ANALOGS OF PYRIMIDINE MONO- AND POLYNUCLEOTIDES

I. DIPHOSPHATES OF N_1-(1,4-DIHYDROXY-2-BUTYL) DERIVATIVES OF URACIL AND THYMINE

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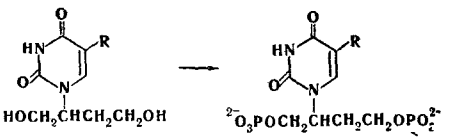
Phosphorylation of N_1-(1,4-dihydroxy-2-butyl) derivatives of uracil and thymine with polyphosphoric acid or β-cyanoethyl phosphate in the presence of dicyclohexylcarbodiimide gives the corresponding diphosphates as the chief reaction products.

If N_1-(1,4-dihydroxy-2-butyl) derivatives of uracil (I) are considered to be analogs of pyrimidine nucleosides in which the glycoside portion is replaced by a dihydroxyalkyl grouping [1], their phosphate esters seem of interest as models of pyrimidine nucleotides and may be used as monomers for the preparation of analogs of oligonucleotides. The present paper is devoted to the synthesis of diphosphates of N_1-(1,4-dihydroxy-2-butyl) derivatives of uracil and thymine (IIa, b). Polyphosphoric acid (PPA) [2] and β-cyanoethyl phosphate in the presence of dicyclohexylcarbodiimide [3, 4] were used as the phosphorylating agents. Considering the presence in I of two nonequivalent hydroxyl groups with close reactivities, one might have expected the formation of a complex mixture of phosphorylation products – a diphosphate (II), isomeric 1'- and 4'-monophosphates, and a cyclophosphate. The reaction conditions were selected in order to achieve formation of primarily diphosphates II. However, analysis of the reaction mixture by paper chromatography showed in addition to diphosphate II, the yield of which was 80%, the presence of other phosphorylation products.* In order to isolate diphosphates II from the reaction mixture we used the low solubilities of their barium salts in water (in contrast to the barium salts of the monophosphates and the cyclophosphate), and we used the difference in their sorption on activated charcoal to separate them from the inorganic phosphate. Diphosphates II were isolated in the form of their sodium salts by the method used in the chemistry of nucleotides [5], and their degree of purity was monitored by means of paper chromatography [6].



Ia, b IIa, b

I, II a R=H; b R=CH_3

The UV spectra of diphosphates II are close to the spectra of uridine and thymidine 3^1-monophosphates [7] and N_1-(5-hydroxy-1-pentyl)uracil phosphate [8].

A characteristic feature of the PMR spectrum of diphosphates II is the substantial decrease in the shielding of the protons of both O-methylene groups and splitting of each of their resonance lines into doub-

*The working out of the conditions of the directed synthesis and separation of the corresponding monophosphates and cyclophosphate is reported in the following publication.

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lets. The splitting (6 Hz) is characteristic for the $^3J_{31POCH}$ spin–spin coupling constants in compounds with pentavalent phosphorus.

EXPERIMENTAL

N_1-(1,4-Dihydroxy-2-butyl)thymine was obtained by the method in [1] and was dried in vacuo over P_2O_5. A solution of β-cyanoethyl phosphate in pyridine was prepared by the method in [3] directly prior to use. The pyridine was purified by treatment with chlorosulfonic acid (20 ml per liter of pyridine), after which it was distilled over KOH and stored over CaH_2.

The purity of the substances was monitored by means of ascending chromatography on "Leningrad medium" paper in isobutyric acid–25% ammonia–water (66 : 1.5 : 33). The electrophoretic mobilities on paper (U_{rel} 5'-UMR) were determined at 700 V in a phosphate buffer (pH 7.5). The PMR spectra of saturated solutions in D_2O were obtained with a Perkin–Elmer R-12A spectrometer (60 MHz). The chemical shifts were measured relative to tert-butyl alcohol as the internal standard (δ 1.23 ppm).

Sodium Salt of N_1-(1,4-Dihydroxy-2-butyl)thymine Diphosphate (IIb). Four 25-ml portions of dry pyridine were added to 32 ml of a solution of β-cyanoethyl phosphate in pyridine (32 mmole), and the mixture was vacuum evaporated to remove traces of water. The residue was dissolved in 25 ml of dry pyridine, and a solution of 1.68 g (8 mmole) of dihydroxybutylthymine Ib in 125 ml of dry pyridine and a solution of 16.0 g (80 mmole) of dicyclohexylcarbodiimide in 25 ml of pyridine were added successively. The mixture was shaken vigorously for 30 min, after which it was allowed to stand at room temperature for 24 h. Water (30 ml) was added, and the mixture was allowed to stand at room temperature for 24 h. The precipitated dicyclohexylurea was removed by filtration and washed with pyridine. A total of 280 ml of 9 N NH_4OH was added to the filtrate, and the mixture was heated at 100° for 3 h. The solution was vacuum evaporated to give a yellowish oil and a small amount of crystals of dicyclohexylurea. Water (50 ml) was added, and the mixture was filtered. A solution of 10.2 g of barium acetate in 50 ml of water was added to the filtrate, and the mixture was allowed to stand at 5° for 24 h. The resulting precipitate was removed by filtration, washed with 100 ml of water and ethanol, and air dried to give 6.35 g of the barium salt of diphosphate IIb containing barium phosphate. The solid was dissolved in 100 ml of water [in order to facilitate dissolving, ~40 ml of Dowex-50 (H^+) was added]. The solution was passed through a column containing Dowex-50 (H^+) (200 ml of the resin) and the column was washed with water. The total eluate (~500 ml) was evaporated at a bath temperature no higher than 30°. The residue was dried by the addition and removal by distillation of absolute ethanol and benzene (three 50-ml portions of each), 100 ml of absolute ether was added, and the mixture was shaken vigorously. The ether layer was decanted in order to remove the phosphoric acid. The operation was repeated three times. The residue was dissolved in 20 ml of ethanol, and a solution of 4.8 g of sodium iodide in 30 ml of acetone was added; 50 ml of acetone was then added. The sodium salt of diphosphate IIb began to precipitate after several days, and the solution took on a dark-red color. Workup gave 2.2 g (62%) of the salt of IIb with mp 221-223° (from aqueous acetone); R_f 0.20, U = 1.16. UV spectrum (in water), λ_{max}, nm ($\varepsilon \cdot 10^{-3}$): pH 2 273 (8.4), pH 12 273 (7.1). IR spectrum, ν, cm^{-1}: 1075 (C–O–P), 1290 (P=O), 1700 (C=O), PMR spectrum, δ: 1.90 (CH_3), 7.52 (6-H), 4.10 (1'-H), 4.8 (2'-H), 2.18 (3'-H), and 3.93 ppm (4'-H). Found: C 26.4; H 4.2; N 6.5%. $C_9H_{15}N_2O_{10}P_2Na \cdot H_2O$. Calculated: C 26.2; H 4.1; N 6.8%.

Sodium Salt of N_1-(1,4-Dihydroxy-2-butyl)uracil Diphosphate (IIa). A 1.05-g (5 mmole) sample of dihydroxybutyluracil Ia was mixed with freshly prepared polyphosphoric acid (15 ml of 85% H_3PO_4 and 20 g of P_2O_5), and the mixture was maintained at 50-60° for 2 h. Water (75 ml) was added, and the mixture was heated at 100° for 15 min, after which it was cooled to room temperature and introduced into a column filled with 50 g of AGN activated charcoal. The phosphoric acid was eluted with water. When the eluate (~1 liter) no longer gave an acid reaction, elution was continued with 50% aqueous ethanol containing 5% NH_4OH. The eluate (700 ml) was evaporated to dryness at 30°. The residue was dissolved in 30 ml of water, and a solution of 2.55 g of $(CH_3COO)_2Ba$ in 20 ml of water was added. The resulting precipitate was separated by centrifugation and washed with ethanol to give 1.95 g (68%) of the barium salt of IIa. The sodium salt was obtained by the method used to obtain the salt of IIb to give 1.9 g (62%) of a product with mp 208-211° [from water–acetone (1 : 10)]; R_f 0.14, U 1.22. UV spectrum (in water), λ_{max}, nm ($\varepsilon \cdot 10^{-3}$): pH 2 267 (9.8), pH 12 267 (7.4). IR spectrum, ν, cm^{-1}: 1075 (C–O–P), 1290 (P=O), and 1700 (C=O). Found: C 23.0; H 3.6; N 6.1%. $C_8H_{12}N_2O_{10}P_2Na_2 \cdot H_2O$. Calculated: C 22.8; H 3.3; N 6.6%.

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