ORGANIC AND BIOLOGICAL CHEMISTRY

[CONTRIBUTION FROM THE LABORATORIES OF THE SLOAN-KETTERING DIVISION OF CORNELL UNIVERSITY MEDICAL COLLEGE, AND FROM THE DEPARTMENT OF MICROBIOLOGY, WESTERN RESERVE UNIVERSITY SCHOOL OF MEDICINE]

Pyrimidine Nucleosides. IV. The Synthesis of 1-β-D-Lyxofuranosylthymine[1]

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To complete the synthesis of all four possible 1-β-D-aldopentofuranosylthymines, 1-β-D-xylofuranosylthymine (II) was epimerized by a series of reactions to 1-β-D-lyxofuranosylthymine (IV). The spectrum of IV between *p*H 12–14 was compared to those of the other three 1-β-D-pentofuranosylthymine isomers. The susceptibility of these four nucleosides and related compounds to enzymic cleavage was examined. Only the 1-β-D-ribofuranosyl isomer (5-methyluridine) was cleaved. These facts were considered in terms of enzyme specificity.

Previous papers from this Laboratory described the synthesis of thymine nucleosides by the condensation of poly-O-acylglycofuranosyl halides with dithyminylmercury with subsequent removal of the protecting acyl groups.[2,3] By this procedure, 1-β-D-ribofuranosylthymine (I) and 1-β-D-xylofuranosylthymine (II) were prepared. Proof that both I and II possessed similar configurations at the glycosyl centers was shown[2] by the fact that both nucleosides gave the same dialdehyde upon treatment with metaperiodate. That this configuration was beta was subsequently demonstrated[3] by the fact that compound I could be epimerized *via* a cyclonucleoside intermediate. This epimerization of I resulted in the synthesis of a third 1-β-D-aldopentofuranosylthymine, 1-β-D-arabinofuranosylthymine (III), which was identical with "spongothymidine" previously isolated by Bergmann and Feeney[4] from certain Caribbean sponges.

The synthesis of the fourth possible 1-β-D-aldopentofuranosylthymine (see Fig. 1), namely, the hitherto-unknown 1-β-D-lyxofuranosylthymine (IV) was of particular interest. Whereas in 5-methyluridine (I), the C'_2–C'_3 hydroxyl groups are in the *cis*-(down) configuration, in IV these functions are *cis*-(up). Stated differently, while in I the relationship of the aglycon to the C_2–C_3 substituents is *trans*, in compound IV this relationship is *cis*. In light of the structural relationships of IV to I (and, indeed, to the ribofuranosyl moieties of the naturally-occurring nucleosides) it is conceivable that these synthetic pentofuranosylthymines might interfere with nucleic acid biosynthesis[5] and, further, might serve as potential anti-cancer agents.

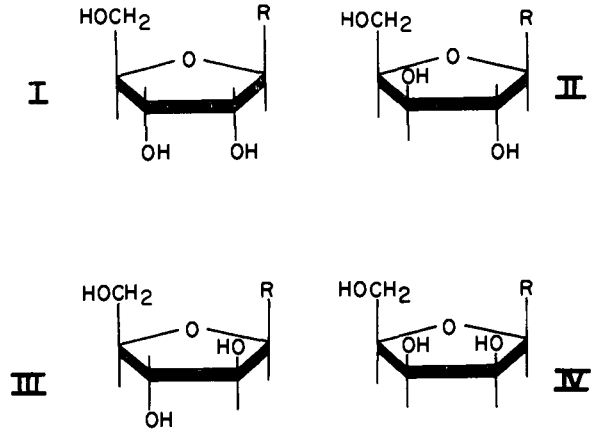

Fig. 1.

A thymine nucleoside was obtained[10] from thymine and D-ribosyl phosphate by enzymic synthesis using a nucleosidase from *E. coli* B. This nucleoside[11] was cleaved at N_1–C'_1 by this enzyme. The availability of II, III and IV would make possible a detailed study of the effects of stereochemical alterations at C_2 and/or C_3 of the sugar moiety upon the ability of nucleosidases to cleave the glycosylic linkage of these pyrimidine nucleosides.

The present paper describes the preparation of 1-β-D-lyxofuranosylthymine (IV) which completes the synthesis of the four possible 1-β-D-aldopentofuranosylthymine nucleosides.

Results and Discussion

The synthesis of 1-β-D-lyxofuranosylthymine by the condensation of a poly-O-acyllyxofuranosyl halide with dithyminylmercury would almost certainly lead to the formation of an α-nucleoside.

(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, Public Health Service (Grant No. C-2329 and CY-3190), and from the Ann Dickler League.

(2) J. J. Fox, N. Yung, J. Davoll and G. B. Brown, THIS JOURNAL, **78**, 2117 (1956).

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

(4) W. Bergmann and R. J. Feeney, *J. Org. Chem.*, **16**, 981 (1951).

(5) Though Gulland and Barker[6] reported the presence of small amounts of L-lyxose in yeast ribonucleic acid, this finding was later retracted.[7] The presence of "spongothymidine" and "spongouridine"[4,8] (III, R = uracil) in *Cryptotethia crypta* would suggest that these nucleosides might be intimately associated with the nucleic acids of this particular sponge. However in a recent report by Bergmann and co-workers[9] on the degradation of the nucleic acids of this as well as fifteen other species of sponges, only the four usual ribonucleosides and 2'-deoxyribonucleosides were detected. Because of the exceptionally low content of ribonucleic acid in *Cryptotethia crypta*, these authors[9] suggest that perhaps a metabolic aberration exists in this particular species which diverts most of the intermediates of ribonucleic acid synthesis to the formation of these arabinonucleosides or perhaps to an unstable substance of greater complexity incorporating these nucleosides.

(6) J. M. Gulland and G. R. Barker, *J. Chem. Soc.*, 625 (1943).

(7) G. R. Barker, K. R. Cooke and J. M. Gulland, *ibid.*, 339 (1944).

(8) W. Bergmann and D. C. Burke, *J. Org. Chem.*, **20**, 1501 (1955)

(9) W. Bergmann, J. C. Watkins and M. F. Stempien, Jr., *ibid.*, **22** 1308 (1957).

(10) J. O. Lampen, in W. D. McElroy and B. Glass, "Phosphorus Metabolism," Vol. II, The Johns Hopkins Press, Baltimore, Md., 1952 p. 368.

(11) The characterization of this enzymically-prepared nucleoside as 1-β-D-ribofuranosylthymine (I) has been reported.[2,3] The presence of a ribosylthymine in small amounts in certain microbial pentosenucleic acids recently has been reported (J. W. Littlefield and D. B. Dunn *Biochem. J.*, **68**, 8P (1958)). This nucleoside is probably I.

Bristow and Lythgoe[12] have shown that α-nucleosides of the purines are obtained by the condensation of silver salts of certain purines with tri-O-acetyl-D-arabinofuranosyl bromide. They reasoned that the halogenose was probably in the β-form (C_1–C_2–*cis* configuration of substituents) and that the purine moiety entered the 1-position of this sugar with Walden inversion.

A more general explanation of this phenomenon, advanced by Baker and co-workers,[13] states that the purinyl group would enter the 1-position of the sugar from a side *trans* to the C_2 substituent regardless of the anomeric configuration of the halogenose and would therefore lead to nucleosides of the C'_1–C'_2–*trans* type. From a C_1–C_2–*cis* halogenose, entry of purine is accompanied by a single Walden inversion of C_1. With a C_1–C_2–*trans* halogenose system, the condensation reaction would involve a double Walden inversion of C_1 either by preliminary attack of a halide ion to give the C_1–C_2–*cis*-halogenose or by the preliminary formation of an orthoester ion from the neighboring C_2-acyl substituent.[13] This "*trans* rule" has made possible the prediction of the configuration at the glycosyl centers of purine nucleosides synthesized by reaction of heavy metal salts of purines with poly-O-acylglycosyl halides.[14]

Recent studies[2,15] point to the fact that both the Hilbert–Johnson method[16] and the mercuri procedure[2] for pyrimidine nucleoside synthesis yield the same nucleoside when identical halogenoses are employed. This would indicate that the stereochemical control of the glycosylic configuration is similar in both of these synthetic processes. With regard to the Hilbert–Johnson process, it has been reported[17] that both α- and β-isomers of tetra-O-acetyl-D-glucopyranosyl chloride (when condensed with 2,4-diethoxypyrimidine) yield the same β-nucleoside, a fact which may be interpreted more adequately in terms of the *trans* rule of Baker and co-workers.

Experimental data are available which would sustain the viewpoint that the configurations at the glycosyl centers of synthetic nucleosides of *both* purines and pyrimidines are similar when prepared from the same halogenose. Synthetically-prepared cytidine (obtained *via* the Hilbert–Johnson[18] method or by the mercuri route[15]) possesses the same configuration at C'_1 as does synthetic adenosine[19] (prepared *via* condensation of 2,8-dichloroadenine silver and acetobromo-D-ribofuranose). A similar situation obtains when 1-D-glucopyranosylthymine[2,20] and 9-D-glucopyranosyladenine[19,21] are compared. It may therefore be concluded that the "*trans* rule" will be operative in the synthetic methods of pyrimidine nucleoside preparation described above. This conclusion militates against the use of these condensation reactions for the synthesis of β-pyrimidine nucleosides of arabinose and lyxose.

It should be possible, however, to prepare the desired lyxofuranosylthymine by epimerization of II *via* a cyclo-nucleoside[22,23] intermediate by a route analogous to that employed for the synthesis of 1-β-D-arabinofuranosylthymine from I.[3] Essential for this synthesis would be the suitably-blocked 2′-sulfonic ester derivative of II.

1-β-D-Xylofuranosylthymine was prepared in improved yields by a modification of procedures previously described.[2] Treatment of II with acetone in the presence of copper sulfate and sulfuric acid gave an isopropylidene derivative (V) in 80% yield (see Fig. 2). The assignment of the positions of attachment of the isopropylidene group in V is based on analogy with methyl D-xylofuranoside which gives the 3,5-isopropylidene derivative under these conditions.[24] In V, all of the functional groups in the sugar moiety are blocked save that of the 2-hydroxyl. Reaction of V with methanesulfonyl chloride in pyridine yielded the 2′-O-mesyl derivative VI in 78% yield.

Whereas previous studies[3,23,25,26] showed that 2′- or 3′-sulfonic ester derivatives of ribonucleosides may be converted to their corresponding cyclonucleosides by treatment with methanolic ammonia at room temperature, the xylo derivative VI proved to be resistant to such treatment. To effect the formation of the cyclo-nucleoside it was necessary to reflux VI in dilute alcoholic sodium hydroxide for several hours whereupon a 70% yield of VII was obtained. The ultraviolet absorption spectrum of VII was unaltered in the *p*H region 5.5–14 in accordance with the absence of potentially-dissociable groups in the molecule (see Table I). The refluxing of VII in aqueous ethanol–hydrochloric acid for several hours hydrolyzed the cyclo bridge and removed the isopropylidene residue. A sirup was obtained which was separated from other components (*e. g.*, thymine) by Celite partition chromatography[27] and yielded a crystalline product (IV) which analyzed for a pentosylthymine.

Proof that IV is the hitherto-unknown 1-β-D-lyxofuranosylthymine is based upon the data (see Table II): IV differs in melting point and in optical rotation from the other three stereoisomers. The ultraviolet absorption spectrum of IV as a function of *p*H (see Fig. 3) is similar to those reported for I, II[2] and III.[28] The acidic pK_a value

(12) N. W. Bristow and B. Lythgoe. *J. Chem. Soc.*, 2306 (1949).

(13) B. R. Baker, J. P. Joseph, R. E. Schaub and J. H. Williams, *J. Org. Chem.*, **19**, 1786 (1954).

(14) B. R. Baker and R. E. Schaub, THIS JOURNAL, **77**, 2396 (1955); **77**, 5900 (1955).

(15) J. J. Fox, N. Yung, I. Wempen and I. L. Doerr, *ibid.*, **79**, 5060 (1957).

(16) G. E. Hilbert and T. B Johnson, *ibid*, **52**, 4489 (1930).

(17) J. J. Fox and I. Goodman, *ibid.*, **73**, 3256 (1951).

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

(19) J. Davoll, B. Lythgoe and A. R. Todd, *ibid.*, 833 (1944).

(20) D. W. Visser, I. Goodman and K. Dittmer, THIS JOURNAL, **70**, 1926 (1948).

(21) E. Fischer and B. Helferich, *Ber.*, **47**. 210 (1914).

(22) V. M. Clark, A. R. Todd and J. Zussman, *J. Chem. Soc.*, 2952 (1951); W. Anderson, D. H. Hayes, A. M. Michelson and A. R. Todd, *ibid.*, 1882 (1954).

(23) A. M. Michelson and A. R. Todd, *ibid.*, 816 (1955).

(24) B. R. Baker, R. E. Schaub and J. H. Williams, THIS JOURNAL, **77**, 7 (1955).

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

(26) D. M. Brown, W. Cochran, E. H. Medlin and S. Varadarajan, *ibid.*, 4873 (1956).

(27) H. M. Kissman, C. Pidacks and B. R. Baker, THIS JOURNAL, **77**, 18 (1955). Celite is a product of Johns–Manville Co.

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

TABLE I

PHYSICAL CONSTANTS OF NUCLEOSIDE DERIVATIVES

Compound	Spectrophotometric: pH	Maxima: λ, mμ	Maxima: A_M	Minima: λ, mμ	Minima: A_M	Polarimetric: $[\alpha]_{589}$	$[\alpha]_{546}$	T, °C.	c
II	1–7.5	267.5	9.89	235	2.58	− 2	− 1	31	1.2, H_2O
	12	267.5	7.45	245	4.65				
V	6.38	267.5	9.74	234.5	2.28	− 8	− 6	22	0.8, H_2O
	11.76	267.5	7.57	243	4.14				
VI	6.8	265	9.83	232.5	2.04	−12	−12	22	1.0, pyridine
	12.2	264	7.10	244	4.50				
VII	5.5	227.5	5.37	233	5.26	−41	−46	23	0.7, H_2O
		255	8.00						
	12	227.5	5.37	233	5.26				
		255	8.00						
IV	1–7.3	267.5	9.85	236	2.32	+60	+71	25	0.8, H_2O
	12	267.5	7.86	245	4.43				

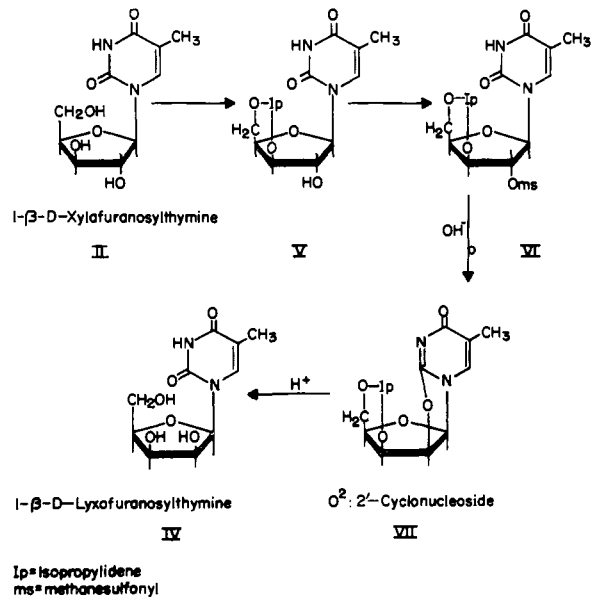

Fig. 2.

TABLE II

PHYSICAL PROPERTIES OF 1-β-D-ALDOPENTOFURANOSYLTHYMINES

	M.p., °C.	$[\alpha]_D$	pK_a[a] Spectrophotometric	pK_a[a] Potentiometric
Lyxo (IV)	186–187.5	+60°	9.92	9.89
Xylo (II)	156–157.5	− 2	9.75	9.68
Arabino (III)	238–242	+93	9.8	..
Ribo (I)	183–185	−10	9.68	..

[a] The pKa values listed refer to the formation of the enolate ion at position 4. Values are accurate to 0.05 pH unit.

of IV, determined spectrophotometrically or potentiometrically, is of the same order of magnitude as those reported for the other aldopentofuranosylthymines.

When treated with metaperiodate, IV consumed one mole of oxidant per mole without the liberation of formic acid, a result in accord with a furanose structure. This consumption of metaperiodate was completed within five minutes. A similarly rapid uptake of oxidant was noted previously for

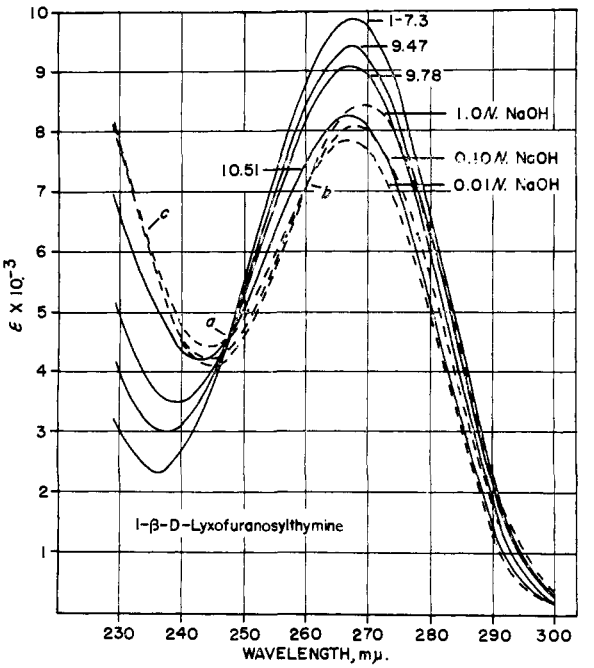

Fig. 3.—Ultraviolet absorption spectrum of 1-β-D-lyxofuranosylthymine in aqueous solutions at pH or normality values indicated; a, b and c refer to isosbestic points.

1-β-D-ribofuranosylthymine (I), which contains *cis*-vicinal hydroxyl groups.[29] The optical rotation of the dialdehyde solution obtained from this oxidative process was essentially the same as that obtained from similar treatment of I, II and III, showing that the glycosylic center of IV was also of the β-form. It must be concluded, therefore, that IV is 1-β-D-lyxofuranosylthymine. By virtue of the epimerization of II to IV, the structures of V, VI and VII (see Fig. 2) are also confirmed.

Spectral Studies.—It has been demonstrated[28] that pyrimidine nucleosides exhibit spectral shifts between pH 12 to 14 attributable to the ionization of the carbohydrate moiety. A mechanism for

(29) It has been shown previously[2,4,15] that nucleosides containing *trans*-hydroxyl groups in the 2'-3'-positions require 1–2 days for oxidation with metaperiodate to run its course, whereas ribosyl nucleosides (*e.g.*, uridine, cytidine or 1-β-D-ribofuranosylthymine) are completely oxidized to their dialdehydes within 5 minutes.

these spectral shifts has been proposed[30] which postulates that in pyrimidine nucleosides hydrogen bonding exists between the *un-ionized* sugar hydroxyl function(s) and the 2-carbonyl group of the pyrimidine. In the high alkaline region, where sugars are known to ionize,[31] the hydrogen bridge is ruptured, thus giving rise to electronic shifts in the pyrimidine moiety which are reflected in the spectrum. It also was demonstrated[30a] that these spectral variations are due, mainly, to ionization of the 2′-hydroxyl group with a limited contribution from the other hydroxyl functions in the sugar moiety.

It was noted further[2,3,28] that spectral shifts manifested by 1-β-D-arabinofuranosylthymine in this region were greater than those exhibited by the xylo and ribo isomers. Since it has been demonstrated that ionization of the 2′-hydroxyl group is mainly responsible for spectral variations of pyrimidine nucleosides in this *p*H region, it is reasonable to expect that the arabino isomer (III) (which differs from I and II in the juxtaposition of the 2′-hydroxyl to the aglycon) would show significant differences in its spectrum (see Fig. 4). It should follow, then, that IV (in which the relationship of the 2′-hydroxyl function to the aglycon is similar to that in III) would also exhibit similar spectral differences. Indeed, the spectrum of IV in this region (see Fig. 4) shows larger spectral shifts (more akin to III) than are given by I or II.

Perhaps of greater significance is the fact that the arabino and lyxo isomers begin to exhibit spectral shifts in the high alkaline range at lower *p*H values (0.005 to 0.01 *N* NaOH), whereas with I and II spectral variations are realized only *above* 0.01 *N* alkali (see Fig. 4). From these data it can be concluded that the sugar residues in III and IV are stronger acids than are the ribo and xylo isomers. With the information available it is difficult to render an explanation encompassing all of these facts; however, it is noteworthy that the phenomena of large spectral shifts and stronger acidity appear to be linked characteristics for those isomers in which the 2-hydroxyl group bears a *cis* relationship to the aglycon.

Though measurable differences in spectra are exhibited by these four pentofuranosylthymine isomers in the high alkaline region, the general similarity of their spectral patterns should not be overlooked (*viz.*, the single isosbestic point, an increase in extinction as the *p*H of the medium is increased accompanied by a bathochromic displacement of the maximum). It is to be noted that uridine and cytidine[28] (the latter of which contains an amino function rather than an enolate ion at position 4) also exhibit a similar spectral pattern in this region. These similarities would indicate that a general mechanism is operative by which ionization of the carbohydrate residue affects the aglycon (*i.e.*, rupture of hydrogen bonding to the 2-carbonyl group[30a]). The effect of alteration of the configuration of the sugar hydroxyl groups (as in I → IV) upon the spectrum in this high *p*H region would appear to be one of degree rather than kind.

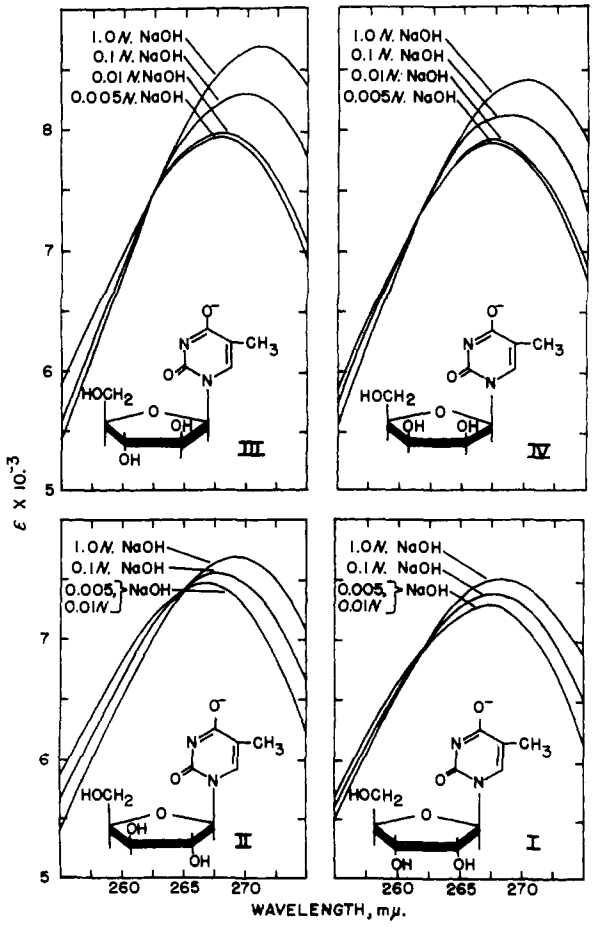

Fig. 4.—Ultraviolet absorption spectrum of 1-β-D-aldopentofuranosylthymines in aqueous solutions in the high alkaline region at normalities indicated.

Table III

Cleavage of 1-β-D-Aldopentofuranosylthymine Derivatives by *E. coli* B[a]

Substrate[b]	Thymine formed, μmole	Substrate recovery, μmoles
Ribo (I)	0.78	..
Xylo (II)	0	1.03
Arabino (III)	0	0.94
Lyxo (IV)	0	1.10

[a] *E. coli* B was grown in Penassay broth medium with aeration at 37° for 18 hr. The cells were harvested by centrifugation, washed twice with cold saline and finally resuspended in 20 ml. of saline solution. The incubation mixture contained 1.0 μmole of substrate, 0.8 ml. of 0.067 *M* phosphate buffer (*p*H 7.2) and 0.1 ml. of washed cells of *E. coli* B in a total volume of 1.0 ml. After 40 minutes incubation at 37°, the reaction was stopped by the addition of cold perchloric acid. After centrifugation, the amount of thymine present in the supernatant was determined by measuring the optical density at 300 mμ at *p*H 13. [b] 1-β-D-Ribopyranosyluracil was not cleaved under these conditions, whereas uridine was hydrolyzed with the liberation of uracil.

Enzymic Studies.—The results in Table III demonstrate that of the four 1-β-D-aldopentofuranosyl nucleosides of thymine, only the 1-β-D-ribofuranosyl isomer is cleaved by intact cell suspensions of *Escherichia coli* B. The suscepti-

(30) (a) J. J. Fox, L. F. Cavalieri and N. Chang, This Journal, **75**, 4315 (1953); (b) in a recent paper dealing with the photochemistry of cytosine nucleosides, K. L. Wierzchowski and D. Shugar (*Biochim. et Biophys. Acta*, **25**, 355 (1957)) also suggest that hydrogen bonding exists between the aglycon and the sugar moiety.

(31) R. Kuhn and H. Sobotka, *Z. physik. Chem.*, **109**, 65 (1924).

TABLE IV

ACTION OF BACTERIAL NUCLEOSIDASES ON PYRIMIDINE NUCLEOSIDES[a]

Nucleoside	μmoles	Free pyrimidine formed: *E. coli* B nucleosidase,[b] μmole	Free pyrimidine formed: *L. pentosus* nucleosidase,[c] μmole
Uridine	1.0	0.55	0.70
Ribo (I)	1.1	.64	.79
Arabino (III)	1.0	.0	.0
1-β-D-Glucopyranosylthymine	1.0	.0	.0
	4.0	.0	.0
1-β-D-Xylopyranosylthymine	1.0	.0	.0
	4.0	.0	.0

[a] Substrate and extract as indicated were present in 0.4 ml. of 0.025 *M* sodium arsenate buffer, *p*H 7.5. At zero time (addition of enzyme) and after 60 min. of incubation at 37°, 0.1-ml. aliquots were removed, diluted to 3.0 ml. and heated 10 min. at 100°. The absorption at 300 mμ in 0.1 *N* NaOH was used as a measure of the free base formed. The extinction coefficients of Hotchkiss[32a] were employed, and all values were corrected for a control incubation without substrate. [b] A cell-free extract of *E. coli* strain B was prepared by the procedure used for strain 15 by Manson and Lampen.[32b] The extract was then fractioned with ammonium sulfate. The material precipitated between 0.30 and 0.70 saturation (about 80% of the original nucleosidase activity) was dialyzed, and the final solution clarified by centrifuging for 20 min. at 18,000 × *g*. Each incubation contained 0.10 ml. of this fraction equivalent to 200 mg. of wet packed cells. [c] Each incubation mixture received 0.05 ml. of a cell-free extract prepared from *Lactobacillus pentosus* strain 124-2 by the method of Lampen and Wang[32c] (alumina used in grinding).

bility of 1-β-D-ribofuranosylthymine to enzymic cleavage (as determined by the amount of thymine liberated) was found to be unaffected by the presence of 1 to 4 equivalents of II, III or IV in the reaction medium. These data are in agreement with the results obtained employing cell-free extracts of *E. coli* B and *Lactobacillus pentosus*. As shown in Table IV, uridine and I are cleaved at N_1–C'_1, whereas III was not (compounds II and IV were not then available).

These cell-free systems were unable to cleave the 1-β-D-glyco*pyranosyl*thymine isomers of xylose and glucose (see Table IV). Although 1-β-D-ribo*pyranosyl*thymine was unavailable for testing, the susceptibility of uridine and 1-β-D-ribo*pyranosyl*uracil to cleavage by cell suspensions was examined (see Table III, footnote). Uridine was readily hydrolyzed (to liberate uracil) under these conditions, whereas 1-β-D-ribo*pyranosyl*uracil was not. These results strongly indicate that the furanosyl structure is a requirement for enzymic action.

It is clear that the 1-β-D-pentofuranosyl structure in these pyrimidine nucleosides cannot explain the specificity of the enzyme(s) since inversion of the hydroxyl groups at C'_2 or at C'_3 (as in II and III) leads to inactive substrates. It is equally apparent that even the C'_2–C'_3-*cis* relationship of sugar hydroxyls (as in I and IV) is not the sole prerequisite for enzymic susceptibility since IV is inactive.

The most obvious explanation is, of course, that the nucleosidase(s) are specific for the 1-β-D-ribofuranosyl[33] structure. However, it is also possible to invoke the postulate that the most essential requirement for enzymic action is a *trans* relationship between the aglycon and the C_2–C_3-*cis*-hydroxyl groups. This relationship is satisfied in I but not in IV. If this postulate is valid (and if the configuration at C'_4 is not critical), it would suggest that the as yet unavailable 1-α-D-lyxofuranosylthymine (VIII) will be cleaved by these nucleosidases since VIII would meet these conditions (see Fig. 5). Also of interest would be 1-α-L-rhamnofuranosylthymine (IX)[34] which possesses the added feature of a C-methyl group.

Acknowledgments.—The authors are indebted to Drs. Aaron Bendich and George B. Brown of this Institute for helpful suggestions and continued interest, and to Mr. C. Pidacks, Drs. H. M. Kissman and M. J. Weiss (Lederle Laboratories) for information on the use of Celite partition chromatographic techniques.

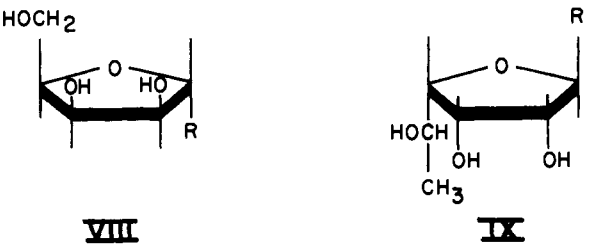

Fig. 5.

Experimental[35]

1-(Tri-O-benzoyl-β-D-xylofuranosyl)-thymine.[2]—This procedure is a modification of the method already reported. A solution of tetra-O-benzoyl-α-D-xylofuranose[36] (0.08 mole) in 1 liter of dry benzene was saturated under anhydrous conditions with hydrogen bromide at 5°. The temperature was lowered to 0° and the solution again saturated with additional hydrogen bromide. The flask was sealed tightly and allowed to stand at room temperature for 7–10 days. Chloroform (*ca.* 100 ml.) was then added to the solution to prevent freezing during the subsequent extraction. The solution was extracted rapidly in turn with ice-water, ice-cold bicarbonate solution and cold water. The organic layer was dried by shaking with sodium sulfate, filtered, and the filtrate concentrated rapidly in a rotary evaporator to 150 ml. (bath temperature not exceeding 30°). This concentrated solution contains the halogenose (2,3,5-tri-O-benzoyl-D-xylosyl bromide).

An excess of dithyminylmercury[2] (24.0 g., 0.053 mole) was added to one liter of dry toluene. The vigorously-stirred suspension was dried further by azeotropic distillation of approximately one-fourth of the solvent. About one-half of the halogenose solution was added to the hot, stirred mixture. The reaction was refluxed and, by the use of a take-off adapter, approximately 75–100 ml. of solvent was removed. The remainder of the halogenose solution was then added and again an equal volume of solvent was removed. The stirred mixture was refluxed for 1.5 hours after which it was concentrated down to 200–300 ml., cooled and filtered from 10 g. of insoluble material. The filtrate was treated with petroleum ether until precipitation was complete, filtered, and the solid dissolved in chloroform. After filtration from a small amount of insoluble material, the chloroform solution was washed with 30% potassium

(32) (a) R. D. Hotchkiss, *J. Biol. Chem.*, **175**, 315 (1948); (b) L. A. Manson and J. O. Lampen, *ibid.*, **193**, 539 (1951); (c) J. O. Lampen and T. P. Wang, *ibid.*, **198**, 385 (1952).

(33) The question of D- or L-isomerism is also to be considered.

(34) An unsuccessful attempt to prepare IX *via* the mercuri procedure has been reported recently by B. R. Baker and K. Hewson, *J. Org. Chem.*, **22**, 966 (1957).

(35) All melting points are corrected unless stated otherwise. Elemental analyses were performed by Mr. J. F. Alicino, Metuchen, N. J.

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

iodide solution and water and dried over sodium sulfate. The chloroform solution was separated from sodium sulfate and concentrated to dryness *in vacuo*. The solid residue was triturated with 75–100 ml. of hot ethyl acetate and cooled. A yield of 24–27 g. (55–60%) of colorless prisms was obtained, m.p. 197–200° (first crop of 20–23 g.). Fox and co-workers[2] report 197.5–198.5° for analytical material.

Conversion of this material to 1-β-D-xylofuranosylthymine (II) was accomplished by the previously-reported procedure.[2]

1-(3′,5′-Isopropylidene-β-D-xylofuranosyl)-thymine (V).—A suspension of anhydrous copper sulfate (13 g.) in 300 ml. of dry acetone containing 0.15 ml. of concentrated sulfuric acid and 8.73 g. (0.034 mole) of 1-β-D-xylofuranosylthymine was stirred at 37° for 48 hours. After filtration from insoluble material, the filtrate was neutralized with anhydrous sodium bicarbonate (*ca.* 8 g.) and filtered. The acetone solution was concentrated down to dryness leaving a solid material in near theoretical yield, m.p. 163–185°. Crystallization from methanol gave colorless needles, m.p. 175–177° (resolidifies and remelts at 185.5–186.5°). This material contains 80–90% of a mole of methanol of crystallization which may be removed after drying for several hours at 100° *in vacuo*. The yield of pure material was 90%, m.p. 186.5–187.5°.

Anal. Calcd. for $C_{13}H_{18}O_6N_2$: C, 52.3; H, 6.0; N, 9.4. Found: C, 51.8; H, 6.1; N, 9.2.

1-(2′-O-Methanesulfonyl-3′,5′-isopropylidene-β-D-xylofuranosyl)-thymine, (VI).—Methanesulfonyl chloride (3.03 g., 0.035 mole) was added to a solution of V (9.0 g., 0.030 mole) in 27 ml. of dry pyridine and stirred for 23 hours at 34°. The amber-colored reaction mixture (containing some pyridinium chloride) was poured into stirred ice-water. The solid was removed and washed well with water, 8.32 g., m.p. 162–165°. An additional crop (0.85 g.) was obtained from the filtrate. The combined precipitates were dissolved in hot absolute ethanol, treated with charcoal, filtered and cooled. Yellow prisms, m.p. 163–165.5° (78%), were collected. The product was recrystallized from absolute ethanol from which colorless prisms, m.p. 163–165.5°, were obtained.

Anal. Calcd. for $C_{14}H_{20}O_8N_2S$: S, 8.5. Found: S, 8.4.

1-(O²:2′-Cyclo-3′,5′-isopropylidene-β-D-lyxofuranosyl)-thymine (VII).—A solution of 7.52 g. (0.020 mole) of VI and 0.021 mole of sodium hydroxide in 760 ml. of 80% ethanol was refluxed for 9 hours. The initially-bright-yellow solution became colorless after 5 hours. The course of the reaction was followed by spectral analysis of aliquots removed from the reaction. The original solution gave a maximum at 265 mμ, corresponding to the maximum of VI. As the reaction progressed, the maximum shifted hypsochromically to 256 mμ corresponding to the formation of VII (see Table I for spectral data).

The solution was taken to dryness *in vacuo* and the residue triturated with a small amount of hot absolute ethanol for 30 minutes. The insoluble sodium mesylate (80% of theory) was filtered from the hot ethanolic solution. A yield of 4.31 g. (78%) of colorless needles, m.p. 254–260° (uncor.) crystallized from the filtrate. Pure material was obtained by recrystallization from water (to remove traces of sodium mesylate), and then from absolute ethanol. Colorless needles were obtained, m.p. 259–262° (uncor.).

Anal. Calcd. for $C_{13}H_{16}N_2O_5$: C, 55.7; H, 5.75; N, 10.0. Found: C, 55.8; H, 5.91; N, 10.3.

1-β-D-Lyxofuranosylthymine (IV).—The cyclonucleoside VII (3.65 g., 0.013 mole) was refluxed in dilute ethanolic hydrochloric acid (0.046 mole in 480 ml. of 95% ethanol). After 6.5 hours at reflux temperature (when the spectrally-observed bathochromic displacement of the maximum was completed), the clear colorless solution was reduced to 15–20 ml. *in vacuo* on a rotary evaporator. The sirup was taken up in absolute ethanol and the volume was again reduced to about 15 ml. This process was repeated several times after which a colorless amorphous residue, essentially free of acid, remained. This crude material was then purified by passage through a Celite column. A typical separation is described below.

A column, 24 cm. in height and 2.5 cm. in diameter, was used in the purification of 202 mg. of crude lyxofuranosylthymine. The column was packed to a height of 17 cm. with 25 g. of Celite which had been mixed well with 12.5 ml. of stationary (lower) phase obtained from a mixture of ethyl acetate, methanol, water and *n*-heptane (10:6:5:3). The sample was dissolved in a minimum amount of stationary phase and was then mixed with twice its weight of Celite. This mixture was packed onto the top of the column. The mobile (upper layer) phase was passed through the column and the effluent collected. Absorption at 267 mμ was determined for each fraction.

From the first major peak a solid was obtained which gave long, colorless needles upon crystallization from absolute ethanol, m.p. 206–209.5° (uncor.). This minor component has not been characterized further. Thymine was recovered in 3–4% yield as the second major peak. It was identified by melting point, paper chromatography and ultraviolet absorption spectrum.[37]

1-β-D-Lyxofuranosylthymine (IV) was eluted from the column after approximately 350 ml. of effluent had been collected. Material from the major peak appeared to be homogeneous as observed by paper chromatography (*n*-butanol–water, 86:14). The combined fractions were concentrated to a yellow gum from which crystalline material was obtained by dissolving the residue in a minimum of absolute ethanol and cooling. Subsequent recrystallization from absolute ethanol gave colorless needles, m.p. 185.5–186.5° (35% based on VII). Further recrystallization from absolute ethanol gave needles, m.p. 186–187.5°.

Anal. Calcd. for $C_{10}H_{14}N_2O_6$: C, 46.5; H, 5.5; N, 10.9. Found: C, 46.6; H, 5.5; N, 11.1.

Spectrophotometric Studies.—Ultraviolet absorption spectra were determined with a Beckman model DU spectrophotometer, using buffers and techniques previously described.[30,37,38] Phosphate buffer was employed for the *p*H 7.30 curve (see Fig. 2) and glycine buffers in the *p*H 9.47–10.51 range. In the high alkaline region, freshly prepared solutions of sodium hydroxide (made up to an ionic strength of 1.00 with additions of sodium chloride) were used. Since the apparent pK_a values of the sugar dissociation were not quantitatively determined in this range, a knowledge of the specific *p*H values of these sodium hydroxide solutions is unnecessary. The apparent pK_a value for the formation of the enolate ion at position 4 in IV was determined spectrally by procedures previously described.[37,38] Spectral data for compounds II, IV, V, VI and VII are given in Table I.

Potentiometric Titrations.—A 0.04 molar solution of the nucleoside in 3.0 ml. of water was titrated with 0.30 *N* sodium hydroxide in an atmosphere of nitrogen with constant stirring. Determinations of *p*H were made with a Beckman model G *p*H meter; pK_a values were calculated from seven equidistant points on the titration curve and averaged.

Polarimetric Determinations.—Optical rotations were determined using equipment and techniques previously described[2] (see Table I for data). For the determination of the rotation of the dialdehyde solution from IV, a solution of known concentration of IV was treated with excess sodium metaperiodate in the polarimetric cell, and readings taken until constancy was reached. The specific rotation of the dialdehyde obtained from IV (17°) was based upon the original concentration of this nucleoside in the aqueous solution.

Metaperiodate Titrations.—Procedures described in the first paper of this series[2] were followed. 1-β-D-Lyxofuranosylthymine (0.001 mmole per ml.) was found to consume 1.0 mole of metaperiodate per mole within 3 minutes and released no titratable acid. No further uptake of oxidant was observed during the ensuing 120 hours.

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(37) D. Shugar and J. J. Fox, *Biochim. et Biophys. Acta*, **9**, 199 (1952).

(38) J. J. Fox and D. Shugar, *Bull. soc. chim. Belges*, **61**, 44 (1952).