decreases the color yield in the phenol–sulfuric acid reaction in an undefined manner.[20] Both of these compounds are converted to volatile derivatives (glycolic aldehyde dimethylacetal and trimethyl borate) by methanolysis and are eliminated by evaporation of the solvent. The complete removal of glycolic aldehyde dimethylacetal may be checked by testing the residue with the diphenylamine reagent.[21] In the event of incomplete removal of the aldehyde, the methanolysis and subsequent distillation is repeated. Glycerol, formed from terminal nonreducing hexopyranose and pentofuranose units, and erythritol, formed from nonterminal (1→4)-linked hexopyranose units, do not interfere with the glucose determination (Fig. 1).

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Polynucleotides. II. Synthesis of (3′→5′)-Linked Diribonucleoside Phosphates Containing Uridine

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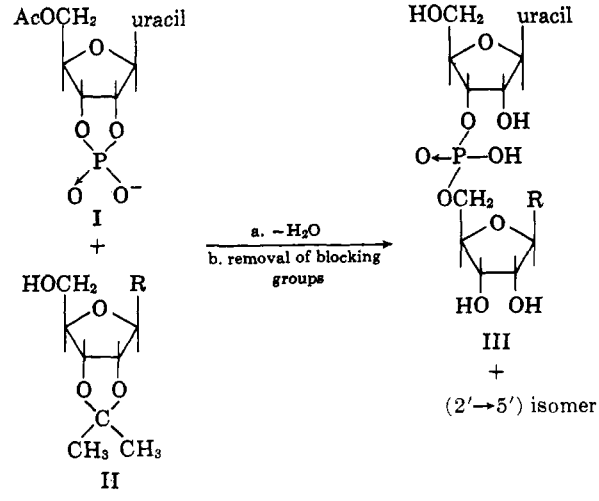
In the past few years, the syntheses of several (3′→5′)-linked diribonucleoside phosphates have been reported.[1-5] These syntheses fall into two groups, namely those which produce the (3′→5′) internucleotide bond and those which produce a mixture of (2′→5′)- and (3′→5′)-linked isomers. The second type of synthesis was utilized by us, and an ion-exchange procedure was developed which resolves the mixed isomers. The internucleotide bond was formed by treating 5′-*O*-acetyluridine-2′,3′ cyclic phosphate with a nucleoside blocked in the 2′- and 3′-positions in the presence of diphenyl phosphorochloridate. The following diribonucleoside phosphates have been prepared: uridylyl(3′→5′)-5-bromouridine, uridylyl(3′→5′)uridine, uridylyl(3′→5′)-6-thioinosine, and uridylyl(3′→5′)inosine.

One of the major problems in the synthesis of (3′→5′)-linked diribonucleoside phosphates stems from the requirement for an intermediate blocked in the 2′- and 5′-positions. The key intermediate can be prepared either by acetylation of the 2′-position of nucleoside-3′ phosphates[6] or by phosphorylation of ribonucleosides blocked in the 2′- and 5′-positions such as the easily prepared 2′,5′-di-*O*-trityluridine.[7] The first approach relies on the ability to obtain pure 3′-ribonucleotides and the second is limited to those nucleosides for which selective blocking of the 2′- and 5′-positions is practical. The difficulties in preparing diribonucleoside phosphates can be ameliorated to some extent if selective blocking of the 2′- or 3′-position is eliminated with the result that the final product becomes a mixture of (2′→5′)- and (3′→5′)-linked isomers. The usefulness of this method, therefore, rests on the ability to resolve the isomers. One of the first exponents of this approach to oligonucleotide syntheses was Michelson,[5] who prepared a series of [2′(3′)′→5′]-linked diribonucleoside phosphates and in some cases was able to separate the isomers. Such an approach to the synthesis of diribonucleoside phosphates results in a reduction of the number of steps and increased over-all yields. We have examined this approach with the intention of ascertaining its general usefulness for the synthesis of oligonucleotides and this paper describes the preparation of diribonucleoside phosphates in which uridylyl-3′ phosphate is linked to the 5′-position of 5-bromouridine, uridine, inosine, and 6-thioinosine.

The internucleotide bond was formed according to the method developed by Michelson[5] in which a ribonucleoside-2′,3′ cyclic phosphate (I) reacts with a 2′,3′-isopropylidene derivative of a ribonucleoside (II) under the influence of diphenyl phosphorochloridate and in the presence of tri-*n*-butylamine. Ready availability of ribonucleoside-2′,3′ cyclic phosphates is essential to



a, R = 5-bromouracil
b, R = uracil
c, R = hypoxanthine
d, R = 6-mercaptopurine

(1) Part I, R. H. Hall, and R. Thedford, *J. Org. Chem.*, **28**, 1506 (1963).

(2) D. H. Rammler and H. G. Khorana, *J. Am. Chem. Soc.*, **84**, 3112 (1962).

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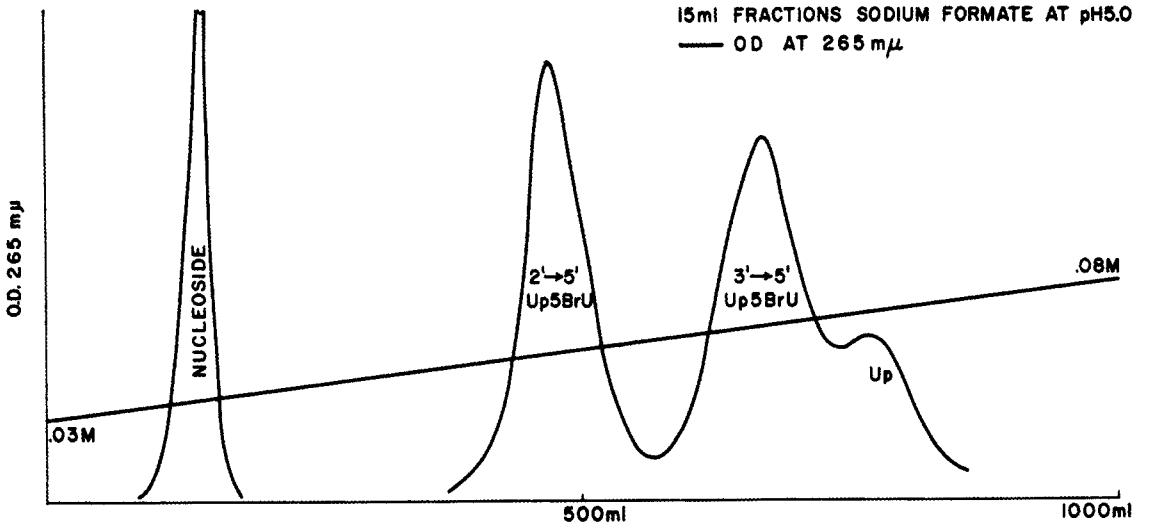

Fig. 1.—Ion-exchange chromatographic separation of uridylyl[2′(3′)→5′]-5-bromouridine—[2′(3′)→5′]Up5BrU, 0.1-mmole scale, using a Dowex-1X2 formate column, 14 × 0.9 cm.

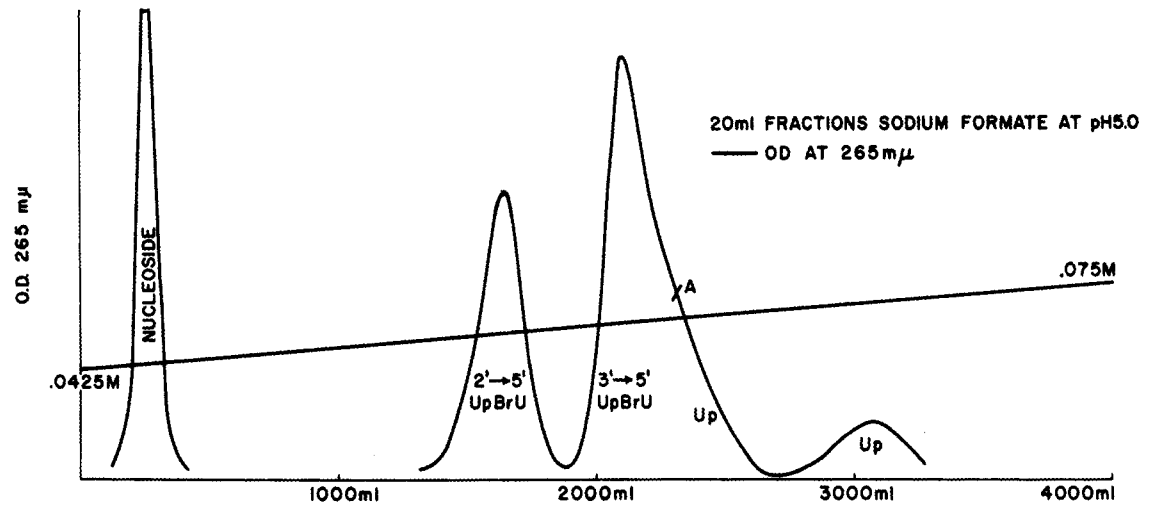

Fig. 2.—Ion-exchange chromatographic separation of uridylyl[2′(3′)→5′]-5-bromouridine—[2′(3′)→5′]Up5BrU, 1.0-mmole scale, using a Dowex-1X2 formate column, 42 × 1.2 cm.

the usefulness of this method and a number of methods have been described for their preparation. For our purposes the ethyl chloroformate method of Michelson[5] with some modifications was found to be the most satisfactory route to the formation of nucleoside-2′,3′ cyclic phosphates. Ethyl chloroformate can be used in aqueous solution, but the hydrochloric acid which is released during the reaction must be effectively neutralized since cyclic phosphates are unstable at pH values of less than 4.0 or more than 9.0.[8] Using the reaction conditions as previously described[5] for the preparation of uridine-2′,3′ cyclic phosphate, only 70% of cyclized phosphate was obtained, but, upon addition of a greater excess of tri-*n*-butylamine, a yield of 100% was obtained. Since tri-*n*-butylamine has only a limited solubility in water, there was no danger of hydrolyzing the cyclic phosphate by making the reaction medium too basic.

The first diribonucleoside phosphate prepared in this series was uridylyl(3′→5′)-5-bromouridine (IIIa). The nucleotide intermediate, 5′-*O*-acetyluridine-2′,3′ cyclic phosphate (I), had been prepared previously by Smrt and Šorm[3] who used dicyclohexyl carbodiimide (DCC) for the preparation of the cyclic phosphate. For our purposes, we found the ethyl chloroformate method more suitable, as it was not necessary to isolate the product before it was treated with IIa. Compound IIa was prepared by the method developed by Hampton[9] and the product was conveniently isolated from the reaction mixture by using partition chromatography on Celite-545 according to the method of Hall.[10] This compound had been prepared previously by Smrt and Šorm,[11] and Ueda.[12] Formation of the internucleotide bond to form uridylyl[2′(3′)→5′]-5-bromouridine (IIIa) was carried out in anhydrous dioxane. In order to ensure that the intermediates of the reaction were scrupulously dry, tri-*n*-butylammonium 5′-*O*-acetyluridine-2′,3′ cyclic phosphate and 2′,3′-isopropylidene-5-bromouridine were dissolved previously in dioxane

(8) D. M. Brown, C. A. Dekker, and A. R. Todd, *J. Chem. Soc.*, 2715 (1952).

(9) A. Hampton, *J. Am. Chem. Soc.*, **83**, 3640 (1961).

(10) R. H. Hall, *J. Biol. Chem.*, **237**, 2283 (1962).

(11) J. Smrt and F. Šorm, *Collection Czech. Chem. Commun.*, **25**, 553 (1960).

(12) T. Ueda, *Chem. Pharm. Bull.* (Tokyo), **8**, 455 (1960).

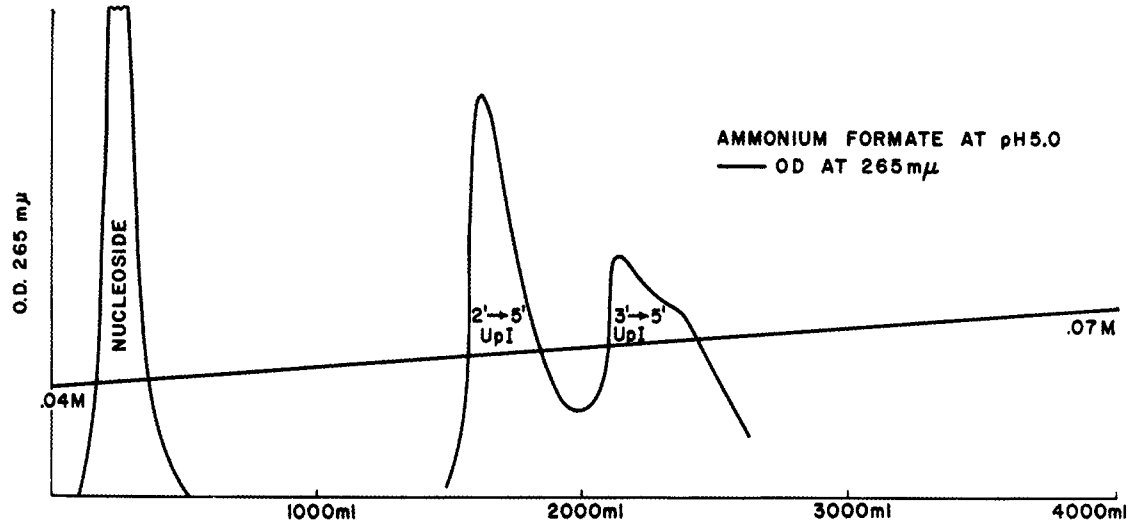

Fig. 3.—Ion-exchange chromatographic separation of uridylyl[2′(3′)→5′]inosine—[2′(3′)→5′]UpI, 1.0-mmole scale, using a Dowex-1X2 formate column, 42 × 1.2 cm.

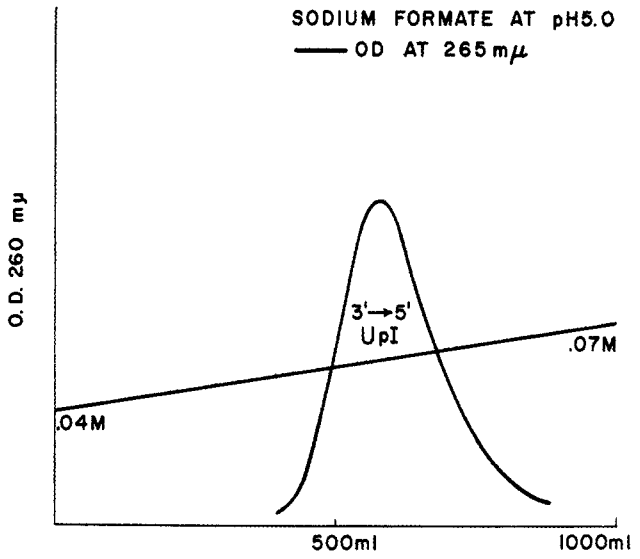

Fig. 4.—Ion-exchange chromatographic purification of uridylyl-(3′→5′)inosine isolated from peak shown in Fig. 3, using a Dowex 1X2 formate column, 25 × 0.9 cm.

and the solution was freeze-dried.[13] Freeze-drying was repeated three times after which the residue was dissolved in dioxane and diphenyl phosphorochloridate was added. The diphenylphosphorochloridate, although a phosphorylating agent, does not appear to phosphorylate the free nucleoside 5′-hydroxyl group but presumably reacts with the cyclic phosphate, providing energy for the desired phosphorylation.[14]

Dowex-1X2 (formate) ion-exchange resin was used for the chromatographic separation. Separation of the reaction products was undertaken on a 0.1-mmole scale, using a small column and a fairly steep formate gradient as shown in Fig. 1. Chromatography was then scaled up to a 1-mmole level using a less steep formate gradient (Fig. 2). Attempts to separate the products of the reaction at pH below 5.0 were made, but the separation was not satisfactory. The optimum pH for this type of separation appears to lie between 5.0 and 5.5, which corresponds to the conditions required for separation of uridine-2′ phosphate and uridine-3′ phosphate.[15] The choice of cation to be used in the buffer solutions, *i.e.*, sodium *vs.* ammonium, appeared to make little difference in the degree of separation. The use of sodium formate, however, did tend to reduce tailing of the peaks, as shown by the resolution of uridylyl[3′(2′)→5′]inosine (IIIc) illustrated in Fig. 3 and 4. When the fraction containing the (3′→5′) isomer (Fig. 3) was rechromatographed using a sodium formate elution gradient, a single bell-shaped peak was obtained (Fig. 4), although the relatively smaller loading of the column during the rerun may have contributed toward this improved elution pattern. It was not possible to separate completely uridylyl(3′→5′)-5-bromouridine from uridylic acid (Fig. 2); therefore, the material eluted after point A was discarded and the material from the major part of the peak was rechromatographed in the same system to give pure uridylyl(3′→5′)-5-bromouridine (IIIa). The total yield of the diribonucleoside phosphates based on uridine-2′(3) phosphate averaged 40–50%. The ratio of the amounts of the two isomers approximated 1:1, so that the effective yield of the desired isomer was 20–25%. The identity of the two isomers of uridylyl[2′-(3′)→5′]-5-bromouridine was established by the action of pancreatic ribonuclease, which brought about the complete hydrolysis of uridylyl(3′→5′)-5-bromouridine to uridine-3 phosphate, and 5-bromouridine, and had no detectable effect on the (2′→5′)-linked isomer. The complete hydrolysis by ribonuclease also indicated that no migration of the phosphate bond from the 3′-position to the 2′-position occurred under the conditions used for the final isolation of the products.

Identical procedures were used for the synthesis of uridylyl(3′→5′)uridine (IIIb), uridylyl(3′→5′)inosine (IIIc), and uridylyl(3′→5′)-6-thioinosine (IIId). Compound IIId, when directly obtained from the column, ran as a single spot on paper chromatography and, upon treatment with ribonuclease, yielded 1 equiv. each of 6-thioinosine and uridylic acid, but, after lyophilization

(13) 2′,3′-Isopropylidene-6-thioinosine was dried at 109° under vacuum, not as described for the other isopropylidene derivatives, and the internucleotide linkage was formed in a mixture of dioxane and *N,N*-dimethylformamide (1:1).

(14) R. Letters and A. M. Michelson, *J. Chem. Soc.*, 71 (1962).

(15) W. E. Cohn, in "Nucleic Acids," Vol. 1, Chargaff and Davison, Ed., Academic Press, New York, N. Y., 1955, pp. 211–242.

of the entire fraction, the product became yellow. An ultraviolet absorbing impurity, the identity of which has not yet been established, was separated from the product by high voltage electrophoresis.

Synthesis of uridylyl(3′→5′)uridine in the present series offers an opportunity for comparison of this general method with those used previously for this synthesis. For example, Hall and Thedford[1] directly obtained the (3′→5′)-linked diribonucleoside phosphate *via* a uridine intermediate specifically blocked at the 2′- and 5′-positions. The synthesis required four steps and the over-all yield was 7.4%, based on uridine as the starting material. Starting from commercially available uridylic acid, the present method also consists of four steps and yields the diribonucleoside phosphate in over-all yield of 23%. Since the specific synthesis of the (3′→5′)-linked isomer requires an ion-exchange column procedure for final purification, the separation of the mixed (2′→5′)- and (3′→5′)-linked isomers in the method reported here represents no additional burden. Thus, depending on the available starting materials, the synthesis of certain diribonucleoside phosphates *via* the mixed (2′→5′)- and (3′→5′)-linked isomers may be the method of choice.

Experimental

Paper Chromatography.—Paper chromatography was carried out on Whatman No. 3 MM chromatograms which were developed in each of two descending solvent systems for 16 hr.: A, isopropyl alcohol–concentrated ammonium hydroxide–water (7:1:4), and B, isopropyl alcohol–aqueous ammonium sulfate, 1% (2:1).

Paper Electrophoresis.—Paper electrophoresis was carried out for 1 hr. in a Gilson Electrophorator on Whatman No. 3 MM chromatography paper in a buffer of 0.05 *M* formic acid adjusted to pH 3.0 with ammonia (4500 volts, 1 hr.). Results are expressed as a fraction of the mobility of uridylic acid [(3′),2′ mixture] which, under the previous conditions, moves as a single spot approximately 25 cm. from the origin.

Unless stated otherwise, all evaporations referred to were carried out in a rotary evaporator at temperatures up to 30°. Samples for analyses were dried at 25° to constant weight over phosphorus pentoxide and under vacuum. Melting points are uncorrected. Spectra were obtained on a Cary Model 14 spectrophotometer.

Uridine-2′,3′ Cyclic Phosphate (Modification of the Method of Michelson[5]).—Uridine-2′(3′) phosphate (324 mg., 1 mole) was dissolved in water (3 ml.) and the solution was treated with tri-*n*-butylamine (1.1 ml., 7.6 mmoles) and ethyl chloroformate (0.2 ml., 2.1 mmoles) and shaken vigorously in a stoppered flask for 5 min. The reaction mixture was allowed to stand for a further 10 min. with occasional agitation before being evaporated to a thick gum. Paper chromatography of the crude gum in the two systems, A and B, and electrophoresis showed 100% conversion to cyclic phosphate; R_f, solvent A, 0.28, and B, 0.43; electrophoretic mobility, 1.12. The gum was washed several times with anhydrous ether and evaporated from anhydrous pyridine solution repeatedly until the product, pyridinium uridine-2′,3′ cyclic phosphate, crystallized.

Anal. Calcd. for $C_{14}H_{16}N_3O_8P$: C, 43.6; H, 4.19; N, 10.90. Found: C, 43.94; H, 4.32; N, 10.94.

Preparation and Purification of 2′,3′-Isopropylidene-6-thioinosine (IId).—This intermediate was prepared according to the method of Hampton.[9] The difficulties in isolating the product were overcome by employing a partition column as follows. Following the reaction on a 1-mmole scale, the solution was chilled to 0°, poured into dilute ammonium hydroxide, and this solution was evaporated to dryness. The residue was dissolved in 10 ml. of the lower phase of the solvent system *n*-butyl alcohol-aqueous ammonium hydroxide, 10% (3:1). This was mixed with 22 g. of Celite-545 and the mixture was packed on top of a previously packed partition column containing 140 g. of Celite-545 and 63 ml. of lower phase (column size, 2.54 × 80 cm.). The column was developed with the upper phase and the desired product was obtained within the first hold-back volume. (See ref. 10 for complete details of this technique for partition columns.) The product which moved as a single spot on paper chromatography (R_f, solvent A, 0.67, and B, 0.65) was crystallized from hot water; m.p. 268°. dec., yield 72%.

Anal. Calcd. for $C_{13}H_{16}N_4O_4S$: C, 48.13; H, 4.93; N, 17.28; S, 9.88. Found: C, 48.24; H, 4.94; N, 16.99; S, 9.89.

Uridylyl(3′→5′)-5-bromouridine (III). A Formation of the Internucleotide Bond.—The product of 1-mmole preparation of 5′-*O*-acetyluridine-2′,3′ cyclic phosphate[16] was dissolved in anhydrous dioxane (10 ml.) containing 2′,3′-isopropylidene-5-bromouridine[9,11,12] (726 mg., 2 mmoles). The mixture was freeze-dried from dioxane (3 × 10 ml.) before being dissolved in 5 ml. of dioxane. To this solution was added tri-*n*-butylamine (0.6 ml., 4.16 mmoles), followed by diphenyl phosphorochloridate (0.3 ml.). The flask was sealed and shaken for 5 min., then set aside for the reaction to continue for 24 hr., after which time the solvent was removed by evaporation. The resulting gum was dissolved in cold water (10 ml.), and the pH of the solution was adjusted to 9.5 with ammonium hydroxide (7.5 *M*). The aqueous solution was extracted with ether (four 20-ml. portions); pH was readjusted to 9.5 with ammonia. The alkaline solution was incubated at 37° for 24 hr. to remove the acetyl group. The acid labile isopropylidene group was removed as follows. A slight excess of Dowex 50-W-X8 (H^+ form) ion-exchange resin was added to the solution to remove all the cations and the solution was stirred at 35° for 1.5 hr. in the presence of the resin. The ion-exchange resin was removed from solution by filtration, and the pH of the solution was adjusted to 8.5 with ammonium hydroxide (7.5 *M*).

Ion-Exchange Chromatography.—Commercially available Dowex-1X2 (chloride) ion-exchange resin (200–400 mesh) was prepared for chromatography by washing in a column (30 × 1 in.) with 2 *N* sodium hydroxide solution until no further trace of chloride ion was found in the effluent. The resin was then washed with distilled water to neutrality followed by 4 *N* formic acid (4:1.). Finally the resin was washed with 80% formic acid (1 l.) and distilled water until the effluent was neutral. After adjustment of the solution to pH 8.5, the solution was absorbed onto the top of a column (40 × 1.2 cm) of Dowex-1X2 formate ion-exchange resin and the column was washed with a little distilled water (100–150 ml.). Gradient elution with formate was commenced (see Fig. 2 for conditions), and 20-ml. fractions were collected. The solutions of formate buffer containing uridylyl-(3′→5′)-5-bromouridine[17] and the (2→5′) isomer were cooled to 0° and run under pressure through a column of Dowex-50-WX8 (H^+ form) ion-exchange resin, 50–100 mesh, to remove the cations. The resulting acidic solutions were immediately frozen and lyophilized to yield white powders. The products were redissolved, frozen and relyophilized. The (2→5′)-linked isomer was chromatographically pure and weighed 128 mg. (20.4%). The (3′→5′)-linked product was contaminated with approximately 3% uridylic acid as shown by paper chromatography. This sample was refractionated on the same size column under conditions identical with those described in Fig. 2. In this manner 126 mg. of analytically and chromatographically pure uridylyl(3′→5′)-5-bromouridine was obtained; paper chromatography gave R_f, system A, 0.51, and B, 0.57; electrophoretic mobility, 0.71.

Anal. Calcd. for $C_{13}H_{22}BrN_4O_{14}P$: C, 34.4; H, 3.57; N, 8.9; Br, 12.7. Found: C, 34.54; H, 3.83; N, 8.67; Br, 13.0.

Uridylyl(3′→5′)uridine.—5′-*O*-Acetyluridine-2′,3′ cyclic phosphate (1 mmole) was treated with 2′,3′-isopropylideneuridine (2 mmoles) in the same way as for the preparation of uridylyl-[2′(3′→5′]-3-bromouridine. The blocking groups were removed under the same conditions as previously described, and the conditions for the ion-exchange chromatography are shown in Fig. 5. The yields of the diribonucleoside phosphate isomers, which were obtained as white lyophilized powders, were 127 mg. (23%) and 134.5 mg. (24.5%) for the (2′→5′)- and (3′→5′) isomers, respectively. The triethylammonium salt was prepared by dissolving the free acid in water and titrating with triethylamine to pH 7.0. The solution was filtered and lyophilized yielding triethylammonium uridylyl(3′→5′)uridine;

(16) Acetylation of uridine-2′,3′ cyclic phosphate was performed according to the method of Smrt and Šorm.[3]

(17) Because the fraction containing the (3′→5′)-linked isomer also contained a small amount of uridylic acid, it was collected only to point A, Fig. 2.

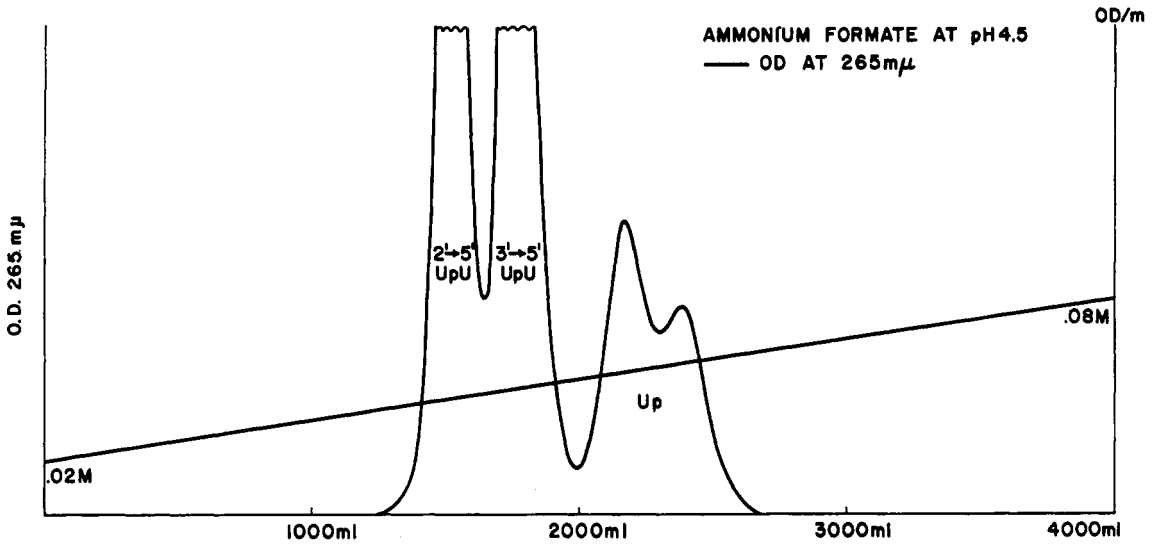

Fig. 5.—Ion-exchange chromatographic separation of uridylyl[2′(3′)→5′]uridine—[2′(3′)→5′]UpU; 1-mmole scale using a Dowex 1X2 formate column, 42 × 1.2 cm.

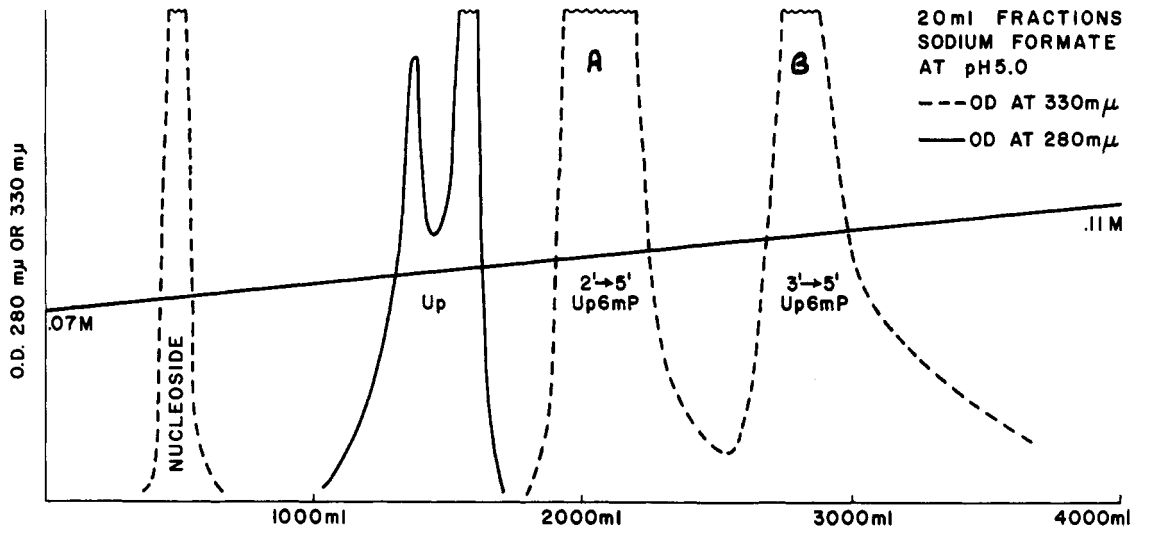

Fig. 6.—Ion-exchange chromatographic separation of uridylyl[2′(3′)→5′]-6-thioinosine—[2′(3′)→5′]Up6MP, using a Dowex 1X2 formate column, 42 × 1.2 cm.

paper chromatography gave R_f, solvent A, 0.26, and B, 0.30; electrophoretic mobility, 0.67.

Anal. Calcd. for $C_{24}H_{28}N_5O_{14}P \cdot 4H_2O$: C, 39.9; H, 6.36; N, 9.68. Found: C, 39.85; H, 6.36; N, 9.96.

Uridylyl(3′→5′)inosine (IIIc).—Uridylyl[2′(3′)→5′]inosine was prepared under conditions described for uridylyl[2′(3′)→5′]-uridine, from 5′-*O*-acetyluridine-2′3′ cyclic phosphate (1 mmole) and 2′,3′-isopropylideneinosine (2 mmoles). Conditions for the separation of the components of the reaction mixture are shown in Fig. 3. Although the (3′→5′) isomer, which was obtained white and amorphous, was homogeneous on paper chromatography in two systems (R_f, A, 0.23, and B, 0.30) and electrophoresis (mobility, 0.67), no satisfactory analysis was obtained (weight 151 mg., 27%).

Uridylyl(3′→5′)6-thioinosine (IIId).—5-*O*-Acetyluridine-2′,5′ cyclic phosphate (2 mmoles) was treated with 2′,3′-isopropylidene-6-thioinosine (1 mmole) in dioxane (2 ml.) and *N*,*N*′-dimethylformamide (2 ml.) as previously described. Only the nucleotide was dried by lyophilization from dioxane; the nucleoside was dried at 109° under vacuum. The hydrolytic removal of the blocking groups was carried out as described earlier. The conditions for the ion-exchange chromatography of the mixed isomers are shown in Fig. 6. The fractions were treated with Dowex-50 as described before and lyophilized in the dark. The yields were (2′→5′) isomer, 48.2 mg., and (3′→5′) isomer, 50.0 mg. Paper chromatography gave R_f, solvent A, 0.058, and B, 0.26; electrophoretic mobility, 0.60. Electrophoresis of the lyophilized material for 1 hr. at 4500 v. (pH 3.0) yielded the desired product and an impurity.[18] No satisfactory analysis for uridylyl(3′→5′)-6-thioinosine was obtained.

TABLE I

ULTRAVIOLET ABSORPTION DATA

	λ_{max}, pH 2.0		λ_{max}, pH 7.0		λ_{max}, pH 11.0	
	mμ	ε	mμ	ε	mμ	ε
(3′→5′)Up5BrU	267	15,000	267	15,000	266	11,500
(3′→5′)UpI	252		252		256	
(3′→5′)UpU	262	19,100	262	19,100	262	14,800
(3′→5′)-Up6MPR	262 321		262 317		260 310	

Ribonuclease Treatment.—One milligram of each of the diribonucleoside phosphates was dissolved in 0.5 ml. of water and

(18) Although the product contained in the fractions corresponding to peak B, Fig. 6, was colorless and homogeneous so far as could be detected by chromatography in systems A and B and by electrophoresis, some degradation occurred during lyophilization and the product was obtained as a yellow powder. Ultraviolet spectral data (Table I) was obtained by eluting the compound from a paper electrophoretogram.

the pH was adjusted to 7.0. Magnesium sulfate (5 λ of a 1 *M* solution) and 0.1 mg. of crystalline ribonuclease were added. The pH was adjusted from time to time as required. After 6 hr., the whole sample was streaked on Whatman 3 MM paper which was developed in solvent A. Each of the (3′→5′)-linked isomers was completely degraded to uridylic acid and the free nucleoside.

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Neighboring Group Participation in the Elimination of the Exocyclic Secondary *p*-Tolylsulfonyloxy Group in D-Glucofuranose Derivatives[1,2]

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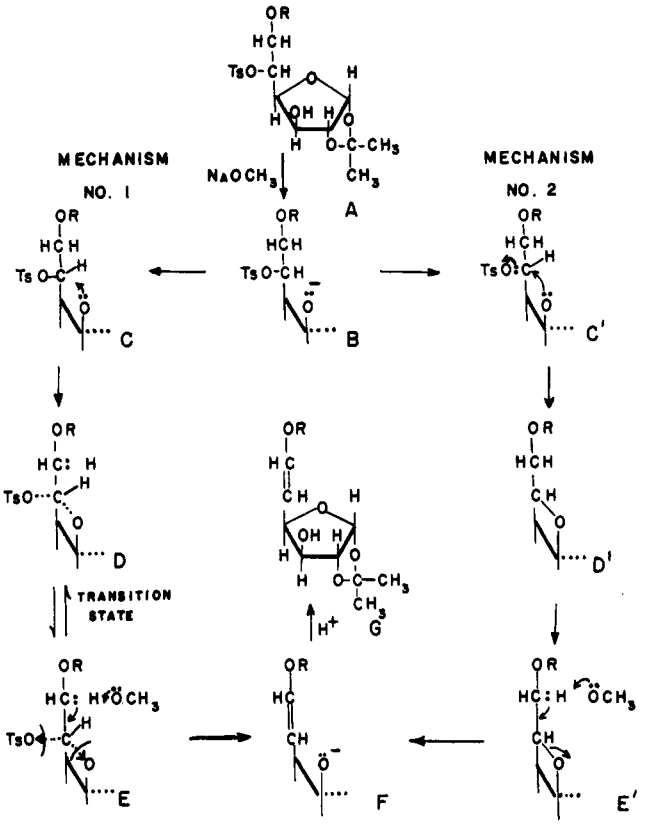
Base-catalyzed elimination of the exocyclic secondary tosyloxy group in a D-glucofuranose structure is dependent on the participation of a neighboring alkoxide group. Model tosylated D-glucofuranose derivatives with a stereochemical configuration similar to 6-*O*-benzyl-1,2-*O*-isopropylidene-5-*O*-*p*-tolylsulfonyl-α-D-glucofuranose are synthesized and treated with sodium methoxide to substantiate an anchimeric assistance in desulfonyloxylation. Tosyloxy elimination is always accompanied by β-proton elimination, with subsequent formation of 5-deoxy-α-D-*xylo*-hexofuran-5-enose derivatives when specific 5-*O*-*p*-tolylsulfonyl-α-D-glucofuranose derivatives contain a free C-3 hydroxyl group. Since increased yield of olefin is observed when the sodium methoxide concentration is raised from 1 to 4 moles per mole of tosylate, it is suggested that tosyloxy elimination is dependent on the concentration of an ionized C-3 hydroxyl group, and that protonization of the β-hydrogen with subsequent formation of olefin is dependent on the concentration of methoxide ion.

Desulfonyloxylation and β-elimination of 6-*O*-benzyl-1,2-*O*-isopropylidene-5-*O*-*p*-tolylsulfonyl-α-D-glucofuranose (V) in the presence of sodium methoxide, with subsequent formation of 6-*O*-benzyl-5-deoxy-1,2-*O*-isopropylidene-α-D-*xylo*-hexofuran-5-enose (XI) has been recently reported.[3] This reaction reveals that, although the stereochemical configuration of V would seem to permit a 3,5-anhydro ring, olefin formation is dominant. This elimination of an exocyclic secondary tosyloxy group, as observed in a selected series of D-glucofuranose derivatives, is now examined in greater detail and two mechanisms for a β-proton elimination in the formation of olefins are proposed (see col. 2).

Four pairs of 5-*O*-*p*-tolylsulfonyl-α-D-glucofuranose derivatives, all of which contain an alkali stable R group substituted for the hydrogen on the C-6 hydroxyl group, were synthesized. One derivative in each pair has its C-3 hydroxyl group protected with an alkali stable R group. Each compound was treated with sodium methoxide, and the products were characterized. A scheme for the synthesis of compounds I to VIII (Table I) is as follows. Unimolar tosylation of 6-deoxy-1,2-*O*-isopropylidene-α-D-glucofuranose[4] gave 6-deoxy-1,2-*O*-isopropylidene-5-*O*-*p*-tolylsulfonyl-α-D-glucofuranose (I). The catalytic hydrogenation of 5,6-anhydro-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-glucofuranose[5] and subsequent tosylation furnished 6-deoxy-1,2-*O*-isopropylidene-3-*O*-methyl-5-*O*-*p*-tolylsulfonyl-α-D-glucofuranose (II). Treatment of 5,6-anhydro-1,2-*O*-isopropylidene-α-D-glucofuranose[6] with sodium methoxide, followed by a monotosylation of the product[7]



therefrom, furnished 1,2-*O*-isopropylidene-6-*O*-methyl-5-*O*-*p*-tolylsulfonyl-α-D-glucofuranose (III). The reaction of 5,6-anhydro-3-*O*-benzyl-1,2-*O*-isopropylidene-α-D-glucofuranose[4] with sodium methoxide and subsequent tosylation of the product obtained produced 3-*O*-benzyl-1,2-*O*-isopropylidene-6-*O*-methyl-5-*O*-*p*-tolylsulfonyl-α-D-glucofuranose (IV). A preparation for the compound which initiated the study of olefin formation, namely 6-*O*-benzyl-1,2-*O*-isopropylidene-5-*O*-*p*-tolylsulfonyl-α-D-glucofuranose (V), is described in an earlier paper.[3] When 5,6-anhydro-3-*O*-benzyl-1,2-*O*-

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(2) Presented before the Division of Carbohydrate Chemistry at the 145th National Meeting of the American Chemical Society, New York, N. Y., Sept., 1963.

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