ANALOGS OF CARBOHYDRATE METABOLISM COENZYMES
VII. Synthesis of Isocytidine Diphosphate Glucose

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We have previously [1, 2] pointed out the importance of the imide grouping \(O=C_2-N_3H-C_4=O\) of the uracil nucleus, which is capable of forming hydrogen bonds, in the biological activity of uridine diphosphate glucose (UDPG). According to a hypothesis in [2], the presence of this grouping is essential for the formation of the secondary structure of a nucleoside diphosphate sugar, determining the specificity of its biochemical properties. In preceding papers of this series the synthesis of analogs of UDPG modified at \(N_9\) and \(C_4\) of the uracil nucleus has been reported: 3-\(N\)-methyl-uridine diphosphate glucose [1], cytidine diphosphate glucose [3], and 4-thiouridine diphosphate glucose [4]. The study of their biochemical and chemical properties has enabled the following conclusions to be drawn:

1) Analogs of UDPG containing the grouping \(N_9H-C_4=X\) (where \(X = O, S\)) in the uracil nucleus, which are capable of forming hydrogen bonds, can replace UDPG in four essentially different enzymatic reactions; a change in this grouping involving a loss of the capacity for forming hydrogen bonds destroys the biological activity of the compound [5, 6];

2) The catalytic hydrogenation of UDPG and its analogs with a nonmodified \(N_9H-C_4=O\) group in the uracil nucleus takes place considerably more slowly than the hydrogenation of the corresponding nucleoside-5'-phosphates. This difference is not observed for analogs which are not capable of forming hydrogen bonds and are inactive in enzymatic reactions [7].

These conclusions agree completely with the hypotheses previously put forward on the existence of a secondary structure in nucleoside diphosphate sugars [2]. However, the answer to the question of the importance of a carbonyl group at \(C_2\) of the uracil nucleus in the formation of the secondary structure and in the biological activity of UDPG requires the investigation of analogs of UDPG modified at \(C_4\) of the pyrimidine nucleus. The present paper describes the synthesis of an analog containing a \(NH_2\) group in the place of the carbonyl group at \(C_4\) of isocytidine diphosphate glucose. Brief information on its synthesis has been published in [8].

The starting material for the preparation of isocytidine derivatives was 2', 8'-O-isopropylidene-\(O_2', 5'-cyclo\)-uridine (I). The synthesis of this compound has been reported by Brown, Todd, and Varadarajan [9], who obtained it by the action of silver acetate on 2', 8'-O-isopropylidene-5'-deoxy-5'-iodouridine in absolute methanol. On repeating this procedure, we found that in addition to compound (I) a considerable amount of a by-product having a lower polarity was formed. It is probable that this product, which we did not investigate in detail, arises through the opening of the ring of (I) under the action of methanol. In actual fact, when the reaction was carried out in acetonitrile the formation of the by-product was completely eliminated and the yield of (I) rose somewhat. Isocytidine diphosphate glucose was synthesized from substance (I) by the following route:

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The conversion of compound (I) into 2', 3'-O-isopropylideneisocytidine (II) was carried out by treating its solution with ammonia in methanol by the procedure described by Brown and co-authors [9]. The absorption maxima of the UV spectrum of the reaction product showed characteristic changes when the pHs varied and were close to those given in the literature.

The phosphorylation of substance (II) encountered considerable difficulties. The use of Tener's method — phosphorylation with 2-cyanoethyl phosphate in the presence of dicyclohexyl carbodiimide [10] — did not give satisfactory yields of isocytidine 5'-phosphate. The reaction of (II) with 2-cyanoethyl phosphate and dicyclohexylcarbodiimide under standard conditions (4 hours, 60°C) [4], followed by hydrolysis to eliminate the isopropylidene group by the action of concentrated formic acid in the cold (under standard conditions of hydrolysis the N-glycoside bond of the isocytidine ruptures completely) and treatment with a dilute solution of ammonia to eliminate the cyanoethyl group led to the formation of isocytidine-5'-phosphate (III) with a yield of 15%. Reducing the reaction time to 1.5 hr increased the yield to 23%. When N-acetylisopropylideneisocytidine (VI) was phosphorylated by means of 2-cyanoethyl phosphate and dicyclohexylcarbodiimide it was possible to isolate substance (III) with a yield of 40%. The product was formed as a result of the action of acetic anhydride and triethylamine on (II) in admixture with dioxane and dimethylformamide (cf. [12]). The behavior of the substance on paper and a study of its UV spectrum with change in pH agree with the structure assigned to compound (VI).

The best method of obtaining (III) proved to be the direct phosphorylation of isopropylideneisocytidine with pyrophosphoryl chloride. This reagent has been used previously with success for the synthesis of natural nucleoside-5'-phosphates [12]. The reaction of (II) with pyrophosphoryl chloride took 16 hr at room temperature; no side reactions were found. The 2', 3'-O-isopropylideneisocytidine-5'-phosphorodichloroate formed as an intermediate was hydrolyzed with water at -30°C. The isopropylidene group was split off by keeping a strongly acid solution at room temperature for a short time; the N-glycoside bond did not hydrolyze during this treatment. The isocytidine-5'-phosphate was separated from isocytidine and phosphoric acid by ion-exchange chromatography on "Dowex-1" anion-exchanger (C1-form) and was isolated in the form of the lithium salt in 50% yield. From its chromatographic, electrophoretic, and spectroscopic properties the substance obtained corresponded to isocytidine-5'-phosphate.

Thus, we have succeeded in developing a method which can be used not only for the synthesis of analogs of UDPG but also in investigations on the relationship of structure and function in natural nucleosides.

The conversion of isocytidine-5'-phosphate into isocytidine-5'-phosphoromorpholidate (IV) was effected by the method of Khorana and coworkers, [13]; a sample of (IV) containing traces of (III) was used without further purification for reaction with a-D-glucose-1-phosphate by a known general method [14] with modifications [1, 3]. The final product of the whole synthesis, isocytidine diphosphate glucose (V), was purified by ion-exchange chromatography on DEAE-cellulose, and was then isolated as the lithium salt. For analysis and for biochemical investigation, compound (V) was converted into the sodium salt. The sample of (V) was homogeneous on chromatography and paper electrophoresis; its structure was shown on the basis of the criteria customary for such compounds: the UV spectrum, which agrees with the UV spectrum of (III), the electrophoretic mobility characteristic for a di-substituted pyrophosphate, and the nucleoside-glucose ratio after acid hydrolysis (found: 1:0.90). The yield of product (V) from (III) was 50%.

Preliminary results of the biochemical investigation of substance (V) showed that it is incapable of replacing UDPG in reaction with saccharosynthetase and UDPG-4-epimerase. This fact shows the improtance of the carbonyl group at C4 in the biological activity of UDPG and agrees with the hypothesis previously expressed on the secondary structure of nucleoside diphosphate sugars [2].

Experimental

The systems used for paper chromatography were: 1) butan-1-ol-water (86: 14); 2) ethanol – 0.5 M ammonium acetate solution, pH 7.5 (5:2). Buffer solutions: pH 7.5, 0.02 M solution of triethylammonium carbonate; pH 4.0, 0.0075 M triethylammonium acetate solution.

2', 3'-O-Isopropylidene-O2P, 5'-cyclouridine (I). a. A mixture of 2.36 g (6.1 mmole) of 5'-deoxy-5'-iodo-2', 3'-O-isopropylideneuridine [15], 4.44 g (26.5 mmole) of silver acetate and 500 ml of absolute methanol was boiled for 15 minutes without the access of moisture and filtered through Celite. Hydrogen sulfide was passed through the filtrate, the precipitate of silver sulfide was separated by filtration through Celite, and the solution was evaporated to dryness. The residue was recrystallized from alcohol. This gave 0.95 g (58%) of 2', 3'-O-isopropylidene-O2P, 5'-cyclouridine. The product was homogeneous on paper chromatography with Rf 0.53 (system 1). UV spectrum (in methanol) \( \lambda_{max} \) 237 m\( \mu \), \( \lambda_{min} \) 219 m\( \mu \), which agrees with literature data [9]. According to paper chromatography in system 1, the mother liquor contained a substance with Rf 0.85, in addition to isopropylideneuridine.

b. A mixture of 1.10 g (2.72 mmole) of 5'-deoxy-5'-iodo-2', 3'-O-isopropylideneuridine and 110 ml of dry acetonitrile was treated with 1.58 g (9.5 mmole) of silver acetate and was stirred for a night at room temperature without the access of moisture and light. The mixture was filtered through Celite and the residue was washed with 10 ml of aqueous acetonitrile was treated with 1.58 g (9.5 mmole) of silver acetate and was stirred for a night at room temperature without the access of moisture and light. The mixture was filtered through Celite and the residue was washed with 10 ml of