0.80, in that order. *Anal.* Calcd. for $C_5H_7O_{14}P_3Li_5 \cdot 5H_2O$: 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 eluents[6] through the column, six-ml. fractions being collected: 60 ml. of 0.1 *M* sodium formate buffer, *p*H 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 *p*H 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-orcinol 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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VANCOUVER 8, B. C., CANADA

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

The Synthesis of 9-α-D-Ribofuranosyladenine

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

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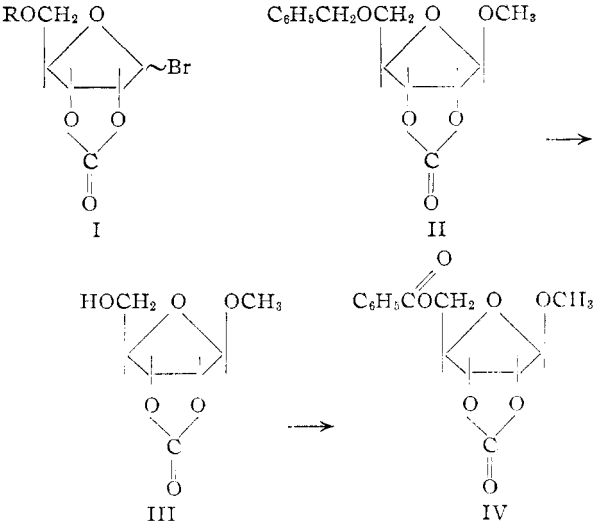
The use of 5-*O*-benzoyl-D-ribofuranosyl bromide 2,3-cyclic carbonate (I, R = benzoyl) in the synthesis of α-nucleosides has been investigated. Condensation with chloromercuri-6-benzamidopurine and then removal of the protecting groups gave a 15% yield of 9-β-D-ribofuranosyladenine (adenosine) and a 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-configuration[1-3] 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 B_{12} 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 II[1] 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 promising and it was decided to vary the substituent at C(5) in I, in order to enhance its solubility.



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

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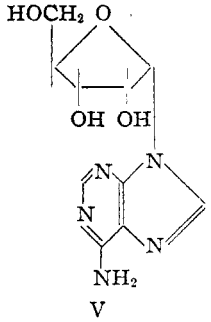
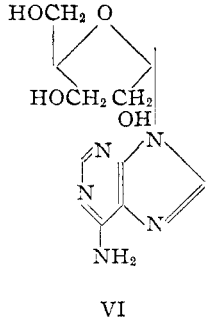
The crystalline methyl β-D-ribofuranoside 2,3-cyclic carbonate (III) was prepared from II by hydrogenolysis and was benzoylated in anhydrous pyridine to give an excellent yield of the crystalline IV. Treatment of the latter with hydrogen bromide–acetic acid mixture gave the oily I (R = benzoyl) which was found to be soluble in xylene at 50° or above.

Chloromercuri-6-benzamidopurine has proved a useful derivative in the synthesis of a number of adenine nucleosides[9,12] and its condensation with I (R = benzoyl) in boiling xylene was investigated in the present work. After a reaction period of one hour, the protecting groups were removed from the products with sodium methoxide in boiling methyl alcohol. Partition chromatography using a cellulose powder column gave, first, a 15% yield of 9-β-D-ribofuranosyladenine (adenosine) and then a 24% yield of a new crystalline compound (m.p. 201°) which has been shown to be 9-α-D-ribofuranosyladenine (V). Thus, the new product was isomeric in molecular composition with adenosine and gave adenine and ribose after mild acidic hydrolysis. It consumed as expected one mole of periodic acid. That the attachment of the ribofuranose moiety is at the 9-position of the adenine ring followed from its ultraviolet light absorption characteristics (λ_{max} 257 mμ in acid; 259 mμ in alkali), which are very similar to those of adenosine. In this connection it may be noted that the isomeric 7-α-D-ribofuranosyladenine, a substance with spectral characteristics different from those of the 9-substituted adenines, has recently been isolated from pseudovitamin B_{12} by Friedrich and Bernhauer.[13]

The anomeric relationship between the new product and adenosine was also supported by its optical rotation ([α]D + 24°) (adenosine, [α]D −60.4°) and was confirmed in the following way. Adenosine and the new compound were separately oxidized with periodic acid and the products, presumably dialdehydes, were reduced with sodium borohydride. The final products (VI and its anomer) had equal and opposite rotations.



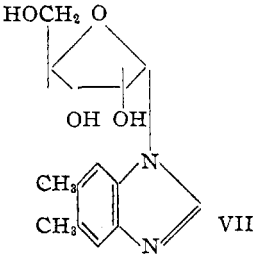
V



VI

The synthetic 9-α-D-ribofuranosyladenine was not attacked[14] by the bacterial nucleoside hydrolase which has been described recently by Takagi and Horecker.[15] This observation is in agreement with the previous studies of these workers who found this enzyme to be specific for β-D-ribofuranosides.

The above results were encouraging in that the desired product, the α-nucleoside, was formed predominantly and that its yield was relatively satisfactory. However, some further experiments on the condensation of I (R = benzoyl) with chloromercuri 5,6-dimethylbenzimidazole were less successful. Only low yields of both anomers of the 1-D-ribofuranosyl-5,6-dimethylbenzimidazole were obtained. In these products, which were separated as their crystalline 2′,3′-isopropylidene derivatives[16] on a silicic acid column, the α-anomer VII appeared to predominate again.



VII

In spite of the low yield obtained in the synthesis of α-D-ribofuranosyl-5,6-dimethylbenzimidazole, we believe that the use of ribofuranosyl halides of the type I is worthy of further study as the basis of a general method for the synthesis of α-nucleosides.

Experimental

Methyl 5-*O*-Benzoyl-β-D-ribofuranoside 2,3-Cyclic Carbonate (IV).—Five grams of methyl 5-*O*-benzyl-β-D-ribofuranoside 2,3-cyclic carbonate[1] was dissolved in 200 ml. of methyl alcohol and the solution shaken with hydrogen in the presence of 0.5 g. of 5% palladium on carbon until the uptake of hydrogen ceased (2.5 hours). The catalyst was then removed by filtration and the solution concentrated *in vacuo*, the last traces of methyl alcohol being removed by two evaporations *in vacuo* with 20-ml. portions of pyridine. The residue was dissolved in 8 ml. of anhydrous pyridine and treated under cooling with 8 ml. of benzoyl chloride. The reaction mixture was kept overnight at room temperature and then heated at 40° for one hour. The excess of benzoyl chloride then was decomposed by the addition of ice and the product extracted with chloroform. The extract was washed first with water, then with sodium bicarbonate and then again with water. Evaporation of the solution gave a gum which was crystallized first from benzene–petroleum ether and then from methyl alcohol to give 4.1 g. (80%) of methyl 5-*O*-benzoyl-β-D-ribofuranoside 2,3-cyclic carbonate with m.p. 88°.

Anal. Calcd. for $C_{14}H_{14}O_7$: C, 57.14; H, 4.79. Found: C, 57.02; H, 5.03.

9-α-D-Ribofuranosyladenine (V).—5-*O*-Benzoyl-D-ribofuranosyl bromide 2,3-cyclic carbonate (I, R = benzoyl) was prepared as follows. Methyl 5-*O*-benzoyl-β-D-ribofuranoside 2,3-cyclic carbonate (IV) (2.5 g.) was dissolved in 10 ml. of 32% hydrogen bromide in acetic acid and acetic anhydride (1 ml.) was added to the solution. The mixture was heated at 75° for four hours with exclusion of moisture and then concentrated to a sirup *in vacuo*. The last traces of the reagents were removed by two co-distillations with toluene *in vacuo*.

A suspension of 4.03 g. of chloromercuri-6-benzamidopurine[12] and 1 g. of acid-washed Celite in 50 ml. of xylene was freed from moisture by azeotropic distillation of half the xylene with rapid mechanical stirring. A solution of the above bromide I in 20 ml. of dry xylene was then added and

(12) B. R. Baker and K. Hewson, *J. Org. Chem.*, **22**, 966 (1957). We wish to thank Dr. J. A. Johnson, Jr., of Southern Research Institute Alabama and Dr. B. R. Baker for communicating their procedure for the preparation of this substance. This procedure is based on a modification introduced by Dr. J. J. Fox (ref. 11).

(13) W. Friedrich and K. Bernhauer, *Chem. Ber.*, **89**, 2507 (1956).

(14) We are grateful to Dr. W. E. Razzell of this Laboratory for the enzymatic test.

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the stirred reaction mixture heated under reflux for one hour. The mixture was then concentrated to dryness *in vacuo* and the powdered residue extracted several times with chloroform. The combined chloroform extracts were washed twice with 30% aqueous potassium iodide, then water and dried over sodium sulfate. Evaporation gave 3.23 g. of a gum which was dissolved in 30 ml. of anhydrous methyl alcohol. Sodium methoxide (2 ml. of 1 *N*) was added and the solution heated under reflux for one-half hour. Water (20 ml.) was then added followed by successive small portions of pyridinium Amberlite IR-120 resin until the solution had reached *p*H 9. It was then evaporated *in vacuo* and a small amount of methyl benzoate remaining was removed by two distillations with water. The residue was dissolved in 70 ml. of a *n*-butyl alcohol–water (86:14, v./v.) mixture with warming and the solution applied to the top of a 45 cm. long × 7 cm. diameter column of Whatman cellulose powder packed with the same solvent. Elution was begun using the same solvent mixture. Ultraviolet absorbing material appeared in the effluent after a total of 1600 ml. of liquid had passed through the column. The eluate was then collected in 9-ml. fractions, the total ultraviolet absorbing material being eluted in 140 fractions. Paper chromatographic examination of suitably chosen fractions in the same butyl alcohol–water system showed that tubes 1–26 contained pure adenine (R_f, 0.39, 0.16 g.). Tubes number 32–80, which contained 9-β-D-ribofuranosyladenine (R_f, 0.20), were combined and evaporated. The residue was dissolved in water, the solution extracted once with a small volume of ether and then evaporated to dryness to give chromatographically pure adenosine (0.34 g., 15%); m.p. after two crystallizations from water, 233°; $[\alpha]_D$ −60.4° (*c* 0.7, water). Tubes 86–140, which contained chromatographically homogeneous material (R_f, 0.17), were combined and concentrated. The residual solid was dissolved in water and the solution extracted with a small volume of ether. Evaporation of the aqueous solution gave 0.54 g. of 9-α-D-ribofuranosyladenine (V) which crystallized from methyl alcohol–ether to give prisms with m.p. 201°; $[\alpha]_D$ +24° (*c* 0.65, water); λ_{max} at neutral *p*H, 259 mμ; ϵ_{max} 14,500; λ_{max} in acid, 257 mμ.

Anal. Calcd. for $C_{10}H_{13}O_4N_5$: C, 45.0; H, 4.9; N, 26.2. Found: C, 45.6; H, 4.9; N, 26.0.

It consumed 1 mole of periodic acid as determined spectrophotometrically[17] and was hydrolyzed by boiling 0.05 *N* hydrochloric acid to give adenine and D-ribose, as shown by paper chromatography. The α-nucleoside formed a crystalline picrate which decomposed at 190°.

Anal. Calcd. for $C_{16}H_{16}O_{11}N_8$: C, 38.7; H, 3.2. Found: C, 38.5; H, 3.5.

About 20 mg. of the above 9-α-D-ribofuranosyladenine and authentic adenosine were separately treated with 1.5 ml. of 0.08 *M* sodium periodate solution and the mixture kept for about ten minutes at room temperature. Forty milligrams of sodium borohydride then was added to each solution, followed after one-half hour by the slow addition of 0.5 ml. of 10% acetic acid. When the evolution of gas ceased the specific rotation of each solution was determined in a Keston Polarimeter. The solution containing the 9-α-D-ribofuranosyladenine had $[\alpha]_D$ −66°, the solution containing the β-anomer had $[\alpha]_D$ +66°, based upon weights of the starting materials.

Anomeric 1-D-Ribofuranosyl-5,6-dimethylbenzimidazoles.—To an azeotropically dried suspension of 0.762 g. of chloromercuri-5,6-dimethylbenzimidazole[18] and 0.2 g. of acid-washed Celite in xylene was added a xylene solution of 5-*O*-benzoyl-D-ribofuranosyl bromide 2,3-cyclic carbonate, prepared from 0.588 g. of methyl 5-*O*-benzoyl-β-D-ribofuranoside 2,3-cyclic carbonate. The mixture was heated under reflux with rapid stirring for one hour and then diluted with three volumes of chloroform. It was filtered from the insolubles and the residue washed with chloroform. The combined filtrates were washed, first, with 30% potassium iodide solution (twice), then water and dried over sodium sulfate. Removal of the solvent *in vacuo* gave 0.40 g. of a gum which was dissolved in 20 ml. of anhydrous methyl alcohol. One milliliter of 1 *N* sodium methoxide was then added and the solution heated under reflux for one-half hour. It was neutralized by the dropwise addition of dilute sulfuric acid and then evaporated. The residue was twice evaporated with acetone and then taken up in 30 ml. of anhydrous acetone. Two milliliters of concentrated sulfuric acid was added and the mixture kept at room temperature for one hour. It was poured into an excess of sodium carbonate solution and the mixture evaporated to remove acetone. The product was extracted into chloroform and applied as a solution in 20 ml. of 1:1 mixture of benzene and ether on to the top of a silicic acid column (25 cm. long × 2 cm.). Elution was begun with benzene containing increasing amounts of ether. The nucleosides came off with benzene containing 40% ether. The first product, 2′,3′-*O*-isopropylidene-1-β-D-ribofuranosyl-5,6-dimethylbenzimidazole crystallized from acetone–light petroleum. The melting point of this sample (10 mg.) after one crystallization was 186° and it was not depressed on admixture with a sample of this substance prepared by the route: treatment of chloromercuri-5,6-dimethylbenzimidazole[18] with 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide,[19] and then treatment with alkali and with acetone and sulfuric acid.

After the elution of the β-anomer, an intermediate fraction (5 mg., m.p. 162°) was obtained and finally, the 2′,3′-*O*-isopropylidene 1-α-D-ribofuranosyl-5,6-dimethylbenzimidazole (12 mg.). The melting point after one crystallization was 176°; Holly, *et al.*,[18] record 181–181.5°.

Paper Chromatography of Ribofuranosyl-5,6-dimethylbenzimidazoles.—The solvent system, apparently new, which was found satisfactory for this purpose was *n*-butyl alcohol–water–concentrated hydrochloric acid (8–1–1, v./v.) The descending technique was employed and the R_f values noted were as follows: 5,6-dimethylbenzimidazole, 0.76; 1-β-D-ribofuranosyl-5,6-dimethylbenzimidazole, 0.57; 1-α-D-ribofuranosyl-5,6-dimethylbenzimidazole, 0.54.

Acknowledgment.—We wish to thank the National Research Council of Canada, Ottawa, for the financial support of this work.

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