ANALOGS OF PYRIMIDINE MONO- AND POLYNUCLEOTIDES. V.*

SYNTHESIS OF MODELS OF DI- AND TRINUCLEOTIDES BY THERMAL POLYCONDENSATION

S. A. Giller, Z. A. Shomshtein, and T. A. Popova

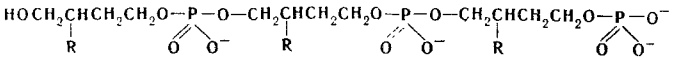
UDC 547.963.32'854.4:543.54

Analogs of di- and trinucleotides were synthesized by thermal polycondensation of N_1-(1,4-dihydroxy-2-butyl)thymime with a mixture of its isomeric monophosphates. The model compounds do not react with polyadenylic acid. The dinucleotide analog suppresses the synthesis of polyphenylalanine in the polyuridylic acid matrix.

The method of thermal fusion of mononucleotides with nucleosides in vacuo has been successfuly used for the preparation of the corresponding di- and trinucleotides [2, 3].

Developing our research on the synthesis of analogs of nucleic acids [1, 4], we used this method for the preparation of models of di- and trinucleotides in which the monosaccharide residues are replaced by dihydroxybutyl groups. In order to achieve this, a lyophilized mixture of N_1-(1,4-dihydroxy-2-butyl)thymine (I) and its monophosphates (II) was heated in vacuo for several minutes, after which the melt was dissolved in water and fractionated by means of gel filtration on Sephadex G-10 and by anion-exchange chromatography on QAE-Sephadex.

The substance that had a distribution coefficient of 0.46 on Sephadex G-10 was identified as starting I. The oligomeric products during gel filtration under the described conditions were eluted in the form of two weakly resolved peaks (fractions 1 and 2) and were rechromatographed on QAE-Sephadex. Fraction 1 was separated into two substances that we eluted with 0.25 and 0.3 N buffer. The first substance was found to be previously characterized dinucleotide analog that we obtained as a side product in the phosphorylation of I [5]. The second substance was chromatographically and electrophoretically homogeneous. Its relative electrophoretic mobility with respect to uridine 5'-monophosphate in neutral media is equal to the corresponding value for N_1-(1,4-dihydroxy-2-butyl)thimine diphosphate (III), whereas in acidic media (pH 2.8) it is higher than the mobility of diphosphate III. Consequently, there is a basis for the assumption that the investigated product contains one secondary and three primary dissociating hydroxyl groups attached to phosphate groups. The results of elementary analysis for this compound are in good agreement with the calculated values for trinucleotide analog IV. However, one cannot form a definitive judgment regarding the structure of this substance, inasmuch as it may exist in the form of a number of isomers.



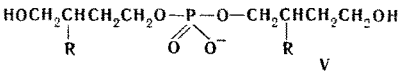
R = thymidyl

IV

*See [1] for communication IV

Institute of Organic Synthesis, Academy of Sciences of the Latvian SSR, Riga. Translated from Khimiya Geterotsiklicheskikh Soedinenii, No. 4, pp. 540-542, April, 1975. Original article submitted February 15, 1974.

By chromatography on QAE-Sephadex fraction 2 can be divided into two compounds, one of which is starting monophosphate II. According to the results of electrophoresis and elementary analysis, the product eluted with 0.05 N buffer may be a mixture of isomeric analogs of dinucleoside monophosphate V.



V

All of the substances indicated above are formed in no more than 6-7% yields. Attempts to raise the yield by raising the temperature or increasing the reaction time were unsuccessful, inasmuch as resinification is observed in this case. Polycondensation of II under similar conditions did not give oligomers with higher molecular weights.

The products of the reaction of IV and V do not react with polyadenylic acid in a 0.015 M sodium citrate buffer with 1 M NaCl at pH 7.8 at 3-10°.

The effect of the dinucleoside monophosphate analog on the biosynthesis of protein in a preincubated protein-synthesizing S-30 *Escherichia coli* system was investigated.* It was found that it suppresses the synthesis of polyphenylalanine in the polyuridylic acid matrix.

EXPERIMENTAL METHODS

Chromatography on QAE-Sephadex and Sephadex G-10 was carried out as in [5]. Compounds I and II were chromatographically pure substances [5].

Polycondensation of N_1-(1,4-Dihydroxy-2-butyl)thymine (I) and Monophosphate II. Compounds I (5 mmole) and II (5 mmole) in the acidic form were dissolved in 10 ml of water and lyophilized in vacuo (3 mm) at room temperature. The mixture was then held at 165° for 7 min. The melt was dissolved in 10 ml of water, and the pH was brought up to 7.5. An analytical sample (0.05 ml) was diluted to 1 ml and applied to a column filled with Sephadex G-10 (the column volume was 70.5 ml, the column height was 65 cm, the buffer was 1 N ammonium carbonate, the fraction volume was 2.3 ml, and the fraction selection time was 5 min). Preparative separation was carried out with a 1480-ml column.

Anion-exchange separation was carried out with a column (height 7 cm, diameter 0.5 cm) filled with QAE-Sephadex (ammonium carbonate buffer pH 7.5, elution rate 120 ml/h, single-fraction selection time 5 min). Fraction 1, obtained by gel filtration, was divided into two peaks, which were eluted with 0.2 and 0.3 N ammonium carbonate buffer. The first peak, constituting 64% of the mixture, contained a substance having R_f values of 0.16, 0.3, and 0.38 in systems A, B, and C [5], respectively. The relative electrophoretic mobilities with respect to uridine 5'-monophosphate (5'-UMP) were 1.1 (pH 7.5) and 1.36 (pH 2.8). The substance eluted with 0.3 N ammonium carbonate buffer had R_f 0.1 in system B and 0.27 in system C, and the relative electrophoretic mobilities with respect to 5'-UMP were 1.19 (pH 7.5) and 1.58 (pH 2.8). Found %: C 35.6; H 5.8; N 15.5. $C_{27}H_{50}N_{10}O_{19}P_3$. Calculated %: C 35.5; H 5.6; N 15.4.†

During chromatography on QAE-Sephadex under conditions similar to those described, fraction 2 was divided into two substances, which were eluted with 0.05 and 0.1 N ammonium carbonate buffers. The first substance had relative electrophoretic mobilities of 0.9 (pH 7.5) and 1.0 (pH 2.8) and R_f values of 0.21, 0.34, and 0.47 in systems A, B, and C [5], respectively; the second substance had relative electrophoretic mobilities of 1.0 (pH 7.5) and 1.0 (pH 2.8). Found %: C 42.6; H 5.7; N 13.7. $C_{18}H_{28}N_5O_{10}P$. Calculated %: C 42.8; H 5.6; N 13.8.

The third substance, which was isolated by gel filtration, had R_f values of 0.72, 0.78, and 0.66 in systems A, B, and C [5], respectively.

---

*The investigation was conducted in the laboratory of nucleic acids of the Institute of Organic Synthesis of the Academy of Sciences of the Latvian SSR under the supervision of Doctor of Chemical Sciences E. Ya. Gren.

†The results of elementary analyses are presented for the ammonium form of the compounds.

LITERATURE CITED

1. S. A. Giller, Z. A. Shomshtein, and T. A. Popova, Khim. Geterotsikl. Soedin., 416 (1975).
2. J. Moravek, J. Kopecky, and J. Škoda, Coll. Czech. Chem. Commun., 33, 4407 (1968).
3. J. Moravek, Tetrahedron Lett., 1707 (1967).
4. S. A. Giller, Z. Z. Shomshtein, and T. A. Popova, Khim. Geterotsikl. Soedin., 409 (1975).
5. S. A. Giller, T. A. Popova, and Z. A. Shomshtein, Khim. Geterotsikl. Soedin., 401 (1975).