CHANGE IN THE INSTRUCTIONS FOR THE SYNTHESIS OF OLIGODEOXYRIBONUCLEOTIDES ON THE VIKTORIYA-2 AND VIKTORIYA-4 M AUTOMATIC SYNTHESIZERS

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Five oligodeoxyribonucleotides have been synthesized on the Viktoriya-2 and Viktoriya-4M automatic synthesizers — d(GATGCTGCCAACC) (a), d(GGCTCTAGCTTCATAC) (b), d(GGTTGGCAGCATC) (c), d(TAGAGCCGGTTGGC) (d), d(GCCAACCGGCTCTA) (e) – and, with their aid, 29- and 30-membered DNA duplexes forming substrates of the restriction endonuclease EcoRII and isoschizomers of it and containing defects in the structure of the double helix.

Oligonucleotides (a–c) were obtained by simultaneous synthesis on paper disks (Whatman 3MM) containing 200 μmole of the first nucleoside per gram of support The weight of one disk was 20 mg. The protected mono- and dinucleotide blocks used in the triester method of synthesizing oligonucleotides [1] were used for the growth of the chain. The approach employed permitted the total number of stages of condensation to be brought down to 14 in place of the 20 necessary for obtaining these oligonucleotides separately with the same blocks.

As the condensing reagent we used a mixture of triisopropylbenzenesulfonyl chloride γ-dimethylaminopyridine N-oxide, and N methylimidazole taken in a molar ratio of 1:3:7. The reaction was performed for 10 min in absolute pyridine. The use of this complex condensing agent improved the solubility of the γ-dimethylaminopyridine N-oxide in pyridine, which led to a decrease in the volume of the solvent in the reactor and, consequently, to a saving of nucleotide material for the creation of a 0.1–0.15 M concentration of the nucleotide.

After the complete elimination of the protective groups and isolation, the oligonucleotides were obtained in the following amounts: a — 25 OU_{260}; b — 11 OU_{260}; c — 154 OU_{260}, which indicated the possibility of using highly loaded paper disks for the synthesis of considerable amounts of DNA fragments.

Oligonucleotides (d and e) were obtained by the phosphite-amide scheme on the polymeric support aminopropyl-CPG with a load of the first nucleoside of 45 μmole/g. The change in the conditions for synthesis in comparison with those described previously [2] consisted in shortening of the time of washing the polymer with a 0.1 M solution of TFA in CH_2Cl_2 from 60 to

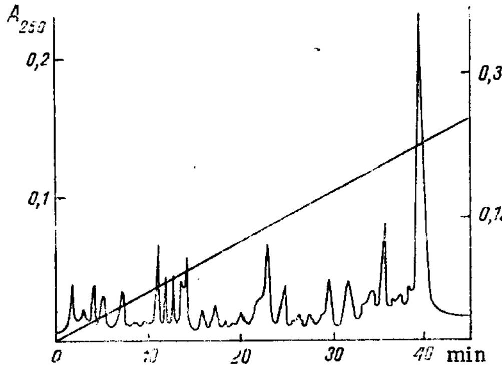

Fig. 1. Ion-exchange HPLC of the reaction mixture obtained in the automatic synthesis of the oligonucleotide GATGCTGCCAACC on a cellulose disk after the elimination of all the protective groups. Column containing the resin Polysil CA (3.2 × 250 nm), gradient of 0.02–0.3 M KH_2PO_4 (pH 6.5) in 30% CH_3CN; rate of elution 1 ml/min.

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30 sec and changing the order of the oxidation and capping stages. Capping was carried out first and then oxidation by the corresponding solutions: a 1 M solution of acetic anhydride in diisopropylethylamine–N-methylimidazole–acetonitrile (4.5:1:30) (capping agent), and a 0.2 M solution of I_2 in pyridine–acetic acid (9:1) (oxidizing agent) without intermediate washing. This change in the conditions led to a shortening of the time of growth of the oligonucleotide chain by one unit to 9.5 min without lowering the yields of final products, which were obtained in an amount of 3 OU_{260} for d and 4.5 OU_{260} for e.

The synthesis on paper disks was carried out in the Viktoriya-2 automatic synthesizer with a modified hydraulic scheme [3], and on the CPG support in the Viktoriya-4M synthesizer.

The oligonucleotides (a-e) were isolated on ion-exchange and reversed-phase high-performance liquid chromatography (HPLC, Fig. 1). The nucleotide sequence was determined by the Maxam–Gilbert method.

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INTERACTION OF THE ANTIBIOTIC FERVENULIN WITH INDOLE

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3-Substituted analogs of the antibiotic fervenulin (I) are transformed into derivatives of xanthine [1] or of 6-azapurine [2] as the result of the nucleophilic attack of their pyrimidotriazine nucleus by formamide at the C_{8a} atom and by the OH^- ion at the C_8 atom, respectively.

We have studied the interaction of (I) with indole (II). When equimolar amounts of saturated butanol solutions of compounds (I) and (II) were mixed, the crystalline molecular complex (III) with a 1:1 composition of the components was obtained with a yield of 65-70%. The frequency of absorption, ν_{NH}, of the electron-donating indole component of the crystals of compound (III) was 92 cm^{-1} lower than for free indole (3405 cm^{-1}). Such a change in the stretching vibrations of a N–H bond in primary and secondary amines indicates the participation of the nitrogen atom in coordination [3].

The peak intensities of the absorption bands at 1586 and 1542 cm^{-1} (C=C and C=N) in the IR spectrum of (III) (tablets with KBr) were, respectively, 45 and 23% lower than those for the antibiotic (I). This shows a decrease in the polarity of the C=C and C=N bonds in (III) because of some transfer of the charge of the indole nitrogen atom to the triazine moiety of the fervenulin molecule.

When compounds (I) and (II) were heated in boiling butanol (3-4 h), no products other than (III) were obtained. At the same time, in the presence of hydrochloric acid indole (II) and 2-methylindole (IV) add to (I) after only 15 minutes' heating in ethanol with the formation of the hydrochlorides of the 4a-indole derivatives of 6,8-dimethyl-2,4,5,6 7,8-hexahydropyrimidol[5,4-el][1,2,4]triazine-5,7-dione (V and VI). By treating aqueous solutions of the hydrochlorides obtained with sodium acetate, the bases (V) and (VI) were isolated in the free state. The yield of (V) was 75%, mp 225-226°C, and that of (VI) 55%, mp 240-241°C (scheme).

A doublet signal of the C_{4a} atom of compound (I) in the high-resolution ^{13}C NMR spectrum ($^3J_{C_{4a}H-3} = 8.6$ Hz) was observed at 133.10 ppm, while the sp^3-hybridized C_{4a} atom of compound (VI) gave a signal at 56.31 ppm with retention of doublet splitting ($^3J_{C_{4a}H-3} = 10$ Hz). The

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