of 1190 mg. (2.0 mmoles) of 2,6-dideoxy-1,3,4-tri-*O*-*p*-nitrobenzoyl-β-D-*ribo*-hexose to the chloride I was carried out according to the original directions.[4] After the removal of the solvent by decantation, the crystalline material was dissolved immediately in 5 ml. of dry dichloromethane. After the addition of 5 ml. of anhydrous pyridine, the solution was boiled until its volume was reduced by two-thirds. The latter was transferred to a separatory funnel containing 400 ml. of ethanol-dichloromethane (1:4) and was extracted successive with *N* sulfuric acid, 5% aqueous sodium bicarbonate, and water. After drying over sodium sulfate, the separated ethanol-dichloromethane extract was evaporated *in vacuo* at 30°. The residue was dissolved in a minimum amount of acetone and the solution was transferred to a small petri dish. The solvent was evaporated carefully by warming and the residue thus obtained was transferred to a small Soxhlet thimble. After extracting continuously with 400 ml. of anhydrous ether for 24 hr., the extract was carefully boiled down to 15 ml., whereupon the desired material separated in crystalline form. By combining the latter with additional material obtained from the mother liquor and recrystallizing from acetone-ether (1:9), there was obtained a total of 385 mg. (45% based on 2,6-dideoxy-1,3,4-tri-*O*-*p*-nitrobenzyl-β-D-*ribo*-hexose) of pure 6-deoxy-3,4-di-*O*-*p*-nitrobenzoyl-D-*ribo*-hexopyranos-1-ene (III), m.p. 143–143.5° $[\alpha]^{20}D$ + 389°. When admixed with a specimen obtained in the preceding preparation, no depression in the melting point was observed.

9-(2-Deoxy-D-arabino-hexopyranosyl)adenine (X). To a solution of 674 mg. (1.0 mmole) of 2-deoxy-3,4,6-tri-*O*-*p*-nitrobenzoyl-α-D-*arabino*-hexosyl bromide (II)[10] in 17 ml. of dry benzene was added 392 mg. (1.13 mmoles) of silver 6-benzamidopurine and the mixture was refluxed with stirring for 12 min. After cooling, the reaction mixture was diluted with 10 ml. of chloroform and was filtered. After evaporation of the filtrate to dryness *in vacuo* at 40°, a crude mixture containing the protected nucleoside IX was obtained and, without further purifications, was dissolved in 50 ml. of warm methanol to which 1 ml. of 3 *N* sodium methoxide had been added. After stirring for 20 hr. the mixture was neutralized with glacial acetic acid and was evaporated *in vacuo* at 40°. The resulting oily residue was dissolved in 10 ml. of methanol and 15 ml. of 10% methanolic picric acid was added. The picrate salt, which formed immediately, was filtered and washed with cold methanol and amounted to 515 mg. The nucleoside X was regenerated from its picrate salt by a procedure similar to that described in the foregoing preparation of 9-(2,6-dideoxy-D-*ribo*-hexopyranosyl)adenine (V). The crude nucleoside X thus obtained was crystallized from 95% ethanol and melted at 136–137°, the temperature varying to a slight extent depending on the rate of heating. On recrystallization from absolute ethanol, some of X was obtained as the unstable, anhydrous nucleoside, softening at 220°, melting with recrystallization at 239–241° and remelting at 242–245°. The remainder of the material, amounting to 68 mg. (21.5% based on the bromide II), was obtained as the dihydrate of 9-(2-deoxy-D-*arabino*-hexopyranosyl)adenine (X), m.p. 141–160° (dec.), $[\alpha]^{27}D$ −4.7 ± 1.3° (*c* 0.50 dihydrate, water), $[\alpha]^{27}D$ + 53.2° (*c* 0.19 dihydrate, pyridine), $\lambda_{max}^{H_2O}$ 260 mμ (4.14).

The nucleoside X travelled as a single spot on papergrams (R_{Ad} = 1.24), giving a blue fluorescence with ultraviolet light and a blue-violet color with boric acid spray reagent. The nucleoside was hydrolyzed in a manner identical to that described in the foregoing preparation of 9-(2,6-dideoxy-D-*ribo*-hexopyranosyl)adenine (V). Paper chromatography of the mixture disclosed two spots, one corresponding to adenine and the other to 2-deoxy-D-*arabino*-hexose.

Anal. Calcd. for $C_{11}H_{15}O_4N_5 \cdot 2H_2O$: C, 41.63; H, 6.04; N, 22.06. Found: C, 41.49; H, 6.15; N, 21.82.

1-(2-Deoxy-D-arabino-hexopyranosyl)thymine (XIII). A mixture of 6.74 g. (10 mmoles) of 2-deoxy-3,4,6-tri-*O*-*p*-nitrobenzoyl-α-D-*arabino*-hexopyranosyl bromide (II) and 7.00 g. (38.5 mmoles) of 2,4-diethoxy-5-methylpyrimidine was heated under a reduced pressure of 20 mm. of mercury for 4 hr. After cooling, the melt was extracted twice with 50-ml. portions of ether and was extracted further with 250 ml. of benzene. The crude protected nucleoside XI thus obtained amounted to 3.30 g. and melted at 230–245°. The latter material, without further purification, was dissolved in 50 ml. of chloroform and to this solution was added 50 ml. of 25% methanolic hydrogen chloride. After stirring overnight, the separated deethylated product XII was filtered and amounted to 1.70 g., softening at 155° with change in crystal form and melting at 241–245°. Without further purification, XII (1.70 g.) was transferred to 100 ml. of absolute methanol to which was added 3 ml. of *N* sodium methoxide and the mixture was stirred for 18 hr. at room temperature. After neutralizing the solution with glacial acetic acid and evaporating to dryness *in vacuo* at 40°, the residue was redissolved in 300 ml. of water and was extracted with 3–100 ml. portions of chloroform. After separating the aqueous phase and evaporating to dryness *in vacuo*, the residue was extracted twice with 100-ml. portions of hot 2-propanol. The 2-propanol extract was evaporated to dryness *in vacuo* and the residue was redissolved in 50 ml. of absolute ethanol. Four drops of concentrated aqueous hydrochloric acid were added, and the sodium chloride which separated was filtered. The filtrate was concentrated by boiling to 25 ml. from which, after cooling, there was obtained a total of 390 mg. (14% based on the bromide II) of 1-(2-deoxy-D-*arabino*-hexopyranosyl)thymine (XIII), subliming at 230° and melting at 231.5–232.5°; $[\alpha]^{27}D$ + 4.6 ± 0.9° (*c*, 0.975 water); $\lambda_{max}^{H_2O}$ 265 mμ (3.99); ν_{max}^{KBr} 3920 cm.$^{-1}$ (conjugated C=O of ring), 1810 cm.$^{-1}$ (broad C—O— band). The nucleoside XIII travelled on papergrams as a single spot (R_{Thy} = 1.14), fluoresced under ultraviolet light, and gave a negative test with boric acid spray reagent.

Anal. Calcd. for $C_{11}H_{16}O_6N_2$: C, 48.55; H, 5.93; N, 10.28. Found: C, 48.82; H, 5.89; N, 10.01.

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Nucleosides. XV. Synthesis of 1-β-D-Lyxofuranosylcytosine *via* Thiation of an Anhydronucleoside[1,2]

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Previous papers in another series[3,4] demonstrated that suitably protected pyrimidine nucleosides

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(2) A preliminary report has appeared. See J. J. Fox, N. Yung, I. Wempen, and R. Duschinsky, Abstracts, *Intern. Union Pure and Applied Chem.* (Symposium on Natural Products), Australia, 1960, p. 66.

(*e.g.*, uridine, thymidine, or their 5-fluoro analogs) may be thiated with phosphorus pentasulfide in pyridine to their 4-thio derivatives which, upon treatment with ammonia, afforded the corresponding cytosine ribo- or deoxy ribonucleosides. In this series,[5] it was shown that certain derivatives of 2,2′-anhydro nucleosides (*e.g.*, 2,2′-anhydro-1-(3′-*O*-mesyl-5′-*O*-benzoyl-β-D-arabinosyl)uracil (I)[6]) are converted smoothly to 1-β-D-lyxofuranosyluracils by merely boiling in water. The mechanism for this rather novel conversion has been fully elaborated.[5] The present paper deals with the application of the thiation process to anhydronucleoside (I) and the subsequent conversion of the thiated intermediate (II, see flow chart) to nucleosides of potential biological interest.

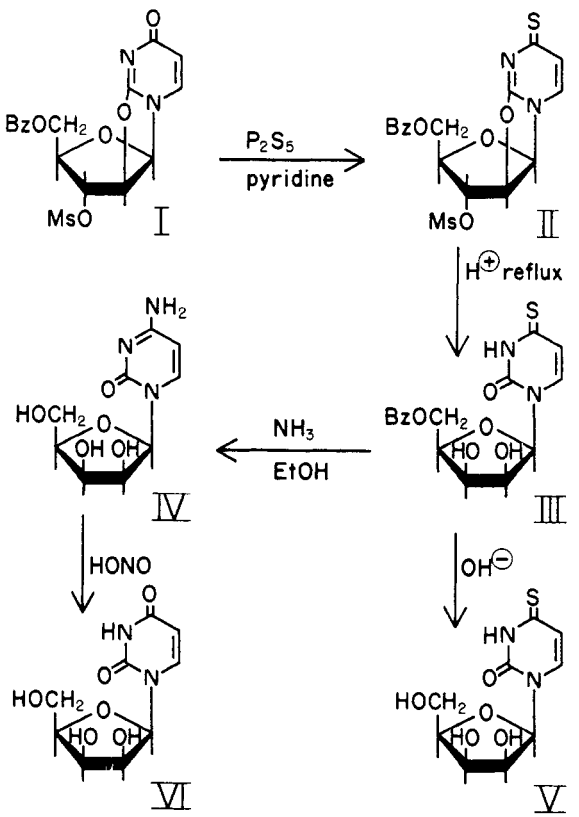

Figure 1

Compound I (whose facile preparation from tri-*O*-mesyluridine has been described[6]) was treated with phosphorus pentasulfide in pyridine at reflux temperature for thirty minutes. A yellow, crystalline intermediate was obtained in good yield with an analysis which corresponded to II. Treatment of II with aqueous ethanol at reflux temperature for several hours did not cleave the anhydro linkage as determined by the absence of formation of methanesulfonic acid[7] in the reaction mixture. When the reaction was treated with acid[8] and refluxed, conversion to the lyxosyl nucleoside III occurred with the concomitant liberation of one equivalent of methanesulfonic acid. Compound III formed an isopropylidene derivative. Treatment of III with alcoholic ammonia afforded the cytosine nucleoside IV, while deacylation of III with alkali yielded the 4-thiouracil nucleoside V.

The cytosine nucleoside IV consumed one mole of metaperiodate per mole rapidly (within three minutes)[9-13] without the liberation of acid in accord with an aldopentofuranosyl structure bearing α-*cis* hydroxyl groups. Compound IV differed in optical rotation from cytidine, as well as from the xylofuranosyl-[11] and arabinofuranosylcytosine.[14] The ultraviolet absorption spectrum of IV was similar to that shown by 1-β-D-aldopentosylcytosines.[4,11,15] Treatment of IV with nitrous acid yielded the known[5] 1-β-D-lyxofuranosyluracil (VI). These data unequivocally establish IV as 1-β-D-lyxofuranosylcytosine.

The conversion of the thiated lyxosyl nucleoside III to the lyxosylcytosine IV establishes position 4 as the site of thiation in the reaction I to II. On this basis, the aglycon of III and V is a 4-thiouracil moiety and V is 1-β-D-lyxofuranosyl-4-thiouracil.

Finally, it is noteworthy that *under the thiating conditions described* herein, the anhydro bond of I remains intact.

EXPERIMENTAL[16]

2,2′-Anhydro-1-(3′-O-mesyl-5′-O-benzoyl-β-D-arabinosyl)-4-thiouracil (II). Phosphorus pentasulfide (0.1 mole) was

(3) J. J. Fox, D. Van Praag, I. Wempen, I. L. Doerr, L. Cheong, J. E. Knoll, M. L. Eidinoff, A. Bendich, and G. B. Brown, *J. Am. Chem. Soc.*, **81**, 178 (1959).

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(5) R. Fecher, J. F. Codington, and J. J. Fox, *ibid.*, **83**, 1889 (1961).

(6) J. F. Codington, R. Fecher, and J. J. Fox, *ibid.*, **82**, 2794 (1960).

(7) The reaction sequence for the conversion of II to III is presumed to be similar to that described previously[5] for the conversion of I to the 5′-*O*-benzoate of 1-β-D-lyxofuranosyluracil. The sequence involves first the rupture of the 2,2′-anhydro bond to form a 3′-*O*-mesyl-arabinosyluracil followed by formation of a 2,3′-anhydro linkage with displacement of the 3′-mesyloxy function. Cleavage of the 2,3′-anhydro nucleoside under acidic conditions generated the lyxosyl nucleoside. In the course of the reaction, one equivalent of methanesulfonic acid is generated.

(8) Acid "treatment" was also found necessary for the conversion of 2,2′-anhydro-1-(3′-*O*-mesyl-β-D-arabinofuranosyl)-5-fluorouracil to 1-β-D-lyxofuranosyl-5-fluorouracil. See N. Yung, J. H. Burchenal, R. Fecher, R. Duschinsky, and J. J. Fox, *J. Am. Chem. Soc.*, **83**, 4060 (1961).

(9) It was demonstrated[10-13] that the 1-β-D-aldopentofuranosylpyrimidines containing α-*cis*-hydroxyl groups consume metaperiodate rapidly (within 5 minutes), whereas with the α-*trans* isomers (arabino or xylo) usually 1–2 days are required for the theoretical uptake of oxidant.

(10) J. J. Fox, N. Yung, J. Davoll, and G. B. Brown, *J. Am. Chem. Soc.*, **78**, 2117 (1956).

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(12) J. J. Fox, N. Yung, and A. Bendich, *ibid.*, **79**, 2775 (1957).

placed in 350 ml. of dry pyridine and heated to reflux temperature. The clear, hot solution was treated with 14 g. of I[6] and refluxed for 30 min. The clear, brown reaction solution was concentrated *in vacuo* and the residue treated with 2 l. of water, stirred, filtered, and the precipitate washed repeatedly with water. The precipitate was recrystallized from 10 l. of preheated (to boiling) 50% ethanol to yield fine, yellow needles (12.5 g.), m.p. 191–194° dec. One further recrystallization (almost quantitative) from 50% ethanol afforded pure material, m.p. 206–207° (to a brown liquid). Ultraviolet light absorption properties: in 50% ethanol, λ_{max} 225, 267, and 330 mμ, λ_{min} 255 and 289 mμ; $[\alpha]^{25}D$ −6° (*c* 0.30, in *N*,*N*-dimethylformamide).

Anal. Calcd. for $C_{17}H_{16}N_2O_7S_2$: C, 48.11; H, 3.80; N, 6.60; S, 15.11. Found: C, 48.04; H, 4.01; N, 6.94; S, 15.09.

1-(5′-O-Benzoyl-β-D-lyxofuranosyl)-4-thiouracil (III). The anhydro nucleoside II (5.0 g. in 400 ml. of 50% ethanol) was treated with 3.0 ml. of 1 *N* methanesulfonic acid and the mixture refluxed for 30 min. whereupon complete solution occurred. The yellow solution was refluxed for a total of 16 hr. Titration of an aliquot with alkali (Methyl Red indicator) indicated that the theoretical amount of methanesulfonic acid had been liberated. The solution was concentrated under vacuum to approximately 50 ml. whereupon yellow needle clusters separated (4.1 g.), m.p. 160° dec. Recrystallization from 25% ethanol gave analytical material, m.p. 169–171° dec. Light absorption properties in 50% ethanol: λ_{max} 230 and 331 mμ, shoulder at 265 mμ, λ_{min} 285 mμ. $[\alpha]^{25}D$ +127° (*c* 0.30, in *N*,*N*-dimethylformamide).

Anal. Calcd. for $C_{16}H_{16}N_2O_6S$: C, 52.74; H, 4.43; N, 7.69; S, 8.80. Found: C, 52.99; H, 4.88; N, 7.89; S, 9.13.

1-β-D-Lyxofuranosyl-4-thiouracil (V). The monobenzoate III (1.5 g.) was shaken in 75 ml. of 50% ethanol containing 5 ml. of *N* sodium hydroxide. After 1 hr., the clear solution was neutralized with acetic acid and concentrated to dryness *in vacuo*. The residue was taken up in water and treated batchwise with Dowex-50 (H^+) to remove sodium ion. The filtrate was then extracted three times with chloroform and the organic layer discarded. The aqueous layer was taken to dryness and the residue crystallized from hot ethanol. Recrystallization from 30 ml. of 90% ethanol gave analytical material, m.p. 198° dec., $[\alpha]^{22}D$ +71° (*c* 0.16, in *N*,*N*-dimethylformamide). Ultraviolet light absorption properties: in water, λ_{max} 250 and 331 mμ, λ_{min} 277 mμ.

Anal. Calcd. for $C_9H_{12}N_2O_5S$: N, 10.76; S, 12.32. Found: N, 10.81; S, 12.55.

1-β-D-Lyxofuranosylcytosine (IV). The monobenzoate III (1.4 g.) was placed in a tube containing *ca.* 50 ml. of ethanol previously saturated with ammonia at 0° and the tube sealed and heated at 100° for 12 hr. The tube was cooled, opened, and the contents concentrated to dryness. The residue was fractionated between water and chloroform and the aqueous layer taken to dryness. The red sirup was dissolved in ethanol with the aid of a few drops of water. Ethanol saturated with picric acid was added to the solution whereupon the *picrate of 1-β-D-lyxofuranosylcytosine* separated (1.34 g.), m.p. 207–208° dec. Recrystallization of 0.5 g. of the picrate from 100 ml. of 85% ethanol yielded a product of m.p. 208–209°.

Anal. Calcd. for $C_{15}H_{16}N_6O_{12}$: N, 17.79. Found: N, 17.20.

The *hydrochloride salt* of 1-β-D-lyxofuranosylcytosine was prepared from the picrate salt. Treatment of an aqueous solution of the picrate with Dowex-1 (Cl^-) batchwise afforded a colorless filtrate. The filtrate was concentrated *in vacuo* to dryness and crystallized from 90% ethanol, melting point begins to brown at 174° dec. and effervesces at 192°. $[\alpha]^{22}D$ +11° (*c* 2.0, in water). Ultraviolet light properties were akin to those reported for cytidine[15]; in *N* hydrochloric acid, λ_{max} 280 mμ, ϵ_{max} 13,110, λ_{min} 240 mμ, ϵ_{min}1080; at *p*H 7–12, λ_{max} 271 mμ, ϵ_{max} 9200, λ_{min} 249 mμ, ϵ_{min} 5600.

Anal. Calcd. for $C_9H_{14}N_3O_5 \cdot HCl$; C, 38.51; H, 5.38; N, 14.97; Cl, 12.63. Found: C, 39.08; H, 4.75; N, 14.82; Cl, 12.86, 12.90.

Conversion of IV *to 1-β-D-lyxofuranosyluracil* (VI). The hydrochloride salt IV (0.28 g.) was treated with an excess of dilute hydrochloric acid and of sodium nitrite and the solution allowed to remain at 40° overnight. Ionophoretic determination (0.1 *M* borate) showed that approximately 50% conversion to the uracil nucleoside had occurred. The aqueous solution was placed on a column (Dowex-50, H^+) and eluted with water until the eluate was free from ultraviolet absorbing material. The eluate was concentrated to a light sirup *in vacuo* (bath temp. ∽ 40°) and the sirup treated with ethanol. The ethanol was removed *in vacuo*. The ethanol addition and evaporation was repeated several times. The remaining light yellow sirup was dissolved in 10 ml. of 95% ethanol, cooled, and crystallized. The product, 1-β-D-lyxofuranosyluracil, (120 mg.) melted at 202.5–203.5° (reported,[5] m.p. 203–204°) and a mixed melting point with an authentic specimen[5] gave no depression.

1-(5′-O-Benzoyl-2′,3′-O-isopropylidene-β-D-lyxofuranosyl)-4-thiouracil. p-Toluenesulfonic acid (0.1 g.) was added to 0.1 g. of III dissolved in 10 ml. of acetone. The clear solution remained at room temperature for 30 min., after which it was concentrated *in vacuo* to *ca.* 5 ml. After addition of 5 ml. ethanol, the clear solution was cooled. Yellow rosettes crystallized (80 mg.), m.p. 195–200°. Recrystallization from 10 ml. of absolute ethanol afforded analytical material, m.p. 199–201.

Anal. Calcd. for $C_{19}H_{20}N_2O_6S$: C, 56.42; H, 4.98; N, 6.93; S, 7.93. Found: C, 56.53; H, 5.01; N, 6.86; S, 7.98.

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Monosaccharide Sulfates. II. The Preparation of Methyl α-D-Glucopyranoside 2-Sulfate[1]

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It has been shown that the sulfation of glucose leads predominantly to the 6-sulfate.[2,3] Similarly, the sulfation of glucose derivatives having a free hydroxyl at the 6- position or at the 6- position and one or more others results in the formation of the

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