m.p. 50–51°; [α]^{20}D −115.6° (c 3.52, ethyl alcohol). Anal. Calcd. for C₇H₁₀O₆: 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 mix-

ture of ether and petroleum ether to give needles with m.p. 74–74.5°; [α]^{20}D +129.6° (c 1.57, in ethyl alcohol). Anal. Calcd. for C₉H₁₂O₇: C, 46.72; H, 5.23. Found: C, 46.66; H, 5.37.

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

BY G. M. TENER, R. S. WRIGHT AND H. G. KHORANA

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.

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 C₂.[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.[6] We take this opportunity to point out that the latter formulation was also adopted by us in our earlier publication.[1]

from the ribopyranose 1-phosphate,[1] showed the absence of the latter in this product.[8]

Examination of the reaction of the pentofuranose 1-phosphates with dicyclohexylcarbodiimide in aqueous pyridine provides a convenient and rapid method for ascertaining their anomeric configuration.[10] Application of this technique to the product obtained above showed that it consisted largely of the α anomer I; however, some β-anomer was also present. These qualitative results were confirmed by fractional crystallization of the dicyclohexylammonium salts. The first major crop obtained represented ca. 75% of the total product and consisted of the pure α-anomer ($[\alpha]^{20}$D +40.3°). The mother liquor from the main crop yielded a small fraction with $[\alpha]^{20}$D −2.4°, obviously a mixture of the cyclohexylamine salts of the α- and β-anomers, from which a small amount of the salt of the pure β-anomer ($[\alpha]^{20}$D −13.6°) could be obtained.

The identity of the major crystalline product with the enzymatically prepared samples[11] was established by paper chromatography, reaction with dicyclohexylcarbodiimide and rate of acidic hydrolysis. This synthetic sample was fully active as a substrate for fish muscle purine nucleoside phosphorylase.[2b]

It should be noted that the over-all yield of the anomeric mixture of the ribose 1-phosphates, as based on the weight of the cyclic carbonate[4] V, is very satisfactory being approximately three times the yield of β-D-ribofuranose 1-phosphate obtained using 2,3,5-tri-O-benzoyl-D-ribofuranosyl bromide.[1] This high yield is to be ascribed to the absence of participation effects in the intermediate II employed. The net yield of the pure, biologically important α-anomer is also satisfactory and since the readily accessible anomeric mixture of the carbonates V may be used as the starting material,[4] the method described affords a convenient and straightforward synthesis of I.

(8) The possible formation of a ribopyranose 1-phosphate was considered on the basis of the following argument: the starting material in the preparation of the bromide II is methyl-5-O-benzyl-D-ribofuranose 2,3-cyclic carbonate, which undergoes (ref. 4) (a) acetolysis of the benzyl group and (b) mutarotation in the presence of hydrogen bromide–acetic acid–acetic anhydride. If the rapid acetolysis reaction involves the two steps (1) debenzylation resulting in the formation of IV (cf. removal of triphenylmethyl group by hydrogen bromide in acetic acid)[9] and (2) acetylation of IV, then during the concomitant

HOCH₂ ... OCH₃ IV
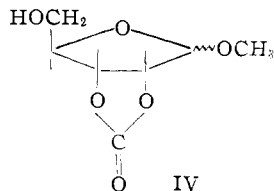
mutarotation, furanose → pyranose conversion might be possible. If so, then the product obtained would contain the pyranosyl bromide and this will then yield a ribopyranose 1-phosphate. Actually, when IV was carried through the reaction sequence, no pyranose 1-phosphate could be detected in the product.

(9) J. L. Barclay, A. B. Foster and W. G. Overend, *J. Chem. Soc.*, 2505 (1955).

(10) See H. G. Khorana, G. M. Tener, R. S. Wright and J. G. Moffatt, This Journal, **79**, 430 (1957), for a detailed discussion of the reactions of sugar phosphates with dicyclohexylcarbodiimide.

(11) We are grateful to Drs. Hayes and Kalckar and Dr. H. L. A. Tarr for the enzymatic samples.

In an alternative approach to the synthesis of I, the use of the crystalline, although rather unstable, 3,5-di-O-benzoyl-D-ribofuranosyl halides[6,7] (III) appeared promising. The replacement reaction at C_1 in these compounds might be expected to be relatively free from the participation effect[12] and,

C₆H₅CH₂OCH₂ ... OCH₃ V
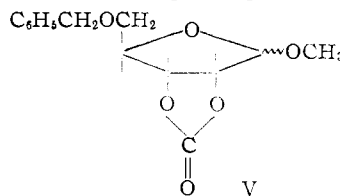
indeed, Ness and Fletcher[7] recently have obtained a 35% yield of 1,3,5-tri-O-benzoyl-α-D-ribofuranose by the reaction of (III, X = Cl) with silver benzoate. In the present work, the freshly prepared halides III were brought into reaction with triethylammonium dibenzyl phosphate and the products were carried through the subsequent standard steps. In both cases, only moderate yields of ribose 1-phosphate were obtained. On the basis of the reaction with dicyclohexylcarbodiimide and the optical rotations of the crystalline cyclohexylamine salts, prepared from the barium salts, the products were found to be anomeric mixtures. So far, it has not been possible to isolate a pure sample of the α-anomer by this method.

Experimental

α-D-Ribofuranose 1-Phosphate, Using Methyl 5-O-Benzyl-D-Ribofuranoside 2,3-Cyclic Carbonate.—Three g. of the crystalline methyl 5-O-benzyl-β-D-ribofuranoside 2,3-cyclic carbonate (or the oily mixture of the α- and the β-anomers[4]) was dissolved in 10 ml. of a mixture of acetic acid and acetic anhydride (commercial glacial acetic acid, 250 ml., acetic anhydride 11 ml.) and to the solution was added another 10 ml. of 32% hydrogen bromide solution in acetic acid. The clear solution was heated for 5 hours at 55° with exclusion of moisture and the resulting dark brown solution concentrated to dryness *in vacuo*. The last traces of the acidic mixture and benzyl bromide were removed by repeated evaporation three times in the presence of xylene and subsequent drying at 50° in a high vacuum for one-half hour. To a solution of the dark oil in 20 ml. of anhydrous benzene was added a solution of 4.16 g. of triethylammonium dibenzyl phosphate (prepared by mixing stoichiometric amounts of dibenzyl hydrogen phosphate and triethylamine) in 20 ml. of benzene. After one hour at room temperature, triethylamine hydrobromide (70% of theory) was removed by centrifugation and the clear supernatant evaporated under vacuum to an oil which was hydrogenated in anhydrous methyl alcohol using freshly prepared palladium (5%) on charcoal catalyst. After 30 minutes when the hydrogen uptake had ceased, the catalyst was removed by filtration and to the filtrate was added immediately 15 ml. of 4 N lithium hydroxide solution. The methanol was removed *in vacuo* and replaced by 25 ml. of water. The solution, which should be alkaline (pH 11 or above) at this stage, was heated in a polyethylene tube for 10 minutes at 100° and then cooled. Tri-lithium phosphate which crystallized was removed by centrifugation and the clear solution was passed through a column of Amberlite IR-120 resin (pyridinium form). The total effluent, after washing the column thoroughly with water, was concentrated *in vacuo* and brought to pH 9 with barium hydroxide; total volume ca. 40 ml. The barium salts of ribofuranose 1-phosphates were precipitated by the addition of five volumes of ethyl alcohol, collected by centrifugation, washed with

(12) The studies of Lemieux and Huber (*Can. J. Chem.*, **31**, 1040 (1953); **33**, 128 (1955)) on the reactions of the anomeric 3,4,6-tri-O-acetyl-1-D-glucosyl chlorides have shown the virtual absence of neighboring group participation in replacement reactions at C_1 in these compounds.

acetone then ether and dried *in vacuo*. The product was taken up in water, a trace of barium phosphate removed by centrifugation and the organic phosphates reprecipitated by the addition of ethyl alcohol and treated further as above to give a yield of 2.3 g. (*ca.* 55%). (The yield in different runs varied between 50–60%.) Descending paper chromatography in the solvent system isopropyl alcohol–ammonia–water (70-10-20, v.v.) showed a single spot (R_f 0.16) corresponding to ribofuranose 1-phosphate. Ribose (R_f 0.65), inorganic phosphate (R_f 0.08) and ribopyranose 1-phosphate[1] (R_f 0.11) all were absent.

The Preparation and Fractional Crystallization of Dicyclohexylammonium α-D-Ribofuranose 1-Phosphate.—The barium salt (1.98 g.), as prepared above, was dissolved in *ca.* 10 ml. of water, freed from a small amount of insoluble barium phosphate,[13] and the clear solution passed through a column (10 cm. × 1.5 cm. diameter) of Amberlite 1R-120 cyclohexylammonium form. The combined effluent and water wash was concentrated to dryness *in vacuo* at 40° and the residue dissolved in 20 ml. of methyl alcohol. Ether was added to turbidity and the mixture set aside at 0°. The crystalline product which separated overnight was collected by filtration and washed with ether containing 25% methyl alcohol; yield 1.5 g., $[\alpha]^{20}$D +40.3° (*c* 2.37, water). *Anal.* Calcd. for $C_{17}H_{37}O_8N_2P$: C, 47.6; H, 8.70; N, 6.54; P, 7.23. $C_{17}H_{37}O_8N_2P \cdot 1H_2O$: C, 45.74; H, 8.81; P, 6.94. Found in air dried material: C, 45.38; H, 8.82; P, 7.4. After drying in a high vacuum at room temperature over phosphorus pentoxide; C, 47.06; H, 9.17; N, 6.51; P, 7.8.

A small sample (10 mg.) of this material was converted to the pyridinium salt, using an ion-exchange resin, and brought into reaction with an excess (25 mg.) of dicyclohexylcarbodiimide in 80% aqueous pyridine at 0°. Paper chromatography[10] showed the complete conversion of the starting material to faster travelling products, thus indicating the complete absence of β-D-ribofuranose 1-phosphate[1] in this material.

The mother liquor from the above crystalline cyclohexylammonium salt gave on concentration and dilution with ether two small crops of crystals, weighing 0.25 and 0.2 g.

(13) The barium salts tend to decompose in the solid state, even on storage at low temperature, to form barium phosphate.

(highly colored) with $[\alpha]^{20}$D −2.4° and −11.3°, respectively. Recrystallization of the first of these crops afforded pure dicyclohexylammonium β-D-ribofuranose 1-phosphate ($[\alpha]^{20}$D −13.6° (*c* 2.0, ethyl alcohol)). No cyclic phosphate formation could be detected when the latter was treated with dicyclohexylcarbodiimide.[10]

D-Ribofuranose 1-Phosphates Using 3,5-Di-O-benzoyl-D-ribofuranosyl Halides. (a) Chloride[7].—3,5-Di-O-benzoyl-D-ribofuranosyl chloride (1.04 g.) in 5 ml. of dry benzene was treated at room temperature with a benzene solution containing one equivalent of triethylammonium dibenzyl phosphate. Triethylamine hydrochloride, which began to separate after 10 minutes, was removed after a total period of 3 hours by filtration (0.299 g., 78%) and the filtrate concentrated *in vacuo* at room temperature. The resulting thick syrup was hydrogenated in 50 ml. of anhydrous methyl alcohol at 0° in the presence of freshly prepared palladium catalyst. After about 1 hour, when the hydrogen uptake was complete, the catalyst was removed, 15 ml. of water added and the solution brought to and maintained at *p*H 11.3 with 1 *N* sodium hydroxide. After 3 hours at room temperature an excess of the pyridinium form 1R-120 ion-exchange resin (10 ml.) was added and the mixture well stirred for 10 minutes. The resin was then removed and washed with water and the combined solution and washings were concentrated *in vacuo* to 5 ml. with occasional additions of a few drops of pyridine. The product was isolated as the barium salt (0.454 g.) and converted to the crystalline cyclohexylammonium salt in the manner described above. The specific rotation of this sample, $[\alpha]^{20}$D +16.4° (*c* 2.7, water) and a study of its reaction with dicyclohexylcarbodiimide showed it to be a mixture of the α- and β-D-ribofuranose 1-phosphates.

(b) Bromide.—3,5-Di-O-benzoyl-D-ribofuranosyl bromide[6] (0.59 g.) gave, in the above method, 0.140 g. of the barium salt of a product which again was a mixture of the α- and the β-D-ribofuranose 1-phosphates.

[CONTRIBUTION FROM THE DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY, UNIVERSITY OF MINNESOTA]

The Structure of Chagual Gum. I. Composition of the Gum and Isolation of 2-O-(D-Glucopyranosiduronic Acid)-D-xylose[1]

BY J. K. HAMILTON, D. R. SPRIESTERSBACH AND F. SMITH

Chagual gum, an exudate from species of *Puya* produced as a result of damage by insects, is the neutral salt of the polysaccharide acid which is shown by hydrolysis to be composed of arabinose, xylose, galactose and glucuronic acid. Graded hydrolysis of the gum gives an aldobiouronic acid, the structure of which has been shown to be 2-O-(D-glucopyranosiduronic acid)-D-xylose.

Chagual gum is obtained from species of *Puya* (*P. chilensis, P. lanuginosa* and *P. lanata*), the best known of which is *Puya chilensis* found on the slopes of the Andes in South America. The gum exudate, produced as a result of damage by the larvae of the insect *Kastnia elegans*, forms clear pale yellow globules which are only partly soluble in water, the remainder swelling to form jelly-like masses.[2] This gum is of interest not only because it is generated by the plant after injury but also

(1) Paper No. 3536 Scientific Journal Series, Minnesota Agricultural Experiment Station, University of Minnesota. This paper forms part of a thesis submitted by D. R. Spriestersbach to the Graduate School of the University of Minnesota in partial fulfillment for the degree of Ph.D., 1954.

(2) F. N. Howes, "Vegetable Gums and Resins," Chronica Botanica Company, Waltham, Mass., 1949.

because unlike other plant gum exudates[3] it contains a relatively large amount of xylose and a small amount of arabinose.

Chagual gum has been reported to contain galactose since it gave mucic acid upon oxidation with nitric acid and the presence of pentose sugars was revealed by the formation of furfural when the gum was treated with hydrochloric acid. Moreover acid hydrolysis furnished xylose and galactose and it was further suggested that some of the galactose belonged to the L-series.[4]

The native gum ($[\alpha]_D − 30°$ (NaOH)) investigated in this work was only partially soluble in water but

(3) J. K. N. Jones and F. Smith, *Advances in Carb. Chem.*, **4**, 243 (1949).

(4) E. Wintersteiner, *Ber.*, **31**, 1571 (1898).