[CONTRIBUTION FROM THE CHEMISTRY DIVISION OF THE BRITISH COLUMBIA RESEARCH COUNCIL]

Phosphorylated Sugars. II. The Preparation of the Anomeric Methyl 5-O-Benzyl-D-ribofuranoside 2,3-Cyclic Carbonates and the Study of their Reactions with Hydrogen Bromide in Acetic Acid

BY G. M. TENER AND H. G. KHORANA

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Methyl 5-*O*-benzyl-2,3-isopropylidene-D-ribofuranoside (I) is converted quantitatively to methyl 5-*O*-benzyl-D-ribofuranoside (II) on treatment with methanolic sulfuric acid. The latter reacts with phosgene in pyridine to give an excellent yield of methyl 5-*O*-benzyl-D-ribofuranoside 2,3-cyclic carbonate (III). The oily product may be separated on a silicic acid column into the crystalline α-(IIIa) and β-(IIIb) anomers. A detailed study has been made of the reactions of the pure anomers with a mixture of hydrogen bromide–acetic acid and acetic anhydride. The reactions observed are (1) rapid acetolysis of the 5-*O*-benzyl groups; (2) anomerization, in *ca.* two hours at 20°, of the products of the reaction 1 to give an equilibrium mixture, consisting of 13% of the α-anomer (Va) and 87% of the β-anomer (Vb); (3) replacement of the methoxyl groups in V by the bromide group, a reaction which has been found to be exceptionally slow. This slowness of reaction 3 which is probably due to the absence of participation effects from the protecting group at C_2 has made possible the study of reaction 2.

The enzymatic phosphorolysis of ribonucleosides yields a ribofuranose 1-phosphate,[1] the configuration of which has been shown to be α.[2] The same configuration has also been concluded[3,4] for the two related biologically important ribose derivatives, ribofuranose 1,5-diphosphate[5] and 5-phosphoryl-ribofuranose 1-pyrophosphate.[6] The problems of the chemical synthesis of these substances, therefore, are similar in their requirement for suitably protected ribofuranose derivatives such that phosphorylation or pyrophosphorylation at C_1 would lead to products having the α configuration. The present communication describes the preparation of new derivatives of D-ribose which promise to fulfill this requirement. The successful synthesis of α-D-ribofuranose 1-phosphate using these derivatives is reported in the following communication.[7]

The importance of "neighboring group participation"[8] in the synthesis of nucleosides using acyl-furanosyl halides is now well established.[9] The acyl group used to protect the hydroxyl function at C_2 exercises the net effect of "shielding" one side of the furanose ring and, consequently, in most of these syntheses, products having a C_2–C_1 *trans* arrangement are obtained. The same effect is also operative in the synthesis of pentofuranose 1-phosphates since in the previously reported synthesis[2] of ribofuranose 1-phosphate from 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide[10] and triethylammonium dibenzyl phosphate, the β-anomer was obtained exclusively.[11] In the hope of synthesizing products having the α configuration, it was, therefore, decided to prepare suitably protected ribofuranosyl halides in which the replacement reaction at C_1 would be free from the neighboring group influence. The preparation of a 5-*O*-substituted-D-ribofuranosyl halide 2,3-cyclic carbonate, which meets this requirement, was undertaken.

Methyl 2,3-*O*-isopropylidene-D-ribofuranoside was prepared in one step from D-ribose according to Levene and Stiller[12] and was benzylated by a modification of the procedure of Kenner, Taylor and Todd[13] to give I in a nearly quantitative yield. The isopropylidene group could be removed selectively from I by heating in methanolic sulfuric acid[14] to give the glycol II in excellent yield. The latter was brought into reaction with phosgene in pyridine and the product, methyl 5-*O*-benzyl-D-ribofuranoside 2,3-cyclic carbonate (III) was obtained, again in an excellent yield, as a distillable viscous oil. Since, as shown below, the oily carbonate III may be separated into the crystalline α- and β-anomers, the intermediates I and II also are, undoubtedly, anomeric mixtures.

When a benzene solution of the oily carbonate III was applied to a silicic acid column and the column washed with the same solvent, a product was eluted which readily crystallized (m.p. 59°). It accounted for almost half of the total material and was levorotatory ($[\alpha]^{20}D$ −54.5°). Continued elution with benzene containing ether gave the remainder of the material as a second peak which also readily crystallized (m.p. 62–63°) and was dextrorotatory, $[\alpha]^{20}D$ +102.6°. From their identical analytical composition and their optical rotations it is clear that these two substances are, respectively, the β- (IIIb) and the α-anomers (IIIa). The β-anomer (IIIb) may also be isolated from the

(1) H. M. Kalckar, *J. Biol. Chem.*, **167**, 477 (1947). For subsequent references on phosphorolysis of nucleosides, see F. Schlenk, "Biosynthesis of Nucleosides and Nucleotides," in "The Nucleic Acids," Vol. II, Academic Press, Inc., New York, N. Y., 1955, p. 309, and also G. Schmidt, Vol. 1, p. 555.

(2) R. S. Wright and H. G. Khorana, THIS JOURNAL, **77**, 3423 (1955); **78**, 811 (1956).

(3) H. G. Khorana, G. M. Tener, R. S. Wright and J. G. Moffatt, *ibid.*, **79**, 430 (1957).

(4) C. N. Remy, W. T. Remy and J. M. Buchanan, *J. Biol. Chem.*, **217**, 885 (1955).

(5) H. Klenow, *Arch. Biochem. Biophys.*, **46**, 186 (1953).

(6) A. Kornberg, I. Lieberman and E. S. Simms, *J. Biol. Chem.*, **215**, 389 (1955).

(7) G. M. Tener, R. S. Wright and H. G. Khorana, THIS JOURNAL, **79**, 441 (1957).

(8) (a) For a comprehensive discussion of the participation effects in the replacement reactions of polyacylglycosyl halides see R. U. Lemieux, *Advances in Carbohydrate Chem.*, **9**, 1 (1954); (b) L. J. Haynes and F. H. Newth, *ibid.*, **10**, 207 (1955).

(9) B. R. Baker, J. P. Joseph, R. E. Schaub and J. H. Williams, *J. Org. Chem.*, **19**, 1786 (1954), and the many subsequent papers in THIS JOURNAL by Baker and co-workers.

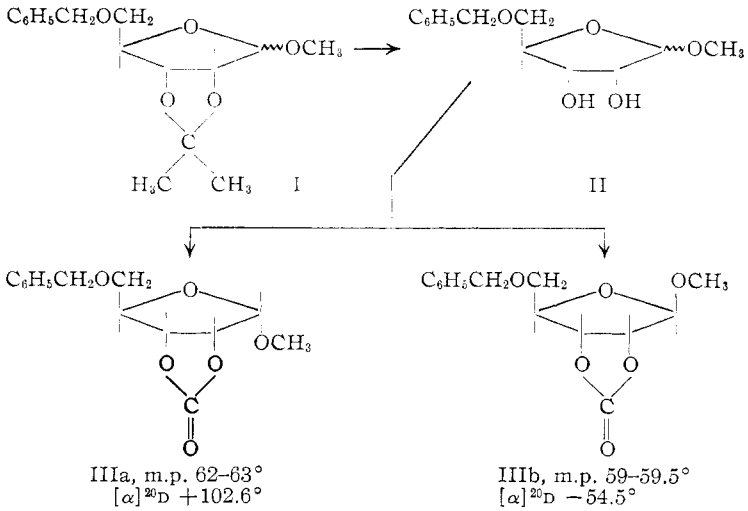
(10) R. K. Ness, H. W. Diehl and H. G. Fletcher, THIS JOURNAL, **76**, 763 (1954).

(11) See, however, the results of experiments on the synthesis of D-arabinofuranose 1-phosphate, R. S. Wright and H. G. Khorana, *ibid.*, in press.

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

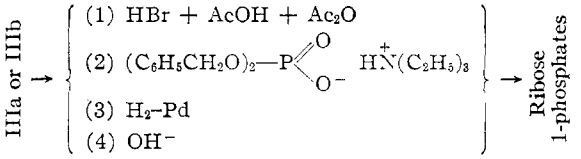
(13) G. W. Kenner, C. W. Taylor and A. R. Todd, *J. Chem. Soc.*, 1620 (1949).

(14) *Cf.* C. H. Shunk, J. B. Lavigne and K. Folkers, THIS JOURNAL, **77**, 2210 (1955).



anomeric mixture simply by fractional crystallization but the α-anomer has, so far, not been obtained crystalline without column chromatography.

Experiments which are described separately,[7] demonstrated the usefulness of these new derivatives, IIIa and IIIb, in the synthesis of α-D-ribofuranose 1-phosphate according to the scheme



The remarkable and, from the practical standpoint, highly useful discovery was made that both anomers (IIIa and IIIb) gave the same product, predominantly the α-1-phosphate. This finding suggested that both anomers lead, probably, to the same ribofuranosyl bromide on treatment with the mixture of hydrogen bromide–acetic acid and acetic anhydride. The detailed study reported below of the reactions which occur when the anomeric carbonates (IIIa, IIIb) are treated with the above mixture has confirmed this conclusion and revealed several features of general interest.

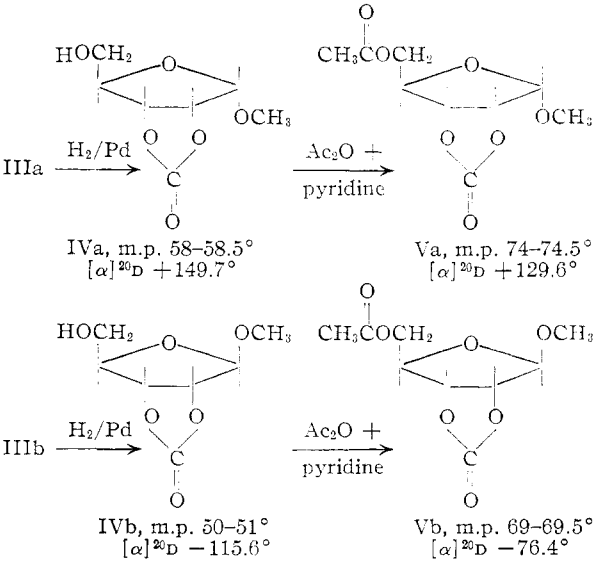
When the anomeric carbonates (IIIa and IIIb) are dissolved in acetic acid containing hydrogen bromide (16%) and a little acetic anhydride, the first reaction which occurs is the "acetolysis" of the 5-*O*-benzyl groups. After some ten minutes at room temperature the corresponding 5-*O*-acetates (Va and Vb) must be the main products since paper chromatography, after mild alkaline hydrolysis to remove the acetyl and the carbonate groups, reveals a single spot corresponding to methyl-D-ribofuranoside. (No spot corresponding to methyl-5-*O*-benzyl-D-ribofuranoside can be detected.) These observations are in accord with the results reported by Allerton and Fletcher[15] on the acetolysis of the benzyl ethers of other carbohydrate derivatives. It may be added that acetic anhydride does not appear to be necessary for the acetolysis reaction.[16]

(15) R. Allerton and H. G. Fletcher, Jr., THIS JOURNAL, **76**, 1757 (1954).

(16) Acetolysis of the benzyl groups is, perhaps, a two-step reaction: (1) debenzylation, (2) acetylation of the liberated hydroxyl group. Rapid debenzylation will be expected to occur in a manner analogous to the removal of triphenylmethyl group in hydrogen bromide–acetic acid. See *e.g.*, J. L. Barclay, A. B. Foster and W. G. Overend, *J. Chem. Soc.*, 2505 (1955); J. M. Anderson and E. Percival, *ibid.*, 814 (1956).

The study of the optical rotation changes during the above treatment of the anomeric carbonates IIIa and IIIb, shows that, in addition to acetolysis, anomerization occurs and that, from both anomers, an equilibrium mixture (*ca.* $[\alpha]^{20}D$ −35°) is obtained after about two hours at room temperature. Since, as shown below, no replacement of the C_1-methoxyl groups can be detected at this stage, only Va and Vb are present and their relative amounts can be calculated from the optical rotations of the pure crystalline anomers to be Va, 13%, Vb, 87%.

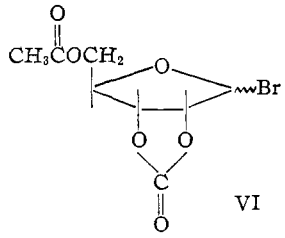
The latter compounds were prepared by acetylation with acetic anhydride and pyridine of the crystalline methyl D-ribofuranoside 2,3-cyclic carbonates (IVa and IVb), which, in turn, were prepared by the hydrogenolysis of the starting materials, IIIa and IIIb. The above observations on the anomerization process were supported by a polarimetric study of a solution of pure methyl 5-*O*-acetyl-β-D-ribofuranoside 2,3-cyclic carbonate (Vb) in hydrogen bromide–acetic acid–acetic anhydride. As shown in Fig. 1 (curve III) the optical rotation changes observed were identical with those obtained with the corresponding 5-*O*-benzyl compound IIIb. Finally, a direct confirmation of the



above reactions, namely, acetolysis and subsequent anomerization in favor of the β-anomer Vb was secured by repeating the experiment with methyl 5-*O*-benzyl-α-D-ribofuranoside 2,3-cyclic carbonate (IIIa) and working up the reaction mixture after one-half hour. The readily crystallizable product was identified as methyl 5-*O*-acetyl-β-D-ribofuranoside 2,3-cyclic carbonate (Vb) by direct comparison (m.p. and mixed m.p.) with a sample of this substance prepared by the alternate route (IIIb → IVb → Vb).

Acid-catalyzed mutarotations of acylated alkyl glycosides have been studied extensively by a number of workers and detailed mechanisms have been postulated, recently, by Lindberg and by Lemieux.[17] However, all the studies reported appear to have been carried out with alkyl pyranosides and, further, in all cases, neighboring group effects[18] were present. The results reported here represent, as far as we are aware, the first study of anomerization of an alkyl glycoside in which the neighboring group effect is absent. However, detailed kinetic studies are required before any exact suggestions regarding the mechanism of anomerization can be made.

The final reaction, the conversion of Va and Vb to VI, is conveniently followed by paper chromatography of the reaction products after mild alkaline



VI

treatment.[19] It could be demonstrated that even after a three hour treatment at room temperature none of the bromide VI was formed. Heating the reaction mixture for several hours at 50° was necessary to complete the conversion of V to VI. The bromide has so far been obtained only as an oil and is probably an anomeric mixture, with, perhaps, the β-anomer predominating.[20]

It is well known that the alkoxyl groups at C_1 in acyl glycosides may often be readily replaced by halides and this is so, especially, in the case of the alkyl acyl furanosides. Thus, for example, methyl 2,3,5-tri-*O*-benzoyl-D-ribofuranoside (VII) is converted completely to the corresponding 1-bromide on treatment with hydrogen bromide in acetic acid for 20 minutes[10] at room temperature. In these reactions the acyloxy group at C_2 may be visualized[8] as facilitating the cleavage of the glycosyl bond as shown in VII. The unusually great stability of the methoxyl groups in V, therefore, may be attributed to the absence of the participation effect from the substituent at the C_2-hydroxyl group.

While the immediate application of VI to the synthesis of α-D-ribofuranose 1-phosphate is reported in the following paper,[7] it is clear that the anomeric carbonates III offer a promising route to the synthesis of other ribose phosphates[4-6] and α-D-ribonucleosides, *e.g.*, 1-α-D-ribofuranosyl-5,6-dimethylbenzimidazole.[21] These synthetic possibilities are under investigation.

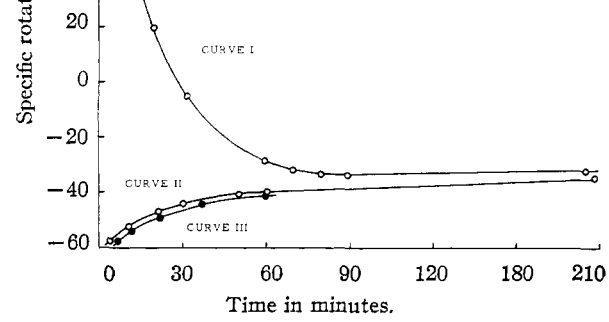
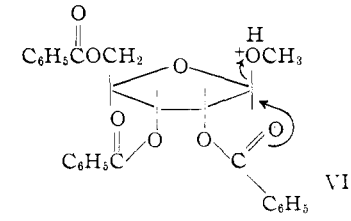


Fig. 1.—Study of the optical rotation changes in hydrogen bromide–acetic acid–acetic anhydride: curve I, methyl 5-*O*-benzyl-α-D-ribofuranoside 2,3-cyclic carbonate (IIIa); curve II, methyl 5-*O*-benzyl-β-D-ribofuranoside 2,3-cyclic carbonate (IIIb); curve III, methyl 5-*O*-acetyl-β-D-ribofuranoside 2,3-cyclic carbonate (Vb).



VII

Sugar carbonates were first prepared by Haworth and co-workers,[22] who demonstrated the usefulness of these acid-stable, alkali-labile derivatives in structural work. However, they appear to have been completely neglected since this early work and, further, there is no record, as far as we are aware, of their use in synthetic work in the glycoside field. The studies reported in this series emphasize the advantage they may offer in certain cases in work on carbohydrate derivatives.

Experimental

Methyl 2,3-*O*-isopropylidene-D-ribofuranoside was prepared in one step from D-ribose according to the procedure of Levene and Stiller.[12]

Methyl 5-*O*-benzyl-2,3-*O*-isopropylidene-D-ribofuranoside (I). (*cf.* Kenner, Taylor and Todd[13]).—To a solution of methyl 2,3-*O*-isopropylidene-D-ribofuranoside (2 g.) in 20 ml. of dry xylene were added 8 g. of benzoyl chloride and 8 g. of finely powdered potassium hydroxide. The mixture was stirred at 80° for four hours, cooled to room temperature and to it was then added 50 ml. of water. After shaking the mixture for ten minutes, the water layer was separated and extracted once with xylene. The combined xylene layers were then extracted back with water and dried over anhydrous sodium sulfate before being concentrated to a sirup *in vacuo*. The residue was distilled under a high vacuum (5×10^{-3} mm.) at 120–140°, giving the product as a colorless oil (2.42 g.); $n^{20}D$ 1.5033.

Methyl 5-*O*-Benzyl-D-ribofuranoside (II).—A solution of I (22.2 g.) in 220 ml. of methyl alcohol containing 30.2 ml.

(17) For a detailed review of the subject see ref. 8a, p. 40.

(18) Anomerization of two compounds, although not alkyl glycosides, in which neighboring group participation is essentially absent, has been recorded. The compounds are 3,4,6-tri-*O*-acetyl-β-D-glucosyl chloride (P. Brigl, *Z. physiol. Chem.*, **116**, 1 (1921); R. U. Lemieux and G. Huber, *Can. J. Chem.*, **31**, 1040 (1953)) and 1,3,4,6-tetra-*O*-acetyl-2-*O*-trichloroacetyl-β-D-gucopyranoside (R. U. Lemieux, C. Brice and G. Huber, *ibid.*, **33**, 134 (1955)).

(19) The expected VI would thus be converted to free ribose, whereas unchanged Va and Vb would be identified on paper chromatograms as methyl ribofuranoside.

(20) In the preparation of ribofuranose 1-phosphate from this bromide the product consists largely of the α-anomer.[7] If, as is not unlikely, a Walden inversion occurs during the replacement of the bromide by the phosphate group, then the bromide VI should be largely of the β-configuration. This conclusion would appear to be supported by the low dextrorotation of the oily bromide.

(21) N. G. Brink, *et al.*, THIS JOURNAL, **72**, 1866 (1950).

(22) W. N. Haworth and C. R. Porter, *J. Chem. Soc.*, 2796 (1929).

of 0.98 *N* sulfuric acid was heated under reflux for 4 hours, then neutralized by the addition of solid sodium bicarbonate. Sodium sulfate which separated and any excess of sodium bicarbonate were removed by filtration and the methanolic solution evaporated under reduced pressure. Ether was added to the residue and some inorganic salts, which separated at this stage, were removed by filtration. The oil obtained after evaporation of ether was dissolved in water (1 liter) and freed from the insoluble oily impurity (probably dibenzyl ether, formed during the last step) by three extractions with light petroleum. The clear aqueous solution was concentrated to dryness and the oil distilled *in vacuo* (0.01 mm.) at a temperature of 120–140° to give a practically quantitative yield of II; $n^{20}D$ 1.5229. *Anal.* Calcd. for $C_{13}H_{18}O_5$: C, 61.42; H, 7.13. Found: C, 61.60; H, 7.19.

Methyl 5-*O*-Benzyl-D-ribofuranoside-2,3-Cyclic Carbonates (IIIa and IIIb).—To a solution of II (2.23 g.) in anhydrous pyridine (40 ml.) was added dropwise a solution of phosgene (2 g.) in toluene (20 ml.) and the reaction mixture stirred at room temperature for one hour with exclusion of moisture. Two hundred ml. of ice-water was then added and the mixture extracted twice with 100-ml. portions of ether. The combined ether extracts were washed with water, dried over anhydrous sodium sulfate and then concentrated to dryness, leaving 2.41 g. of an orange brown liquid. Distillation in a short path apparatus at 0.01 mm. pressure (bath temperature, 190°) gave the mixture of IIIa and IIIb as a pale yellow oil which slowly solidified on standing.

Separation of IIIa and IIIb on a Silicic Acid Column.—The column (30 cm. × 4 cm.) was prepared by pouring a slurry of silicic acid[23] (120 g.) in petroleum ether into a chromatographic tube and packing under air pressure (30 cm. mercury). The petroleum ether was drained to the surface of the column and a benzene solution (150 ml.) of the mixture (23 g.)[24] of IIIa and IIIb was applied to the top. Elution was commenced with the same solvent, 100-ml. fractions being collected at a flow rate of 5–10 ml./minute. The maximum of the first peak, corresponding to IIIb, was reached after 500 ml. of the eluent had passed through, a total of 1700 ml. being required to completely elute this peak. The combined fractions were evaporated to dryness leaving an oily residue (10.3 g.) which soon crystallized. Recrystallization from aqueous methyl alcohol and then a mixture of ether and petroleum ether gave dense needles of m.p. 59–59.5°, $[\alpha]^{20}D$ −54.5° (*c* 1.06, ethyl alcohol). *Anal.* Calcd. for $C_{14}H_{16}O_6$: C, 59.95; H, 5.75. Found: C, 59.90; H, 5.82.

Continued elution of the column with benzene containing 6% ether gave the α-anomer IIIa (peak after 200 ml.), a total of 700 ml. of the eluent being required. Evaporation of the eluate gave IIIa as a crystalline residue (11.1 g.). This product, after recrystallizations first from aqueous methyl alcohol and then a mixture of ether and petroleum ether, was obtained as small feathery needles of m.p. 62–63°; $[\alpha]^{20}D$ +102.6° (*c* 2.52, ethyl alcohol). *Anal.* Calcd. for $C_{14}H_{16}O_6$: C, 59.95; H, 5.75. Found: C, 59.99; H, 5.84.

A further amount of an oily material (1.25 g.) was obtained on subsequent elution of the column with a mixture of ether and benzene (1:1). This material, which did not crystallize, has not been examined further.

Isolation of IIIb by Direct Crystallization.—The semi-solid material, obtained on keeping a distilled mixture of IIIa and IIIb for several days, was cooled to 0° and triturated with chilled methyl alcohol. The crystalline material was collected by filtration and washed with more cold methyl alcohol. Recrystallization from aqueous methyl alcohol and then ether–petroleum ether afforded pure IIIb in *ca.* 35–40% yield, based on the total weight of the mixture. The α-anomer could not be obtained crystalline through fractional crystallization.

The Reactions of the Anomeric Methyl 5-*O*-Benzyl-D-ribofuranoside 2,3-Cyclic Carbonates (IIIa and IIIb) with Hydrogen Bromide–Acetic Acid–Acetic Anhydride. (A) **Paper Chromatographic Study.**—A solution of 100 mg. of IIIa or IIIb in 5 ml. of hydrogen bromide (16%)–acetic acid–acetic anhydride (2%) was heated under anhydrous conditions and 1-ml. aliquots were withdrawn at intervals. The aliquots were concentrated to dryness *in vacuo* at 50° and the residue hydrolyzed in 1 ml. of saturated aqueous barium hydroxide at 80° for 10 minutes. The barium carbonate which separated was removed by centrifugation and the clear supernatants neutralized by the addition of the pyridinium form of Amberlite IR-120. The reaction products were examined by paper chromatography on Whatman 4 paper, using water-saturated *n*-butyl alcohol[25] as the solvent system. The spots were located by the periodate–benzidine spray.[26] The R_f's of the control samples, which were applied side by side, were as follows: D-ribose, 0.19; methyl D-ribofuranoside, 0.44; methyl 5-*O*-benzyl-D-ribofuranoside, 0.86. The following results were obtained. (1) At room temperature, in less than three minutes, two spots (of equal intensity) corresponding to methyl D-ribofuranoside and methyl 5-*O*-benzyl-D-ribofuranoside were detected. After 15 minutes, the only spot detectable was that corresponding to methyl D-ribofuranoside. No change was observed during the next three hours. (2) At 55°, in 30 minutes, the strong spot corresponding to methyl-D-ribofuranoside was present; however, a weak spot, corresponding to free ribose had appeared. After four hours the spot corresponding to free ribose was strong, a weak spot corresponding to methyl D-ribofuranoside being still present. (3) At 100°, almost all the methyl D-ribofuranoside had disappeared within 45 minutes and over longer reaction periods, the only spot visible on the paper chromatograms was that of D-ribose. The reaction at this temperature was accompanied by much darkening and the separation of an insoluble tar.

Essentially similar results were obtained in the above study when acetic anhydride was omitted from the acidic mixture.

(B) **Polarimetric Study.**—Solutions of the pure anomers (IIIa and IIIb) in the hydrogen bromide–acetic acid–acetic anhydride mixture were prepared separately and the changes in the optical rotations were followed at intervals. The results of this study covering a period of 210 minutes are shown in Fig. 1. After this period, the rotations of the two solutions were indistinguishable. Subsequently, the rotations slowly increased in the dextrorotatory direction until after four days the value was almost zero. (Paper chromatography, after working up the reaction mixture as described above, showed that the conversion to the bromide was largely complete.)

Curve III (Fig. 1) shows the optical rotation changes observed starting with methyl 5-*O*-acetyl-β-D-ribofuranoside 2,3-cyclic carbonate (Vb). As expected, these changes[27] are very similar to those observed with (IIIb) (curve II).

(C) **Isolation of Methyl 5-*O*-Acetyl-β-D-ribofuranoside 2,3-Cyclic Carbonate (Vb).** (1) **Using IIIb.**—A solution of 212 mg. of IIIb in the hydrogen bromide–acetic acid–acetic anhydride mixture (10 ml.) was kept at room temperature for 30 minutes. The solvent was then evaporated in a vacuum and the last traces removed by repeated co-evaporation with toluene. The residual oil was crystallized from ether–petroleum ether and then from aqueous methyl alcohol to give Vb (90 mg.) as needles of m.p. 69–69.5°; $[\alpha]^{20}D$ −76.4° (*c* 2.75, ethyl alcohol). *Anal.* Calcd. for $C_9H_{12}O_7$: C, 46.72; H, 5.23. Found: C, 46.26; H, 5.34.

This material was identical (m.p. and mixed m.p.) with a sample prepared by the acetylation of IVb (see below).

(2) When (IIIa) was treated in the manner described above the crystalline material isolated on working up was again found to be Vb.

Methyl α-D-Ribofuranoside 2,3-Cyclic Carbonate (IVa).—Methyl 5-*O*-benzyl-α-D-ribofuranoside 2,3-cyclic carbonate (3 g.) was hydrogenated in methyl alcohol using freshly-prepared 5% palladium-on-charcoal catalyst. The product readily crystallized from an ether–petroleum ether mixture as small diamonds with m.p. 58–58.5°; $[\alpha]^{20}D$ +149.7 (*c* 3.08, ethyl alcohol). *Anal.* Calcd. for $C_7H_{10}O_6$: C, 44.21; H, 5.30. Found: C, 43.91; H, 5.49.

Methyl β-D-Ribofuranoside 2,3-Cyclic Carbonate (IVb).—This was prepared as above from IIIb. The substance crystallized from carbon tetrachloride as long needles with

(23) Mallinckrodt silicic acid, prepared by the method of Ramsay and Patterson for chromatography, was used.

(24) The mixture obtained in the last step may be used for column chromatography without distillation.

(25) L. Hough, J. K. N. Jones and W. H. Wadman, *J. Chem. Soc.*, 1702 (1950).

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

(27) Corrected for molecular weight difference from IIIb.

m.p. 50–51°; $[\alpha]^{20}D$ −115.6° (*c* 3.52, ethyl alcohol). *Anal.* Calcd. for $C_7H_{10}O_6$: C, 44.21; H, 5.30. Found: C, 44.09; H, 5.37.

Methyl 5-*O*-Acetyl-α-D-ribofuranoside 2,3-cyclic carbonate. (Va).—A solution of methyl α-D-ribofuranoside 2,3-cyclic carbonate (IVa) (110 mg.) in pyridine (1.2 ml.) was treated with acetic anhydride (0.5 ml.) for 12 hours at room temperature. The solution was then evaporated to dryness *in vacuo* and the product crystallized from aqueous methyl alcohol. It was recrystallized from a mixture of ether and petroleum ether to give needles with m.p. 74–74.5°; $[\alpha]^{20}D$ +129.6° (*c* 1.57, in ethyl alcohol). *Anal.* Calcd. for $C_9H_{12}O_7$: C, 46.72; H, 5.23. Found: C, 46.66; H, 5.37.

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[CONTRIBUTION FROM THE CHEMISTRY DIVISION OF THE BRITISH COLUMBIA RESEARCH COUNCIL]

Phosphorylated Sugars. III. Syntheses of α-D-Ribofuranose 1-Phosphate

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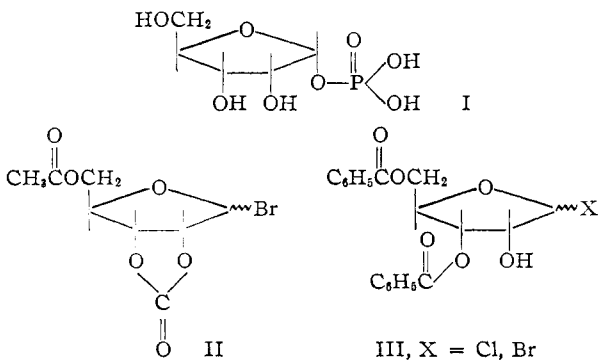
The treatment of 5-*O*-acetyl-D-ribofuranosyl bromide 2,3-cyclic carbonate with triethylammonium dibenzyl phosphate followed by hydrogenation and alkaline hydrolysis of the product to remove the protecting groups affords a 55–60% yield of an anomeric mixture of D-ribofuranose 1-phosphates, which consists predominantly of the α-anomer. The latter substance, which may be isolated readily in a satisfactory yield as the pure crystalline dicyclohexylammonium salt, has been shown to be identical in all respects with the product obtained by the enzymatic phosphorolysis of ribonucleosides. Alternative syntheses using 3,5-di-*O*-benzoyl-D-ribofuranosyl halides gave moderate yields of mixture of anomeric ribofuranose 1-phosphates.

In the first paper of this series[1] the synthesis of β-D-ribofuranose 1-phosphate was reported and evidence was presented which indicated that the product obtained by the enzymatic phosphorolysis of ribonucleosides[2] has the α configuration. The present communication records two syntheses[3] of this anomeric compound, identical in all respects with the enzymatically prepared samples. The present work, therefore, provides confirmation of the structure of the enzymatic samples[2] and, furthermore, makes this substance available in quantity in a pure state.



I

II

III, X = Cl, Br

In attempting the synthesis of α-D-ribofuranose 1-phosphate (I), our underlying aim was to investigate the use of a ribofuranosyl halide in which the replacement of the halide by the phosphate group would be free from the participation effect of the group at C_2.[4] Two derivatives[5] appeared promising in this respect; (1) the 5-*O*-acetyl-D-ribofuranosyl bromide 2,3-cyclic carbonate (II), the preparation of which has been recorded in the preceding paper[4] and (2) 3,5-di-*O*-benzoyl-D-ribofuranosyl halides (III).[6,7]

The standard method for the synthesis of sugar 1-phosphates involves the reaction of a glycosyl halide with a metal salt of phosphoric acid or its derivatives. In adapting this method to the synthesis of the highly labile pentofuranose 1-phosphate, Wright and Khorana[1] utilized the benzene-soluble triethylammonium dibenzyl phosphate and carried out the reaction at low temperature in an anhydrous medium. The same method has proved useful in the present work. The addition of one equivalent of triethylammonium dibenzyl phosphate to a benzene solution of II at room temperature resulted in the separation of triethylammonium hydrobromide. Palladium-catalyzed hydrogenolysis of a one-hour reaction product followed by alkaline hydrolysis gave ribofuranose 1-phosphate, isolated in 55–60% yield as the barium salt. Paper chromatography in the solvent system, isopropyl alcohol–ammonia–water (70-10-20, v./v.), which has been shown to separate the ribofuranose 1-phosphates

(1) R. S. Wright and H. G. Khorana, THIS JOURNAL, **77**, 3423 (1955); **78**, 811 (1956).

(2) (a) H. M. Kalckar, *J. Biol. Chem.*, **167**, 477 (1947); (b) H. L. A. Tarr, *Federation Proc.*, **14**, 291 (1955); **15**, 369 (1956).

(3) A preliminary report of a part of this work has already appeared: G. M. Tener, R. S. Wright and H. G. Khorana, THIS JOURNAL, **78**, 506 (1956).

(4) For a fuller discussion see the preceding paper: G. M. Tener and H. G. Khorana, *ibid.*, **78**, 437(1956).

(5) The only two ribofuranosyl halides known until recently and previously used in synthetic work were the 2,3,5-tri-*O*-acetyl-D-ribofuranosyl chloride, and the 2,3,5-tri-*O*-benzoyl-ribofuranosyl bromide. For references see "The Nucleic Acids," Vol. I, Academic Press., Inc., New York, N. Y., 1955.

(6) R. K. Ness and H. G. Fletcher, Jr., THIS JOURNAL, **76**, 1663 (1954). We are grateful to Dr. Fletcher for kindly suggesting the use of these halides in the present work.

(7) R. K. Ness and H. G. Fletcher, Jr., THIS JOURNAL, **78**, 4710 (1956). As shown by these authors, the product with m.p. 142–143° is 1,3,5-tri-*O*-benzoyl-α-D-ribose and not an ortho acid derivative as previously suggested.[4] We take this opportunity to point out that the latter formulation was also adopted by us in our earlier publication.[1]