

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

Phosphorylated Sugars. VI.¹ Syntheses of α -D-Ribofuranose 1,5-Diphosphate and α -D-Ribofuranose 1-Pyrophosphate 5-Phosphate

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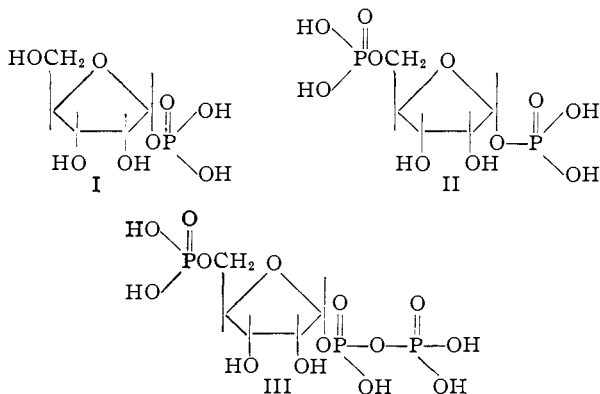
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Phosphorylation of benzyl β -D-ribofuranoside, or the mixture of benzyl glycosides obtained by treatment of D-ribose with benzyl alcohol and hydrogen chloride, with diphenylphosphorochloridate, followed by mild alkaline treatment gave benzyl 5-diphenylphosphoryl- β -D-ribofuranoside (X). Treatment of the latter with phosgene in pyridine gave the crystalline benzyl 5-diphenylphosphoryl- β -D-ribofuranoside 2,3-cyclic carbonate (VIII). α -Ribose-1,5-diphosphate (II) was synthesized from VIII by the following sequence of steps: (1) conversion to the corresponding ribofuranosyl halide (V) by treatment with hydrogen bromide-acetic acid mixture, (2) reaction of V with triethylammonium dibenzylphosphate followed by hydrogenation of the product, first in the presence of palladium and then of platinum, and finally (3) alkaline treatment to remove the carbonate group. The synthetic ribose 1,5-diphosphate was characterized as its crystalline tetracyclohexylammonium salt and shown to have the α -configuration. α -D-Ribofuranose 1-pyrophosphate 5-phosphate (III) was synthesized from V by an analogous series of reactions except that triethylammonium tribenzylpyrophosphate was substituted for triethylammonium dibenzylphosphate. Both synthetic products were indistinguishable in their properties from the corresponding samples prepared enzymatically.

Recent enzymatic studies have led to the discoveries of a number of biologically important phosphate esters of D-ribose. Of these, ribose 1-phosphate (I) was the first to be isolated and was shown to be a product of the enzymatic phosphorolysis of ribonucleosides.² Later, this ester I was shown to be reversibly formed also from ribose 5-phosphate by the action of the enzyme phosphoribomutase, a reaction in which the ester, ribose 1,5-diphosphate³ (II) serves as the coenzyme. Another derivative of D-ribose was discovered more recently and shown to be 5-phosphoryl- α -D-ribofuranose 1-

to the development of methods for the synthesis of the above phosphate esters of D-ribose as part of a broader program of researches on biologically important phosphate esters. It was hoped that these synthetic studies would lead to further characterization of these substances and would at the same time make them available more readily in a pure state. The syntheses of the anomeric D-ribofuranose 1-phosphates⁹⁻¹¹ were reported previously in this series and it was shown that the α -anomer was identical with the product obtained by the enzymatic phosphorolysis of ribonucleosides. The present communication records the extension of this work to syntheses of the more recently discovered esters, II and III. A brief report of this work already has appeared.¹²

In the earlier work, the reaction of 2,3,5-tri-O-benzyl β -D-ribofuranosyl bromide with triethylammonium dibenzylphosphate in benzene was found to give the β -D-ribofuranose 1-phosphate exclusively.⁹ This result was not unexpected in view of the well-known participation effect of the acyloxy group at C₂ in the replacement reactions at C₁. For the synthesis of the anomeric ribofuranose 1-phosphate (I), it was necessary to use a suitably protected ribofuranosyl halide in which the participation effect from C₂ would be absent. The intermediate investigated in this work was 5-O-acetyl-D-ribofuranosyl bromide 2,3-cyclic carbonate (IV) and it led to a highly successful synthesis of



pyrophosphate 5-phosphate. This ester, which is formed by the pyrophosphorylation of ribose 5-phosphate at C₁ by adenosine 5'-triphosphate,⁴⁻⁶ is involved in the enzymatic synthesis of purine and pyrimidine nucleotides from the corresponding bases,⁷ in the *de novo* synthesis of purine nucleotides⁷ and in the enzymatic synthesis of indole from anthranilic acid.⁸

Attention has been devoted in this Laboratory

(1) Paper V. R. S. Wright and H. G. Khorana, *THIS JOURNAL*, **80**, 1994 (1958).

(2) H. M. Kalckar, *J. Biol. Chem.*, **167**, 477 (1947).

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

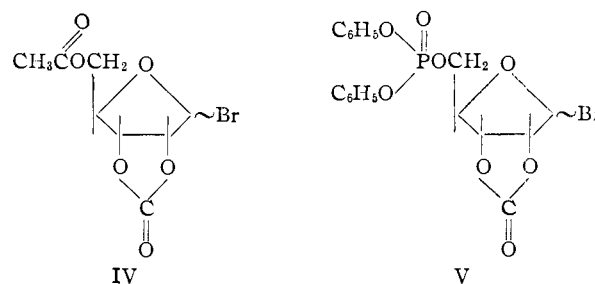
(4) A. Kornberg, I. Lieberman and E. S. Simms, *J. Biol. Chem.*, **215**, 389 (1955).

(5) C. N. Remy, W. T. Remy and J. M. Buchanan, *ibid.*, **217**, 885 (1955).

(6) H. G. Khorana, J. Fernandez and A. Kornberg, *ibid.*, in press.

(7) For a comprehensive review of literature see A. Kornberg in "The Chemical Basis of Heredity," Johns Hopkins Press, Baltimore, Md., 1957, p. 579.

(8) C. Yauoisky, *J. Biol. Chem.*, **223**, 171 (1956).



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

(10) G. M. Tener and H. G. Khorana, *ibid.*, **79**, 437 (1957).

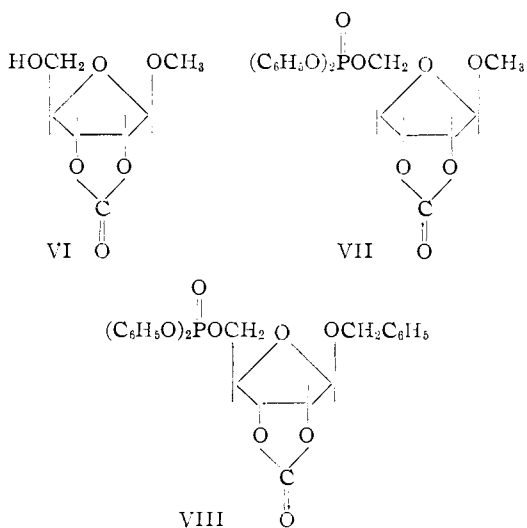
(11) G. M. Tener, R. S. Wright and H. G. Khorana, *ibid.*, **78**, 506 (1956); **79**, 441 (1957).

(12) G. M. Tener and H. G. Khorana, *Chemistry and Industry*, 562 (1957).

α -D-ribofuranose 1-phosphate^{10,11} (I). These results encouraged us to investigate the use of the cyclic carbonate grouping for protecting the 2- and 3-hydroxyl groups in D-ribose in the present work, since both II and III also have been shown to have the α -configuration.^{5,13}

The scheme envisioned for the syntheses of II and III required the common intermediate V, which already carried a suitably protected phosphoryl group at C₅. Replacement of the bromide group by a phosphate or pyrophosphate group followed by the removal of protecting groups would then give, respectively, II or III.

In the first approach to the synthesis of V, the crystalline methyl β -D-ribofuranoside 2,3-cyclic carbonate (VI), whose preparation has previously been recorded,¹⁰ was phosphorylated with diphenyl phosphorochloridate and the product VII was obtained as a crystalline substance. Hydrogenation to remove the phenyl groups and successive alkali



line and acidic treatments to remove, respectively, the cyclic carbonate and the methyl groups gave a product indistinguishable from D-ribose 5-phosphate. However, attempts to convert VII to the desired V by treatment with hydrogen bromide in acetic acid met with only limited success. The methoxyl group was unusually stable and the conditions necessary for its displacement by the bromide ion brought about considerable decomposition.¹⁴ The difficulty experienced in the conversion of VII to V led us to investigate the preparation of the corresponding benzyl ribofuranoside (VIII), which would be expected to be more labile¹⁵ than VII to hydrogen bromide-acetic acid treat-

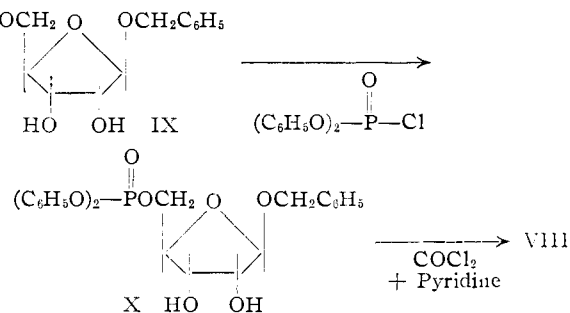
(13) H. G. Khorana, G. M. Tener, R. S. Wright and J. G. Moffatt, *THIS JOURNAL*, **79**, 430 (1957).

(14) This is due mainly to the presence of the tertiary phosphate group in VII. Thus diphenyl phosphate is one of the products which is formed on hydrogen bromide-acetic acid treatment. It may be noted that no harmful effect of the unusually harsh treatment with hydrogen bromide-acetic acid necessary for the preparation of IV was observed in the previous work.

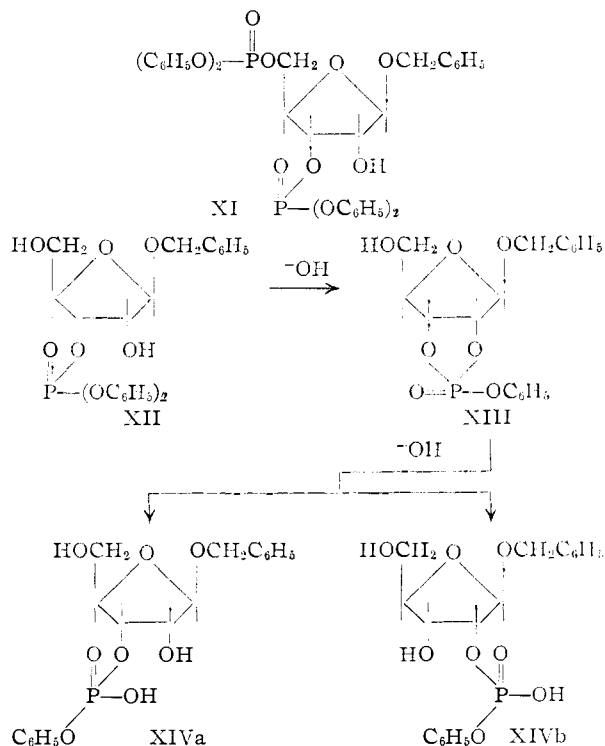
(15) Benzyl groups have been shown to undergo very rapid acetyloysis (see for example ref. 9 and R. Allerton and H. G. Fletcher, Jr., *THIS JOURNAL*, **76**, 1757 (1954)). In the present case the product would be 1-O-acetyl-5-diphenylphosphoryl-D-ribose 2,3-cyclic carbonate. The acetate group at C₁ would be expected to be replaced by the bromide ion more readily than the methoxyl group in VII.

ment. This alternative approach to the synthesis of the key intermediate V did, in fact, prove much more successful, especially in view of the following short and satisfactory route to the preparation of VIII itself.

The steps employed for the preparation of VIII involved the direct phosphorylation of IX to give X, followed by carbonation in pyridine. No element of ambiguity in the structure of the desired



product was expected to be introduced for reasons discussed below. Firstly, phosphorylation of the primary hydroxyl group in IX was expected to occur in preference to that of the secondary hydroxyl groups, using approximately one equivalent of the phosphorylating agent. Further, it was considered that the side products arising out of phosphorylation of the hydroxyl groups at C₂ or C₃ (e.g., XI and

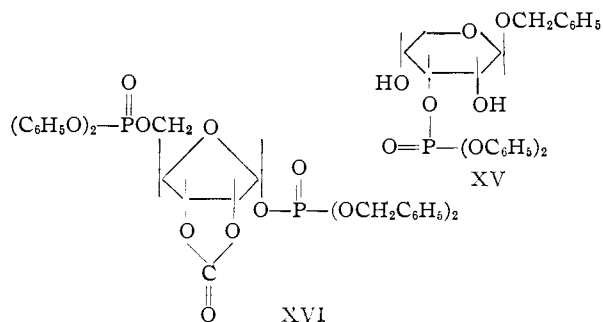


XII) would be extremely labile under alkaline conditions by virtue of the *cis*-hydroxyl groups present in them.¹⁶ A very mild alkaline treatment would thus effect their hydrolysis as illustrated in the case of XII to give the water-soluble products

(16) D. M. Brown, D. I. Magrath and A. R. Todd, *J. Chem. Soc.*, 4396 (1955); J. G. Moffatt and H. G. Khorana, *THIS JOURNAL*, **79**, 1194 (1957).

of the type XIV.¹⁷ Since the desired product X would be expected to be relatively stable under these conditions, the mild alkaline treatment would provide a simple means of isolating the neutral X in a pure state. Using this procedure, X was in fact obtained in about 70% yield from IX. It was then converted to the highly crystalline VIII by treatment with phosgene in pyridine. The essentially quantitative yield of VIII from X provided further characterization of the latter substance.

In the above synthesis of X, the crystalline IX obtained according to Fletcher and co-workers¹⁸ was first used. Subsequently, the isolation of IX was found unnecessary and the crude mixture of benzyl ribofuranoside and ribopyranoside(s) obtained by treatment of D-ribose with benzyl alcohol containing 1% hydrogen chloride was phosphorylated directly. The arguments concerning the alkaline lability discussed above would also apply to products arising out of phosphorylation of benzyl ribopyranoside¹³ (XV and its isomers) and a control experiment on purified benzyl ribopyranoside showed this to be the case (complete decomposition of phosphorylation product(s) within two minutes in 0.5 N sodium hydroxide at room temperature).



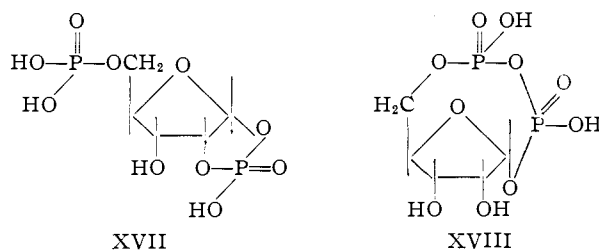
Treatment of VIII with hydrogen bromide in acetic acid for three days at room temperature gave the furanosyl bromide (V) as a light colored oil which was directly treated with one equivalent of triethylammonium dibenzyl phosphate in anhydrous benzene. The product, presumably XVI, was hydrogenated in anhydrous methyl alcohol, first, in the presence of 5% palladium on charcoal, and then in the presence of Adams platinum catalyst to remove, respectively, the benzyl and the phenyl groups. After removal of the carbonate group by treatment with lithium hydroxide, ribofuranose 1,5-diphosphate was isolated first as its water-insoluble barium salt (70% yield from VIII) and then converted to the crystalline tetracyclohexylammonium salt.

On paper chromatograms in suitable solvents the synthetic product had R_f 's identical with those of a sample obtained enzymatically and kindly furnished by Dr. Klenow. Further, both samples showed identical behavior on treatment with dicyclohexylcarbodiimide in aqueous pyridine, a reaction which was used to derive the α -configuration for the natural sample.¹³ Thus, the major spot on short treatment was the faster-travelling ribofura-

(17) The esters, XIVa and XIVb, would also be labile to further treatment with alkali, by virtue of the adjacent hydroxyl groups.

(18) R. K. Ness, D. W. Dichl and H. G. Fletcher, Jr., *THIS JOURNAL*, **76**, 763 (1954).

nose 1,2-cyclic phosphate 5-phosphate (XVII), which, as expected, was negative to periodic acid test and identical with the product formed from III by alkaline treatment.^{5,6} In addition, a minor spot (periodate positive) travelling just ahead of the cyclic phosphate XVII was present, which is per-



haps the pyrophosphate XVIII. Longer reaction periods gave spots travelling close to the solvent front and corresponding, presumably, to phosphorylureas.¹³

No evidence was obtained for contamination of the synthetic α -ribose 1,5-diphosphate by the β -anomer. Thus, the tetracyclohexylammonium salt, prepared from the crude barium salt, was fractionated into three crystalline crops. No difference was observed between the optical rotations of these fractions. Similarly, none of the β -anomer could be detected in the synthetic α -ribofuranose 1-pyrophosphate 5-phosphate (see below). It is recalled that in the previous synthesis of ribose 1-phosphate from IV, the product while consisting largely of the α -anomer was contaminated by some of the β -anomer. The apparent exclusive formation of the α -products in the present work may be due to the more effective participation of the diphenylphosphoryl group in V in replacement reaction at C₁ than that of the acetyl group in IV.

The synthetic α -ribose 1,5-phosphate simulated the phosphoribomutase-catalyzed interconversion of ribose 1- and 5-phosphates in fish muscle extracts. Further, using it as the phosphate group donor, Dr. Tarr was able to demonstrate the synthesis of deoxyribose 1,5-diphosphate¹⁹ from deoxyribose 1-phosphate. In further tests of its enzymatic properties, the synthetic α -ribose 1,5-diphosphate was inactive as a precursor in the synthesis of uridine 5-phosphate from orotic acid in the presence of orotidylic acid pyrophosphorylase and decarboxylase.⁶ This result is in agreement with the previous observations of Kornberg, *et al.*,²⁰ and Buchanan⁵ and co-workers.

For the synthesis of α -ribose-1-pyrophosphate 5-phosphate (III) from V, the latter was brought into reaction with triethylammonium tribenzylpyrophosphate²¹ and the product, presumably XIX, was hydrogenated as above. An enzymatic assay after a short alkaline treatment of the hydrogenation product XX indicated a 13% yield of the desired product III at this stage. The total mixture of the products was separated on a Dowex 1-

(19) H. L. A. Tarr, *Chemistry and Industry*, 562 (1957), and unpublished work.

(20) I. Lieberman, A. Kornberg and E. S. Simms, *J. Biol. Chem.*, **215**, 403 (1955).

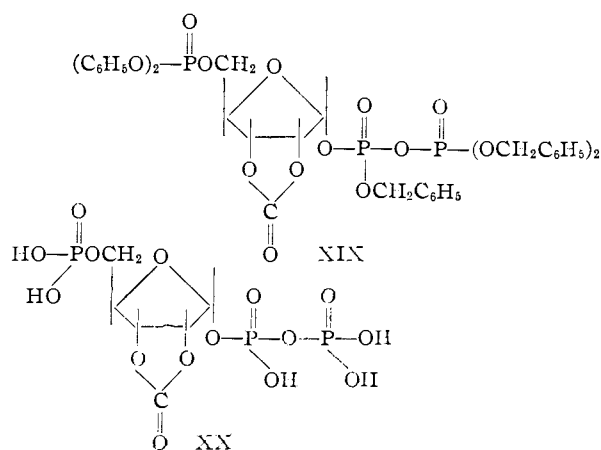
(21) (a) J. Baddiley, V. M. Clark, J. J. Michalski and A. R. Todd, *J. Chem. Soc.*, 815 (1949); (b) L. Zervas and I. Dilaris, *Chem. Ber.*, **89**, 925 (1956).

formate column and III finally was isolated as its amorphous lithium salt.

Paper chromatography showed this material to be identical with the major component of a sample of III prepared enzymatically.^{4,6} Analyses carried out on the amorphous salt showed a ratio of ribose to phosphorus of 1:3.10 and indicated it to be a pentahydrate. The uptake of periodic acid (one mole) was in agreement with this formulation. An enzymatic assay indicated the material to be at least 80% active.

Evidence to show the absence of the β -anomer of III in the synthetic product was obtained by treating this sample with 0.1 *N* sodium hydroxide at 100° for 10 minutes, a treatment which would be expected to hydrolyze III but which would not be expected to degrade the β -anomer. Subsequent ion-exchange chromatography⁶ revealed no ribose containing material appearing in the place expected for a triphosphate derivative.

The reasons for the low yield of III in the above synthesis, in contrast with the very satisfactory yield of ribose 1,5-diphosphate, are not very clear. The replacement of the bromide group in V by tri-benzylpyrophosphate group seemed to proceed fairly satisfactorily as judged by the separation of triethylammonium bromide (50% yield in place of 70% in the synthesis of II). It is possible that the glycosyl bonds in the intermediates, XIX and XX, were even more labile than those in the corresponding intermediates in the synthesis of ribose 1,5-diphosphate. Further, complication was to be anticipated in the removal of the carbonate grouping in XX, because of the alkaline lability of III.



However, degradation of the desired product III, during the brief alkaline treatment of XX does not appear to be the sole cause of the low yield. Thus, in separate experiments, the alkaline degradation of III was followed by (a) loss of enzymatic activity, (b) appearance of inorganic phosphate (III $\text{OH}^- \rightarrow \text{XVII} + \text{inorganic phosphate}^{5,6,13}$ and (c) loss of ability to consume periodic acid, which is a consequence of the formation of XVII. These results showed that III was fairly stable in dilute alkali at room temperature (about 30% decomposition in 50 minutes at room temperature in 0.25 *N* sodium hydroxide). It should be emphasized that these observations pertain only to hydrolysis by mono-

valent alkali metal hydroxides. The presence of divalent ions, e.g., barium,⁶ catalyzed markedly the degradation of III to XVII.

Experimental

Analytical Methods.—Paper chromatography was carried out on Whatman No. 1 filter paper using the solvent systems, *n*-propyl alcohol–concd. ammonia–water (6–3–1, v./v.)²² (solvent A) and 1 *M* ammonium acetate (*pH* 7.5)–ethyl alcohol (3–7.5, v./v.)²³ (solvent B). The spots were located by spraying the chromatograms with the molybdate spray^{22,24} for phosphorus or with the aniline phthalate spray for ribose,²⁵ into which some concentrated hydrochloric acid (0.5 ml./100 ml.) was incorporated. The presence of vicinal hydroxyl groups was shown on paper chromatograms by the periodate–benzidine spray.²⁶ Periodic acid uptake was determined quantitatively by the method of Morrison.²⁷

Total phosphorus was determined by the method of King,^{28a} inorganic and labile phosphorus by the method of Lowry and Lopez.^{28b} Pentose was determined by the orcinol method.²⁹

α -D-Ribose 1-pyrophosphate 5-phosphate was assayed enzymatically using the orotidylic acid pyrophosphorylase, and decarboxylase present in yeast. The preparation used was the “ethanol fraction” in the procedure of Lieberman, *et al.*²⁰

Methyl 5-Diphenylphosphoryl- β -D-ribofuranoside 2,3-Cyclic Carbonate (VII).—To a solution of 1 g. of methyl β -D-ribofuranoside 2,3-cyclic carbonate¹⁰ (VI) in anhydrous pyridine (10 ml.) was added dropwise with stirring diphenyl phosphorochloridate (1.85 g.). The clear solution was kept for 18 hours at room temperature and then concentrated *in vacuo* to an oil. This was taken up in ether (100 ml.) and the ethereal solution washed with four 100-ml. portions of water. The ethereal solution was then dried over anhydrous sodium sulfate and evaporated to give an oil which crystallized from an ether–petroleum ether mixture to give white needles (1.97 g., 89%) of m.p. 66–67°; $[\alpha]_D^{20} -31.6^\circ$ (*c* 3.42, ethyl alcohol).

Anal. Calcd. for $C_{19}H_{19}O_9P$: C, 54.05; H, 4.54. Found: C, 53.04; H, 4.36.

Benzyl 5-Diphenylphosphoryl- β -D-ribofuranoside (X).—To a cooled solution of benzyl β -D-ribofuranoside¹⁸ (3.32 g.) in anhydrous pyridine (50 ml.) was added 2.55 ml. (3.38 g., one equivalent) of diphenylphosphorochloridate with vigorous stirring. Cold water (2 ml.) was added after a reaction period of 2 hours at room temperature and the solution was concentrated *in vacuo* to a thick oil. This was then shaken vigorously for 15 minutes in the presence of 20 ml. of 1 *N* sodium hydroxide and then extracted with 100 ml. of ether. The ethereal extract was washed twice with 50-ml. portions of 5% aqueous sodium sulfate solution and then dried over anhydrous sodium sulfate. Evaporation of the solvent gave a colorless oil which was held under vacuum overnight. The yield of the sirupy product, which could not be crystallized, was 4.138 g. (73%).

Benzyl 5-Diphenylphosphoryl- β -D-ribofuranoside 2,3-Cyclic Carbonate (VIII).—A solution of 10.5 g. of X in 100 ml. of anhydrous pyridine was treated at 0° with 25 ml. of a solution of phosgene in toluene (0.26 g./mole). Cold water was added after a reaction period of two hours at room temperature, and the total solution was concentrated to dryness at 30° *in vacuo*. The oil was taken up in 200 ml. of ether and the solution washed repeatedly with water to remove last traces of pyridine. The dried ethereal solution deposited fine white needles (8.15 g.) after concentration (to about 25 ml.) and cooling. Concentration of the mother

(22) C. S. Hanes and F. A. Isherwood, *Nature*, **164**, 1107 (1949).

(23) A. C. Paladini and L. F. Leloir, *Biochem. J.*, **61**, 426 (1952).

(24) R. S. Bandurski and B. Axelrod, *J. Biol. Chem.*, **193**, 405 (1951).

(25) S. M. Partridge, *Nature*, **164**, 443 (1949).

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

(27) M. Morrison, G. Rouser and E. Stotz, *THIS JOURNAL*, **77**, 5156 (1955).

(28) (a) E. J. King, *Biochem. J.*, **26**, 292 (1932); (b) O. H. Lowry and J. A. Lopez, *J. Biol. Chem.*, **162**, 421 (1946).

(29) W. Mejsbaum, *Z. physiol. Chem.*, **268**, 117 (1930), as modified by H. G. Albaum and W. W. Umbreit, *J. Biol. Chem.*, **167**, 309 (1947).

liquor afforded a further amount (0.41 g.) of the same material. After one recrystallization from carbon tetrachloride the product VIII melted at 86–86.5°; $[\alpha]_D^{20}$ –68.7° (*c* 0.72, chloroform). *Anal.* Calcd. for $C_{25}H_{23}O_9P$: C, 60.2; H, 4.6; P, 6.20. Found: C, 60.19; H, 5.04; P, 6.19.

The Preparation of VIII from D-Ribose.³⁰—Commercial ribose (50 g.) was suspended in 380 ml. of benzyl alcohol and treated at room temperature with 42 ml. of freshly prepared hydrogen chloride solution (11%) in benzyl alcohol. Most of the ribose dissolved with occasional shaking. After two hours the reaction was terminated by the addition of excess of silver carbonate. The insoluble salts were removed by filtration and benzyl alcohol distilled off *in vacuo* (10^{-2} mm.) using a rotary evaporator and two infrared lamps as heat source. The resulting viscous oil (57 g.) was taken up in 300 ml. of anhydrous pyridine and 60 ml. of diphenylphosphorochloridate added slowly with cooling. After being kept at room temperature overnight, 10 ml. of water was added to the reaction mixture and the solution concentrated. The heavy oil remaining was shaken with an excess of 0.5 *N* sodium hydroxide (200 ml.) for 25 minutes at room temperature and then extracted into ether (150 ml.). The ethereal solution was washed first with 0.5 *N* sodium hydroxide and then with water. After drying over anhydrous sodium sulfate, ethereal solution was evaporated and the oil dissolved in 200 ml. of anhydrous pyridine and treated with a twofold excess of phosgene dissolved in toluene. The mixture was left overnight at room temperature and water (10 ml.) was then added. The solution was concentrated to a heavy oil which was taken up in 300 ml. of ether. The ethereal solution was washed repeatedly with 5% sodium sulfate solution and then dried over anhydrous sodium sulfate. The ethereal solution was concentrated to about 80 ml. and carbon tetrachloride (100 ml.) then added. Further concentration to about 100 ml. and cooling produced a heavy microcrystalline precipitate (14 g., m.p. 84–85°). Recrystallization from carbon tetrachloride gave 12.5 g. of white needles with m.p. 85.5–86.5°.

α -D-Ribofuranose 1,5-Diphosphate (II).—Two grams of benzyl 5-diphenylphosphoryl ribofuranoside 2,3-cyclic carbonate (VIII) was dissolved in a mixture of (a) 20 ml. of acetic acid containing 10% acetic anhydride and (b) 20 ml. of 32% hydrogen bromide in acetic acid. The clear solution was left for three days at room temperature and then concentrated to a gum *in vacuo* at 30°. Last traces of acetic acid and benzyl bromide were removed by two evaporations in the presence of anhydrous xylene. The resulting pale yellow oil was dissolved in 20 ml. of anhydrous benzene, and to the solution was added 10 ml. of a 0.4 *M* benzene solution of triethylammonium dibenzylphosphate. After two hours at room temperature the triethylammonium bromide which had separated was removed by centrifugation and the supernatant solution concentrated under reduced pressure at room temperature. The resulting oil was dissolved in methyl alcohol (40 ml.) and the solution hydrogenated, first, in the presence of 5% palladium on charcoal (0.7 g.) and then 0.5 g. of Adams platinum oxide catalyst, the reaction vessel being immersed in an ice-bath during hydrogenation. After the hydrogen uptake ceased the catalysts were removed by filtration and 10 ml. of 4 *N* lithium hydroxide added to the alcoholic solution. The strongly basic solution was concentrated to a sirup *in vacuo* and then 40 ml. of water added. The alkaline solution was heated at 50° for five minutes and then cooled. The precipitate of trilithium phosphate was removed by centrifugation and the clear supernatant passed through a pyridinium Amberlite 1R-120 resin column (20 × 2.5 cm.). The effluent and water washings (total volume, about 150 ml.) were concentrated to about 15 ml. *in vacuo* at room temperature and then brought to pH 8 with barium hydroxide. The heavy precipitate was collected by centrifugation, washed successively with water, ethyl alcohol and ether and dried *in vacuo*. The weight of the white powder thus obtained was 1.51 g., a further amount (0.14 g.) of the barium salt being recovered from the mother liquors. The total product travelled as a single spot (R_f , 0.06) on paper chromatograms in solvent A and was free from inorganic phosphate (R_f , 0.16) and ribose

5-phosphate (R_f , 0.18). An enzymatically prepared sample of the ribose diphosphate³ had an R_f , 0.07.

The barium salt was converted to the cyclohexylammonium salt by stirring it (1.5 g.) in 50 ml. of water with 25 ml. of wet cyclohexylammonium Amberlite 1R-120 resin until it dissolved. The clear supernatant was then passed through a small column of the same resin (1 × 10 cm.) and the total resin used was washed thoroughly with water. The combined effluent was concentrated under reduced pressure at room temperature to a gum which was dissolved in 30 ml. of warm methyl alcohol. An equal volume of ether was added and the resulting turbid solution stored at 0°. The tetracyclohexylammonium salt, which separated as fine white needles, was collected and dried at room temperature for 24 hours in a high vacuum; m.p. 171–172° dec., $[\alpha]_D^{20} +20.8^\circ$ (*c* 0.43, in water).

Anal. Calcd. for $C_{29}H_{64}O_{11}N_4P_2 \cdot 4H_2O$: C, 44.72; H, 9.30; N, 7.19; P, 7.95. Found: C, 44.54; H, 9.16; N, 6.85; P, 8.37.

On treatment with 0.01 *N* hydrochloric acid at room temperature for three hours 52% of the acid labile phosphorus in II was released as inorganic phosphate. Under these conditions α -ribose 1-phosphate was hydrolyzed to the extent of 54%.

The Reaction of α -D-Ribose 1,5-Diphosphate with Dicyclohexylcarbodiimide.—The procedure used was that described earlier,¹³ except that solvent A was employed for paper chromatography. The following results were obtained. After one hour some of the starting material (R_f , 0.06) was present and a major spot (R_f , 0.17; periodate negative) and a minor spot (R_f , 0.28; periodate positive) appeared. After seven hours, only a very faint spot corresponding to original material was present. The spot with R_f 0.17 was still fairly strong and the weak spot with R_f 0.28 was still present. In addition, a strong spot (R_f , 0.66) and a minor spot (R_f , 0.81) had appeared.

α -D-Ribofuranose 1-Pyrophosphate 5-Phosphate (III).—V was prepared from 1 g. of VIII as described and was dissolved in 20 ml. of anhydrous benzene. The solution was added to a solution of triethylammonium tribenzylpyrophosphate^{24,31} (1.09 g.) in the same solvent (20 ml.). The solution was kept at room temperature for four hours and concentrated to a heavy oil *in vacuo* at low temperature without removal of the separated triethylammonium bromide. A solution of the oil in anhydrous methyl alcohol (30 ml.) was hydrogenated, first, in the presence of 0.5 g. of 5% palladium on charcoal and, then, of 1.5 g. of Adams platinum oxide catalyst. The hydrogenation vessel was immersed in an ice bath throughout. When the uptake of hydrogen ceased³² (4 hours), the catalysts were removed by filtration and the filtrate quickly adjusted to pH 7 with 1 *N* lithium hydroxide. Methyl alcohol was then removed *in vacuo* and the solution made alkaline (pH, about 12) by the further addition of lithium hydroxide. After four minutes at room temperature, the solution was neutralized with dilute acetic acid. An enzymatic assay showed 268 micromoles of III to be present (theory, 2000 micromoles). The solution was applied to the top of a column (12 cm. long × 2.4 cm. diameter) of Dowex 1-2% cross linked (chloride form) resin. Elution was carried out at 5° with 0.2 *M* lithium chloride solution containing 10 ml./liter of 1 *M* lithium acetate buffer, pH 6.5. Fifteen milliliter fractions were collected and the elution was followed by ribose and enzymatic assay. The fractions (11–25) containing the product were pooled, concentrated *in vacuo* at room temperature to about 10 ml. and the lithium salt (147 mg.) was precipitated by the addition of four volumes of ethyl alcohol. Further purification was accomplished by rechromatographing 117 mg. of this material on a similar column and eluting with 0.15 *M* lithium chloride solution. After a minor peak (tubes 35–39), III emerged in tubes 52–66. Using the procedure described above for isolation of the lithium salt, 75 mg. of a white powder was obtained. Analyses of this material indicated molar ratios for ribose:phosphorus:periodic acid uptake: enzymatic activity of 1:3.10:1.07:

(30) This direct synthesis was carried out when the present work was practically complete. Only one run has been performed and the procedure described probably can be refined in details with considerable improvement in the final yield.

(31) Tetrabenzyl pyrophosphate was prepared by the reaction of dibenzyl hydrogen phosphate with dicyclohexylcarbodiimide (H. G. Khorana and A. R. Todd, *J. Chem. Soc.*, 2257 (1953)). It was converted to sodium tribenzyl pyrophosphate by the method of Zervas and Dilaris (ref. 21b).

(32) Owing to the instability of the product, it is desirable to complete the hydrogenation in the shortest time possible.

0.80, in that order. *Anal.* Calcd. for $C_6H_7O_{14}P_3Li_5 \cdot 5H_2O$: P, 18.25. Found: P, 18.5.

Alkaline Degradation of Synthetic III.—A solution of the lithium salt of III obtained from the first column described above was prepared by dissolving 8 mg. in 2 ml. of water. Half of this solution was chromatographed on a small analytical column (9 × 50 mm.) of Dowex 1-formate form. Stepwise elution was carried out by passing the following eluents⁶ through the column, six-ml. fractions being collected: 60 ml. of 0.1 *M* sodium formate buffer, pH 5.0, to remove ribose monophosphates; 60 ml. of 0.25 *M* sodium formate buffer to remove ribose diphosphates and finally 60 ml. of 0.5 *M* sodium formate buffer of the same pH to remove III and any of the anomeric product. The fractions were analyzed for their ribose content. A small peak in the

ribose diphosphate region and a major peak using the 0.5 *M* eluent actually were obtained. The remaining half of the above solution was heated at 100° for ten minutes after adding 1 ml. of 0.1 *N* sodium hydroxide solution, and then neutralized and chromatographed under identical conditions. The major peak now obtained was in tube 14, the ribose diphosphate region. Only a trace of ribose containing material (optical density in ferric chloride-ornicol test, 0.081 against the figure 2.37 obtained for the major peak) was eluted in the ribose triphosphate (III) region.

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

The Synthesis of 9- α -D-Ribofuranosyladenine

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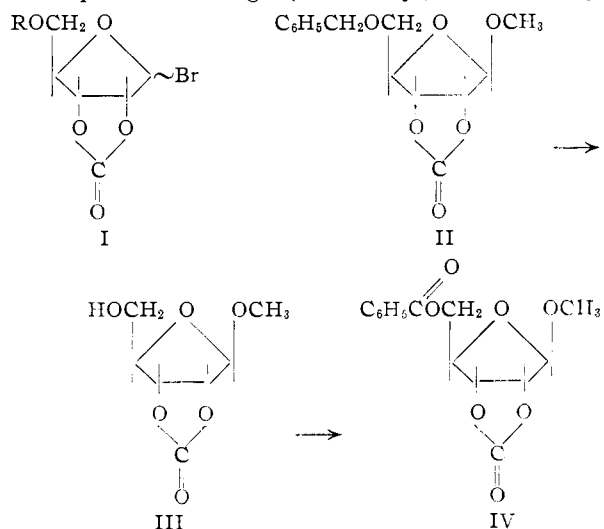
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The use of 5-*O*-benzoyl-D-ribofuranosyl bromide 2,3-cyclic carbonate (I, R = benzoyl) in the synthesis of α -nucleosides has been investigated. Condensation with chloromercuri-6-benzamidopurine and then removal of the protecting groups gave a 15% yield of 9- β -D-ribofuranosyladenine (adenosine) and a 24% yield of the new 9- α -D-ribofuranosyladenine. However, a similar procedure using chloromercuri 5,6-dimethylbenzimidazole gave only low yields of the anomeric 1-D-ribofuranosyl-5,6-dimethylbenzimidazoles.

Recent communications from this Laboratory have described syntheses of a number of phosphate esters of D-ribose, in which the substituents at C(1) have the α -configuration.¹⁻³ The key intermediates investigated in these studies were ribofuranosyl halides of the general type I, in which the 2- and 3-hydroxyl functions were protected by a cyclic carbonate group. This protecting group was chosen in place of the conventional acyl groups with a view to eliminating the well-known participation effect of the acyloxy group at C(2) in the replacement reactions at C(1), which leads very frequently to products having C(2)-C(1) *trans* configuration.⁴⁻⁶ The products obtained using intermediates of the type I were, in fact, found to be predominantly of the desired α -configuration¹⁻³ and these results encouraged us to investigate their use in the synthesis of α -nucleosides. No satisfactory method has been described hitherto for the preparation of this class of compounds which are of potential interest in metabolic studies and certain members of which occur as components of the vitamin B₁₂ group.^{7,13} In the present communication we record the experiments which have led to the synthesis of the hitherto undescribed 9- α -D-ribofuranosyladenine, anomeric with the well-known

ribonucleoside, adenosine. A brief account of a part of this work has been published.⁸

5-*O*-Acetyl-D-ribofuranosyl bromide 2,3-cyclic carbonate (I, R = acetyl), which was obtained by the treatment of II¹ with hydrogen bromide in acetic acid, was found to be insoluble in toluene or xylene, solvents which have proved suitable for the synthesis of nucleosides from acylglycosyl halides and mercuri derivatives of purines and pyrimidines.⁹⁻¹¹ Probably because of its insolubility, the experiments using I (R = acetyl) were not very



(1) G. M. Tener and H. G. Khorana, *THIS JOURNAL*, **79**, 437 (1957).

(2) G. M. Tener, R. S. Wright and H. G. Khorana, *ibid.*, **78**, 506 (1956); **79**, 441 (1957).

(3) G. M. Tener and H. G. Khorana, *Chemistry and Industry*, 562 (1957); *THIS JOURNAL*, **80**, 1999 (1958).

(4) For a comprehensive review of earlier literature on participation effects see R. U. Lemieux, *Advances in Carbohydrate Chem.*, **9**, 1 (1954).

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

(6) R. S. Wright and H. G. Khorana, *THIS JOURNAL*, **78**, 811 (1956).

(7) See for example, K. Folkers and D. E. Wolf, "Vitamins and Hormones," Vol. XII, Academic Press, Inc., New York, N. Y., 1954, p. 1; S. K. Kon, "The Biochemistry of Vitamin B₁₂," Cambridge University Press, 1955, p. 17.

promising and it was decided to vary the substituent at C(5) in I, in order to enhance its solubility.

(8) R. S. Wright, G. M. Tener and H. G. Khorana, *Chemistry and Industry*, 954 (1957).

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

(10) H. M. Kissman, C. Pidacks and B. R. Baker, *ibid.*, **77**, 18 (1955), and related papers in this series by Baker and co-workers.

(11) J. J. Fox and co-workers, *ibid.*, **78**, 2117 (1956); **79**, 5060 (1957).