hydrazinolysis the number 2 position of propylhygric acid is completely racemized. It, therefore, is likely that the tritium lost to the hydrazine solvent originated in this position of the lincomycin. Thus, 13.7% and 24.5% of the molecular tritium can be assigned to the number 2 position of the propylhygric acid moiety in the cases of the noncatalytic and catalytic samples, respectively.

In regard to ease of purification, yield of product, and incorporation of stable tritium, the catalytic method was superior to the conventional Wilzbach method for tritiating lincomycin. On the other hand, the noncatalytically tritiated lincomycin had a more uniform distribution of tritium making it more suitable for drug metabolism studies. If metabolism resulted in hydrolysis of lincomycin at the amide linkage, the methyl thiolincosaminide moiety could not be traced in the case of the catalytic sample because of its lack of tritium.

REFERENCES

(1) Mason, D. J., Dietz, A., and DeBoer, C., *Antimicrobial Agents Chemotherapy-1962*, **1962**, 554.
(2) Herr, R. R., and Bergy, M. E., *ibid.*, **1962**, 560.
(3) Hoeksema, H., Bannister, B., Birkenmeyer, R. D., Kagan, F., Magerlein, B. J., MacKellar, F. A., Schroeder, W., Slomp, G., and Herr, R. R., *J. Am. Chem. Soc.*, **84**, 4223 (1964).
(4) Herr, R. R., and Slomp, G., *ibid.*, to be published.
(5) Schroeder, W., Bannister, B., and Hoeksema, H., *ibid.*, to be published.
(6) Magerlein, B. J., Birkenmeyer, R. D., Herr, R. R., and Kagan, F., *ibid.*, to be published.
(7) Slomp, G., and MacKellar, F. A., *ibid.*, to be published.
(8) Daikos, G. K., Kontomichalou, P., Papadoyannakis, N., and Tourkantonis, A., *Antimicrobial Agents Chemotherapy-1963*, **1964**, 197; Holloway, W. J., Kahlbaugh, R. A., and Scott, E. G., *ibid.*, **1964**, 200; Harnecker, J., Contreras, J., Gilabert, B., and Ubilla, V., *ibid.*, **1964**, 205; Walters, E. W., Romansky, M. J., and Johnson, A. C., *ibid.*, **1964**, 210; Trakas, J. C., and Lind, H. E., *ibid.*, **1964**, 216.
(9) Wilzbach, K. E., *J. Am. Chem. Soc.*, **79**, 1013(1957).
(10) Thomas, R. C., and Ikeda, G. J., *J. Pharm. Sci.*, **55**, 112(1966).
(11) Meshi, T., and Takahashi, T., *Bull. Chem. Soc. Japan*, **35**, 1510(1962).
(12) Meshi, T., and Sato, Y., *ibid.*, **37**, 683(1964).
(13) Herberg, R. J., *Anal. Chem.*, **32**, 42(1960).

Synthesis of Tetrose Nucleosides I

Adenine Nucleosides of Erythrose and Threose

By DANIEL H. MURRAY and JOHN PROKOP

D- and L-Erythrose and D- and L-threose were individually converted to their triacetates which were condensed with chloromercuri-6-benzamidopurine in the presence of titanium tetrachloride. After deacylation, the four crude mixtures of anomeric nucleosides were each resolved on a strong anion exchange resin, leading to the isolation of all eight possible 9-tetrafuranosyladenines. The anomeric configurational assignments were made by consideration of the mechanism of nucleoside condensation (Baker's *trans* rule) and by Hudson's rules of isorotation. Preliminary results of tests for biological activity with *Streptococcus faecalis* 8043 and with adenosine deaminase are reported.

In recent years substantial attention has been directed toward the synthesis of potential inhibitors of nucleic acid metabolism. This has led to the preparation of a wide variety of 5- and 6-carbon sugar nucleosides, of which several of the compounds containing pentose sugar moieties have exhibited antibacterial and/or antitumor activity, for example, cordycepin, xylofuranosyladenine, and spongoadenosine. The majority of such nucleosides have been shown to act as nucleotides *via* phosphorylation at the 5′-position. On the other hand, some compounds have been shown to act in their nucleoside form, for example, decoyinine (1) and 5′-deoxyxylofuranosyladenine (2). In these, lack of a hydroxyl group at a terminal position removes the possibility of nucleotide formation. The 9-tetrofuranosyladenines are further examples of this class of nucleosides. Only the 9-β-D-erythrofuranosyladenine (III) has been prepared previously (3). It has been of interest in the present study to prepare all eight of the possible 9-tetrosyladenines. The biological activity of these is reported in a separate communication (2).

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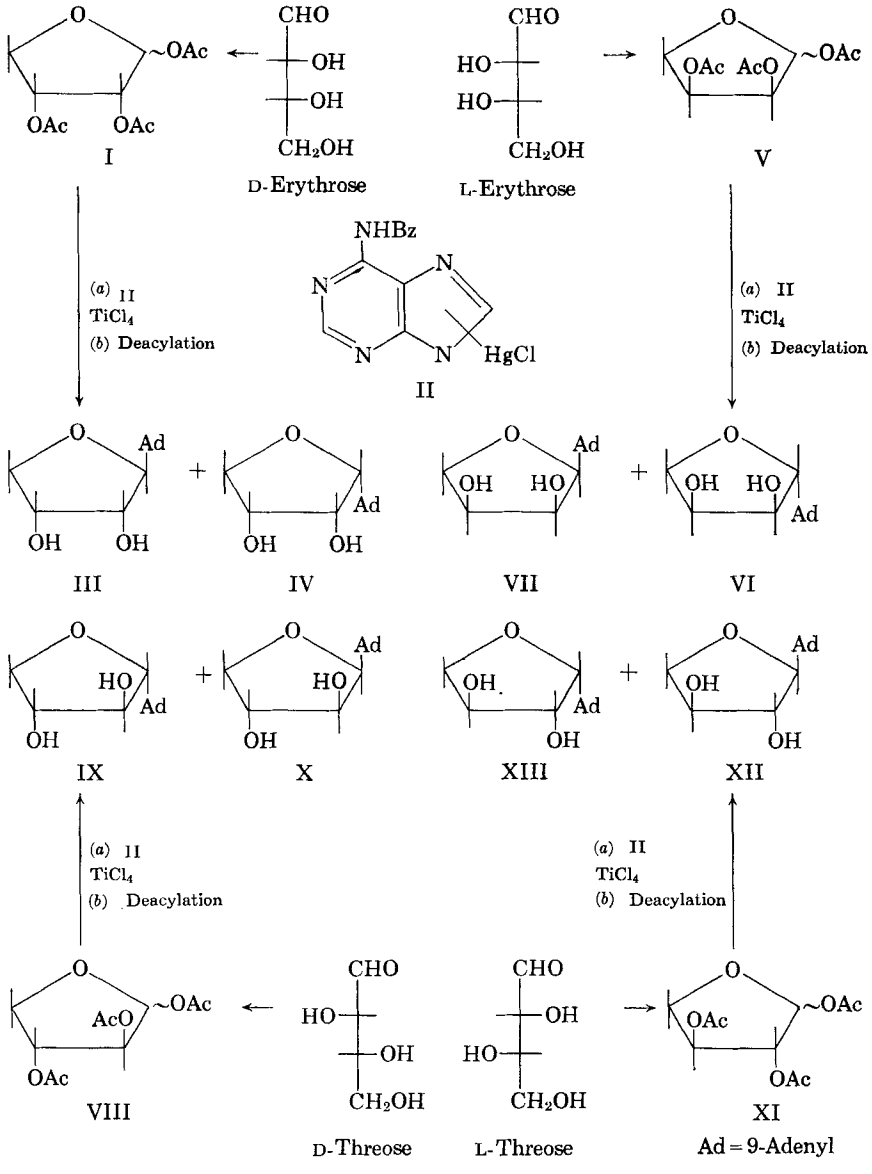
DISCUSSION

The general method of synthesis involved coupling of an acetylated sugar with chloromercuri-6-benzamidopurine (II) (Scheme I) in the presence of titanium tetrachloride. Previous experience with this method (4, 5) has indicated that higher yields of nucleoside are frequently obtained as compared with the more usual procedure of condensation in which the acylated sugar is first converted to the corresponding halide. Furthermore, it has been reported that the use of titanium tetrachloride has led to the formation of significant quantities of the *cis* nucleoside[1] (6, 7). It was anticipated that at least one of the *cis* nucleosides in the present series, 9-β-D-threofuranosyladenine (X), might have significant biological activity. Hence, the authors

[1] The terminology *cis* or *trans* nucleoside refers to the configuration of substituents on carbons 1 and 2 of the sugar moiety.



Scheme I

have sought a procedure which would yield sufficient of a desired *cis* nucleoside for limited biological testing. The titanium tetrachloride coupling step was a desirable element in the procedure.

A second desirable element was a routine method for separating the anomeric pairs of nucleosides. Although such anomeric mixtures of nucleosides, particularly those resulting from condensation of 2-deoxysugars, have been resolved satisfactorily through fractional crystallization procedures, this method is tedious and becomes of more limited use when one of the anomers in the mixture is present in only a relatively small proportion. Chromatographic separations involving adsorption techniques are also limited because of their inability to handle practical amounts of compounds, and, in some instances, because of poor resolution necessitating rechromatography. A recent method, which is reported to give good resolution and has large capacity, involves chromatography on a strong anion exchange resin, and utilizes the differences in pKa's of the sugar hydroxyl groups at high pH (8). Application of this procedure to the mixtures of nucleosides obtained by the condensation of acetylated tetroses led to the isolation of all eight possible 9-tetrafuranosyladenines.

The four tetroses required for these syntheses were obtained through established routes. D-Erythrose was prepared by periodate cleavage of 4,6-*O*-ethylidene-D-glucose followed by acid hydrolysis (9). The resulting syrupy sugar was acetylated using acetic anhydride in pyridine to give the syrupy acetate (I) which was probably an anomeric mixture. Condensation of I with chloromercuri-6-benzamidopurine (II) in the presence of titanium tetrachloride, deacylation with methanolic ammonia,

TABLE I—MELTING POINTS AND SPECIFIC ROTATION FOR THE NUCLEOSIDES

Nucleoside	M. p.	$[\alpha]_D^{20}$
III	239–240° dec.	−114°
VI	238.5–239.5° dec.	+112°
IV	217–218°	+45°
VII	216.5–218°	−44°
IX	214.5–215°	+66°
XII	214.5–215°	−66°
X	239.5–240.5° dec.	−5°
XIII	240–241° dec.	+5°

and chromatography on anion exchange resin of the quaternary ammonium type[2] led to the isolation of crystalline 9-β-D-erythrofuranosyladenine (III) and 9-α-D-erythrofuranosyladenine (IV) in yields of 31 and 13%, respectively. Sixty per cent aqueous methanol[3] was used as eluent throughout these separations.

L-Erythrose was obtained by the procedure starting from rhamnose (10). The sugar was acetylated as before to give the syrupy triacetate (V) in 81% yield. Similar condensation with II and deacylation with methanolic sodium methoxide, followed by chromatographic separation on the anion exchange resin gave the crystalline β- and α-anomers (VI and VII) in yields of 36 and 7.4%, respectively, based on the crude triacetate (V). That these nucleosides (VI and VII) were the enantiomers of III and IV can be seen by comparison of their melting points and specific rotations as shown in Table I. In addition, the infrared spectra of these mirror-image pairs were superimposable.

D- and L-Threose were obtained by periodate oxidation of 1,3-*O*-benzylidene-D- and -L- arabitol, respectively, followed by acid hydrolysis (11). These sugars were readily converted to their triacetates using acetic anhydride in pyridine. The product in each case consisted of both a crystalline isomer and a noncrystallizing syrup obtained from the liquors, and were probably the anomeric forms (12). Either of these products could be converted to the desired nucleosides. Crystalline D-threose triacetate (VIII) was converted to the anomeric adenine nucleosides as described above for D- and L-erythrose. Chromatography of the crude product on the anion exchange resin gave only two ultraviolet absorbing peaks which proved to be 9-α-D-threofuranosyladenine (IX) and 9-β-D-threofuranosyladenine (X) in yields of 55 and 10%, respectively, based on the triacetate. When the crude, syrupy triacetate (VIII), obtained from the mother liquors, was condensed and chromatographed, additional α- (IX) and β- (X) nucleosides resulted in yields of 31 and 3.1%, respectively, along with several minor ultraviolet absorbing peaks which were not characterized.

Crystalline L-threose triacetate (XI) was similarly converted to 9-α-L-threofuranosyladenine (XII) and 9-β-L-threofuranosyladenine (XIII) in yields of 57% and 12%, respectively. When the syrupy triacetate (XI) was employed, the respective yields of α- and β-anomers were 34 and 4.3%. That the α-nucleosides (IX and XII) and the β-nucleosides (X and XIII) represented enantiomeric pairs can be seen by comparison of the constants shown in Table I. The infrared spectra of these pairs were superimposable.

Periodate consumption studies established the furanoside structure of these nucleosides. In addition, the D-erythrosides (III and IV) oxidized rapidly with 1 *M* equivalent of periodate being consumed within 5 min. In contrast, oxidation of the L-threosides (XII and XIII) was considerably slower with 1 *M* equivalent of periodate being consumed only after 5 days. These results are consistent with those reported earlier for the periodate consumption of 2′,3′-*cis* and -*trans* hydroxyl groups in nucleosides containing furanosyl sugars (13).

The anomeric configurations of these nucleosides could be implied by their relative yields. So far as is known, the *trans* nucleoside predominates in all nucleoside condensations including those involving the titanium tetrachloride procedure (6, 7, 14). Application of Hudson's rules of isorotation (Table I) supports these assignments.

Biological Activity—The effect of these nucleosides on growth was examined in *Streptococcus faecalis* 8043. Only compounds X and XII were inhibitory, preventing 50% of growth at 2×10^{-5} *M*. All the others were negative. Compounds X, XII, and VII were significantly inhibitory in an adenosine deaminase system. These results are discussed in detail elsewhere (2).

EXPERIMENTAL[4]

D-Erythrose Triacetate (I)—To a stirred solution of 7.09 Gm. (54.8 mmoles) of D-erythrose syrup (9) in 50 ml. of reagent pyridine was added 23.5 ml. (250 mmoles) of acetic anhydride dropwise below 20°. The flask was stoppered and the reaction was allowed to remain at room temperature overnight. The dark amber reaction was then poured into 200 ml. of an ice water mixture with stirring and, after 30 min., the mixture was extracted with chloroform (3 × 100 ml.). These extracts were combined and washed with aqueous saturated sodium bicarbonate (3 × 100 ml.) and water (100 ml.). After drying over magnesium sulfate, the extract was treated with activated charcoal[5] and filtered through diatomaceous earth[6] giving a pale yellow filtrate which was evaporated to dryness *in vacuo* (60°). The last traces of pyridine were removed by evaporative distillation *in vacuo* with 25 ml. of toluene yielding 12.0 Gm. (89%) of a light amber syrup which was sufficiently pure for condensation. For analysis, a sample was distilled in a sublimation tube and a clear, colorless liquid was collected at 105–110°/0.06 mm.; $[\alpha]_D^{28}$ −66.2° (c 4.8, $CHCl_3$); $\nu_{max.}^{film}$ (cm.$^{-1}$) 1750 (acetate C=O), 1375 (methyl), 1220 (acetate C—O—C).

Anal.—Calcd. for $C_{10}H_{14}O_7$: C, 48.78; H, 5.73. Found: C, 48.55; H, 5.81.

[2] Marketed as Bio-Rad AG1×8(OH) by Bio-Rad Laboratories, Richmond, Calif.

[3] Practical grade methanol was entirely satisfactory for this purpose.

[4] Melting points were obtained using a Mel-Temp instrument and are uncorrected. The infrared and ultraviolet spectra were determined on Perkin-Elmer models 337 and 202, respectively, and optical rotations were obtained using a Perkin–Elmer 141 polarimeter. The column chromatograms were monitored with an ISCO model UA analyzer. Galbraith Laboratories, Inc., Knoxville, Tenn., performed the elemental analyses.

[5] Marketed as Norit by American Norit Co., Inc., Jacksonville, Fla.

[6] Marketed as Celite by Johns-Manville, New York, N. Y.

9-α- and β-D-Erythrofuranosyladenine (IV and III, Respectively)—A mixture of 9.27 Gm. (38 mmoles) of crude erythrose triacetate (I), 17.9 Gm. (38 mmoles) of chloromercuri-6-benzamidopurine (II) (4, 5), 18 Gm. of diatomaceous earth, and 650 ml. of ethylene dichloride was distilled until 80 ml. of distillate had been collected. After cooling to room temperature, a solution of 4.13 ml. (38 mmoles) of titanium tetrachloride in 45 ml. of ethylene dichloride was added dropwise and the mixture was then heated under reflux overnight while protected from moisture. The mixture was cooled to room temperature and 40 ml. of aqueous saturated sodium bicarbonate was added while stirring vigorously. Additional solid sodium bicarbonate was added until a neutral reaction was obtained. The mixture was filtered through diatomaceous earth and the cake was washed with additional ethylene dichloride (3 × 35 ml.). After combining the filtrate and washings and removal of the solvent *in vacuo*, the residue was redissolved in 300 ml. of chloroform, filtered free of some insolubles, and washed with 300 ml. of 30% aqueous potassium iodide and 300 ml. of water. The organic solution was dried over magnesium sulfate, treated with activated charcoal, and filtered, and the filtrate was evaporated to dryness *in vacuo* (60°) leaving crude 2',3'-di-*O*-acetyl-9-α- and β-D-erythrofuranosyl-6-benzamidopurine as an amber glass; yield, 11.3 Gm. (71%); $\bar{\nu}_{max.}^{KBr}$ (cm.$^{-1}$) 1750 (acetate C=O), 1700 (amide C=O), 1375 (methyl), 1220 (acetate C—O—C), 710 (monosubstituted benzene).

A solution of 11.2 Gm. of the above blocked nucleosides in 360 ml. of anhydrous methanol was saturated with ammonia gas while maintaining the temperature at about 5°. After standing in a stoppered flask at 5° overnight, the ammonia and solvent were removed *in vacuo* and the residue partitioned between 300 ml. of water and 100 ml. of chloroform. The organic phase was separated and the aqueous phase was washed further with chloroform (2 × 100 ml.). The organic phases were combined and back-washed with 100 ml. of water which was then added to the original aqueous phase. A small amount of insolubles was removed by filtration and the aqueous phase was then concentrated *in vacuo* to about 100 ml. After addition of 120 ml. of methanol, the nucleoside-containing solution was transferred to the top of a column (8.0 × 50 cm.), packed with the anion exchange resin, which was previously equilibrated with 60% aqueous methanol. The nucleosides were eluted with the same solvent at a rate of approximately 475 ml./hr. with the fractions collected at 1-hr. intervals. Fractions 23–28 were combined and evaporated to dryness giving the α-anomer (IV) as needles; yield, 1.15 Gm. (13%), m.p. 212–217°. Two recrystallizations from 95% ethanol gave the analytical sample, m.p. 217–218°; $[\alpha]_D^{20}$ + 45° (c 0.67, H_2O); $\lambda_{max.}^{pH\ 1}$ (mμ) 257.5 (ε 14,500); $\lambda_{max.\ 259.5}^{pH\ 7\ and\ 13}$ (ε 15,000); $\nu_{max.}^{KBr}$ (cm.$^{-1}$) 3400–3100 (broad OH and NH), 1605, and 1575 (C=C and C=N). Periodate consumption (moles of periodate/mole of compound) (13, 17, 18): 1.00 (5 min. and constant).

Anal.—Calcd. for $C_9H_{11}N_5O_3$: C, 45.57; H, 4.67; N, 29.52. Found: C, 45.54; H, 4.60; N, 29.70.

The β-anomer (III) was obtained by combining fractions 42–58 and removing the solvent *in vacuo* to give a nearly white, crystalline solid; yield, 2.74 Gm. (31%), m.p. 224.5–225.5° dec. The analytical sample was obtained by recrystallizing twice from 50% aqueous ethanol, m.p. 239–240° dec.; $[\alpha]_D^{20}$ −114° (c 0.40, H_2O); $\lambda_{max.}^{pH\ 1}$ (mμ) 257.5 (ε 14,000); $\lambda_{max.}^{pH\ 7\ and\ 13}$ 260 (ε 14,500); $\bar{\nu}_{max.}^{KBr}$ (cm.$^{-1}$) 3355, 3130 (OH and NH), 1605, 1575 (C=C and C=N). Periodate consumption (moles of periodate/mole of compound) (13, 17, 18): 0.95 (5 min.), 0.99 (1 hr.), 1.00 (48 hr.).

Anal.—Calcd. for $C_9H_{11}N_5O_3$: C, 45.57; H, 4.67; N, 29.52. Found: C, 45.69; H, 4.78; N, 29.54.

L-Erythrose Triacetate (V)—To a stirred solution of 3.64 Gm. (30 mmoles) of syrupy L-erythrose (10) in 30 ml. of reagent pyridine was added 12.9 ml. (136 mmoles) of acetic anhydride dropwise while maintaining the temperature below 20°. The reaction was allowed to remain at room temperature in a stoppered flask overnight, then poured into 100 ml. of an ice water mixture and worked up as described above for the D-isomer; yield of light amber syrup was 6.05 Gm. (81%) and was sufficiently pure for condensation. For analysis, a sample was distilled in a sublimation tube and a clear, colorless liquid was collected at 105°/0.06 mm.; $[\alpha]_D^{28}$ +63.7° (c 3.6, $CHCl_3$); $\bar{\nu}_{max.}^{film}$ (cm.$^{-1}$) 1750 (acetate C=O), 1375 (methyl), 1220 (acetate C—O—C).

Anal.—Calcd. for $C_{10}H_{14}O_7$: C, 48.78; H, 5.73. Found: C, 49.29; H, 6.04.

9-α- and β-L-Erythrofuranosyladenine (VII and VI, Respectively)—A mixture of 5.59 Gm. (23 mmoles) of crude L-erythrose triacetate (V), 10.8 Gm. (23 mmoles) of chloromercuri-6-benzamidopurine, 11 Gm. of diatomaceous earth, and 400 ml. of ethylene dichloride was distilled until 60 ml. of distillate had been collected. The reaction was cooled to room temperature and a solution of 2.5 ml. (23 mmoles) of titanium tetrachloride in 25 ml. of ethylene dichloride was added dropwise. The reaction was then heated under reflux for 18 hr. while protected from moisture. After cooling the mixture to room temperature, 25 ml of aqueous saturated sodium bicarbonate was added followed by additional solid sodium bicarbonate while stirring vigorously until a neutral reaction was obtained. The mixture was then processed as described for the D-isomers leaving crude 2',3'-di-*O*-acetyl-9-α- and β-L-erythrofuranosyl-6-benzamidopurine as a light amber glass; yield, 7.05 Gm. (73%); $\bar{\nu}_{max.}^{KBr}$ (cm.$^{-1}$) 1750 (acetate C=O), 1700 (amide C=O), 1375 (methyl), 1220 (acetate C—O—C), 710 (monosubstituted benzene).

A solution of 6.82 Gm. of the crude, blocked nucleosides in 160 ml. of 0.1 *N* methanolic sodium methoxide was allowed to stand at room temperature in a stoppered flask for 24 hr. After neutralizing the reaction with glacial acetic acid and removal of the solvent *in vacuo*, the residue was partitioned between 100 ml. of water and 100 ml. of chloroform. The organic phase was separated and the aqueous phase washed further with chloroform (2 × 50 ml.), filtered free of insolubles, and evaporated to near dryness *in vacuo*. The residue was redissolved in 100 ml. of 50% aqueous methanol and transferred to the top of a column (3.8 × 50 cm.) packed with the anion exchange resin. The nucleosides were eluted using 60% aqueous methanol (to which the column had been equilibrated originally) at a rate of approximately 80 ml./fraction. Fractions were

collected at 1-hr. intervals. The α-anomer (VII) was obtained by combining fractions 31–38. These fractions also contained a small amount of a contaminating substance which immediately preceded the desired peak from which it was incompletely separated. Subsequent rechromatography of these combined fractions did allow complete removal of the interfering substance. Removal of the solvent *in vacuo* left the desired nucleoside (VII) as a crystalline solid; yield 385 mg. (7.4% from L-erythrose triacetate), m.p. 207–211°. Three recrystallizations from 95% ethanol gave the analytical sample, m.p. 216.5–218°; $[\alpha]_D^{20}$ −44° (c 0.61, H_2O); $\lambda_{max.}^{pH\ 1}$ (mμ) 257.5 (ε 14,200), $\lambda_{max.}^{pH\ 7\ and\ 13}$ 259.5 (ε 14,800). The infrared spectrum of this substance was superimposable with that of the D-isomer (IV). Mixed melting point with IV was depressed.

Anal.—Calcd. for $C_9H_{11}N_5O_3$: C, 45.57; H, 4.67; N, 29.52. Found: C, 45.81; H, 4.75; N, 29.74.

Combination of fractions 55–74 and removal of the solvent *in vacuo* gave the β-anomer (VI) as a crystalline solid; yield, 1.88 Gm. (36% from L-erythrose triacetate), m.p. 229–233°. For analysis, a sample was recrystallized twice from 50% aqueous ethanol, m.p. 238.5–239.5° dec.; $[\alpha]_D^{20}$ +112° (c 0.42, H_2O); $\lambda_{max.}^{pH\ 1}$ (mμ) 257.5 (ε 14,000), $\lambda_{max.\ 260}^{pH\ 7\ and\ 13}$ (ε 14,300). The infrared spectrum was superimposable with that of the D-isomer (III). Mixed melting point with III was depressed.

Anal.—Calcd. for $C_9H_{11}N_5O_3$: C, 45.57; H, 4.67; N, 29.52. Found: C, 45.62; H, 4.80; N, 29.71.

D-Threose Triacetate (VIII)—To a stirred solution of 3.52 Gm. (29 mmoles) of syrupy D-threose (11) in 30 ml. of reagent pyridine was added dropwise 20 ml. of acetic anhydride below room temperature. The reaction was allowed to remain at room temperature in a stoppered flask overnight, then poured into 100 ml. of an ice water mixture to decompose the remaining anhydride. The mixture was extracted with chloroform (3 × 50 ml.) and the combined extracts were washed with aqueous saturated sodium bicarbonate (4 × 50 ml.) and water (50 ml.). The extract was dried over magnesium sulfate, treated with activated charcoal, and filtered, and the filtrate evaporated to dryness *in vacuo* (50°). The last traces of pyridine were removed by repeated distillations *in vacuo* of toluene (3 × 25 ml.) yielding 6.79 Gm. (94%) of a partially crystalline syrup. After trituration with 30 ml. of absolute ethanol, the crystalline portion was collected on a filter, washed with absolute ethanol, and dried; yield, 1.43 Gm. (20%), m.p. 115.5–117°. One recrystallization from absolute ethanol gave m.p. 117–118.5°; $[\alpha]_D^{28}$ +35.3° (c, 4.1, $CHCl_3$). [Lit. (12) m.p. 117–118°, [α] +35.55 (c 4, $CHCl_3$).]

The above filtrate was evaporated to dryness *in vacuo* (50°) yielding 5.31 Gm. (74%) of the noncrystalline anomer of D-threose triacetate. This material was also suitable for condensation.

9-α- and β-D-Threofuranosyladenine (IX and X, Respectively)—A mixture of 1.23 Gm. (5 mmoles) of crystalline D-threose triacetate, 2.37 Gm. (5 mmoles) of chloromercuri-6-benzamidopurine (II), 3 Gm. of diatomaceous earth, and 100 ml. of ethylene dichloride was distilled until 25 ml. of distillate had been collected. The reaction was cooled to room temperature and a solution of 0.55 ml. (5 mmoles) of titanium tetrachloride in 6 ml. of ethylene dichloride was added dropwise. The mixture was then heated under reflux overnight while protected from moisture. To the cooled mixture was added 16 ml. of aqueous saturated sodium bicarbonate with vigorous stirring, followed by additional solid sodium bicarbonate until a neutral reaction was obtained. After filtration through diatomaceous earth, the cake was washed with 35 ml. of ethylene dichloride, the washing combined with the filtrate, and the solvent removed *in vacuo*. A solution of the residue in 50 ml. of chloroform was filtered free of some insolubles, then washed with 50 ml. of 30% aqueous potassium iodide and 50 ml. of water. The chloroform solution was dried over magnesium sulfate, treated with activated charcoal and filtered through diatomaceous earth, and the filtrate evaporated to dryness *in vacuo* (50°) leaving crude 2′, 3′-di-*O*-acetyl-9-α and β-D-threofuranosyl-6-benzamidopurine as an amber glass; yield 1.77 Gm. (83%); $\bar{\nu}_{max}^{KBr}$ ($cm.^{-1}$) 1750 (acetate C=O), 1700 (amide C=O), 1375 (methyl), 1220 (acetate C—O—C), 710 (monosubstituted benzene).

A solution of 1.75 Gm. of the above blocked nucleosides in 40 ml. of 0.1 *N* methanolic sodium methoxide was allowed to stand at room temperature in a stoppered flask for 24 hr. Without further processing, the solution was transferred to the top of a column (3.8 × 50 cm.) containing the anion exchange resin which had previously been equilibrated with 60% aqueous methanol. The nucleosides were eluted using 60% aqueous methanol at a rate of approximately 80 ml./hr. Fractions were collected at 1-hr. intervals. The β-isomer (X) was isolated by evaporation of fractions 60–70 which yielded 121 mg. (10.4% from D-threose triacetate) of a crystalline solid, m.p. 234–236° dec. Two recrystallizations from 95% ethanol gave the analytical sample, m.p. 239.5–240.5° dec.; $[\alpha]_D^{20}$ −5° (c 0.40, H_2O); $\lambda_{max.}^{pH\ 1}$ (mμ) 257.5 (ε 14,000), $\lambda_{max.\ 260}^{pH\ 7\ and\ 13}$ (ε 14,600); $\bar{\nu}_{max.}^{KBr}$ ($cm.^{-1}$) 3400–3100 (broad OH and NH), 1605, 1575 (C=C and C=N).

Anal.—Calcd. for $C_9H_{11}N_5O_3$: C, 45.57; H, 4.67; N, 29.52. Found: C, 45.66; H, 4.71; N, 29.66.

The α-isomer (IX) was isolated from fractions 74–91 as a crystalline solid; yield, 641 mg. (54.6% from D-threose triacetate), m.p. 213–215°. A pure sample was obtained by recrystallizing twice from 95% ethanol and thorough drying at 100°, m.p. 214.5–215°; $[\alpha]_D^{20}$ + 66° (c 0.58, H_2O); $\lambda_{max.}^{pH\ 1}$ (mμ) 258 (ε 14,000), $\lambda_{max.\ 260}^{pH\ 7\ and\ 13}$ (ε 14,500); $\bar{\nu}_{max.}^{KBr}$ ($cm.^{-1}$ 3500–3150 (broad OH and NH), 1605, 1575 (C=C and C=N).

Anal.—Calcd. for $C_9H_{11}N_5O_3$: C, 45.57; H, 4.67; N, 29.52. Found: C, 45.84; H, 4.82; N, 29.72.

When the crude, noncrystallizing portion of D-threose triacetate (above) was condensed, deacylated, and chromatographed in a similar manner, additional nucleosides were obtained amounting to 31% of the α- and 3.1% of the β-isomer.

L-Threose Triacetate (XI)—To a stirred solution of 3.75 Gm. (31 mmoles) of syrupy L-threose (15) in 35 ml. of reagent pyridine was added 24 ml. (255 mmoles) of acetic anhydride dropwise below 30°. After standing overnight at room temperature in a stoppered flask, the solvents were removed *in vacuo* (50°). The remaining traces of pyridine and acetic acid were removed by repeated distillation *in vacuo*

of toluene (3 × 30 ml.). A solution of the residue in 200 ml. of chloroform was treated with activated charcoal, filtered through diatomaceous earth, and the filtrate evaporated to dryness *in vacuo* (60°) leaving a partially crystalline, amber syrup; yield, 7.18 Gm. (93%). The crystalline anomer was obtained by triturating the residue with 10 ml. of absolute ethanol and collecting the product on a filter; yield 2.22 Gm. (29%), m.p. 114–117°. One recrystallization from 10 ml. of absolute ethanol gave 2.17 Gm. of the pure compound, m.p. 117–118°; $[\alpha]_D^{28}$ −35.5° (c 4.2, $CHCl_3$). [Lit. (12) m.p. 117–118°, $[\alpha]_D$ +35.55° (c 4, $CHCl_3$) for the D-isomer.]

Evaporation of the filtrate to dryness gave 4.95 Gm. (64%) of the crude, noncrystalline anomer of L-threose triacetate which was also suitable for condensation.

9-α- and β-L-Threofuranosyladenine (XII and XIII, Respectively)—Crystalline L-threose triacetate (XI), 1.23 Gm. (5 mmoles), was condensed with chloromercuri-6-benzamidopurine (II) in exactly the same manner as described for the D-isomer. This resulted in the isolation of 1.70 Gm. (80%) of crude 2′,3′-di-*O*-acetyl-9-α- and β-L-threofuranosyl-6-benzamidopurine as a yellow glass; $\bar{\nu}_{max.}^{KBr}$ (cm.$^{-1}$) 1750 (acetate C=O), 1700 (amide C=O), 1375 (methyl), 1220 (acetate C—O—C), 710 (monosubstituted benzene).

A solution of 1.03 Gm. of the crude, blocked nucleosides in 25 ml. of 0.1 *N* methanolic sodium methoxide was allowed to remain at room temperature for 24 hr. in a stoppered flask. Without further processing, the solution was transferred to the top of a column (3.8 × 50 cm.) of the anion exchange resin previously equilibrated with 60% aqueous methanol. The elution procedure was identical to that described for the D-isomers except that the rate was somewhat slower, approximately 70 ml./hr. Fractions 68–81 were combined and evaporated to dryness *in vacuo* to give 84 mg. (11.7% from L-threose triacetate) of crystalline β-anomer (XIII), m.p. 236.5–238° dec. The analytically pure compound was obtained after two recrystallizations from 95% ethanol, m.p. 240–241° dec.; $[\alpha]_D^{20}$ +5° (c 0.49, H_2O); $\lambda_{max.}^{pH\ 1}$ (mμ) 258 (ε 14,100), $\lambda_{max.\ 260}^{pH\ 7\ and\ 13}$ (ε 14,500). The infrared spectrum was superimposable with that obtained for the β-D-isomer (X). Mixed melting point with the D-isomer (X) showed a depression. Periodate consumption (moles of periodate/mole of compound) (13, 17, 18): 0.05 (5 min.), 0.12 (1 hr.), 0.60 (24 hr.), 0.83 (72 hr.), 0.91 (96 hr.), 0.98 (120 hr.).

Anal.—Calcd. for $C_9H_{11}N_5O_3$: C, 45.57; H, 4.67; N, 29.52. Found: C, 45.61; H, 4.70; N, 29.46.

The crystalline α-isomer (XII) was obtained by combining fractions 85–107 and evaporating to dryness *in vacuo;* yield, 409 mg. (57.0% from L-threose triacetate), m.p. 211.5–213°. Two recrystallizations from 95% ethanol and thorough drying *in vacuo* at 100° for at least 24 hr. gave the pure compound, m.p. 214.5–215°; $[\alpha]_D^{20}$ −66° (c 0.60, H_2O); $\lambda_{max.}^{pH\ 1}$ (mμ) 258 (ε 13,800); $\lambda_{max.}^{pH\ 13}$ 260 (ε 14,200), $\lambda_{max.}^{H_2O}$ 260 (ε 14,200). The infrared spectrum was superimposable with that obtained for the α-D-isomer (IX). Mixed melting point with IX was depressed. Periodate consumption (moles of periodate/mole of compound) (13, 17, 18): 0.02 (5 min.), 0.08 (1 hr.), 0.59 (24 hr.), 0.80 (48 hr.), 0.89 (72 hr.), 0.94 (96 hr.), 0.98 (120 hr.).

Anal.—Calcd. for $C_9H_{11}N_5O_3$: C, 45.57; H, 4.67; N, 29.52. Found: C, 45.43; H, 4.58; N, 29.72.

Condensation of crude, noncrystalline L-threose triacetate (above), deacylation, and chromatography as described above gave the α-anomer (XII) in 34% yield and the β-anomer (XIII) in 4.3% yield.

REFERENCES

(1) Bloch, A., and Nichol, C. A., *Biochem. Biophys. Res. Commun.*, **16**, 400(1964).
(2) Murray, D. H., Prokop, J., and Bloch, A., unpublished data.
(3) Shah, R. H., Schaeffer, H. J., and Murray, D. H., unpublished data.
(4) Prokop, J., and Murray, D. H., *J. Pharm. Sci.*, **54**, 359(1965).
(5) Murray, D. H., and Prokop, J., *ibid.*, **54**, 1468(1965).
(6) Baker, B. R., Schaub, R. E., and Kissman, H. M., *J. Am. Chem. Soc.*, **77**, 5911(1955).
(7) Goldman, L., Marsico, J. W., and Angier, R. B., *ibid.*, **78**, 4173(1956).
(8) Dekker, C. A., *ibid.*, **87**, 4027(1965).
(9) Schaffer, R., *ibid.*, **81**, 2838(1959).
(10) Baxter, J. N., and Perlin, A. S., *Can. J. Chem.*, **38**, 2217(1960).
(11) Neish, A. C., *ibid.*, **32**, 334(1954).
(12) Hockett, R. C., *J. Am. Chem. Soc.*, **57**, 2260(1935).
(13) Fox, J. J., Yung, N., Davoll, J., and Brown, G. B., *ibid.*, **78**, 2117(1956).
(14) Baker, B. R., "Chemistry and Biology of Purines," in "Ciba Foundation Symposium," Wolstenholme, G. E. W., and O'Connor, C. M., eds., Little, Brown and Co., Boston, Mass., 1957, p. 120.
(15) Steiger, M., and Reichstein, T., *Helv. Chim. Acta*, **19**, 1016(1936).
(16) Gätzi, K., and Reichstein, T., *ibid.*, **21**, 195(1938).
(17) Jackson, E. L., and Hudson, C. S., *J. Am. Chem. Soc.*, **59**, 994(1937).
(18) Lythgoe, B., and Todd, A. R., *J. Chem. Soc.*, **1944**, 592.