and 11% cyclohexene, respectively) and *cis*- and *trans*-3,4-dihydroxytetrahydrofuran (yielding 58 and 47% of dihydrofuran, respectively) bear this out. Mono ethers and esters of 1,2-glycols, chlorohydrins and amino-alcohols might be amenable to a similar "dehydroxylation" reaction.

Acknowledgment.—H. W. gratefully acknowledges a Fullbright Research Fellowship at the University of Leiden, as well as stimulating discussions with Prof. Dr. E. C. Kooyman.

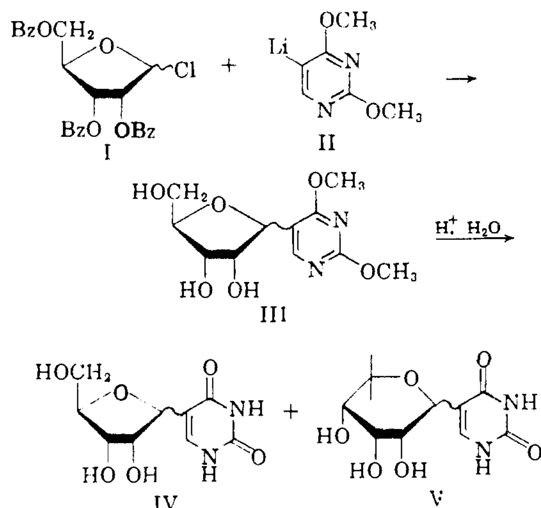
DEPARTMENT OF ORGANIC CHEMISTRY
THE UNIVERSITY
GRONINGEN, HOLLAND
HANS WYNBERG
A. KRAAK

RECEIVED JULY 24, 1961

SYNTHESIS OF PSEUDOURIDINE

Sir:

Pseudouridine, a naturally occurring nucleoside,[1] is particularly interesting because of its unique structure (IV). We wish to report the first chemical synthesis of pseudouridine and to confirm the 5-D-ribofuranosyluracil structure recently assigned to it.[2]



n-Butyllithium[3] (18 mmoles) was added dropwise to 18 mmoles of 2,4-dimethoxy-5-bromopyrimidine in 50 ml. of tetrahydrofuran under rigorously anhydrous conditions at −75°. The resulting clear, yellow to reddish-brown solution of 2,4-dimethoxypyrimidine-5-lithium[4] (II) was allowed to stand for 5 minutes and then 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl chloride (I),[5] (prepared from 2 mmoles of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribose)[5] was added slowly (10 minutes) with stirring. The reaction mixture was stirred for 1 hour at −75° and then allowed to warm to room temperature overnight.

Water and ether were added to the solution and the two phases were separated. The aqueous layer was reextracted with ether and then neutralized with Amberlite IRC-50-H^+ ion exchange resin. After removal of the resin by filtration, the filtrate (4,800 optical density units at 260 mμ and pH 6.5, 1 cm. light path) was concentrated and poured onto a column of Dowex-1-OH^- (200-400 mesh, 8% cross-linked, 4 × 16 cm.) and fractionated by elution with water. The first fraction (lithium hydroxide and a small, but variable, amount of ultraviolet-absorbing material) was discarded. Continued elution with water gave a single ultraviolet-absorbing peak. Evaporation of this solution gave a colorless gum which was assumed to be a mixture of α- and β-2,4-dimethoxy-5-D-ribofuranosylpyrimidine (III, 550 optical density units, λ_{max} 261.5 mμ, λ_{min} 240, OD_{max}/OD_{min} 2.3, R_f 0.75 in n-butyl alcohol-acetic acid-water (4:1:5),[6] positive periodate test for vicinal hydroxyl groups).[7] No further ultraviolet-absorbing material was eluted with water.

III (750 optical density units) was taken up in 20% (v./v.) dichloroacetic acid and hydrolyzed for 4 hours at 100° to remove the blocking groups. The residue was dissolved in 0.02 M H_3BO_3 adjusted to pH 10 with NH_4OH and poured onto a Dowex-1-HCO_3^- column (200–400 mesh, 8% cross-linked, 1 × 8 cm.). Linear gradient elution, modified from the procedure of Cohn,[2b] with 0.02 M H_3BO_3 (adjusted to pH 9 with NH_4OH) in the mixing flask and 0.05 M NH_4HCO_3 in the reservoir, gave five major peaks. These were identified by their ultraviolet spectra as unhydrolyzed 2,4-dimethoxy-5-D-ribofuranosylpyrimidine followed in order by the pseudouridine isomers A_F, A_S, B and C described by Cohn.[8] The fraction containing the C isomer was freed from NH_4HCO_3 with Dowex-50W-H^+ and from borate by repeated addition and evaporation of methanol. The solid then was purified by paper chromatography on Whatman 40 paper in n-butyl alcohol–acetic acid–water.[6] The major ultraviolet-absorbing band (R_f 0.24) was eluted with water and the solution was evaporated to dryness. The white solid (12 mg.) was recrystallized twice from 95% ethanol yielding 5.2 mg. (needles) of pseudouridine C. The synthetic material was identical with natural pseudouridine (isolated from urine[9]) by the following criteria: m.p. 223–224° (uncorr., reported 220–221°[2b]), mixed melting point, paper chromatography in four solvent systems,[10] paper electrophoresis in borate buffer and ultraviolet spectra compared at pH 2, 12 and 14.

(1) See C. A. Dekker in *Ann. Rev. Biochem.*, **29**, 453 (1960), for a recent review.

(2) (a) C. Yu and F. W. Allen, *Biochem. Biophys. Acta*, **32**, 393 (1959); J. P. Scannell, A. M. Crestfield and R. W. Allen, *ibid.*, 406 (1959); W. Cohn, *ibid.*, 569 (1959); (b) W. Cohn, *J. Biol. Chem.*, **235**, 1488 (1960).

(3) R. G. Jones and H. Gilman, *Organic Reactions*, **VI**, 339 (1951). The butyllithium solution should be clear before use.

(4) B. W. Langley, *J. Am. Chem. Soc.*, **78**, 2136 (1956).

(5) H. M. Kissman, C. Pidacks and B. R. Baker, *ibid.*, **77**, 18 (1955).

(6) S. M. Partridge, *Biochem. J.*, **42**, 238 (1948).

(7) M. Viscontini, D. Hoch and P. Karrer, *Helv. Chim. Acta*, **38**, 642 (1955).

(8) Cohn[2b] has established that naturally occurring pseudouridine (C isomer) is converted to an equilibrium mixture of pseudouridine isomers (designated A_F, A_S, B and C) by heating with acid at 100°. The periodate titration data he reports leave little doubt that the A isomers are 5-α- and 5-β-D-ribo*pyranosyl*uracil (V) and B and C are the 5-ribo*furanosyl* anomers (IV).

(9) We are indebted to Dr. Waldo Cohn for this sample (estimated by him as 75% pure). We purified it further by paper chromatography (ref. 10a) and two recrystallizations from 95% ethanol.

(10) (a) n-Butyl alcohol–acetic acid–water,[6] R_f 0.26; (b) isopropyl alcohol–ammonia–water (7:1:2), R. Markham and J. D. Smith, *Biochem. J.*, **52**, 552 (1952), R_f 0.40; (c) n-butyl alcohol–saturated with water, R. D. Hotchkiss, *J. Biol. Chem.*, **175**, 315 (1948), R_f 0.11; (d) isopropyl alcohol–1% ammonium sulfate (2:1), N. Anand, V. M. Clarke, R. H. Hall and A. R. Todd, *J. Chem. Soc.*, 3665 (1950), R_f 0.54;

Anal.[11] Calcd. for $C_9H_{12}N_2O_6$ (244.20): C, 44.26; H, 4.95; N, 11.47. Found: C, 44.54; H, 5.20; N, 11.71. λ_{max} 262 mμ, ϵ_{max} 7,900; λ_{min} 232.5, ϵ_{min} 2,090, OD^{290}/OD^{260} 0.06 at *p*H 2. λ_{max} 287, ϵ_{max} 7,960; λ_{min} 244.5, ϵ_{min} 1,970; OD^{290}/OD^{260} 2.13 at *p*H 12.[12]

The yield of pseudouridine C based on the sugar derivative (I) was 2%. The total yield of all four pseudouridine isomers was 3.5%.

This synthesis conclusively establishes the 5-D-ribosyluracil structure of natural pseudouridine, but the important question concerning the configuration of the anomeric carbon still remains unanswered. Hydrolysis experiments have indicated that the A isomers are produced, as expected, during acidic hydrolysis of the methoxyl groups. Since the benzoyl groups are removed from the sugar by a side reaction,[13] the β-directing influence of the 2-benzoyl group[14] may be lost. From a consideration of the spectral data and by analogy with other nucleosides it seems likely that pseudouridine C is 5-β-D-ribofuranosyluracil and the B isomer is 5-α-D-ribofuranosyluracil. Attempts to settle this structural point and to improve the yield of pseudouridine by utilizing the less reactive cadmium reagent are in progress.

Acknowledgments.—We are indebted to Mr. Viktor Kurkov for technical assistance and to Mr. Peter Lengyel for preparing 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribose. This work was supported by grants from the National Science Foundation (No. 1602 C4) and the United States Public Health Service (No. RG-7262 C1).

DEPARTMENT OF BIOCHEMISTRY ROBERT SHAPIRO
NEW YORK UNIVERSITY SCHOOL OF MEDICINE
NEW YORK 16, NEW YORK ROBERT WARNER CHAMBERS

RECEIVED JUNE 30, 1961

(11) Schwarzkopf Microanalytical Laboratory, Woodside, New York.

(12) These ϵ_{max} values are in fair agreement with those reported by Yu and Allen,[2a] but they are about 10% lower than those reported by Cohn.[2b] The sample provided by Dr. Cohn and purified as described in ref. 9 gave values corresponding to those given above.

(13) Presumably by reaction of the lithium derivative with the ester groups to form bis-(2,4-dimethoxy-5-pyrimidyl)-phenylmethanol.

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

BOOK REVIEWS

The Enzymes. Second Edition, Completely Revised. Volume 3. Prosthetic Groups and Cofactors (Part B). Edited by PAUL D. BOYER, Department of Physiological Chemistry, University of Minnesota, Minneapolis, Minnesota, HENRY LARDY, Institute for Enzyme Research, University of Wisconsin, Madison, Wisconsin, and KARL MYRBÄCK, Institute for Organic Chemistry and Biochemistry, University of Stockholm, Sweden. Academic Press Inc. 111 Fifth Avenue, New York 3, N.Y., 1960. xiv + 497 pp. 15.5 × 23 cm. Price, $16.00.

The first edition of "The Enzymes" has proved to be a useful well of information; undoubtedly the second edition will turn out likewise. The information in these volumes is presented in a streamlined fashion which should provide painless reading with maximal absorption for the reader.

The biologist of today is presented with numerous volumes which are published for the dual purposes of keeping him abreast of diverse fields of interest, not necessarily his own, and to present an appraisal of the current status of these fields. In reading Volume 3 (Part B) of "The Enzymes," this reviewer had the impression that he was reading a super volume of an annual review work. One wonders if we really need or can afford such comprehensive treatises which in so many ways duplicate each other, duplicate the efforts of the varied annual summarizing publications and do away so completely with the personal aspects of scientific writing. It would seem that more effort could be devoted toward publishing memoirs or monographs, contributed by individuals who wish to sum up a point of view or provide a record of their investigations. Such publications need not be subject to the rigid time relations that are needed for multi-volume treatise publication and could benefit thereby.

Volume 3 (Part B) of "The Enzymes" contains eleven contributions of varying length; all contributions suffer from irritating editorial troubles. In this volume there is considerable unevenness among the eleven contributions, an unevenness that does not reflect the spirit of a revised edition of an advanced treatise on enzymology, the contributions to which should be authoritative treatments of each topic and remain so for some years.

Jaenicke and Lynen provide a lengthy and thorough discussion on almost all aspects of coenzyme A. Of particular interest to biochemists will be the compilation, into a very useful table, of all known enzyme reactions that require either coenzyme A or one of its derivatives. Botanically inclined readers will be interested to know that royal jelly is a plant (Table, 2 p. 14). On the whole, this is a masterly review and one which more than adequately summarizes Lynen's investigations and thoughts on coenzyme A. The chapter was originally written in German, was admirably translated by Dr. Helmut Beinert, but somehow a discrepancy between the numbered equations, figures and tables and their corresponding numbers in the text did appear in the final printed version.

Most aspects of the chemistry and enzymology of the pyridine coenzymes are discussed by N. O. Kaplan. This discussion summarizes, and usefully so, recent investigations on the chemical and physical properties of DPN and TPN. Possible reaction mechanisms of DPN and TPN, as studied with these coenzymes or with one of their derivatives, are also discussed. The chapter points out again the complexities introduced by the discovery of different isomeric forms of the pyridine coenzymes. This reviewer found the discussion of the enzymatic destruction of DPN most helpful, but was disappointed that plants weren't included in the section on the distribution of pyridine nucleotides.

E. M. Kosower presents a series of interesting considerations on what is known about charge transfer complexing. Even though little is known about such reactions in biological systems, Kosower does consider the evidence and theories for such reactions in pyridinium systems and possible applications of these theories to reactions of pyridine nucleotides. This chapter is a solid discussion of things that are either partially known or need to be investigated.

L. J. Reed contributes a chapter on lipoic acid. The chapter comprises a comprehensive review of the chemistry and biological role, in terms of reaction mechanisms, of lipoic acid.

"Metal and Enzyme Interactions: correlation of composition, function, and structure" is the title of a very ambitious chapter contributed by B. L. Vallee. The main emphasis of this chapter is on the non-heme metal-coenzymes and consequently centers on Vallee's own contributions. After a brief introductory discussion of metal containing