ORGANIC AND BIOLOGICAL CHEMISTRY

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Pyrimidine Nucleosides. I. A New Route for the Synthesis of Thymine Nucleosides[1]

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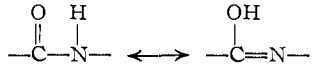
A simplified method is given for the synthesis in good yields of thymine nucleosides by the condensation of a mercury derivative of thymine directly with poly-*O*-acylglycosyl halides followed by removal of protecting acetyl or benzoyl groups. Glycopyranosylthymines obtained by this "mercuri" procedure are shown to be identical with those obtained by the Hilbert-Johnson method. The mercuri procedure is extremely useful for the preparation of pentofuranosyl nucleosides of thymine. The synthesis and characterization of 1-β-D-ribofuranosylthymine and 1-β-D-xylofuranosylthymine are reported and the former shown to be identical with an enzymically prepared material. 2,3,5-Tri-*O*-benzoylpentosyl halides are utilized for pentofuranosylthymine synthesis and their superiority to their corresponding tri-*O*-acetylpentofuranosyl halides is indicated. The synthesis of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-α-D-xylose is reported.

The successful application of metal derivatives of some purines to the synthesis of purine nucleosides was accomplished several decades ago by Fischer and Helferich[4] who prepared glucosyladenine from the reaction product of the silver salt of 2,8-dichloroadenine with tetra-*O*-acetyl-D-glucopyranosyl bromide ("acetobromoglucose") in boiling xylene. They were less fortunate, however, in their attempts to apply this procedure to the synthesis of pyrimidine nucleosides.[5] The silver salts of various pyrimidines, when reacting with acetobromoglucose, gave substances which were hydrolyzed easily and which reduced Fehling solution, thus indicating that the sugar moiety was probably linked through an oxygen atom at the 2- or 4-position of the pyrimidine ring.

In 1925, Levene and Sobotka[6] utilized pyrimidines substituted in various positions. The reaction of the silver or alkali metal salts of 3-methyluracil, 5-nitrouracil, 3-methyl-5-nitrouracil and 2-ethylthio-4-hydroxypyrimidine with acetobromoxylose in xylene led to the formation of glycosides between the sugar and the pyrimidine as was evident by the ease with which these products were hydrolyzed in acidic or alkaline media.[7] Cytosine did not react at all.

It was generally conceded, therefore, that N^1-glycosylation of the pyrimidine ring to nucleosides was not a feasible approach if lactam–lactim tautomerism of the type



were structurally possible, and that instead these approaches would result in the formation of glycosides.[8]

Indeed, Fischer and co-workers[4,9] prepared N-glycosylpurines from the metal salts of the corresponding purines only in those cases in which tautomerism of the type discussed above was excluded, as in the cases of the silver salts of theophylline and 2,8-dichloroadenine.

In accord with this reasoning, a method for pyrimidine nucleoside synthesis was developed by Hilbert and Johnson[8] which involves the condensation of a poly-*O*-acylglycosyl halide with a 2,4-dialkoxypyrimidine. This method has been used universally for the preparation of pyrimidine nucleosides for the past twenty-five years.

It has been reported recently[10] that monochloromercuri derivatives of certain purines may be employed to greater advantage than the silver salts in the Fischer–Helferich type synthesis with halogenoses resulting in higher yields of purine nucleosides. With the hope of providing a simplified route for the preparation of pyrimidine nucleosides, the usefulness of the mercuri derivatives of the pyrimidines for direct N^1-glycosylation to nucleosides was investigated.

In this paper the syntheses of thymine nucleosides from a mercury derivative of thymine are reported. The results of our investigations with cytosine derivatives will be reported later.

Results and Discussion

A mercury derivative of thymine (stated to be $C_5H_4N_2O_2Hg$) has been reported by Myers.[11] In our hands the reaction of thymine with molecular proportions of alkali and mercuric chloride gave mixtures of insoluble mercury pyrimidines which contained varying amounts of halogen. With the proper proportion of reactants, however, a dithyminylmercury derivative is obtained in essentially quantitative yields. It is to be noted that the possibility of lactam–lactim tautomerism of the type previously described surely exists in this molecule.

Treatment of dithyminylmercury with acetobromoglucose in refluxing toluene yielded 1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-thymine in 60% yield. Hydrolysis of this crystalline intermediate with methanolic hydrogen chloride gave N^1-β-D-

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(2) Fellow of the Damon Runyon Memorial Fund (1952–1954).

(3) Fellow of the National Cancer Institute of the Public Health Service (1949–1951).

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

(5) E. Fischer, *ibid.*, **47**, 1377 (1914).

(6) P. A. Levene and H. Sobotka, *J. Biol. Chem.*, **65**, 469 (1925).

(7) The silver salt of thymine, when condensed with acetobromoglucose, yielded a thymine glycoside intermediate which upon hydrolysis with methanolic hydrogen chloride produced thymine (Fox and Yung, unpublished observations).

(8) G. E. Hilbert and T. B. Johnson, THIS JOURNAL, **52**, 4489 (1930).

(9) E. Fischer and K. von Fodor, *Chem. Ber.*, **47**, 1058 (1914); B. Helferich and M. von Kuhlewein, *ibid.*, **53**, 17 (1920).

(10) J. Davoll and B. A. Lowy, THIS JOURNAL, **73**, 1650 (1951).

(11) V. C. Myers, *J. Biol. Chem.*, **7**, 249 (1909–1910).

glucopyranosylthymine,[12] identical with that previously obtained[13] by the Hilbert procedure.[8]

Similarly, when tri-*O*-acetyl-D-xylopyranosyl bromide or tri-*O*-acetyl-L-arabinopyranosyl chloride is condensed with dithyminylmercury and the protecting acetyl groups subsequently removed, D-xylopyranosylthymine and L-arabinopyranosylthymine are isolated which prove to be identical with those obtained[13,14] by the Hilbert–Johnson procedure. These results demonstrate that identical nucleosides of thymine are obtained by the Hilbert procedure (using 2,4-dialkoxy-5-methylpyrimidine) and by the mercuri method when those halogenoses are employed.[15]

1-β-D-Ribofuranosylthymine.—Since previous studies with microörganisms indicated that synthetic pyrimidine nucleosides containing sugars of the pyranose structure are biologically inactive,[14,16] attention was devoted toward the synthesis of glycofuranosyl derivatives of thymine. Of special interest would be the preparation of a ribofuranosylthymine which may be regarded as a "5-methyluridine" or as a 2'-hydroxy analog of thymidine. The preparation of such a compound had been reported previously by Roberts and Visser using the Hilbert–Johnson procedure.[17] More recently, Lampen[18] obtained a thymine nucleoside from thymine and D-ribosyl phosphate by enzymic synthesis using a nucleosidase from *Escherichia coli* B. The synthetic preparation of Roberts and Visser (A) was not split by nucleosidase preparations from *E. coli* whereas the biologically-formed nucleoside (B) was cleaved by this enzyme.[18] Further, though the ultraviolet absorption spectra of these samples were essentially similar and resembled that for thymine nucleosides,[19] their melting points differed (see Table I) and a mixed-melting point gave a depression of approximately twenty degrees. Neither of these thymine nucleosides had been further characterized.

We prepared ribofuranosylthymine *via* the mercuri method by the condensation of sirupy tri-*O*-acetyl-D-ribofuranosyl chloride[20] with dithyminylmercury in toluene. A sirup was obtained which did not crystallize (presumably containing 2',3',5'-tri-*O*-acetyl-D-ribofuranosylthymine) but which, upon deacetylation with methanolic hydrogen chloride, gave crystalline product in yields ranging from 5 to 25%. Because of the low and erratic yields obtained (which may be ascribed to the instability of the "halogenose") it was decided to utilize 2,3,5-tri-*O*-benzoyl-D-ribosyl halide as a reactant with the hope of improving yields as well as of obtaining a crystalline intermediate. 2,3,5-Tri-*O*-benzoyl-D-ribosyl chloride and bromide were prepared from 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribose[21] and condensed with dithyminylmercury to give crystalline intermediates in 50 and 36% yields, respectively.[22] Debenzoylation of this intermediate with alcoholic ammonia in a sealed tube proceeded smoothly to D-ribofuranosylthymine (*cf.* Table I).

TABLE I

PHYSICAL PROPERTIES OF RIBOSYLTHYMINES

Ribosylthymine[a]	M.p.,[b] °C.	$[\alpha]_D$	Moles IO_4^- consumed per mole of cmpd.	HCOOH liberated	$[\alpha]_D$ of dialdehyde produced[c]
A	175–177	−54°	1	0	−29°
B	182–184	−10	1	0	+16
C	183–185	−10	1	0	+16

[a] A = sample from Roberts and Visser[17] prepared *via* the Hilbert–Johnson procedure; B = sample prepared enzymically by Lampen[18]; C = reported herein *via* mercuri method. [b] Melting points are corrected. [c] Rotation of dialdehyde solution from periodate oxidation of 1-β-D-glucopyranosylthymine, $[\alpha]_D$ +16°.

As shown in Table I, the enzymically prepared material B and the sample prepared by the mercuri procedure give essentially similar melting points and optical rotations. The melting point of a mixture of the two shows no depression. Admixture of ribosylthymine B or C with A produces a depression in the melting point of approximately twenty degrees. Both the material from Lampen and our own sample are readily cleaved by the nucleosidase from *E. coli* B.,[23] whereas sample A is not. All three nucleosides consume one mole of metaperiodate per mole without the liberation of formic acid, in accord with a furanose structure.

In order to determine the configuration at the glycosidic centers of these three nucleosides, 1-β-D-glucopyranosylthymine was used as the reference compound.[12] This nucleoside consumes two moles of metaperiodate per mole with the liberation of one mole of formic acid.[24] Of the three ribosylthymines discussed, only those which are of the β-furanosyl structure will give the same dialdehyde after consumption of one mole of metaperiodate per

(12) The β-configuration of glucopyranosylthymine is assigned on the following basis: D-glucopyranosyluracil, prepared by the Hilbert–Johnson method, gives the same dialdehyde as does uridine when treated with metaperiodate (J. Davoll, B. Lythgoe and A. R. Todd, *J. Chem. Soc.*, 833 (1946)). The configuration at the glycosidic center of cytidine (and thereby uridine) has been rigorously established as beta by X-ray crystallographic analyses (S. Furberg, *Acta Chem. Scand.*, **4**, 751 (1950)) and by cyclonucleoside formation (V. M. Clark, A. R. Todd and J. Zussman, *J. Chem. Soc.*, 2952 (1951)). These data provide a firm basis for the assignment of the β-configuration to glucopyranosyluracil. Since glucopyranosylthymine may be prepared by the Hilbert–Johnson procedure using the same halogenose, it is reasonable to conclude that the configuration of this nucleoside is also of the β-form. For independent proof of the validity of this assumption see reference 26.

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

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

(15) The similarity of nucleoside products from the Hilbert and from the mercuri procedures also obtains with the pyrimidine cytosine (Fox and Yung, unpublished observations).

(16) K. Dittmer, I. Goodman, D. Visser and H. P. McNulty, *Proc. Soc. Exptl. Biol. Med.*, **69**, 40 (1948).

(17) M. Roberts and D. W. Visser, THIS JOURNAL, **74**, 668 (1952).

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

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

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

(21) This intermediate was first prepared by us by the procedure of F. Weygand and F. Sigmund (*Chem. Ber.*, **86**, 161 (1953)) from crude guanosine, and later from D-ribose by the method of R. K. Ness, H. W. Diehl and H. G. Fletcher, Jr., (THIS JOURNAL, **76**, 763 (1954)), including the recent modifications of H. M. Kissman, C. Pidacks and B. R. Baker, *ibid.*, **77**, 18 (1955)).

(22) The systematically higher yields obtained with 2,3,5-tri-*O*-benzyl-D-ribosyl halides as compared with the corresponding acetyl derivative is in agreement with the recent findings of Kissman, *et al.*,[21] who obtained higher yields of purine nucleosides using 2,3,5-tri-*O*-benzoyl-D-ribosyl chloride for condensation with appropriate purines.

(23) J. O. Lampen, personal communication.

(24) M. Z. Newmark, I. Goodman and K. Dittmer, THIS JOURNAL, **71**, 3847 (1949).

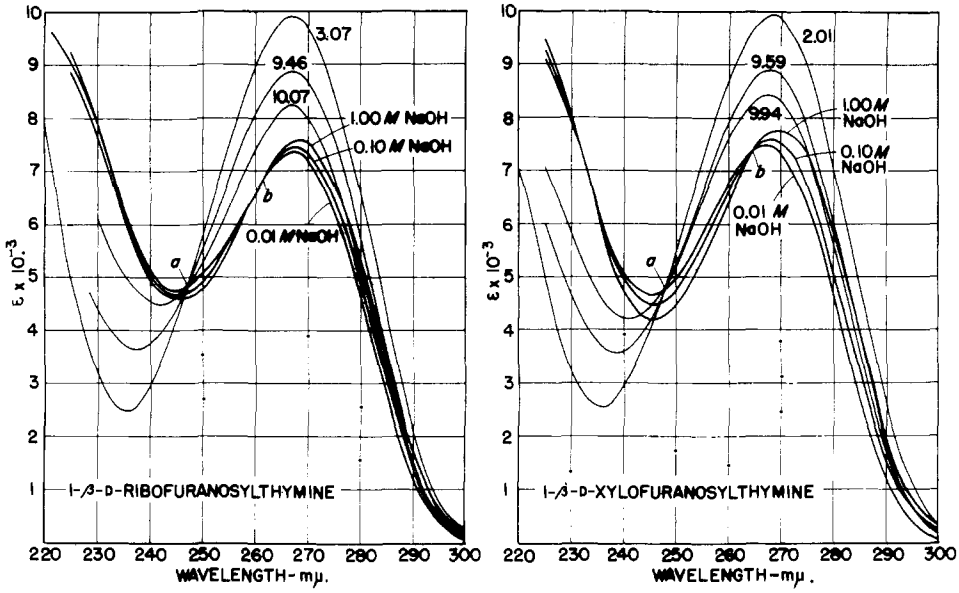

Fig. 1.—Ultraviolet absorption spectra in aqueous solutions at *p*H values indicated on the curves; pK_a values: for ribofuranosylthymine 9.68; for xylofuranosylthymine 9.75.

mole as 1-β-D-glucopyranosylthymine. As shown in Table I, the enzymically-prepared material and the synthetic nucleoside prepared *via* the mercuri procedure when treated with periodate produced the same rotation for the resulting dialdehyde solution as does 1-β-D-glucopyranosylthymine.[25] Therefore, the enzymically-prepared material from Lampen and our own synthetic D-ribofuranosylthymine are one and the same compound and must be designated as a β-nucleoside,[26] that is, as true 5-methyluridine. This fact, however, does not permit the designation of the Roberts–Visser material as an α-nucleoside since the specific rotation of the available sample was far more negative than 1-β-D-ribofuranosylthymine. Thus, the character of their material is still unclear.

1-β-D-Xylofuranosylthymine.—For the synthesis of xylofuranosylthymine, crystalline 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-α-D-xylose[27] (I) was prepared from amorphous 2,3,5-tri-*O*-benzoyl-D-xylose[28] by acetylation in pyridine. Treatment of I with hydrogen chloride in ether yielded a sirup (presumably 2,3,5-tri-*O*-benzoyl-D-xylosyl chloride) which when reacted in toluene with dithyminylmercury gave crystalline 2′,3′,5′-tri-*O*-benzoyl-D-xylofuranosylthymine (II) in 28% yield. Tetra-*O*-benzoyl-α-D-xylofuranose[28] (III) may be used directly for conversion to a benzobromoxylofuranose. Thus, treatment of III with hydrogen bromide in anhydrous methylene dichloride gave a sirup (2,3,5-tri-*O*-benzoyl-D-xylosyl bromide) which condensed with dithyminylmercury to give II in 37% yield. Debenzoylation of II with alcoholic ammonia in a sealed tube proceeded essentially quantitatively to IV, xylofuranosylthymine. This nucleoside consumed one mole of metaperiodate per mole without the liberation of formic acid in accord with a pentofuranose structure. The rotation of the dialdehyde solution resulting from IV was identical with that produced by 1-β-D-glucopyranosyl- and 1-β-D-ribofuranosylthymine.[26] The ultraviolet absorption spectrum of IV (see Fig. 1) was similar to that for glycosylthymines previously reported.[19] It is concluded, therefore, that IV is 1-β-D-xylofuranosylthymine. The same configurational assignment at the glycosidic center also applies to II.

Spectrophotometric Studies.—In previous studies,[19] dealing with the ultraviolet absorption spectra of N-glycosylthymines as a function of *p*H, spectral variations attributable to the influence of the sugar moiety on the chromophore of the pyrimidine were demonstrated in the high alkaline range (between *p*H values 12.0 to 14). It was shown further that the spongothymidine of Bergmann and Feeney[29] gave appreciable spectral shifts in this range which were of greater magnitude than that calculated for a hypothetical 5-methyluridine (D-ribofuranosylthymine), and, on this spectrophotometric basis, a D-ribose residue was excluded as the sugar moiety of spongothymidine.

The spectra of 1-β-D-ribofuranosylthymine and 1-β-D-xylofuranosylthymine are given in Fig. 1.

(25) We have provided a sample of 1-β-D-glucopyranosylthymine to W. Bergmann and D. F. Burke who have recently reported (*Angew. Chem.*, **67**, 127 (1955)) an identity of the rotation of the metaperiodate oxidation product of this nucleoside with that from D-arabinofuranosylthymine (spongothymidine) and have assigned the β-configuration to the latter. See references 12 and 26.

(26) D-Ribofuranosylthymine prepared by the mercuri procedure has since been converted to crystalline spongothymidine (J. J. Fox and N. Yung, to be published) *via* a cyclonucleoside intermediate by the use of a procedure based upon the studies of M. Michelson and A. R. Todd, *J. Chem. Soc.*, 816 (1955), with thymidine. For this epimerization to occur, ribofuranosylthymine must be of the β-configuration. This provides rigorous proof of the β-configuration of D-glucopyranosylthymine, of spongothymidine, and of D-xylofuranosylthymine and its tri-*O*-benzoyl derivative.

(27) The α-configuration is assigned to this sugar derivative on the basis of its highly positive specific rotation.

(28) H. G. Fletcher, Jr., THIS JOURNAL, **75**, 2624 (1953), prepared this compound from III.

(29) W. Bergmann and R. J. Feeney, *J. Org. Chem.*, **16**, 981 (1951). The sugar moiety of spongothymidine had not been characterized at that time. It was later shown to be β-D-arabinofuranose by Bergmann and Burke.[25]

The spectral shifts in the high alkaline range are greater for the xylose nucleoside. However, the shifts in this same region manifested by spongothymidine[19] are far greater than either of the two in Fig. 1. It is to be noted that of these three thymine nucleosides, only spongothymidine possesses a *cis* relationship (in the Fischer projection) of the aglycone to the 2-hydroxyl group of the sugar. It would be of interest to know whether equally large spectral shifts in the high alkaline range will also be given by 1-β-D-xylofuranosylthymine[30] where a similar *cis* relationship obtains.

General Considerations.—With regard to the synthesis of thymine nucleosides, a comparison with the Hilbert–Johnson procedure shows the mercuri method to be the simpler and more efficient process. Dithyminylmercury is prepared easily and in quantitative yields from thymine, whereas in the Hilbert procedure a two-step conversion to the dialkoxypyrimidine is required. The condensation reaction of halogenoses with dithyminylmercury does not require an excess of either reactant and a goodly portion of unreacted pyrimidine is easily recovered (either in the form of free thymine or as its metal derivative) which is a matter of importance where the use of isotopically labeled thymine may be contemplated.

The dithyminylmercury reaction with halogenoses is far more rapid, requiring only 0.5 to one hour duration. Aside from considerations of reaction mechanisms, the rapidity of the mercuri reaction with halogenoses is probably of great importance where (a) the purity of the sugar derivatives used is questionable (as in the case of the sirupy poly-*O*-acylglycofuranosyl halides where traces of acid are difficult to remove) and (b) where the stability of these halogenoses at elevated temperatures is likely to be low.

Experimental[31]

Dithyminylmercury.—Thymine (0.10 mole, 12.6 g.) was dissolved in 400 ml. of hot water containing 4.00 g. of sodium hydroxide and the clear solution treated with an alcoholic solution of 13.5 g. (0.05 mole) of mercuric chloride. Precipitation occurred immediately. After cooling and filtration (the neutral filtrate was free from Hg^{++} ion and contained only traces of thymine), the white, amorphous precipitate was washed well with water until the washings were free from halide ion and finally washed with ethanol followed by ether. The dried material weighed 22.0 g. (theory, 22.5) and gave an indefinite decomposition point above 320°. The product did not contain chlorine and analyzed for a dithyminylmercury compound.

Anal. Calcd. for $C_{10}H_{10}N_4O_4Hg$: C, 26.64; H, 2.23; N, 12.43. Found: C, 26.49; H, 2.25; N, 12.49.

Dithyminylmercury dissolved in dilute alkali with the slow formation of a cloudy, white suspension, neutralization of which with acetic acid regenerated dithyminylmercury. Treatment of dithyminylmercury with dilute mineral acid gave thymine.

Several attempts were made to obtain a monochloromercuri derivative of thymine using 1:1:1 proportions of thymine, base and mercuric chloride in varying order of addition. Invariably, difficultly filterable precipitates were formed which, after copious washing with water, contained chlorine in amounts ranging from 5 to 9%, indicating that mixtures were formed. Though these precipitates gave condensates in subsequent reactions, the yields were much lower.

1-(Tetra-*O*-acetyl-β-D-glucopyranosyl)-thymine.—Powdered dithyminylmercury (4.5 g., 0.01 mole) was added to 150 ml. of dry toluene. The vigorously stirred suspension was dried by azeotropic distillation of approximately one-fourth of the solvent. Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (8.2 g., 0.02 mole) was added and the stirred mixture was refluxed. Within 0.5 hour almost all of the suspension had dissolved. Refluxing was continued for an additional one-half hour. The warm, turbid solution was filtered from 0.4 g. of dithyminylmercury and the cooled filtrate was treated with petroleum ether. The precipitate was collected, dissolved in chloroform and filtered from some mercuric bromide. The chloroform solution was washed with 30% aqueous potassium iodide and with water and dried over sodium sulfate. Removal of solvent on a water-bath *in vacuo* left a viscous sirup which was dissolved in 100 ml. of warm methanol and the alcohol removed under vacuum. Methanol addition and removal were repeated three times whereupon crystallization occurred in the concentrated methanolic solution. Cooling and filtration gave 4.7 g. (m.p. 145–148° to a viscous sirup) and an additional 1.2 g. was obtained by concentrating the mother liquor for a total yield of 64% based either on mercury-pyrimidine or upon halogenose. The substance was sufficiently pure at this stage for conversion to glucopyranosylthymine. Recrystallization from methanol gave pure material in the form of its hemihydrate, m.p. 156–158° (cor.), $[\alpha]^{30}D$ −10° (*c* 1.9, $CHCl_3$). The product was somewhat hygroscopic. Ultraviolet light absorption in dilute ethanol showed a maximum at 262.5 mμ, minimum at 232.5 mμ

Anal. Calcd. for $C_{19}H_{24}N_2O_{11}\cdot{}^1/_2H_2O$: C, 49.03; H, 5.41; N, 6.01; H_2O, 1.70. Found: C, 49.21; H, 5.18; N, 5.97; H_2O, 1.70.

1-β-D-Glucopyranosylthymine.—Unrecrystallized 1-(tetra-*O*-acetyl-β-D-glucopyranosyl)-thymine (5.8 g.) was dissolved in 250 ml. of methanol and the solution saturated in the cold with hydrogen chloride. The stoppered flask remained at room temperature for two days after which time the solvents were removed and the residue crystallized from alcohol-ether. The product melted at 269–270° dec. A sample prepared by the Hilbert–Johnson procedure[8,13] melted one degree lower and a mixed melting point of the two gave no depression. The ultraviolet absorption spectra at various *p*H values were identical with those previously reported for glucopyranosylthymine.[19] The yields were nearly quantitative $[\alpha]^{25}D$ +16° (Visser, *et al.*,[13] report +14.6°).

1-(Tri-*O*-acetyl-L-arabinopyranosyl)-thymine.—This derivative was prepared from tri-*O*-acetyl-β-L-arabinopyranosyl chloride and dithyminylmercury using the same molar proportions and procedure as with tetra-*O*-acetylglucopyranosylthymine (see above) with the exception that xylene was used in place of toluene. From 0.01 mole of dithyminylmercury and 0.02 mole of halogenose, 1.68 g. (22%) of product was obtained, m.p. 137–141°. Recrystallization from methanol did not alter the melting point. Light absorption in dilute ethanol, maximum 262.5, minimum 232.5 mμ, $[\alpha]^{31}D$ +28° (*c* 2.5, $CHCl_3$). Elemental analyses agreed with a hemihydrate.

Anal. Calcd. for $C_{16}H_{20}N_2O_9\cdot{}^1/_2H_2O$: C, 48.87; H, 5.38; N, 7.12. Found: C, 48.45; H, 5.36; N, 6.83.

1-L-Arabinopyranosylthymine.—Hydrolysis of the above material with methanolic hydrogen chloride for two days at room temperature yielded crystalline nucleoside, m.p. 248–250° (Visser, *et al.*, report 250–251°[13]) and admixture with a sample prepared by the Hilbert–Johnson procedure[13] showed no depression. The absorption spectrum agreed with the detailed spectrum as a function of *p*H previously reported.[19]

1-D-Xylopyranosylthymine.—The reaction of 0.005 mole of dithyminylmercury with 0.01 mole of tri-*O*-acetyl-D-xylopyranosyl bromide in refluxing xylene gave (after filtration and removal of 0.45 g. of thymine) a sirup, presumably tri-*O*-acetylxylopyranosylthymine, which did not crystallize but which was hydrolyzed with methanolic hydrogen chloride. Xylopyranosylthymine (0.93 g., 36%) was obtained, m.p. 285–286°. Admixture with authentic xylopyranosylthymine prepared by the Hilbert–Johnson procedure[14] gave no depression; light absorption identical with that previously reported,[19] $[\alpha]^{21}D$ +3° (*c* 1.2, H_2O).

1-(Tri-*O*-acetyl-β-D-ribofuranosyl)-thymine.—1-*O*-Acetyl-2,3,5-tri-*O*-benzoyl-D-ribose[21] (0.02 mole) was added to 200

(30) The synthesis of 1-β-D-xylofuranosylthymine from 1-β-D-xylofuranosylthymine is under way in these laboratories.

(31) Melting points were determined on a heated microscope stage and are uncorrected unless otherwise specified. Analyses were performed by Dr. J. F. Alicino.

ml. of anhydrous ether previously saturated with hydrogen chloride at 0°. The stoppered flask was allowed to remain at 4° for five days. The solvent was removed *in vacuo* and to the sirup 50 ml. of anhydrous benzene was added and removed under vacuum three times. The benzene solution (30 ml.) of the halogenose (2,3,5-tri-*O*-benzoyl-D-ribosyl chloride) was added to an azeotropically-dried and stirred suspension of 0.01 mole of dithyminylmercury in hot xylene. After a reflux period of one hour, the hot, turbid solution was filtered from 0.64 g. of crystalline precipitate. (This precipitate was almost pure thymine, m.p. 315–318° undepressed by admixture with authentic material. It crystallized from hot water as tiny platelets and its ultraviolet absorption spectrum as a function of *p*H[32] was identical with that for thymine.) The chilled filtrate was treated with low-boiling petroleum ether and the precipitate separated and taken up in chloroform and treated in a manner similar to that used in the preparation of 1-(tetra-*O*-acetyl-β-D-glucopyranosyl)-thymine (see above). A sirup was obtained which was taken up in 15 ml. of hot ethyl acetate. Low-boiling petroleum ether was added to incipient cloudiness and cooled. An oil separated which slowly crystallized overnight. The filtered precipitate was washed with ether (the ether not only removes the color but also removes any unreacted 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribose) giving 4.7 g. of product, m.p. 162.5–164°. An additional 1.0 g. was recovered from the mother liquor (yield, 50% based upon dithyminylmercury or halogenose or 66% based upon thymine recovered). Recrystallization from ethyl acetate gave pure material, m.p. 167–168° (cor.); light absorption in dilute alcohol, maximum 265 mμ, minimum 255 mμ, $[\alpha]^{31}D$, −83° (*c* 2, $CHCl_3$).

Anal. Calcd. for $C_{31}H_{26}N_2O_9$: C, 65.27; H, 4.59; N, 4.91. Found: C, 65.35; H, 4.58; N, 4.90.

2,3,5-Tri-*O*-benzoyl-D-ribosyl bromide may be used instead of the corresponding chloro derivative for the preparation of this intermediate, but in lower yields. Twenty grams of hydrogen bromide was introduced into a flask containing 0.02 mole of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribose in 250 ml. of anhydrous ether. The clear solution remained at room temperature for two hours after which time the solvent was removed *in vacuo*. The procedure given above for the chloro analog was then followed. Yield of tri-*O*-benzoylated nucleoside was 36%.

1-β-D-**Ribofuranosylthymine**.—Two grams of 1-(tri-*O*-benzoyl-β-D-ribofuranosyl)-thymine and 70 ml. of ethanolic ammonia (saturated at 0°) were heated overnight in a sealed tube at 100°. The tube was opened and the solution concentrated *in vacuo* to a sirup to which water was added and ethyl benzoate removed by steam distillation under vacuum. The residue was taken up in water and extracted several times with ether to remove benzamide. The water layer was taken down to dryness and ethanol added and removed several times under vacuum until crystallization occurred in the concentrated alcoholic solution. Filtration gave 1-β-D-ribofuranosylthymine in essentially quantitative yields; m.p. after one recrystallization from ethanol, 183–185° (cor.), $[\alpha]^{31}D$ −10° (*c* 2, H_2O).

Anal. Calcd. for $C_{10}H_{14}N_2O_6$: C, 46.50; H, 5.46; N, 10.85. Found: C, 46.46; H, 5.32; N, 10.57.

1-*O*-**Acetyl-2,3,5-tri-*O*-benzoyl-α-D-xylose** (I).—Sirupy 2,3,5-tri-*O*-benzoyl-D-xylose[28] (34.8 g., 0.075 mole) was added to a solution of 100 ml. of anhydrous pyridine in an equal volume of methylene dichloride and cooled to *ca.* 15°. Acetic anhydride (27 g., 0.26 mole) was added and the stoppered flask remained at room temperature for two days. Concentration of the solution *in vacuo* on a water-bath yielded a fluid sirup which was poured into a mixture of 175 g. of ice-water and 75 ml. of chloroform. The organic layer was separated and washed with aqueous bicarbonate and with water. After drying over sodium sulfate, the solvent was removed *in vacuo* leaving a heavy sirup which was dissolved in a hot solution of ethanol–ethyl acetate (5:2), treated with Norite, filtered, and, after several days, rosettes of tiny white needles formed in the solution, yield 12.6 g. (33%), m.p. 126–128°. One recrystallization from ethanol–ethyl acetate (10:1) gave feathery crystals, m.p. 127–128.5°, $[\alpha]^{31}D$ +147° (*c* 1, $CHCl_3$).

Anal. Calcd. for $C_{28}H_{24}O_9$: C, 66.66; H, 4.80. Found: C, 66.36; H, 4.80.

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

The mother liquors yielded a sirup, probably rich in the β-anomer, which resisted all attempts at crystallization, $[\alpha]^{29}D$ +42° (*c* 1, $CHCl_3$). Both the crystalline material and the residual sirup gave product in condensation reactions (see below).

1-(**Tri-*O*-benzoyl-β-D-xylofuranosyl**)-**thymine** (II).—A mixture of 5.0 g. of I in 200 ml. of anhydrous ether was saturated with hydrogen chloride at 0°. After four days at 5°, the solvent was removed leaving an amber sirup to which anhydrous benzene was added and removed *in vacuo* several times. The benzene solution (30 ml.) of the halogenose (presumably 2,3,5-tri-*O*-benzoyl-D-xylosyl chloride) was then added to a previously dried, stirred mixture of 2.25 g. of dithyminylmercury in hot xylene. After 30 minutes of refluxing with stirring, the reaction mixture was filtered from 0.7 g. of thymine and the filtrate treated with petroleum ether. The semi-solid precipitate was taken up in chloroform and washed with aqueous potassium iodide and water as in the preparation of the corresponding ribose analog. Five grams of a yellow sirup was obtained which was taken up in a minimum of warm ethyl acetate. Petroleum ether was added to incipient turbidity. Upon cooling, a sirup formed which slowly crystallized to give 1.8 g. of crude II, m.p. 185–190°. Recrystallization from ethyl acetate yielded pure material, 1.6 g. (28%), m.p. 197.5–198.5° (cor.), $[\alpha]^{31}D$ +58° (*c* 2, $CHCl_3$). The ultraviolet absorption spectrum was similar to that for the 2,3,5-tri-*O*-benzoyl derivative of ribofuranosylthymine.

Anal. Calcd. for $C_{31}H_{26}N_2O_9$: C, 65.27; H, 4.59; N, 4.91. Found: C, 65.35; H, 4.85; N, 4.95.

2,3,5-Tri-O-benzoyl-D-xylosyl Bromide.—It was found that II may be prepared in better yields *via* the bromo halogenose directly from the more easily accessible tetra-*O*-benzoyl-D-xylofuranose. 1,2,3,5-Tetra-*O*-benzoyl-α-D-xylose[28] (0.02 mole) was taken up into 200 ml. of dry methylene dichloride and the solution saturated with hydrogen bromide at 0°. The tightly stoppered flask remained at room temperature for 20 hours during which time the color turned to a light amber. After pouring in a thin stream into ice-water with vigorous stirring, the organic layer was removed and washed rapidly with *ice-cold* bicarbonate to remove the acids and finally with ice-water. The dried solution was concentrated down *in vacuo* to a sirup and treated twice with anhydrous benzene in a manner previously described. Treatment of the benzene solution of this halogenose (2,3,5-tri-*O*-benzoyl-D-xylosyl bromide) with dithyminylmercury (0.01 mole) for one hour in refluxing xylene gave 1.5 g. of dithyminylmercury (recovered) and 4.1 g. of product II, m.p. 196–198°, 36% based upon starting materials, or 54% based upon dithyminylmercury recovered.

1-β-D-**Xylofuranosylthymine** (IV).—The conversion of II to xylofuranosylthymine was accomplished using the same procedure as with the ribose analog. Product crystallized out after several days in the form of clusters of prisms from a concentrated solution of absolute ethanol, m.p. 156–157.5°, $[\alpha]^{31}D$ −2° (*c* 2, H_2O).

Anal. Calcd. for $C_{10}H_{14}N_2O_6$: C, 46.51; H, 5.46; N, 10.86. Found: C, 46.37; H, 5.31; N, 10.93.

Spectrophotometric Studies.—Measurements were made with a Beckman spectrophotometer, model DU, using techniques and buffers previously described.[33] Glycine buffers were employed between *p*H values 8.80 and 10.41. The apparent pK_a values were determined spectrally by procedures previously employed.[32,34]

Polarimetric Investigations.—Optical rotations were determined with a Polarimetric unit model D attachment[35] to the Beckman model DU spectrophotometer calibrated with standard sucrose solutions. For the determination of the rotations of the dialdehydes, solutions of nucleosides of known concentrations were treated in the polarimetric cell with excess sodium metaperiodate. Readings were taken at frequent intervals until a constancy of values was reached. The specific rotation of the dialdehydes produced were based upon the original concentrations of the nucleoside solutions.

(33) J. J. Fox, L. F. Cavalieri and N. Chang, THIS JOURNAL, **75**, 4315 (1953).

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

(35) Standard Polarimeter Co., New York, N. Y. See A. S. Keston, Abstr. 125th Meeting Am. Chem. Soc., Cincinnati, Ohio, p. 18C (1955).

TABLE II

METAPERIODATE OXIDATION DATA

	Moles IO_4^- consumed per mole of compound							
	Within 3 minutes	1 hr.	4 hr.	8 hr.	24 hr.	48 hr.	72 hr.	96 hr.
Uridine	0.92	1.00	1.00	..	1.05	..	..	..
Xylopyranosylthymine	..	0.15	..	0.73	1.58	2.10	2.20	..
Xylofuranosylthymine	.02	0.08	0.14	..	0.58	0.79	1.01	1.05
"Ribofuranosylthymine" A	.90	..	1.03	..	1.03	..	..	..
Ribofuranosylthymine B	..	1.05	..	1.05	1.08	..	..	..
Ribofuranosylthymine C	.80	0.96	..	..	1.01	..	..	..
Glucopyranosylthymine	..	.29	..	..	2.02	2.06	..	..
Thymidine	..	.00	..	..	..	..	..	0.04

Metaperiodate Oxidation Studies.—Concentrations of nucleosides ranging between 0.001 to 0.002 mM/ml. were treated with excess metaperiodate and aliquots titrated iodometrically according to the usual procedures.[36,37] The acidity produced was determined according to Jackson and Hudson.[38] The results are listed in Table II. The extent of the oxidation within three minutes is noteworthy, and would seem to correlate with the presence of *cis*-hydroxyls.

(36) E. L. Jackson and C. S. Hudson, THIS JOURNAL, **59**, 994 (1937).
(37) B. Lythgoe and A. R. Todd, *J. Chem. Soc.*, 592 (1944).
(38) E. L. Jackson and C. S. Hudson, THIS JOURNAL, **61**, 1530 (1939).

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The Molecular Weights and Dimensions of Some Human Serum Lipoproteins

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Three fractions of low density human serum lipoproteins have been examined by means of light scattering and velocity ultracentrifugation. The molecular weights at *p*H 6.7 were 2.77×10^6, 2.80×10^6 and 3.08×10^6 for fractions of flotation constants 5.9, 6.4 and 8.1 svedbergs respectively. One of the fractions was also studied over the *p*H range 3–9.6 and showed little or no change in molecular weight. The densities of the salt solutions in which two of the fractions have zero sedimentation velocity were determined and the molecular volumes derived therefrom correlated with the molecular weight and dissymmetry data from light scattering. The available data are most satisfactorily fitted by assuming ellipsoidal molecules of small axial ratios. A spherical model provides a less satisfactory fit while rod-like or coil-like molecules are ruled out.

Introduction

In 1949 Gofman, Lindgren and Elliot[4] showed that the boundary anomaly which McFarlane[5] and Pedersen[6] had observed in the ultracentrifugation of human serum could be interpreted as a piling up of lipoproteins at the albumin boundary. Later, Gofman and his associates[7] gave methods for isolating fractions of low density lipoproteins (density less than d^{26}_4 1.063) by centrifugal techniques and used ultracentrifugal data to estimate the molecular weights of these fractions.

In view of the significant statistical correlations between the concentrations of certain low density serum lipoproteins and the clinical manifestations of coronary disease,[8,9] we thought it worthwhile to characterize some of these fractions more precisely than has been done previously. To this end we have applied the techniques of light scattering to determine the molecular weights and dissymmetries of the scattering envelopes of three fractions of human serum lipoproteins and compared these data with ultracentrifugal data on the same fractions.

Materials.—The lipoprotein fractions were isolated by a method described by Lindgren, Elliot and Gofman.[7] Plasma from blood which had been discarded by a blood bank because of positive serology was centrifuged in 9-ml. tubes in a Spinco preparative centrifuge for 24 hr. at 80,000*g*. A layer of material which contained most of the lipoproteins with flotation coefficients[10] between S_f 4 and S_f 10 was pipetted out. Enough solution from the bottoms of the tubes was added to this to give a resulting solution whose concen-

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(2) Supported in part by the United States Atomic Energy Commission.
(3) Supported in part by the Office of Naval Research, Contract NR 121-175.
(4) J. W. Gofman, F. T. Lindgren and H. Elliot, *J. Biol. Chem.*, **179**, 973 (1949).
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(6) K. O. Pedersen, *J. Phys. Colloid Chem.*, **51**, 156 (1947).
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(9) H. B. Jones, J. W. Gofman, F. T. Lindgren, T. P. Lyon, D. Graham, B. Strisower and A. V. Nichols, *Am. J. Med.*, **11**, 358 (1951).
(10) Following Gofman, we use the term flotation coefficient to denote the negative of the sedimentation coefficient in Svedberg units of species undergoing centripetal migration in a sodium chloride solution of density 1.0630 g./ml. at 26°. The symbols S_f and S_f° will be used to denote the observed flotation coefficient and the flotation coefficient corrected to infinite dilution, respectively. Centrifuge data for all other media will be expressed as sedimentation coefficients.