ment of the amino acids in the polypeptide chains. Because the ring system imposes such restrictions on proline, this amino acid will have a profound effect on the direction of the polypeptide chain whenever a prolyl residue is present in the sequence. Indeed, it is unlikely that the polypeptide chains in feather keratin would assume any of the pleated sheet or simple helical configurations[28,29] which have been suggested thus far. Is there any evidence from the present data or from other data that the amino acids adjacent to proline are restricted in any way? In the present investigation, 15 prolyl peptides have been identified. Of those peptides of determined sequence, gly-pro, and thr-pro-(i)leu are the most predominant. One may conclude tentatively that, in a sequence such as -X-pro-, X tends to be an amino acid with a relatively short side chain, for example, glycine, serine and threonine and that amino acids with longer side chains such as glutamic acid are less likely to be found in this sequence. This suggestion receives some support from the literature. Thus, in gelatin[12] and collagen,[30] -gly-pro- is an important sequence and -ala-pro- and -ser-pro- have been found. Likewise, in a melanophore-stimulating peptide, the sequences -gly-pro- and -ser-pro-pro- are present.[31,32] By comparison, a much different situation obtains in the corticotropins[33,34] which contain the sequences -lys-pro-val-, -arg-pro-val-, -tyr-pro-asp-, and -phe-pro-leu-. If, indeed, the proline residues are important in determining the spatial configuration of the polypeptide chains, it may be suggested that the adjacent residues in conjunction with the proline residues assist in maintaining certain specific arrangements which, in turn, are responsible for some of the characteristic properties of the proteins themselves.

Acknowledgments.—This investigation was supported in part by a contract between the Quartermaster Corps., U. S. Army, and the California Institute of Technology.

(28) L. Pauling, R. B. Corey and H. R. Branson, *Proc. Nat. Acad Sci.*, **37**, 205 (1951).
(29) L. Pauling and R. B. Corey, *ibid.*, **37**, 251 (1951).
(30) T. D. Kroner, W. Tabroff and J. J. McGarr, THIS JOURNAL, **77**, 3356 (1955).
(31) J. I. Harris and P. Roos, *Nature*, **178**, 90 (1956).
(32) I. I. Geschwind, C. H. Li and L. Barnafi, THIS JOURNAL, **78**, 4494 (1956).
(33) P. Bell, *ibid.*, **76**, 5565 (1954).
(34) W. F. White and W. A. Landmann, *ibid.*, **77**, 1711 (1955).

PASADENA, CALIFORNIA

[CONTRIBUTION FROM THE LABORATORIES OF THE SLOAN-KETTERING DIVISION OF CORNELL UNIVERSITY MEDICAL COLLEGE]

Pyrimidine Nucleosides. II. The Synthesis of 1-β-D-Arabinofuranosylthymine ("Spongothymidine")[1]

BY JACK J. FOX, NAISHUN YUNG AND AARON BENDICH

RECEIVED DECEMBER 19, 1956

1-D-Ribofuranosylthymine (I) was converted by a series of reactions to 1-β-D-arabinofuranosylthymine. Since this epimerization can occur only with nucleosides containing the β-D-glycosyl structure, the configuration at the glycosyl center of I and of other thymine nucleosides was firmly established as beta. 1-β-D-Arabinofuranosylthymine was shown to be identical with the naturally occurring nucleoside, "spongothymidine."

In Part I of this series[2] a method was described for the facile synthesis of thymine nucleosides by the condensation of poly-O-acylglycosyl halides with dithyminylmercury followed by removal of the protecting acetyl or benzoyl groups. In this manner 1-D-ribofuranosylthymine (I) and 1-D-xylofuranosylthymine (II) were prepared. The former was identical with a product prepared enzymically by Lampen[3] and also was shown to be similar to a compound isolated recently by Fink and co-workers[4] from rat liver slices incubated with radiothymine.

Spectral and metaperiodate-oxidation studies[2] with I and II showed that the sugar–pyrimidine linkage was at position 1 of thymine and, further, that the lactol ring structures were of the furanose type. It was demonstrated that I, II and 1-D-glucopyranosylthymine gave the same dialdehyde upon oxidation with metaperiodate. On the basis of the reasonable assumption that 1-D-glucopyranosylthymine (III) is a β-nucleoside,[5] it was tentatively concluded that I and II are nucleosides of the β-configuration.

An unequivocal determination of the structure at the glycosyl center of I would serve not only for the assignment of configuration to the biochemically produced materials of Lampen[3] and Fink,[4] but it would also help to provide a basis for future generalizations about the structure of nucleosides obtained by this new synthetic procedure with other pyrimidines. In this paper, a proof is given for the structure of 1-D-ribofuranosylthymine. As a corollary to this proof, a first synthesis and unequivocal proof of structure of "spongothymidine" are presented. A preliminary report on this study has appeared.[6]

A rigorous method for the determination of the

(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 C-471), 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. O. Lampen, in W. D. McElroy and B. Glass, "Phosphorus Metabolism," Vol. II, The Johns Hopkins Press, Baltimore, Md., 1952, p. 368.
(4) K. Fink, R. E. Cline, R. B. Henderson and R. M. Fink, *J. Biol. Chem.*, **221**, 425 (1956).
(5) See discussion in footnote 12 of Part I of this series.
(6) J. J. Fox and N. Yung, *Federation Proc.*, **15**, 254 (1956).

glycosylic configuration of certain nucleosides has been reported[7] which involves the formation of *cyclo*-nucleosides by intramolecular alkylation when 5′-tosyl derivatives of adenosine or cytidine are heated in inert solvents. An examination of molecular models shows that, for the formation of these *cyclo*-nucleosides to occur, the configuration at the glycosyl center must be beta. Michelson and Todd[8] have prepared $O^2,3'$-*cyclo*thymidine and converted it to a 2′-deoxynucleoside (almost certainly the "deoxyxylosyl" analog) indicating that for the formation of this *cyclo*-nucleoside Walden inversion occurred at $C^{3'}$.

An examination of molecular models shows that if, indeed, ribofuranosylthymine is a β-D-nucleoside, then the carbonyl oxygen atom at C^2 of the pyrimidine is favorably located for a rearward, nucleophilic attack upon either C^2, C^3 or C^5 of the sugar moiety. It should be possible, therefore, to isomerize I at $C^{2'}$ or at $C^{3'}$ *via* a *cyclo*-nucleoside intermediate starting from the appropriate sulfonic ester derivative of I.

1-D-Ribofuranosylthymine was converted into its 5′-trityl derivative IV by reaction with trityl chloride in pyridine.[9] Treatment of IV with methanesulfonyl chloride gave a sirup which resisted attempts at crystallization. This sirup should contain a 2′-O-mesyl derivative (V) and/or a 3′-O-mesyl isomer (VI). A 2′,3′-di-O-mesyl derivative also may be postulated as one of the reaction products along with some unreacted IV.

It should be noted that with structures V and VI (see Fig. 1) the vicinal hydroxyl functions are *cis* and the 5′-position is blocked by a trityl group so that elimination of the mesyloxy group with inversion at $C^{2'}$ or at $C^{3'}$ by a rearward attack by a sugar hydroxyl group is excluded. Treatment of this sirup with ammonia in aqueous methanol should effect *cyclo*-nucleoside formation with Walden inversion as a result of nucleophilic attack of O^2 of the pyrimidine upon either $C^{2'}$ (in the case of V) or $C^{3'}$ (from VI) to give VII or VIII, respectively (or both). Subsequent hydrolysis of the anhydro nucleoside(s) with acid (*cyclo*-ring opening) should also result in detritylation and give rise either to 1-β-D-arabinofuranosylthymine (IX) from VII or of 1-β-*D*-xylofuranosylthymine (X) from VIII (or both).[10] The latter nucleoside should be identical with II previously prepared directly *via* the mercuri procedure[2]; whereas IX would be 1-β-D-arabinofuranosylthymine, a new synthetic aldopentofuranosylthymine. It is clear from the foregoing discussion that (aside possibly from the recovery of starting material I) the only nucleosides which will be formed from this reaction sequence will possess a *trans* relationship between the 2′- and 3′-hydroxyl groups. Thus 1-β-D-lyxofuranosylthymine[11] cannot arise as a product of these reactions.

The experimental facts are that a product was isolated in crystalline form which was neither I nor X (II). The elemental analyses agreed with that for a pentosylthymine. This product was cleaved slowly by metaperiodate[12] with an uptake of one mole of oxidant per mole and without the liberation of formic acid in accord with a pentofuranosyl structure. The specific rotation of the dialdehyde solution resulting from this oxidation was identical with that obtained by similar treatment of I, II and III[2] demonstrating quite conclusively that the configuration at the glycosyl centers of all four compounds was similar (see Fig. 2).

On the basis of the non-identity of this nucleoside with I or X (or II), and since a lyxofuranosylthymine cannot arise from this synthesis, it is therefore obvious that the product obtained from these reactions is 1-β-D-arabinofuranosylthymine (IX). Consequently, the β-formulation must also be assigned to the glycosyl centers of I, II and III.

By virtue of the epimerization of I to IX, it is reasonable to assume that structures V and VII (not isolated) were intermediates in the synthesis of IX. Indeed, Brown and co-workers[13] have very recently reported the conversion of uridine to 1-β-D-arabinofuranosyluracil and have isolated a *cyclo*-uridine intermediate in crystalline form from a 2′-*O*-tosyl derivative of 5′-O-acetyluridine.

Bergmann and Feeney[14] have isolated a pentofuranosylthymine from certain Caribbean sponges. Spectral studies[15] as well as optical rotational data[14] indicated strongly that the sugar moiety was not D-ribose. On the basis of chemical and physicochemical studies, Bergmann and Burke[16] have assigned the 1-β-D-arabinofuranosyl structure to this thymine nucleoside.

We have compared IX with a sample of spongothymidine (kindly furnished by Drs. Bergmann and Burke) with regard to melting points, mixed melting points, optical rotation and detailed spectrophotometric behavior as a function of *p*H (including the high alkaline range[15]) and find them to be

(7) 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).

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

(9) IV gave a positive spray test for vicinal hydroxyls (J. G. Buchanan, C. A. Dekker and A. G. Long, *ibid.*, 3162 (1950)) thus confirming that tritylation had occurred at the 5′-position.

(10) This statement rests on the assumption that opening of the *cyclo*-nucleoside involves cleavage of the linkage between the oxygen atom of the *cyclo*-bridge and C^2 of the pyrimidine ring. Cleavage could occur also at the glycosyl linkage (≥C—N<) which, as pointed out by Michelson, *et al.*,[8] would give rise to glycosides. The latter type of compound would be unstable to the acidic conditions of the hydrolysis, and, indeed, in one of our experiments small amounts of thymine were isolated.

If a 2′,3′-di-O-mesyl derivative of IV had been formed and converted into a 3′- or 2′-mesylated derivative of VII or VIII, respectively it may be postulated that in the course of these reactions a second rearward attack could occur with the formation of a 2′,3′-epoxide. The argument for the formation of IX or X is not altered since only *trans* isomers of I could arise from epoxide-ring opening.

(11) Reference 30 in Part I of this series[2] contains these typographical errors; it should refer to 1-β-D-lyxofuranosylthymine instead of 1-β-D-xylofuranosylthymine. Reference 30 itself should read: "The synthesis of 1-β-D-lyxofuranosylthymine from 1-β-D-xylofuranosylthymine is underway in these laboratories."

(12) The slow uptake of metaperiodate is consistent with the presence of a *trans*-glycol system (C. C. Price and M. Knell, THIS JOURNAL, **64**, 552 (1942)). II and III also consume metaperiodate slowly, whereas with I the uptake is completed within a few minutes.[2]

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

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

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

(16) W. Bergmann and D. F. Burke, *Angew. Chem.*, **67**, 127 (1955); *J. Org. Chem.*, **20**, 1501 (1955).

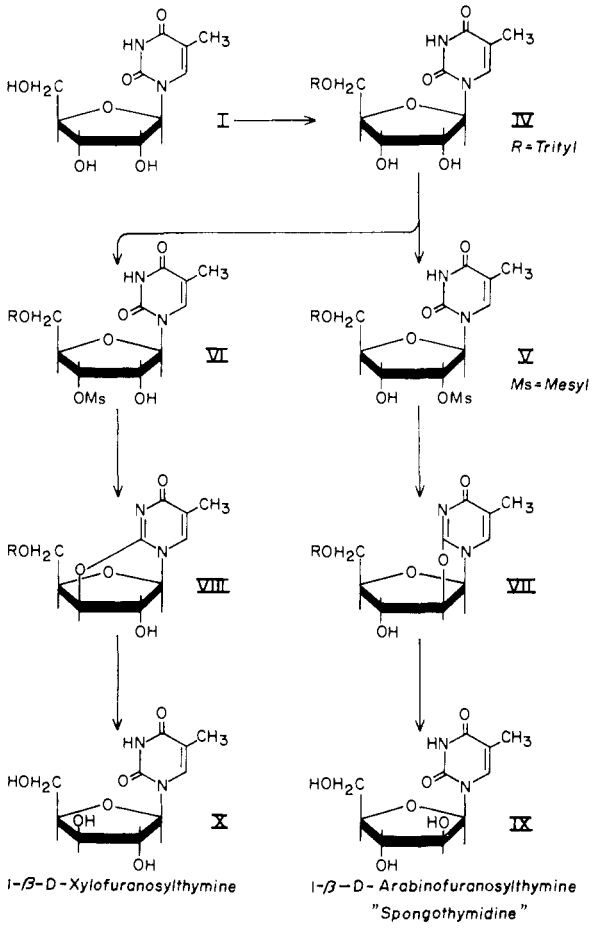

Fig. 1.

identical. Thus the epimerization sequence of I → IX constitutes a synthesis and, concomitantly, an independent proof of structure of this naturally occurring sponge nucleoside.

Acknowledgment.—The authors are indebted to Dr. George Bosworth Brown for helpful discussions and continued interest.

Experimental[17]

1-(5′-Trityl-β-D-ribofuranosyl)-thymine (IV).—The procedure of Levene and Tipson[18] for the tritylation of uridine was employed with modifications. A solution containing 6.0 g. (0.023 mole) of 1-β-D-ribofuranosylthymine (I) in 100 ml. of anhydrous pyridine was treated with 7.0 g. (0.025 mole) of triphenylchloromethane. After one day at 10°, the reaction mixture was warmed on a steam-bath for 2 hr., cooled and poured into ice-water. Upon vigorous stirring, a yellow, viscous sirup formed. The water was decanted and the sirup taken up in warm acetone. After filtration from a small residue, the filtrate was concentrated to a solid *in vacuo*. This residue was heated with 500 ml. of boiling water for 30 minutes, filtered and the precipitate taken up in hot ethanol from which it crystallized as knobby clusters. The precipitate was filtered, washed with benzene and dried (6.2 g.), m.p. 153–160°. Elemental analyses indicated a contamination of this product with approximately 20% of triphenylcarbinol. Purification was accomplished by two recrystallizations from benzene–alcohol followed, finally, by recrystallization from absolute alcohol to give needles, m.p. 155° (to a viscous sirup which clears at 165°). Triphenylcarbinol was isolated from the mother liquors.

Anal. Calcd. for $C_{29}H_{28}N_2O_6$: C, 69.60; H, 5.60; N, 5.60. Found: C, 69.46; H, 5.74; N, 5.52.

Conversion of IV to 1-β-D-Arabinofuranosylthymine (Spongothymidine).—Unrecrystallized IV (2.0 g.) in 35 ml. of cold, anhydrous pyridine was treated with methanesulfonyl chloride (0.8 ml.) and kept at 10° for 24 hr. One milliliter

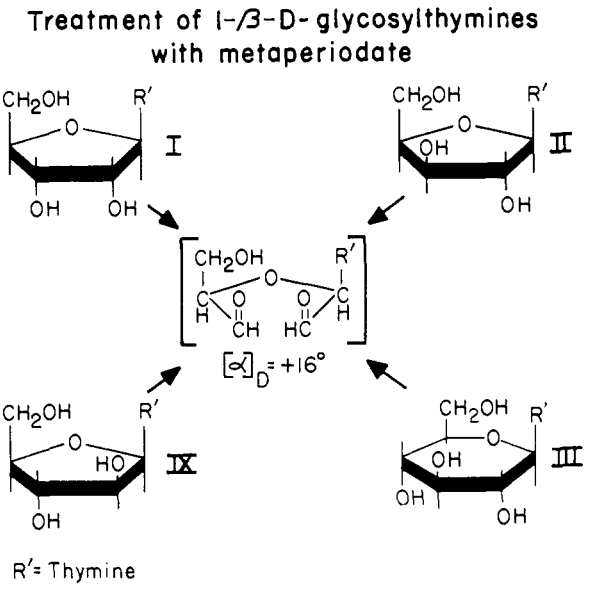

Fig. 2.

of water was then added to the cold mixture, and after 1 hr. the reaction mixture was poured into stirred ice-water. A precipitate formed which was washed well with water and dried.[19] This solid was added to 50 ml. of 95% methanol saturated with ammonia and let stand overnight at room temperature.

The solution was concentrated under vacuum, treated with water and acidified with sulfuric acid to *p*H 1 and refluxed for 2 hr. Upon cooling, triphenylcarbinol was removed by filtration and the filtrate made alkaline with barium hydroxide. The precipitated barium sulfate was removed and the filtrate treated with carbon dioxide (to *p*H 5). After filtration from barium salts, the filtrate was concentrated to approximately 10 ml. whereupon more inorganic salts precipitated. The filtrate obtained by separation from inorganic material was concentrated to 3 ml. Upon cooling, the nucleoside IX precipitated as clusters of needles, 0.15 g., m.p. 238–242° (undepressed by admixture with authentic spongothymidine). An additional 0.06 g. was obtained from the mother liquor. Recrystallization from 25% ethanol did not alter the melting point.

Anal. Calcd. for $C_{10}H_{14}N_2O_6$: C, 46.50; H, 5.46; N, 10.85. Found: C, 46.54; H, 5.62; N, 10.80.

The ultraviolet absorption spectrum of this product in aqueous solutions of various *p*H values (including the high alkaline range[16,20] was identical to that previously reported for spongothymidine.[15] As with spongothymidine,[14] IX consumed one mole of metaperiodate per mole without the liberation of formic acid. When IX was treated with metaperiodate in the same manner as were I, II and III,[2] a similar rotation ([α]D +16°) was obtained for the dialdehyde solution produced (see Fig. 2). The optical rotational prop-

(17) All melting points are uncorrected. Analyses performed by Dr. J. F. Alicino, Metuchen, N. J.

(18) P. A. Levene and R. S. Tipson, *J. Biol. Chem.*, **104**, 385 (1934); **105**, 419 (1934).

(19) This precipitate melts at approximately 100° and gives a low sulfur analysis (theory, 5.54: found, 1.48) indicating, at best, only 25% of mesylated product. A larger excess of methanesulfonyl chloride does not seem to improve the sulfur content of the crude product.

(20) The spectrum of spongothymidine in the high alkaline range (*p*H 12–14) shows large shifts,[16] far greater than those noted for I or II.[2]

erties of the natural and the synthetic nucleoside were also similar[21]:

	$[\alpha]_{5890}$ Å.	$[\alpha]_{5460}$ Å.
Spongothymidine	+94°[22]	+111°
1-β-D-Arabinofuranosylthymine	+93°	+110°

(21) As in Part I of this series, optical rotations were determined with a polarimetric unit model D attachment to a Beckman model DU spectrophotometer. Concentrations were 0.5 g./100 ml. of aqueous solution, temperature 24°.

(22) Bergmann and Feeney[14] give $[\alpha]_D$ +80° (in 8% sodium hydroxide) and $[\alpha]_D$ +92° (in pyridine).

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF VIRGINIA]

Analogs of Nucleotides. II. Phosphonate Esters of Ribose and Glucopyranosyl Purine Derivatives[1]

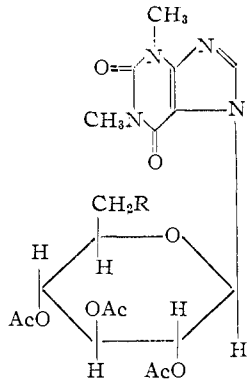
BY JEKISHAN R. PARIKH, MANFRED E. WOLFF AND ALFRED BURGER

RECEIVED NOVEMBER 29, 1956

Syntheses of 1,2,3-tri-*O*-acetyl-D-ribofuranose-5-deoxy-5-(diethyl phosphonate), 7-[2,3,4-tri-*O*-acetyl-6-deoxy-6-(diethyl phosphonate)-β-D-glucopyranosyl]-theophylline, 6-benzamido-9-[2,3,4-tri-*O*-acetyl-6-deoxy-6-(diethyl phosphonate)-β-D-glucopyranosyl]-purine and related compounds are described.

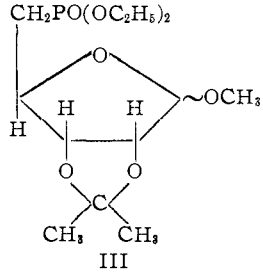
In continuation of studies of phosphonate analogs of nucleotides[2] and of carbohydrate phosphonates[3] as antimetabolites for biological screening against neoplasms and enzyme systems requiring the corresponding metabolites, the synthesis of phosphonate esters of substituted glycosidyl purines has been investigated.

7-(2,3,4-Tri-*O*-acetyl-6-deoxy-6-bromo-D-glucopyranosyl)-theophylline (I) had already been synthesized by Emil Fischer from silver theophylline and 2,3,4-tri-*O*-acetyl-6-deoxy-6-bromo-D-glucopyranosyl bromide.[4] It has now been found to undergo a Michaelis–Arbuzov reaction with triethyl phosphite, 7-[2,3,4-tri-*O*-acetyl-6-deoxy-6-(diethyl phosphonate)-β-D-glucopyranosyl]-theophylline (II) being formed in 40% yield. In attempts to introduce a 2,3-di-*O*-acetyl-5-deoxy-5-(diethyl phosphonate)-D-ribofuranosyl group into the 7-position of theophylline

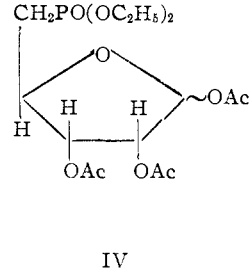


I, R = Br
II, R = $PO(OC_2H_5)_2$

methyl 2,3-isopropylidene-5-deoxy-5-iodo-D-ribofuranoside[5] was heated with triethyl phosphite, and the resulting methyl 2,3-isopropylidene-5-deoxy-5-(diethyl phosphonate)-D-ribofuranoside (III) was hydrolyzed and acetylated to 1,2,3-tri-*O*-acetyl-D-ribofuranose-5-deoxy-5-(diethyl phosphonate) (IV).

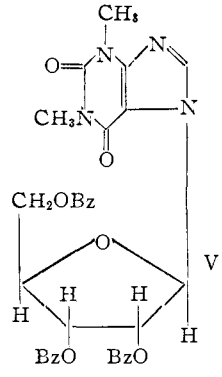


III



IV

The amorphous 1-chloro derivative obtained by treating IV with ethereal hydrogen chloride at 0° could not be condensed with either silver theophylline, or with 6-chloropurine mercuri chloride, although the reactivity of the 1-chlorine atom in a highly substituted ribose derivative was demonstrated by condensing 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride[6] with silver theophylline to yield 7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-theophylline (V).



V

Following the model experiment leading to II,

(1) This work was supported by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.

(2) J. R. Parikh and A. Burger, THIS JOURNAL, **77**, 2386 (1955).

(3) B. S. Griffin and A. Burger, *ibid.*, **78**, 2336 (1956).

(4) E. Fischer, B. Helferich and P. Ostmann, *Ber.*, **53**, 873 (1920). The body of evidence in the literature suggests that the products from the condensation of purine metal adducts with 1-halogenoglucose and -ribose derivatives assume the β-configuration. It is likely, therefore, that I as well as other glycosidyl purines so designated in this article are β.

(5) P. A. Levene and E. T. Stiller, *J. Biol. Chem.*, **104**, 299 (1934).

(6) H. M. Kissman, C. Pidaks and B. R. Baker, THIS JOURNAL, **77**, 18 (1955).