.1 mm.), $[\alpha]^{24}D$ +17.6° (2% in H_2O), yield 4.9 g. (33%). The Vigreux column was replaced by a Claisen head and the β-anomer (IIIb) distilled at 108–110° (0.1 mm.), $[\alpha]^{24}D$ −64.2° (2% in H_2O), yield 3.2 g. (21%). Both compounds were colorless oils, soluble in water and had OH absorption at 2.90 μ and C–Me absorption at 7.25 μ in the infrared. The β-anomer was considerably more viscous than its anomer.

Anal. Calcd. for $C_9H_{16}O_5$: C, 52.8; H, 7.91. Found: (α) C, 53.1; H, 8.06. (β) C, 52.3; H, 7.93.

The same results can be obtained by substituting 50 mg. of *p*-toluenesulfonic acid for the sulfuric acid. The sulfonic acid catalyzed reaction gave from 266 g. of D-xylose, 120 g. (33%) of α-anomer and 75 g. (21%) of β-anomer. When the reaction time for acetonation was increased to 60 hours, 41% of α- and 31% of β-anomer were obtained. The chloroform extraction from a water solution leaves the non-acetonated xylosides in the aqueous layer. Unless removed these decomposed during the distillation with consequent lowering of the yield of β-anomer. The presence of VI, which boils about the same as IIIa, can readily be seen since it is insoluble in water and III is miscible with water in all proportions.

The above conditions are the result of 22 runs studying the acid concentration variable in this reaction. Percival and Zobrist[11] have vaguely described a procedure of acetonation where the exact amount of sulfuric acid is not stated. They described their product, obtained in 70% yield, as having a b.p. of 110° (0.1 mm.) and $[\alpha]D$ −26° (H_2O). Hence, either their b.p. range or their rotation is incorrect. The acetaldehyde which they reported to be an accelerator was found to be without effect on reactions run with or without added acid catalyst.

Methyl 2-*O*-Mesyl-3,5-*O*-isopropylidene-α-D-xylofuranoside (Va).—To a solution of 14.6 g. of IIIa in 40 cc. of reagent pyridine was added with cooling 8.7 cc. of methanesulfonyl chloride in portions at such a rate that the temperature was 20–25°. After the reaction was no longer exothermic (about 15 minutes), the solution was allowed to stand in a stoppered flask for 13–20 hours. Within 15 minutes pyridine hydrochloride had begun to separate. The mixture was poured into 200 cc. of ice-water and the solution extracted with two 100-cc. portions of chloroform. The combined extracts, dried with magnesium sulfate, were evaporated to dryness *in vacuo*. The residue was dissolved in 50 cc. of toluene and the evaporation repeated to remove pyridine; yield 18.6 g. (92%) of an orange gum, $[\alpha]^{24}D$ +65.7° (1.3% in MeOH). This compound had C–Me absorption at 7.28 μ, sulfonate absorption at 8.48 μ and no OH absorption in the infrared.

Anal. Calcd. for $C_{10}H_{18}O_7S$: C, 42.6; H, 6.43; S, 10.4. Found: C, 42.2; H, 6.30; S, 10.7.

Tosylation of IIIa in a similar manner gave a 94% yield of a gum which could not be crystallized.

Methyl 2-*O*-Mesyl-3,5-*O*-isopropylidene-β-D-xylofuranoside (Vb).—From 8.0 g. of IIIb as described for Va was obtained 11.2 g. (100%) of product as a gum, $[\alpha]^{24}D$ −34.5° (1.4% in MeOH), which could not be crystallized and was not quite pure. This compound has C-Me absorption at 7.27 μ, sulfonate absorption at 8.47 μ and no OH absorption in the infrared.

Anal. Calcd. for $C_{10}H_{18}O_7S$: C, 42.6; H, 6.43. Found: C, 40.1; H, 5.75.

Tosylation of IIIb in a similar manner gave 92% yield of a gum which could not be crystallized. Percival and Zobrist[11] have described a crystalline tosylate of III prepared from the α,β-mixture which rotated −45° (MeOH) and therefore has the β-configuration.

Methyl 2,3-Anhydro-α-D-lyxofuranoside (VIIa).—A solution of 13.0 g. of Va in 26 cc. of acetic acid and 11 cc. of water was heated in a bath at 50° for two hours,[14] and then evaporated to dryness *in vacuo* (bath 50°). The residue was suspended in 30 cc. of toluene and just enough absolute alcohol was added to form one layer, then the solution was evaporated to dryness *in vacuo*. The toluene treatment was repeated twice more to remove the traces of acetic acid thoroughly. The gummy residue of IVa was dissolved in 21 cc. of warm methanol, cooled in an ice-bath and treated with an ice-cold solution of 2.9 g. of sodium methoxide (Mathieson) in 29 cc. of methanol. Within 10 minutes sodium methanesulfonate began to separate. After 17 hours at 0–3°, the mixture was slurried with 3 g. of Celite and filtered. Acidified with 2 cc. of acetic acid, the combined filtrate and washings were evaporated to dryness *in vacuo*. The residue was dissolved in 6.5 cc. of water and extracted with three 13-cc. portions of chloroform. The combined magnesium sulfate dried extracts were evaporated to dryness *in vacuo*. Distillation of the residue (7.4 g.) gave 3.5 g. of product, b.p. 100° (0.1 mm.), which solidified in the receiver, m.p. 55–60°.

Anal. Calcd. for $C_6H_{10}O_4$: C, 49.3; H, 6.90. Found: C, 49.0; H, 7.26.

If the reaction time was increased to 3 days at 0°, the yield was 76% (65 g.), b.p. 98–100° (0.1 mm.), m.p. 72–75°, $[\alpha]^{26}D$ +44° (2% in H_2O). Recrystallization from benzene gave white crystals, m.p. 80–82°, $[\alpha]^{26}D$ +67° (2% in H_2O).

Anal. Calcd. for $C_6H_{10}O_4$: C, 49.3; H, 6.90. Found: C, 48.9; H, 6.72.

If a one-hour reflux of Va in methanol containing 1% hydrogen chloride was employed to remove the isopropylidene group to IVa,[11] then oxide formation was executed at 0° for 17 hours as above, the yield of product was 40% (2.7 g.) and the quality was poorer. The use of boiling methanolic sodium methoxide for oxide formation was complete in 5 minutes, but the yield of distilled product was lowered from 40 to 32%. Percival and Zobrist[15] described VIIa as having $[\alpha]^{15}D$ +51° (H_2O) and m.p. 81°.

Methyl 2,3-Anhydro-β-D-lyxofuranoside (VIIb).—From 14.8 g. of Vb by hydrolysis to IVb with 70% acetic acid at 50°, then ring closure to the oxide at 0° for 17 hours as described for VIIa, was obtained 4.3 g. (55%) of product, b.p. 95° (0.1 mm.) which solidified in the receiver to a hygroscopic waxy solid.

When the reaction time was increased to 3 days at 0°, the yield was 71% (37.8 g.), b.p. 98–100° (0.1 mm.), which solidified to a semi-solid and had $[\alpha]^{26}D$ −77° (2% in H_2O).

Anal. Calcd. for $C_6H_{10}O_4$: C, 49.3; H, 6.90. Found: C, 48.9; H, 7.11.

Recrystallization from ether gave white hygroscopic crystals, m.p. 74–75°, $[\alpha]^{25}D$ −102° (2% in H_2O).

Anal. Found: C, 49.5; H, 7.07.

Percival and Zobrist[15] obtained an α,β-mixture of VII wherein the β-anomer was not isolated.

Methyl N-Isopropylidene-3-amino-3-deoxy-α-D-arabinofuranoside (Xa).—A solution of 2.9 g. of crude, distilled VIIa in 15 cc. of concentrated ammonium hydroxide was heated in a steel bomb at 100° for 22 hours. The solution was shaken with Norit and filtered through Celite. The filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in 20 cc. of absolute alcohol and the evaporation repeated. The uncrystallizable gum was dissolved in 30 cc. of hot acetone, then chilled to 0°. The crystals were collected and washed with acetone; yield 2.1 g. (52%), m.p. 155–158°. Recrystallization from acetone gave white crystals, m.p. 157–159°, $[\alpha]^{26}D$ +98.5° (2% in H_2O). This compound had C-Me absorption at 7.25 μ, C=N absorption at 6.02 μ and OH absorption at 2.93 μ in the infrared.

Anal. Calcd. for $C_9H_{17}NO_4$: C, 53.2; H, 8.42; N, 6.88. Found: C, 53.1; H, 7.96; N, 7.12.

Similarly, a 54% yield was obtained from 65 g. of VIIa.

Methyl N-Isopropylidene-3-amino-3-deoxy-β-D-arabinofuranoside (Xb).—By heating 37.8 g. of crude distilled VIIb with 190 cc. of concentrated ammonium hydroxide for 19 hours as described for Xa was obtained 24.9 g. (47%) of product, m.p. 150–152°. Recrystallization from acetone gave white crystals, m.p. 155–157°, $[\alpha]^{25}D$ −96.0° (2% in H_2O). This compound gave a 30° depression in the m.p. when mixed with Xa and had OH absorption at 2.92 μ, C=N absorption at 6.02 μ and C-Me absorption at 7.27 μ in the infrared.

Anal. Calcd. for $C_9H_{17}NO_4$: C, 53.2; H, 8.42; N, 6.88. Found: C, 52.9; H, 8.69; N, 7.05.

3-Amino-3-deoxy-D-arabinose Hydrochloride (XI). (A).—A solution of 300 mg. of Xa in 6 cc. of 1% and 0.16 cc. of concentrated hydrochloric acid was refluxed for 3 hours, then evaporated to a sirup *in vacuo* (bath 40°). The sirup rapidly crystallized on cooling. Trituration with 2 cc. of acetic acid gave 220 mg. (64%) of product, m.p. 156–158° dec. Recrystallization from 0.5 cc. of water by addition of 5.5 cc. of acetic acid gave white crystals, m.p. 159° dec., $[\alpha]^{24}D$ −112° (2% in H_2O), which was positive to Benedict reagent. The rotation strongly suggests that this is a β-form.

Anal. Calcd. for $C_5H_{11}NO_4 \cdot HCl$: C, 32.4; H, 6.52; N, 7.55. Found: C, 32.7; H, 6.69; N, 7.76.

This compound gave no color when heated with ninhydrin in 1.5% sodium hydroxide, but gave a purple color with ninhydrin in 3% sodium bicarbonate. The same results were obtained with 3-amino-3-deoxy-D-ribose hydrochloride.[3] D-Glucosamine gave a deep blue color with ninhydrin in 1.5% sodium hydroxide reagent and a purple color with ninhydrin in 3% sodium bicarbonate.

(B).—Hydrolysis of 300 mg. of pure Xb as described in preparation A afforded 225 mg. (82%) of product, m.p. 158° dec. Recrystallization as in A gave white crystals, m.p. 160° dec., $[\alpha]^{25}D$ −110° (2% in H_2O). The compound gave an infrared spectrum identical with that of the product obtained by procedure A.

Anal. Calcd. for $C_5H_{11}NO_4 \cdot HCl$: C, 32.4; H, 6.52; N, 7.55. Found: C, 32.7; H, 6.67; N, 7.84.

Methyl 3-Acetamido-3-deoxy-α-D-arabinofuranoside (XIVa).—To a stirred solution of 25 g. of Xa in 125 cc. of water was added 18 cc. of acetic anhydride in one portion. The mixture was maintained at 30–35° by occasional cooling. After 7 minutes the solution was evaporated to dryness *in vacuo*. The hot residue was crystallized from 50 cc. of ethyl acetate; yield 22.7 g. (90%), m.p. 115–116°. Recrystallization from ethyl acetate–absolute alcohol afforded white crystals, m.p. 115–116°, $[\alpha]^{24}D$ +102° (2% in H_2O).

Anal. Calcd. for $C_8H_{15}NO_5$: C, 46.8; H, 7.36; N, 6.83. Found: C, 47.2; H, 7.30; N, 6.88.

Further acetylation in acetic anhydride–pyridine gave the triacetate as a sirup in 85% yield.

Methyl 3-Acetamido-3-deoxy-β-D-arabinofuranoside (XIVb).—Acetylation of 15 g. of Xb as described for XIVa gave, on evaporation of the water solution, 14.8 g. (98%) of product, m.p. 150–152°. Recrystallization of a pilot run (96% yield) from ethyl acetate–absolute alcohol afforded white crystals, m.p. 155°, $[\alpha]^{24}D$ −119° (1.7% in H_2O).

Anal. Calcd. for $C_8H_{15}NO_5$: C, 46.8; H, 7.36; N, 6.83. Found: C, 47.2; H, 7.56; N, 6.41.

Methyl 3-Acetamido-3-deoxy-5-*O*-benzoyl-α-D-arabinofuranoside (XVa).—To a solution of 3.0 g. of XIVa in 15 cc. of reagent pyridine and 15 cc. of ethylene dichloride cooled to 3° in an ice-bath was added 1.86 cc. of benzoyl chloride. After 19 hours at 3° the mixture was washed with 120 cc. of water. The aqueous layer was extracted with two 25-cc. portions of ethylene dichloride. The combined extracts, washed with water and dried with magnesium sulfate, were evaporated to dryness *in vacuo*. The residue was twice dissolved in toluene and the evaporation repeated. The oil (4.1 g.) was dissolved in 10 cc. of wet ethyl acetate and

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]

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Puromycin. Synthetic Studies. IX. Total Synthesis

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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.