Nucleosides. XVI. Further Studies of Anhydronucleosides[1]

JACK J. FOX AND NAISHUN C. MILLER

Division of Nucleoprotein Chemistry, Sloan-Kettering Institute for Cancer Research, Sloan-Kettering Division of Cornell University Medical College, New York 21, New York

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Syntheses are described for the preparation of the 2′-deoxylyxofuranosyl isomers of thymidine and 5-fluoro-2′-deoxyuridine *via* 2,3′-anhydronucleoside intermediates. The conversions of 3′-*O*-mesylthymidines with sodium benzoate in N,N-dimethylformamide (DMF) are conclusively shown to proceed through 2,3′-anhydronucleoside intermediates under acid-catalyzed conditions. The reaction of certain anhydro-1-(β-D-pentofuranosyl)uracils with sodium benzoate in DMF has been studied and the role of acid catalysis as a factor in the introduction of nucleophiles into the sugar moiety of 2,2′- or 2,3′-anhydronucleosides is considered. In preliminary screening studies with mice, 1-(2′-deoxy-β-D-lyxofuranosyl)-5-fluorouracil (Vb) was effective against leukemia B82T and B82A but Vb showed no chemotherapeutic effect against leukemia L1210 or Ehrlich ascites tumor.

Previous studies from this and other laboratories[2] demonstrated that 2,2′- or 2,3′-anhydro nucleosides derived from uridine,[3–5] 1-β-ribofuranosylthymine,[6] or 5-fluorouridine[7] are useful chemical precursors for the synthesis of 1-β-D-aldopentofuranosyl epimers of these nucleosides. The present study deals with reactions of 2,3′-anhydronucleosides derived from thymidine and related analogs as part of our program in the synthesis of nucleosides of general biochemical interest and of potential value as anti-tumor agents.

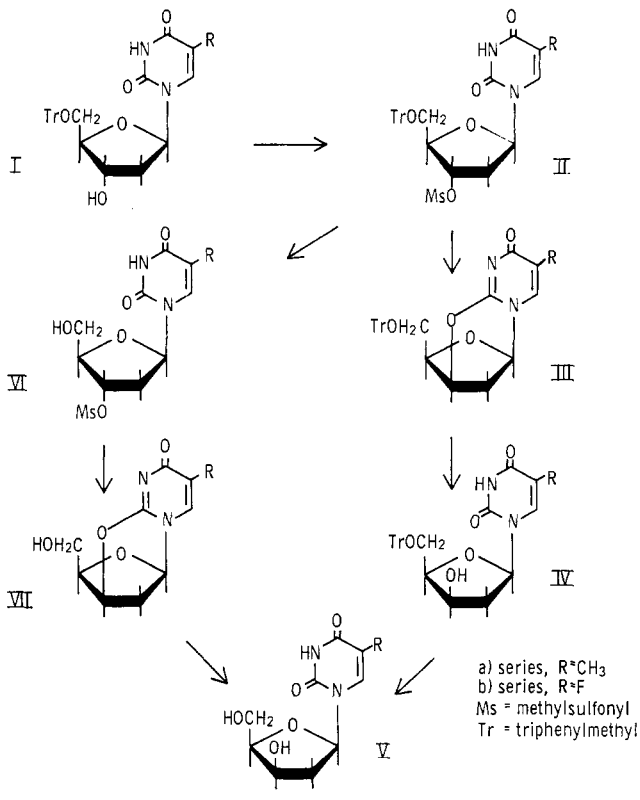

Figure 1

Michelson and Todd,[8] in a comprehensive study on anhydronucleosides derived from thymidine, indicated the formation of a 2′-deoxylyxosyl (-xylosyl) epimer Va (see Fig. 1) isolated as a chromatographic spot by treatment of the 2,3′-anhydro derivative (IIIa) with alkali. The synthesis of crystalline Va in good yield has now been accomplished (see Fig. 1) from IIa. Treatment of IIa[8] with one equivalent of alkali in aqueous ethanol afforded the 5′-*O*-tritylanhydro derivative (IIIa) which (*in situ*) was refluxed with an excess of alkali. The 2,3′-anhydro linkage was cleaved under these conditions (as determined by ultraviolet absorption spectral monitoring of the reaction) affording the 5′-*O*-trityl derivative (IVa) of 2′-deoxylyxosylthymine. Detritylation of IVa afforded 2′-deoxylyxofuranosylthymine (Va) in 63% over-all yield based on thymidine. In this reaction sequence (Ia→Va) neither of the intermediates IIIa nor IVa was isolated in pure form.

Compound Va gave an elemental analysis and absorption spectrum similar to thymidine. It differed from thymidine in melting point and optical rotation. As expected for a 2′-deoxypentofuranosyl structure and like thymidine, it did not consume metaperiodate.

Va was prepared from IIa by an alternate route. Detritylation of IIa according to Michelson and Todd[8] afforded VIa which was refluxed in water (during which time the liberated acid was neutralized with one equivalent of base). This procedure afforded a simplified synthesis of VIIa[8] which was then converted to Va by treatment with excess alkali at room temperature.

1-(2′-Deoxy-β-D-lyxofuranosyl)-5-fluorouracil, Vb, was prepared from 5-fluoro-2′-deoxyuridine.[9] Tritylation of fluorodeoxyuridine afforded the 5′-*O*-trityl derivative (Ib) in 80%[10] yield. Mesylation of Ib afforded IIb which was detritylated to VIb. Dilute alkali converted VIb to VIIb. The latter, upon treatment with 1 *N* alkali at room temperature gave crystalline Vb, 1-(2′-deoxy-β-D-lyxofuranosyl)-5-fluorouracil, in 40% over-all yield from 5-fluoro-2′-deoxyuridine.

Michelson and Todd[8] prepared 3′-halogeno-3′-deoxy nucleosides by treatment of IIa (see Fig. 2) with lithium bromide or sodium iodide in acetone at 100°. The configuration of the 3′-halogen was assumed to be of

(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, Public Health Service (grant no. 3190).

(2) See J. J. Fox and I. Wempen, *Advan. Carbohydrate Chem.* **14**, 283 (1959), for a general review of early works with anhydronucleosides.

(3) J. F. Codington, R. Fecher, and J. J. Fox, *J. Am. Chem. Soc.*, **82**, 2794 (1960).

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

(5) N. Yung and J. J. Fox, *ibid.*, **83**, 3060 (1961).

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

(7) N. Yung, J. H. Burchenal, R. Fecher, R. Duschinsky, and J. J. Fox, *ibid.*, **83**, 4060 (1961).

(8) A. M. Michelson and A. R. Todd, *J. Chem. Soc.*, 816 (1955).

(9) R. Duschinsky, E. Pleven, J. Malbica, and C. Heidelberger, Abstracts of 132nd National Meeting of the American Chemical Society, 1957, p. 19c; M. Hoffer, R. Duschinsky, J. J. Fox, and N. Yung, *J. Am. Chem. Soc.*, **81**, 4112 (1959).

(10) H. J. Thomas and J. A. Montgomery, *J. Med. Pharm. Chem.*, **5**, 24 (1962), prepared Ib in 49% yield reported as a chloroform addition product.

TABLE I[a]

Starting material	Mmoles	DMF, ml.	Sodium benzoate, mmoles	Benzoic acid, mmoles	Product	% Yield
IX	2.5	85	18.0	...	XI (R = Bz)	68
X	2.0	85	16.0	...	XI (R = Bz)	30
X	2.0	85	14.0	2.0	XI (R = Bz)	77
IIa	1.0	20	4.0	...	XI (R = tr)	73
IIIa	0.8	25	3.6	...	mixture[b]	18
IIIa	1.0	30	4.0	1.0	XI (R = tr)	79
IIIa	0.5	20	3.0[c]	...	...[d]	..
IIIa	0.48	20	2.8[c]	0.48	XII	75
IIIa	0.48	20	2.8[c]	1.5	XII	77

[a] All reactions refluxed for 10 hr. [b] Contains starting material and XI (R = Tr) (was not separated). [c] Sodium *p*-nitrobenzoate used instead of sodium benzoate. [d] A small amount of an inseparable gummy material which could not be crystallized.

the "down" or ribo form since the 3′-iodo analog (VIII) could be converted to the anhydronucleoside VIIa. The authors postulated that "A change in configuration at position 3′ appears to be involved (and may have occurred during the preparation of the 3′-iodo derivative) . . . " so that the anhydronucleoside VIIa is a 2′-deoxylyxo(xylo)syl rather than a deoxyribosyl derivative. It is almost certain from their experiments that in the formation of the halogeno derivatives (VIII) a double Walden inversion had occurred *via* the anhydronucleoside intermediate IIIa (see Fig. 1).

Similarly, they obtained a 3′,5′-dibromo-2′,3′,5′-trideoxynucleoside by treatment of 3′,5′-di-*O*-mesylthymidine with lithium bromide. Here, also, the 2′-deoxyribosyl configuration is to be expected for the halogeno nucleoside if, as is most likely, the reaction proceeded *via* a 2,3′-anhydronucleoside intermediate.

We refluxed 3′,5′-di-*O*-mesylthymidine (IX, Fig. 3) with sodium benzoate in N,N-dimethylformamide[11] (DMF) for ten hours and isolated 3′,5′-di-*O*-benzoylthymidine in 70% yield. This dibenzoate (XI, R = benzoyl) is identical with that obtained previously[12] by direct benzoylation of thymidine so that it is certain that in the reaction of IX→XI the 3′-mesyloxy function has been replaced by benzoate without *net* inversion. Here, too, it is reasonable to postulate a double Walden inversion at C-3′ in the course of the reaction of IX to XI preceeding *via* anhydronucleoside X (first inversion) followed by attack at C-3′ by benzoate (second inversion) to form XI.

Accordingly, it should be possible to convert X to XI under similar reaction conditions used for the conversion of IX to XI. The synthesis of X by treatment of IX with saturated methanolic ammonia in dilute ethanol had been reported[8] but convincing evidence as to the position of the anhydro bridge (*i.e.*, 2,3′ or 2,5′) and the mesyloxy function (*i.e.*, C-3′ or C-5′) was lacking. Therefore, VIIa (prepared from IIa→VIa, Fig. 3) was mesylated directly. We obtained a product (X) identical with that synthesized from IX according to Michelson and Todd,[8] thus providing rigid proof of the structure X. Compound X may be prepared more efficiently by refluxing IX in water and neutralizing the methanesulfonic acid as it is liberated by the reaction.

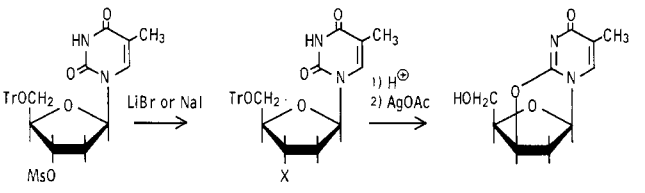

Figure 2

Treatment of X with sodium benzoate in DMF (reflux for ten hours) afforded XI, but in only 30% yield.

In the over-all conversion of IX to XI with sodium benzoate, two equivalents of mesylate are liberated.[13] If the elimination of C-3′ mesyloxy group is effected by intramolecular displacement by attack of the 2-carbonyl group of the pyrimidine (anhydronucleoside formation), then, in the reaction milieu, benzoic acid would form. It is noteworthy that this phenomenon would occur upon the formation of intermediate X so that benzoic acid is present during the ensuing inversion to XI (R = benzoyl). Such a situation would not obtain when X itself is treated with sodium benzoate in DMF. For if the attack by benzoate ion is first on C-3′, the sodium salt of 3′-*O*-benzoyl-5′-*O*-mesylthymidine would form. If the first attack were at C-5′ (displacement of mesyloxy by benzoate) sodium mesylate would form in the reaction medium but not benzoic acid. Upon the addition of one equivalent of benzoic acid[14] to the reaction mixture of X in sodium benzoate–DMF, the yield of di-*O*-benzoylthymidine was raised to 80% (see Fig. 3).

A similar situation obtains when the 5′-*O*-trityl-3′-*O*-mesylthymidine (IIa) is used. Thus when IIa reacted with sodium benzoate–DMF for ten hours under reflux, an 80% yield of XI (R = trityl) is obtained. However, when the 5′-*O*-tritylanhydro derivative (IIIa) was treated similarly, only a small amount of a mixture containing XI (R = trityl, Table 1) was obtained. When this reaction of IIIa was run in the presence of a molar equivalent of benzoic acid, the yield of XI (R = trityl) was raised to 80%. Compound XI (R = trityl) was converted to thymidine by alkaline hydrolysis followed by detritylation with acid. Treatment of

(11) E. J. Reist, L. Goodman, and B. R. Baker, *J. Am. Chem. Soc.*, **80**, 5775 (1958); E. J. Reist, R. R. Spencer, and B. R. Baker, *J. Org. Chem.*, **24**, 1618 (1959).

(12) 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).

(13) Sodium mesylate has been isolated from this reaction.

(14) Recently, R. K. Ness, *J. Org. Chem.*, **27**, 1155 (1962), reported the use of sodium benzoate in benzoic acid (the latter, in large excess, was also used as solvent) as an effective reagent for the displacement of the 2-*O*-(*p*-nitrophenylsulfonyl) groups of fully acylated D-ribofuranose derivatives to yield tetra-*O*-acylated arabinofuranoses.

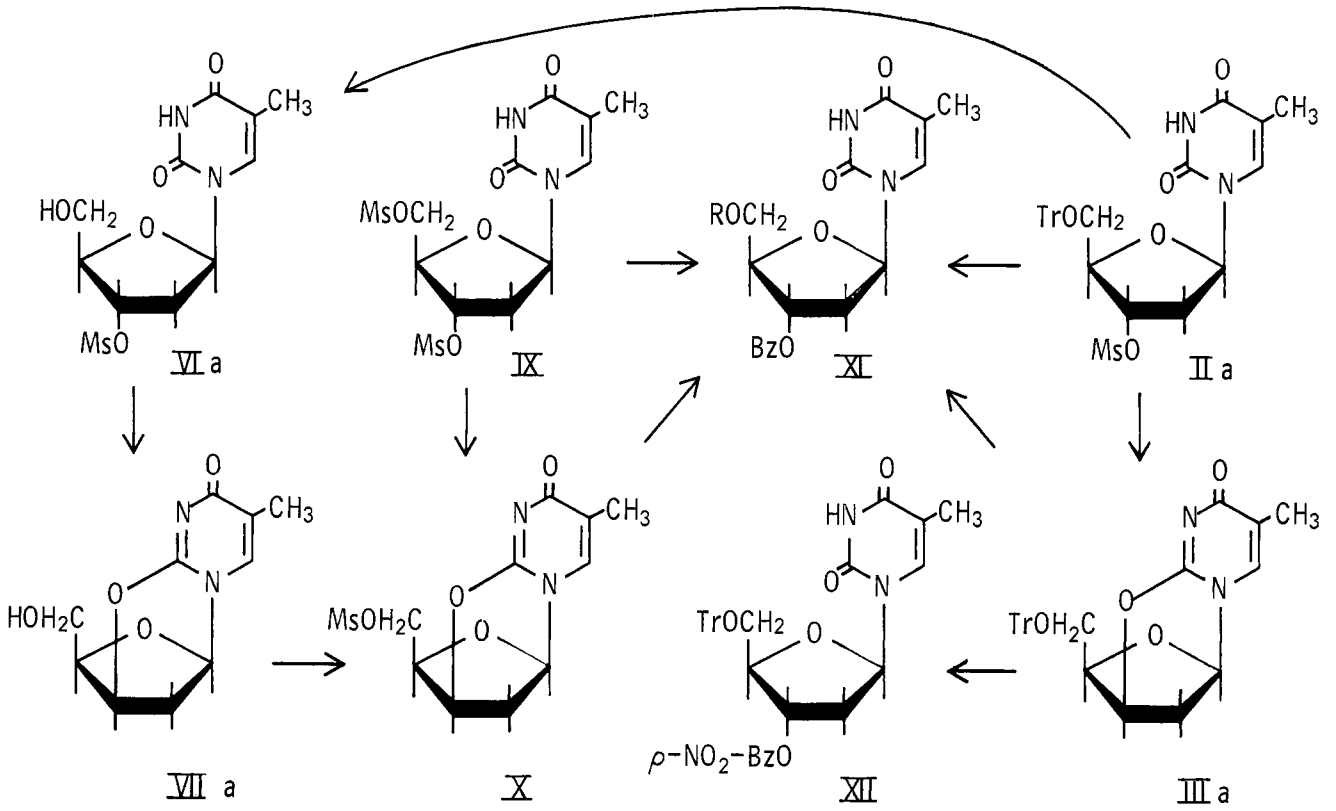

Figure 3

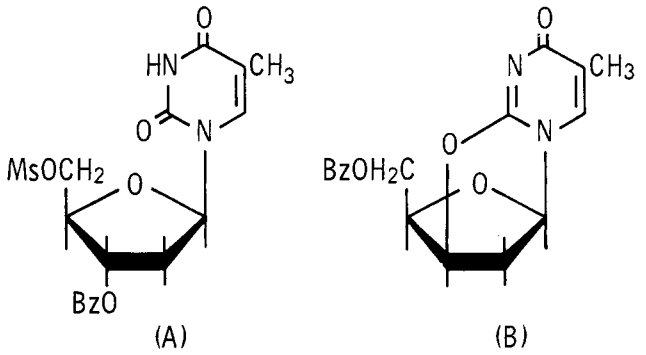

Figure 4

IIIa with sodium *p*-nitrobenzoate in DMF with one molar equivalent of benzoic acid at reflux temperature for ten hours afforded 5′-*O*-trityl-3′-*O*-*p*-nitrobenzoylthymidine in high yield. Without benzoic acid, no product could be isolated from the reaction (see Table 1).

There can be no question that X is an intermediate in the reaction of IX to XI (R = benzoyl), namely that the reaction proceeded first by an intramolecular attack on C-3′ rather than on C-5′. This is shown by the fact that short-time treatment of IX (twenty minutes at 95°) with sodium benzoate–DMF afforded X in crystalline form.[15] Similarly, in the reaction of IIa → XI (R = trityl) chromatographic evidence for anhydronucleoside (IIIa) formation was obtained.

A plausible explanation of this catalysis is that benzoic acid (liberated *in situ* in the reaction of IX or IIa to XI or when benzoic acid is added to X or IIIa to form XI) protonates the conjugated system of the aglycon in X or IIIa which thereby renders C-3′ of the conjugate acid more susceptible to nucleophilic attack by benzoate ion.

These experiments do not establish the sequence of reactions from X→XI (R = benzoyl) which could have proceeded by an attack at C-3′ (catalyzed by acid) to yield the 3′-benzoate [(A), see Fig. 4] of 5′-mesyloxythymidine followed by replacement of the 5′-mesylate[16] of (A) by benzoate to form XI (R = benzoyl). Alternatively, this reaction (X → XI) could proceed first by displacement of the 5′-mesylate by benzoate to form (B) followed by the acid-catalyzed attack at C-3′ of (B) to form XI. Since neither (A) nor (B) were isolated in the reaction of IX or X to XI no decision can be made between them.

These results with thymidine have bearing upon previous work from this laboratory with uridines. It was demonstrated[3] that refluxing of the benzoylmesylanhydronucleoside (XIII, see Fig. 5) with sodium benzoate–DMF for two hours at ~150° afforded two crystalline products, XVII and XVIII (plus an amorphous solid which was a mixture of difficultly separable benzoylated nucleosides). Separate pathways were proposed at that time[3] for the formation of XVII and XVIII from XIII.

This over-all reaction of XIII→XVII and XVIII was repeated, this time *with* added benzoic acid as catalyst. It was now possible to isolate the 2,3′-anhydronucleoside XV in crystalline form (in addition to XVII and XVIII) and to establish its identity by comparison with an authentic specimen prepared previously by an alternate route.[5] It is most reasonable to expect that XV arose

(15) It is noteworthy that under these conditions the 5′-mesylate was not replaced by benzoate. On the other hand it was shown previously[3] that 2′,3′,5′-trimesyloxyuridine treated with the same reagent for 2 hr. at 100° afforded 2,2′-anhydro-1-(5′-*O*-benzoyl-3′-*O*-mesyl-β-D-arabinosyl)-uracil (compound XIII of Fig. 5).

(16) The replacement of the 5′-mesylate by benzoate could proceed directly or possibly *via* a 2,5′-anhydronucleoside intermediate followed by benzoate attack on C-5′.

via intermediate XIV (not isolated) by benzoate attack at C-2′ of XIII. Such a reaction (XIII→XIV) would resemble that for the acid-catalyzed formation of XI from X or IIIa (Fig. 3) in the thymidine studies described above. The formation of XV from XIV with sodium benzoate–DMF reagent has already been reported along with the novel transformation of XV to XVII *via* ortho ester ion XVI.[5]

This pathway (XIII→XVII), and especially the isolation of XV, suggests that the tri-*O*-benzoylated-xylonucleoside (XVIII) and -ribonucleoside XIX could also be products of the over-all reaction of XIII with sodium benzoate–DMF–benzoic acid. As mentioned, crystalline XVIII has been isolated from this reaction. The formation of this benzoylated isomer (XVIII) is easily ascribed to a C-3′ attack on XVI by benzoate ion. The formation of tri-*O*-benzoyluridine (XIX), previously suspected[3,17] (not isolated) could be ascribed to a benzoate attack on C-2′ of XVII (as has been postulated[3]) or a benzoate attack on C-3′ of XV. That attack on C-2′ of XVII could be the case is now shown by the fact that refluxing XVII in sodium benzoate–DMF with benzoic acid catalyst for ten hours gave a high yield of crystalline XIX. These results strongly suggested a *unified* pathway for the formation of XVII, XVIII and XIX, proceeding via the 2,3′-anhydronucleoside XV.

General Considerations.—The present study suggests that under acid-catalyzed conditions other anions may be introduced in the ribo or "down" configuration in the sugar moiety of anhydronucleosides (or their sulfonyloxy precursors) of 1-β-D-aldosylpyrimidines bearing a carbonyl group at position 2 of the aglycon.[17b] Indeed, the formation of 3′-halogeno-2′,3′-dideoxythymidines as described by Michelson and Todd,[8] the synthesis of 2′-iodo-2′-deoxyuridine by Brown, *et al.*[18] and other 2′-halogenouridines recently reported from this laboratory[19] may all be viewed as general examples of acid catalysis (protonation of the anhydronucleoside followed by nucleophilic attack on the sugar moiety). An essentially similar mechanism was implied recently by Murdock and Angier[20] for the formation of a halogenated 1-cyclopentane derivative of thymidine (a thymidine isostere) from an "anhydronucleoside" intermediate.

Screening Studies.[21]—In preliminary studies, 1-(2′-deoxy-β-D-lyxofuranosyl)-5-fluorouracil (Vb) markedly

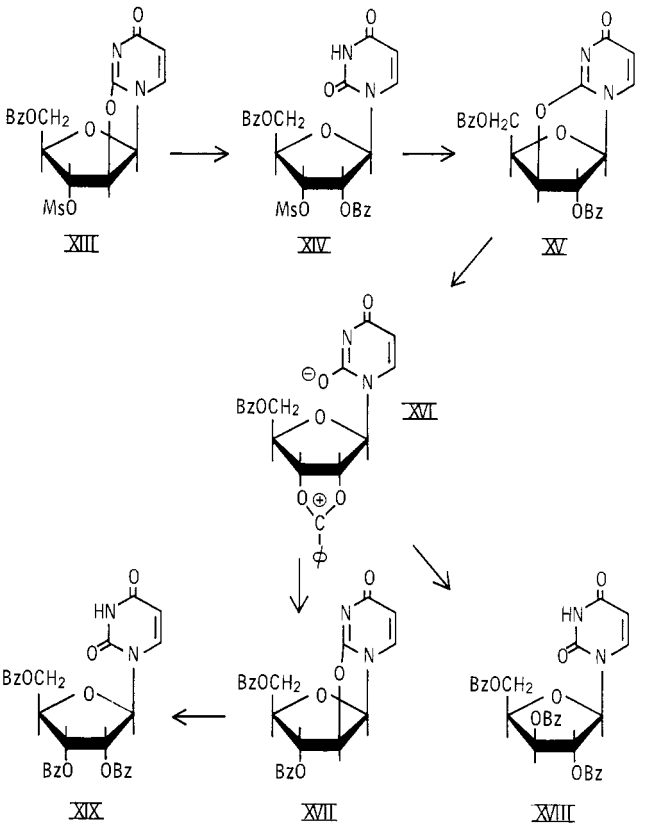

Figure 5

inhibited the growth of leukemia B82T at 369 mg./kg. daily for 10 doses and produced 54% increase in survival time at the same dose in leukemia B82A.[21a]

On the other hand, daily IP administration of Vb at 228 and 500 mg./kg. for seven and ten days, respectively, had no chemotherapeutic or toxic effects on mice implanted with the L1210 leukemia.[21b] Nor was any significant, repeatable anti-tumor activity observed at doses of 500 mg./kg. in tests using mice bearing Ehrlich ascites tumor. This result is in contrast to the significant inhibition produced against this tumor by 5-fluoro-2′-deoxyuridine at 40 mg./kg.[21c]

Experimental[22]

1-(2′-Deoxy-β-D-lyxofuranosyl)-thymine (Va) from IIa.—Unrecrystallized 3′-*O*-mesyl-5′-*O*-tritylthymidine[8] IIa, 6.0 g., 0.01 mole) was dissolved in 100 ml. of ethanol. The warm solution was treated with 50 ml. of water slowly (to prevent precipitation). Sodium hydroxide (10 ml., 1 *N*) was added and the solution allowed to remain at room temperature overnight with stirring. (At the end of this time, anhydronucleoside IIIa is formed as shown by the absorption spectrum of an aliquot—see below for preparation of IIIa.) Additional alkali (20 ml., 1 *N* sodium hydroxide) was added and the solution refluxed for 5 hr. The pH of the solution was brought to ~6 and the volume reduced *in vacuo* to ~25 ml., whereupon precipitation of a white solid occurred. Water (400 ml.) was added and the solids collected by filtration and washed repeatedly with water to remove salts. This residue (IVa) was refluxed for 10 min. in 50 ml. of 80% acetic acid to remove the trityl group. The solvent was removed *in vacuo* and the sirupy residue was triturated three times with warm chloroform to remove triphenylcarbinol. The remaining sirup was crystallized from acetone to yield 1.8 g. (75% based on IIa) of Va, m.p. 153–157°. One recrystallization from ethanol afforded pure material, m.p. 168–169°, $[\alpha]^{23}D$

(17) (a) The presence of a benzoylated nucleoside of the ribo configuration among the products of the reaction of XIII with sodium benzoate–DMF was previously indicated since alkaline hydrolysis of the chloroform-soluble amorphous residue obtained after the isolation of XVII and XVIII showed the presence of uridine along with all other 1-β-D-aldopentofuranosyluracils.[3] (b) NOTE ADDED IN PROOF.—The successful application of these results has been achieved recently (see J. J. Fox and N. C. Miller, Abstracts of the 144th National Meeting of the American Chemical Society, Los Angeles, Calif., March, 1963, p. 4C). Treatment of III (Fig. 1) with potassium thiolbenzoate–DMF–benzoic acid introduced the thiolbenzoate group at C-3′. Subsequent detritylation and deacylation yielded the disulfide of 3′-deoxy-3′-mercaptothymidine.

(18) D. M. Brown, D. B. Parihar, C. B. Reese, and A. R. Todd, *J. Chem. Soc.*, 3035 (1958); D. M. Brown, D. P. Parihar, and A. R. Todd, *ibid.*, 4242 (1958).

(19) J. F. Codington, I. Doerr, D. Van Praag, A. Bendich, and J. J. Fox, *J. Am. Chem. Soc.*, **83**, 5030 (1961).

(20) K. C. Murdock and R. B. Angier, Abstracts of 141st National Meeting of the American Chemical Society, Washington, D. C., March, 1962, p. 23n.

(21) The authors are indebted to the following investigators from this institute for kindly providing their preliminary results: (a) J. H. Burchenal, J. R. Purple, and E. Bucholz; (b) D. J. Hutchison and D. L. Robinson; and (c) P. C. Merker and F. Schmidt.

(22) All melting points were taken on a Thomas–Hoover capillary melting point apparatus and are corrected. Microanalyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich.

+14° (*c*, 0.56 in water). Ultraviolet absorption properties: at pH 5–7: maximum at 267 mμ, ϵ_{min} 9450; minimum at 235 mμ, ϵ_{min} 2250; in 0.01 *N* sodium hydroxide: maximum at 267 mμ, ϵ_{max} 7360; minimum at 245.5 mμ, ϵ_{min} 4460.

Anal. Calcd. for $C_{10}H_{14}N_2O_5$: C, 49.58; H, 5.82; N, 11.56. Found: C, 49.14; H, 4.66; N, 11.30.

3′-*O*-Mesylthymidine[8] (VIa).—A suspension of 5.0 g. (8.9 mmoles) of IIa in ether was saturated at 0° with hydrogen chloride and allowed to remain at 5° for 1 hr. The pink, crystalline solid was washed with ether and filtered. One recrystallization from hot ethanol (preheated before recrystallization) yielded 2.0 g. (72%) of VIa, m.p. 153–155° dec. (reported[8] m.p. 116°, 50% yield).[23]

2,3′-Anhydro-1-(2′-deoxy-β-D-lyxofuranosyl)thymine (VIIa) from VIa.—Compound VIa (1.0 g., 0.003 mole) in 50 ml. of water (treated with 2 drops of methyl red indicator solution) was refluxed. As methanesulfonic acid was liberated, a 1 *M* solution of triethylamine in 50% ethanol was added slowly so that the pH of the reaction solution was maintained at ~4–5. When the liberation of acid ceased, the solvent was removed *in vacuo* and the residue treated with ethanol. Solvent was removed again and the ethanol addition and removal was repeated. A crystalline residue was obtained (needles) which was filtered and washed with ethanol, 550 mg., m.p. 225–227°. An additional 120 mg. (total yield 95%) of slightly lower melting material was obtained from the mother liquor. The two crops were combined and a sample recrystallized from 90% ethanol for analysis m.p. 230–231° (reported[8] 230°), $[\alpha]^{23}D$ −14° (*c*, 0.45 in water).

1-(2′-Deoxy-β-D-lyxofuranosyl)thymine (Va) from VIIa.—Compound VIIa (200 mg., 0.88 mmole) was dissolved in 9.0 ml. of 1 *N* sodium hydroxide and the solution kept at room temperature for 3 hr. Aliquots were examined spectrophotometrically during this period. The spectra showed the gradual disappearance of the maximum at ~254 mμ and the concomitant formation of a new maximum at ~266 mμ akin to that for thymidine.[24] The solution was treated batchwise with Dowex 50 (H^+) and the filtrates combined and evaporated to dryness. The crystalline residue was recrystallized from 90% ethanol, 170 mg., (81%), m.p. 169–170°. A mixture melting point with the product obtained from IIa (*vide supra*) was 168–170°.

1-(2′-Deoxy-5′-*O*-trityl-β-D-ribosyl)-5-fluorouracil (Ib).—5-Fluoro-2′-deoxyuridine[9] (7.5 g., 0.03 mole) in 100 ml. of anhydrous pyridine was treated with 9.2 g. of triphenylmethyl chloride and the solution heated on a steam bath for 2 hr. The solution was poured into well stirred ice–water (2 l.). The water-washed gummy material was dissolved in 150 ml. of ethyl acetate and dried over sodium sulfate. After filtration, the solution was concentrated *in vacuo* to a sirup. The sirup was treated with 3 ml. of benzene and 200 ml. of diethyl ether. With scratching, crystallization occurred. The crystals were collected and washed well with ether and dried (11.7 g., 80%), m.p. 188–189°. This product is easily obtained in analytically pure, unsolvated form by recrystallization from ethanol, m.p. 196–197°. $[\alpha]^{23}D$ +49° (*c*, 0.60 in ethanol). Ultraviolet absorption in ethanol: maximum, 257 mμ; minimum, 235 mμ.

Anal. Calcd. for $C_{28}H_{25}FN_2O_5$: C, 68.81; H, 5.16; N, 5.73. Found: C, 68.77; H, 5.48; N, 5.83.

1-(2′-Deoxy-3′-*O*-mesyl-β-D-ribofuranosyl)-5-fluorouracil (VIb).—Methanesulfonyl chloride (0.6 ml.) was added to a cooled solution of anhydrous pyridine (20 ml.) containing Ib (2.40 g., 0.0049 mole) and the mixture stored for 16 hr. at 5°. Ethanol (2 ml.) was added and, after 1 hr. at 5°, the mixture was poured into 1 l. of stirred ice–water. The granular precipitate (IIb) was collected on a filter and washed repeatedly with water. (Attempts to crystallize this granular precipitate were unsuccessful.) The crude 3′-mesylate (IIb) was dried and dissolved in 150 ml. of a 1:1 mixture of chloroform–ether. The solution was cooled and saturated with gaseous hydrogen chloride with cooling in an ice bath. After 3 hr. at 0°, detritylation was essentially complete and crystalline VIb (1.6 g.) precipitated. Product was collected on a filter and washed well with anhydrous ether. Two recrystallizations from ethanol gave colorless needles (1.25 g., 80%) of analytically pure VIb, m.p. 161–162° dec., $[\alpha]^{23}D$ +32° (*c*, 0.57 in ethanol). Ultraviolet spectrum in ethanol: maximum, 267 mμ; minimum, 234 mμ.

Anal. Calcd. for $C_{10}H_{13}FN_2O_7S$: C, 37.04; H, 4.04; N, 8.64; S, 9.89. Found: C, 37.39; H, 4.41; N, 8.48; S, 9.94.

2,3′-Anhydro-1-(2′-deoxy-β-D-lyxofuranosyl)-5-fluorouracil (VIIb).—A solution of VIb (200 mg., 0.62 mmole) in 5 ml. of water (containing 2 drops of methyl red indicator solution) was refluxed. As methanesulfonic acid was liberated, a 1 *M* solution of triethylamine in 50% ethanol was added so that the pH was maintained at ~4–5. When the liberation of acid ceased, the solvent was removed *in vacuo* and the residue treated with ethanol. The ethanol was removed under vacuum and the crystalline residue treated again with ethanol. After removal of the ethanol the crystalline mass was washed well with ethanol and collected by filtration (130 mg., 93%). Recrystallization afforded analytical material, m.p. 197–198° (efferv.). Ultraviolet properties: maximum, 230 and 256 mμ, slight shoulder at 280 mμ; minimum, 220 and 238 mμ (in water).

Anal. Calcd. for $C_9H_9FN_2O_4 \cdot {}^1/_2\ C_2H_5OH$: C, 47.81; H, 4.82; N, 11.15. Found: C, 48.09; H, 3.84; N, 11.00.

1-(2′-Deoxy-β-D-lyxofuranosyl)-5-fluorouracil (Vb).—Compound VIIb (2.0 mmoles) was dissolved in 10 ml. of 0.5 *N* sodium hydroxide and allowed to stand at room temperature for 1.5 hr. Spectra taken during this time showed the disappearance of the anhydro spectrum (loss of maximum at 228 mμ) and the appearance of a 5-fluorouracilnucleoside spectrum. The solution was treated batchwise with Dowex 50 (H^+) and the combined filtrates concentrated to a sirup *in vacuo*. Ethanol was added and removed *in vacuo* several times until crystallization of product occurred. After filtration, the product was washed with absolute ethanol. Colorless crystals, 350 mg. were obtained, m.p. 192–193°. Recrystallization from 95% ethanol gave a pure sample, m.p. 199–199.5°, (sintering at 198°), $[\alpha]^{23}D$ +40° (*c*, 0.85 in water). Ultraviolet properties: pH 5.6: ϵ_{max} at 270 mμ = 8980, ϵ_{min} at 235 mμ = 1580; in 0.01 *N* sodium hydroxide: ϵ_{max} at 268.5 mμ = 6880, ϵ_{min} at 247 mμ = 4320.

Anal. Calcd. for $C_9H_{11}FN_2O_5$: C, 43.91; H, 4.50; F, 7.72; N, 11.38. Found: C, 44.11; H, 4.31; F, 7.59, 7.66; N, 11.18, 11.19.

2,3′-Anhydro-1-(5′-*O*-mesyl-2′-deoxy-β-D-lyxosyl)thymine (X) from IX.—The di-*O*-mesylate (IX, 4.0 g.) in 180 ml. water with 2 drops of methyl red indicator solution was brought to reflux. The liberated acid was neutralized with 1.0 *N* sodium hydroxide in such a manner that the pH of the reaction never rose above 5. (About one equivalent of alkali was consumed.) When the liberation of acid ceased, the solution was concentrated to ~10 ml., cooled, and the crystalline precipitate (2.35 g.) filtered. Recrystallization from 90% ethanol gave a m.p. 177–178°, dec. (reported[8] 176°, dec.), $[\alpha]^{23}D$ −61° (*c*, 1.23 in DMF). Ultraviolet properties: maximum, 243 mμ, minimum, 218 mμ (in ethanol).

2,3′-Anhydro-1-(5′-*O*-mesyl-2′-deoxy-β-D-lyxosyl)thymine (X) from VIIa.—Compound VIIa (350 mg.) in 40 ml. of anhydrous pyridine was treated with 0.23 ml. of methanesulfonyl chloride. After 40 min. at room temperature, the precipitated material was filtered, 60 mg., and washed thoroughly with pyridine and ether. This substance was starting material. The filtrate was treated with a large volume of ether until precipitation ceased. The solvent was decanted from the oily precipitate and discarded. The oily precipitate was triturated with ethanol which caused crystallization, m.p. 165–167° dec., 150 mg. Recrystallization from 90% ethanol gave rosettes, m.p. 176–177° dec. A sample prepared by an alternate route (see above) melted at 177–178° (reported[8] 176°) and a mixture melting point did not depress.

2,3′-Anhydro-1-(5′-*O*-trityl-2′-deoxy-β-D-lyxosyl)thymine (IIIa).—Three grams of IIa (5.35 mmoles) in 100 ml. of 90% ethanol with 5.1 ml. of 1 *N* sodium hydroxide was refluxed until completion of the reaction (in ~6 min.) was indicated by the alteration of the absorption spectrum to that of an anhydronucleoside. (IIa shows a maximum at 264 mμ, minimum at 243 mμ. IIIa has only shoulders at 230 and 250 mμ.) Solvent was removed *in vacuo* and the residue triturated with water to remove sodium mesylate. The water-insolubles were recrystallized from ethanol. Two crops were obtained, 2.1 g. (85%), m.p.

(23) During the preparation of this manuscript for publication, a report by J. P. Horwitz, J. A. Urbanski, and J. Chua, *J. Org. Chem.*, **27**, 3300 (1962), appeared in which they also were unable to duplicate the 116° melting point and reported 152–153° dec. for VIa in 52% yield from IIa using the detritylation procedure of Michelson and Todd.[8] We have found that recrystallization of 3′-*O*-mesylthymidine from water[8] invariably produces a mixture containing anhydronucleoside VIIa. Even repeated recrystallization from ethanol will cause formation of some VIIa. This behavior is not wholly unexpected in light of previously reported work from our laboratory[4] on the ready formation of lyxonucleosides (*via* 2,3′-anhydro nucleosides) from certain 3′-*O*-mesyl-nucleosides.

(24) J. J. Fox and D. Shugar, *Biochim. Biophys. Acta*, **9**, 369 (1952).

214–217°. Recrystallization from ethanol gave m.p. 226–227°, $[\alpha]^{23}D$ ~+7° (*c*, 0.68 in ethanol.) Ultraviolet properties (in ethanol): shoulders at 230 and 250 mμ, ratio 230/250 = 1.8.

Anal. Calcd. for $C_{29}H_{26}N_2O_4 \cdot C_2H_5OH$: C, 72.63; H, 6.28, N, 5.46. Found: C, 72.46; H, 5.60; N, 5.62.

General Procedures for Reactions in Table I. Example: Conversion of IX to XI (R = Benzoyl).—Di-*O*-Mesylthymidine (IX, 1.0 g.) with sodium benzoate (2.5 g.) was heated under reflux in 85 ml. of DMF for 10 hr. Solvent was removed under vacuum and the residue treated with 250 ml. of water. After filtration, the solid was washed with water and recrystallized from ethanol. Needles, 650 mg., were obtained, m.p. 193–194° (reported[12] for di-*O*-benzoylthymidine, 192.5–193.5°), and a mixture melting point with authentic material was undepressed. An additional 110 mg. was obtained from the mother liquor.

3′-*O*-Benzoyl-5′-*O*-tritylthymidine (XI, R = Trityl).—Compound IIIa (465 mg.) was refluxed in 30 ml. of DMF with 576 mg. of sodium benzoate and 122 mg. of benzoic acid for 10 hr. The subsequent work-up of the reaction was similar to that described for XI (R = benzoyl) above. A total of 460 mg. of XI (R = trityl), m.p. 183–184°, was obtained. $[\alpha]_D^{23}$ ~ −5° (c, 0.78 in chloroform). Ultraviolet properties: maximum, 266 mμ; minimum, 249 mμ (in ethanol).

Anal. Calcd. for $C_{36}H_{32}N_2O_6$: C, 73.44; H, 5.48; N, 4.76. Found: C, 73.75; H, 5.21; N, 4.76.

Without benzoic acid, the yields dropped to ~18%. The material obtained was a mixture containing some product along with other unidentified substances.

XI (R = trityl) was converted to thymidine as follows: 200 mg. of XI (R = trityl) in 10 ml. of ethanol was treated with 5 ml. of 0.1 *N* sodium hydroxide. After refluxing for 2 hr., the reaction was neutralized with acetic acid and evaporated to dryness. The residue was triturated with water to remove salts. The water-insoluble material was filtered and treated with 5 ml. of 80% acetic acid and refluxed for 15 min. After concentration to dryness *in vacuo*, the residue was triturated three times with ether. To the remaining sirup, 1 ml. of ethanol was added whereupon crystallization occurred, 50 mg., m.p.185–186°. Recrystallization from 95% ethanol gave m.p. 186.5–187°, mixture melting point with thymidine was not depressed.

3′-*O*-(*p*-Nitrobenzoyl)-5′-*O*-tritylthymidine (XII) from IIIa.—A mixture of IIIa (220 mg.), sodium *p*-nitrobenzoate (530 mg.) and benzoic acid (58 mg.) was refluxed for 10 hr. in 20 ml. of DMF. After treatment of the reaction in the usual manner (*vide supra*) XII was obtained (75%). Recrystallized from ethanol, m.p. 131–133° (to a viscous orange liquid), $[\alpha]^{23}D$ ~ −9° (*c*, 0.58 in chloroform). Ultraviolet properties: maximum, 263 mμ; minimum, 239 mμ (in ethanol).

Anal. Calcd. for $C_{36}H_{31}N_3O_8 \cdot C_2H_5OH$: C, 67.15; H, 5.48; N, 6.18. Found: C, 67.08; H, 5.17; N, 6.39.

Hydrolysis of XII by the same procedure used for the conversion of XI (R = trityl) above afforded thymidine.

Short-term Treatment of IX. Isolation of X as an Intermediate.—Di-*O*-mesylthymidine (IX, 1.0 g.) was added to a mixture of sodium benzoate (1.3 g.) in 75 ml. of DMF at 100° and the stirred reaction mixture maintained at that temperature for only 0.5 hr. The mixture was cooled to room temperature and the precipitated salts separated by filtration. The filtrate was evaporated *in vacuo* to a dry residue which was crystallized from 90% ethanol, 0.7 g., m.p. 177–178° dec. A mixture melting point with X prepared by an alternate route gave no depression.

2,2′-Anhydro-1-(3′,5′-di-*O*-benzoyl-β-D-arabinosyl)uracil[3] (XVII).—2,2′-Anhydro-1-(β-D-arabinofuranosyl)uracil[25,26] (200 mg., 0.88 mmole) in 16 ml. of anhydrous pyridine at 40–45° was treated with 0.3 ml. of benzoyl chloride. As the stirred mixture slowly dissolved, precipitation began. The stirred mixture was kept at 40–45° overnight after which it was filtered, and the precipitate washed well with ethanol followed by ether. Yield, 250 mg. (66%), m.p. 268–270°. One recrystallization from 90% ethanol gave a m.p. 271–272° (reported[5] 270–272°). Mixture melting point with authentic material[1] showed no depression.

Reaction of XIII with Sodium Benzoate–DMF–Benzoic Acid. Isolation of XV, XVII and XVIII.—XIII[3] (2.0 g., 0.005 mole) in 400 ml. of DMF was treated with 8.6 g. of sodium benzoate (0.06 mole) and 0.5 g. of benzoic acid and the stirred mixture refluxed for 2 hr. After cooling to room temperature, the solids were removed by filtration and washed with a small amount of DMF. The solids were discarded and the combined filtrates were evaporated *in vacuo* to dryness. The residue was triturated with ether, then with chloroform, and finally with water. The water triturate was discarded but the ether and chloroform triturates were saved.

The residue remaining after triturations was crystallized from 90% ethanol. The 2,3′-anhydronucleoside XV (180 mg.) was obtained by filtration. The melting point characteristics were: sintering at 253° with melting and resolidification at 255° followed by melting at 270–271°. Recrystallization of this material from 90% ethanol gave an almost quantitative recovery with no change in melting point properties. A mixture melting point of this material with XV obtained by an independent route[5] showed no depression.

The mother liquor from XV yielded two crops of crystalline material (80 and 50 mg.). The 80-mg. crop melted as follows: slight sintering at 255° (without melting) followed by melting at 268–269°. The 230/260 mμ ratio of its ultraviolet absorption spectrum was 4.8 (reported[3] for XVII = 4.59 and for XV = 5.40[5]) indicating that this crop (80 mg.) was a mixture rich in XVII. One recrystallization of this material from 90% ethanol afforded pure XVII, m.p. 270–272° (without prior sintering or melting) and a mixture melting point with authentic XVII[3,5] was not depressed. The second crop (50 mg.) was also a mixture of XV and XVII as shown by the similarity to the 80 mg. crop in melting point characteristics and spectral ratios.

The ether triturate (*vide supra*), on standing, deposited ~20 mg. of XV. After removal of this material by filtration, the ether mother liquor was concentrated to a sirup and triturated with water. The insolubles were collected by filtration (using diatomaceous earth as a pad on the filter paper) and crystallized from 80% ethanol. Tri-*O*-benzoylxylosyluracil (XVIII, 250 mg.) was obtained, m.p. 110–118° (efferv.) (reported[3] 112–118°). Spectral properties, maximum at 234 mμ, 230/260 mμ = 3.10, also agreed with those reported for XVIII.[3]

The chloroform triturate was evaporated to dryness. The residue was dissolved in hot 90% ethanol and upon cooling 210 mg. (in four crops) of crystalline material was obtained. Melting point and spectral ratios of these fractions suggested them to be mixtures of XV, XVII, and XVIII. Since these fractions had little preparative value they were not investigated further.

2′,3′,5′-Tri-*O*-benzoyluridine (XIX)[12] from XVII.—Compound XVII prepared above (100 mg., 0.23 mmole) in 10 ml. of DMF was treated with 144 mg. of sodium benzoate and 30 mg. of benzoic acid. The mixture was refluxed for 10 hr., after which the clear, faint yellow reaction mixture was concentrated to dryness *in vacuo* and the amorphous residue triturated several times with chloroform. The triturates were combined and the solvent removed *in vacuo*. The residual sirup was dissolved in 1–2 ml. of hot ethanol and allowed to remain at room temperature for 48 hr. Two types of crystals were noted, needle-rosettes and short stubby needles. Separation was accomplished by adding an additional 2–3 ml. of ethanol and heating the mixture to boiling. The short stubby needles (~15 mg.) which were insoluble were separated by filtration of the hot mixture. This precipitate was starting material XVII. Upon cooling of the filtrate, needles (100 mg.) were obtained in two crops, m.p. ~105–140°. Recrystallization from benzene gave m.p. 140–141°. A mixture melting point with authentic tri-*O*-benzoyluridine[12] was not depressed.

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(25) D. M. Brown, A. R. Todd, and S. Varadarajan, *J. Chem. Soc.*, 2388 (1956).

(26) Prepared by an alternate procedure.[19] The authors are indebted to Miss I. L. Doerr for this sample.