ANALOGS OF COENZYMES OF CARBOHYDRATE METABOLISM

VII. Synthesis of 2-Thiouridine Diphosphate Glucose

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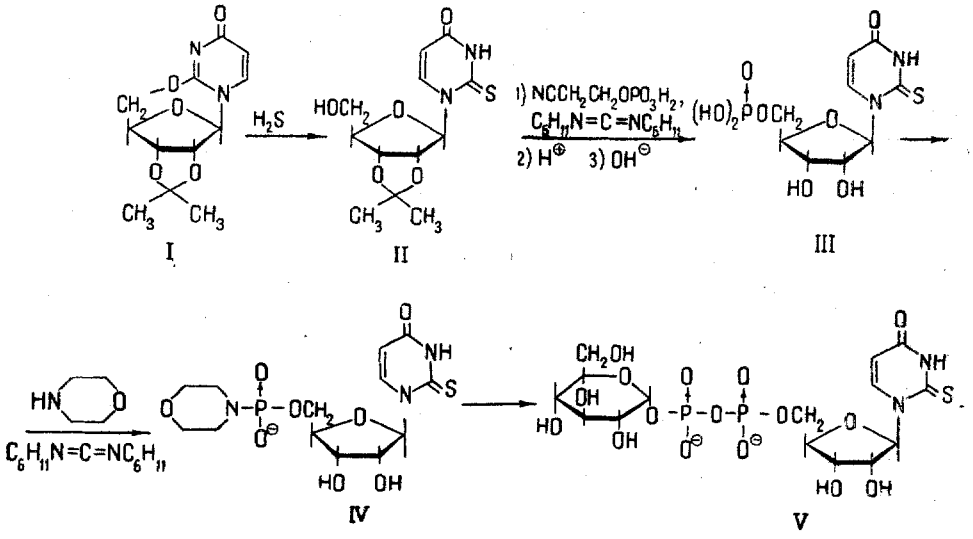
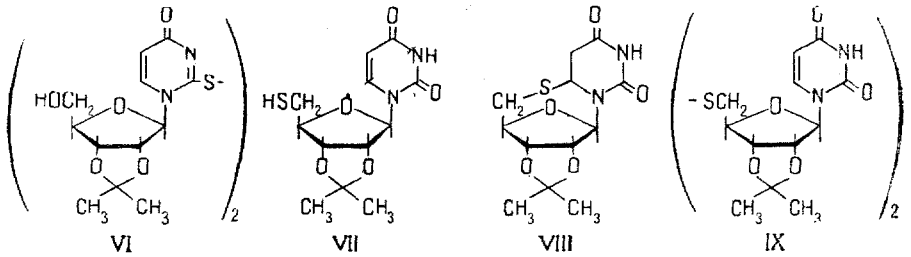
In the preceding communication [1] we pointed out the necessity for the synthesis and biological investigation of analogs of uridine diphosphate glucose (UDPG) modified at C_2 of the pyrimidine nucleus in order to determine the importance of the carbonyl group at C_2 of the uracil nucleus for the formation of a secondary structure [2] and the biological activity of UDPG. Supplementing the previously reported method of obtaining isocytidine diphosphate glucose [1], the synthesis of 2-thiouridine diphosphate glucose is considered in the present work. Brief information on its synthesis has been published previously [3].

The 2-thiouridine diphosphate glucose was obtained from 2', 3'-O-isopropylidene-O_2, 5'-cyclouridine (I) [1, 4] by the following route:



The reaction of substance (I) with hydrogen sulphide in the presence of triethylamine in dimethylformamide has been studied by Todd and his coworkers [5]. It was shown that this reaction gives 2', 3'-O-isopropylidene-2-thiouridine (II) and its disulfide (VI) – a white crystalline substance.

On repeating this reaction, to separate the products we used chromatography on alumina, and isolated three substances: compound (II), a substance identical with the product obtained by Todd, and a substance to which, on the basis of the UV spectrum and the results of elementary analysis, we have ascribed the structure 2', 3'-O-isopropylidene-5'-deoxy-5'-mercaptouridine (VII) [3]. However, the investigations of American authors [6, 7] have shown that a compound with this structure undergoes spontaneous cyclization into 5'-deoxy-5', 6-epithio-5, 6-dihydro-2', 3'-O-isopropylideneuridine (VIII) and that Todd's "disulfide" actually has the structure of substance (VIII). Moreover, the properties of the third substance that we isolated are identical with the properties of the disulfide of (VII), substance (IX), whose synthesis from substance (VII) has recently been effected [7]. Thus, in the reaction of (I) with hydrogen sulfide a mixture of substances (II), (VIII), and (IX) is formed and not (II), (VI), and (VII), as we thought previously [3].



According to our results, the yield of 2', 3'-O-isopropylidene-2-thiouridine (II) in this reaction varies considerably, generally amounting to 25-35%. In one of the experiments, we obtained this substance in 50% yield. In a search for more reproducible reaction conditions, we studied the interaction of (I) with hydrogen sulfide in dry pyridine. Under these conditions, the reaction goes somewhat more slowly, taking 6 days for completion at room temperature. The ratio of (II) to (VII) in the reaction products is practically unchanged, and the yield of substance (II) is 24%. The samples of (II) obtained were homogeneous with respect to paper chromatography and thin layer chromatography on alumina. The IR spectrum of substance (II) agrees well with literature data.

To convert compound (II) into 2-thiouridine-5'-phosphate (III) we used phosphorylation with 2-cyanoethyl phosphate in the presence of dicyclohexylcarbodiimide [8]. There is a brief report in the literature of the synthesis of substance (III) by this method [9], but the conditions of the alkaline and acid hydrolysis for removing the protecting groups are not given. Nevertheless, the correct choice of these conditions is of decisive importance, particularly for obtaining labile derivatives of thionucleosides (cf. [10]).

We carried out the synthesis of (III) under the same conditions as for the synthesis of 4-thiouridine-5'-phosphate [10]. The phosphorylation reaction took place at 60°C (4 hours) in anhydrous pyridine, and the acid hydrolysis to split off the isopropylidene group was performed under the action of 70% acetic acid (45 min, 100°C). To prevent the oxidation of substance (III) to the disulfide, the alkaline hydrolysis was effected in the presence of mercaptoethanol. Under these conditions, 2-thiouridine-5'-phosphate is formed in high yield. Side-reactions, conversion into uridine and the formation of the disulfide, take place to only a small extent. Substance (III) was isolated from the reaction mixture by means of ion-exchange chromatography on "Dowex-1" (HCO_3 form) with elution by triethylammonium hydrogen carbonate. The triethylammonium salt of (III), isolated with a yield of 64%, was converted into the morpholinium salt for the subsequent synthesis.

Then compound (III) was converted into 2-thiouridine-5'-phosphoromorpholidate (IV) by the usual method [11]. In contrast to 4-thiouridine-5'-phosphate, no side-reaction involving the replacement of the sulfur atom by a morpholine residue [11] takes place in this case. The 4-morpholino-N, N'-dicyclohexylcarbamidinium salt of (IV) obtained was used without further purification for the synthesis of 2-thiouridine diphosphate glucose (V).

Substance (V) was obtained under the conditions described previously [3, 10]. The reaction product was separated by ion-exchange chromatography on DEAE-cellulose; the lithium salt of (V) was converted into the sodium salt. The yield of (V) from substance (III) amounted to 32%. The sample of (V) obtained was homogeneous on paper chromatography and electrophoresis, and its electrophoretic behavior corresponded to the structure of a disubstituted pyrophosphate. The UV spectrum closely corresponded to the UV spectrum of 2-thiouridine-5'-phosphate. The ratio of base to glucose after acid hydrolysis was found to be 1:0.99. These results show fully that compound (V) has the structure of 2-thiouridine diphosphate glucose. Preliminary results of biological investigations of the 2-thiouridine diphosphate glucose carried out in the biological testing laboratory of OKhPS [Institute for the Chemistry of Natural Compounds] show that this substance, in contrast to isocytidine diphosphate glucose, is capable of replacing UDPG in the biosynthesis of sugars and the epimerization of UDPG with the formation of galactose derivatives.

Experimental

The paper chromatography and electrophoresis were carried out in the systems described previously [1]. Neutral alumina (Brockman activity II) was used for thin-layer chromatography.

2-Thiouridine-5'-phosphate

2', 3'-O-Isopropylidene-2-thiouridine (II). A. A solution of 371 mg (1.34 mmole) of 2', 3'-O-isopropylidene-O_2, 5'-cyclouridine [1, 4] in 4 ml of freshly distilled dimethylformamide containing 0.2 ml of triethylamine was cooled to −30°C and saturated with hydrogen sulfide. The dark green solution obtained was warmed to room temperature (the excess of hydrogen sulfide was evaporated off) and was left for 48 hours without the access of moisture. The hydrogen sulfide was displaced by a current of nitrogen, the solution was evaporated under vacuum, and the residue was dried at 50°C. The yellowish amorphous substance isolated (weight 374.1 mg) was dissolved in 3 ml of acetone and passed through a column (18 × 2 cm) of alumina. The column was eluted with 450 ml of a mixture of acetone and methanol (10:1) and 250 ml of a mixture of acetone and methanol (1:1), 50-ml fractions being collected. For checking, 0.5-ml aliquots were taken from the fractions and were freed from acetone by evaporation with methanol under vacuum three times. The residue was dissolved in 5 ml of methanol, and the absorption at 230, 260, and 290 mμ was measured.

In fractions 2-5, $\varepsilon_{230} > \varepsilon_{260} > \varepsilon_{290}$; when they were evaporated to dryness, white crystals of 5'-deoxy-5', 6-epithio-5, 6-dihydro-2', 3'-O-isopropylideneuridine were obtained, yield 170 mg, mp 197-200°C (decomp.); the UV spectrum contained no maxima in the range 220-290 mμ.

According to literature data, the mp of this substance is 205-206°C [5], 200-215°C [6], 235-237° [7], UV spectrum λ_{max} 210-212 mμ [5].

In fractions 10-12, $\varepsilon_{260} \approx \varepsilon_{230} \approx \varepsilon_{290}$; their evaporation gave glasslike 2', 3'-O-isopropylidene-2-thiouridine, yield 115 mg (29%), R_f 0.86 [butanol-water (86:14) on paper], 0.30 [chloroform-CH_3OH (30:1), in a thin layer of alumina], 0.85 [acetone-CH_3OH (1:1), in a thin layer of alumina]. UV spectrum (in CH_3OH): λ_{max} 272 mμ, λ_{min} 244 mμ.

In fractions 14-15, $\varepsilon_{260} > \varepsilon_{230} > \varepsilon_{290}$; when they were evaporated, white crystals of the disulfide from 2', 3'-O-isopropylidene-5'-deoxy-5'-mercaptouridine were formed, yield 58 mg, mp 185-187°C (from benzene), R_f 0.25 [chloroform-CH_3OH (30:1)], 0.20 [acetone-CH_3OH (1:1)]; UV spectrum (in CH_3OH): λ_{max} 260 mμ, λ_{min} 230 mμ.

According to literature data, the mp of this substance is 183-185°C and its UV spectrum: λ_{max} 259 mμ, λ_{min} 229 mμ [7].

B. A solution of 475 mg (1.72 mmole) of 2', 3'-O-isopropylidene-O_2, 5'-cyclouridine in 20 ml of dry pyridine was cooled to −30°C and saturated with hydrogen sulfide as described above. The dark yellow solution was kept for 6 days at room temperature without the access of moisture, and then the hydrogen sulfide was eliminated in a current of nitrogen, the solution was evaporated under vacuum, and the residue was treated with 10 ml of absolute alcohol. The precipitate of 5'-deoxy-5', 6-epithio-5, 6-dihydro-2', 3'-O-isopropylideneuridine which deposited (270.7 mg) was separated by filtration, and the filtrate was evaporated to dryness. The residue (202 mg) was dissolved in 3 ml of acetone and chromatographed as described above (A). This gave 124 mg (24%) of 2', 3'-O-isopropylidene-2-thiouridine.

2-Thiouridine-5'-phosphate (III). A mixture of 96.1 mg (0.32 mmole) of 2', 3'-O-isopropylidene-2-thiouridine and 1.48 ml of a 1 M solution of 2-cyanoethyl phosphate in aqueous pyridine [8] was dried by distilling off dry pyridine three times, and the residue was dissolved in 10 ml of dry pyridine, treated with 656 mg (3.2 mmole) of dicyclohexylcarbodiimide and kept for 4 hours at 60°C. Then 20 ml of water was added and after half an hour the precipitate of dicyclohexylurea was filtered off at room temperature, the filtrate was evaporated to dryness, and traces of pyridine were eliminated by distillation with water. The residue was treated with 20 ml of 70% acetic acid and heated for 45 min at 100°C. The solution was again evaporated to dryness, and traces of acetic acid were eliminated by evaporation with water. The residue was dissolved in 10 ml of 1 N caustic potash containing 0.5 ml of 2-mercaptoethanol, and was heated for 15 min at 100°C. After cooling, the solution was filtered and passed through a column (6 × 4 cm) of "Dowex-50" (H^+ form), brought to pH 8 by the addition of alkali, and passed through a column (14 × 2 cm) of "Dowex" 1 × 8 (HCO_3^- form). The column was washed with water (650 ml) and with a 0.05 N solution of triethylammonium hydrogen carbonate with pH 7.5 (1000 ml); uridine-5'-phosphate ($TOD_{260-300}$) was eluted with 0.3 N buffer solution (2000 ml), and 2-thiouridine-5'-phosphate with 0.5 N buffer solution (800 ml) (TOD_{268} 2100 = 0.204 mmole, which corresponds to a yield of 64%). The fractions containing the 2-thiouridine-5'-phosphate were combined and concentrated under vacuum, and the residue was evaporated with water (3 × 100 ml) and dissolved in 20 ml of water, and the solution was passed through a column (6 × 4 cm) of "Dowex-50" (H^+ form). The bulk of the resulting solution of 2-thiouridine-5'-phosphate was neutralized with morpholine and evaporated; the morpholinium salt of 2-thiouridine-5'-phosphate obtained was used for the synthesis of 2-thiouridine-5'-phosphoromorpholidate.

Part of the solution of 2-thiouridine-5'-phosphate was passed through "Dowex-50" (Na^+ form), and the solution was lyophilized, giving the sodium salt of 2-thiouridine-5'-phosphate. The preparation was homogeneous to paper chromatography and electrophoresis. R_f 0.25 (system 1), P_{UMP} 1.2 (pH 7.5), 1.0 (pH 4.0). UV spectrum: 0.1 N HCl and water λ_{max} 273, 219 mμ, ε_{max} 11100, 13800, λ_{min}, 243 mμ, ε_{min} 4950; 0.01 N KOH; λ_{max} 270, 240 mμ, ε_{max} 10500, 14900; λ_{min} 259 mμ, ε_{min} 9800. Isosbestic point 268 mμ, ε 10400; $\varepsilon_{290}/\varepsilon_{268}$ = 0.82 (0.01 N HCl and water), 0.45 (0.01 N KOH). The values of the molar extinction coefficients were calculated for

$$C_9H_{11}N_2O_8SPNa_2 \cdot 3H_2O,$$

which corresponds to a molecular weight of 438.2

2-Thiouridine Diphosphate Glucose

2-Thiouridine-5'-phosphoromorpholidate (IV). A solution of 0.204 mmole of the morpholinium salt of 2-thiouridine-5'-phosphate (TOD_{290} 1890) in 2 ml of water, 2 ml of tert-butanol and 0.06 ml of morpholine was heated to the boil and a solution of 210 mg of dicyclohexylcarbodiimide in 4 ml of tert-butanol was added over 4 hr, after which the reaction mixture was boiled for another 3 hr. Paper electrophoresis (pH 7.5) showed that the initial 2-thiouridine-5'-phosphate (R_{UMP} 1.20) had completely disappeared and 2-thiouridine-5'-phosphoromorpholidate (R_{UMP} 0.58) had been formed. The reaction mixture was treated as in the synthesis of isocytidine-5'-phosphoromorpholidate, and the 4-morpholino-N, N'-dicyclohexylcarbamidinium salt of 2-thiouridine-5'-phosphoromorpholidate was used without purification for the pyrophosphate synthesis.

2-Thiouridine diphosphate glucose (V). The synthesis was carried out by the standard method [1, 3] from 0.20 mmole of 2-thiouridine-5'-phosphoromorpholidate and 0.60 mmole of the trioctylammonium salt of glucose-1-phosphate. After the completion of the reaction, the pyridine was distilled off, traces were eliminated by evaporation with alcohol twice, and the residue was dissolved in ether. The precipitate was separated off, dissolved in 100 ml of water, and passed through a column (13.5 × 1 cm) of DEAE-cellulose (Cl^- form). The column was washed with 100 ml of water, until the pyridine had been eliminated and then with 0.003 N hydrochloric acid (400 ml) and 0.003 N HCl + 0.02 N LiCl (500 ml). The first solvent eluted 2-thiouridine-5'-phosphate (TOD_{290} 265 = 0.028 mmole), and the second eluted 2-thiouridine diphosphate glucose (TOD_{290} 600 = 0.064 mmole = 32%, calculated on the 2-thiouridine-5'-phosphate). The fractions containing the 2-thiouridine diphosphate glucose were neutralized with a 10% solution of triethylamine in alcohol to pH 6.0 and evaporated to small bulk. The syrupy residue was treated with 10 ml of alcohol and 40 ml of acetone. The precipitate which was deposited was separated off by centrifuging and was washed with acetone and ether. The lithium salt of 2-thiouridine diphosphate glucose obtained was converted into the sodium salt on a column of "Dowex-50" (Na^+ form). After lyophilization, the sodium salt of 2-thiouridine diphosphate glucose was obtained with a yield of 60.1 mg. The preparation was homogeneous on paper chromatography [R_f 0.32 ethanol-0.5 M solution of ammonium acetate, pH 7.5 (5:2)] and on electrophoresis [R_{UMP} 1.6 (pH 4, triethylammonium acetate buffer), 1.0 (pH 7.5, triethylammonium hydrogen carbonate buffer)]. The ratio of nucleoside to glucose after acid hydrolysis was found to be 1:0.99.

UV spectrum: 0.01 N HCl – λ_{max} 273 mμ, λ_{min} 243 mμ. The value of the extinction per unit weight shows that the sample is 60% pure.

Summary

The synthesis of 2-thiouridine diphosphate α-D-glucopyranose, a new analog of uridine diphosphate glucose, has been described.

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