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PYROPHOSPHATE TETRAESTER INTERMEDIATE OF COUPLING REACTIONS IN THE PHOSPHOTRIESTER APPROACH TO THE SYNTHESIS OF DEOXYOLIGORIBONUCLEOTIDES

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Abstract. The intermediate in phosphotriester formation in the presence of ~~$ARSO_2Cl$, $ArSO_2Tet$~~, $ArSO_2NT$ and mixtures of $ArSO_2Cl$ + Tet (NT, MeIm, DMAP) was established to be pyrophosphate tetraester II.

The pulsed ^{31}P NMR technique is an extremely powerful method for studying the coupling reactions in oligonucleotide synthesis since transformations involving not only starting and final products but also intermediates may be observed directly in the reaction mixture. Results of ^{31}P NMR investigations of condensation reactions of p-chlorophenyl nucleotides with an OH-components in pyridine solution in presence of well-known [1-7] condensing reagents are presented here. The reagents under investigation are arylsulfonyl chlorides /$ArSO_2Cl$/, arylsulfonyl tetrazoles /$ArSO_2Tet$/, arylsulfonyl 3-nitro-1,2,4--triazoles /$ArSO_2NT$/ and the mixtures of $ArSO_2Cl$ with I-H-tetrazole /Tet/, 3-nitro-1,2,4-triazole /NT/, N-methylimidazole /MeIm/, and 4-N,N-dimethylaminopyridine /DMAP/. The reagents 5'-O-tritylthymidine /(Tr)T/**, thymidine 5'--(bis-2-cyanoethyl)-phosphate /$(CNEt)_2pT$/, triisopropylbenzenesulfonyl chloride /TPS/, NT, 3'-O-levulinyl- or acetyl- thymidine 5'-(p-chlorophenyl)-phosphates /$(ClC_6H_4)pT(Lev)$ or $(ClC_6H_4)pT(Ac)$, respectively/ and polystyrenesulfonyl chloride produced by the pilot plant of the Institute of Organic Chemistry (Novosibirsk), triisopropylbenzenesulfonyl 3-nitro-1,2,4-triazole /TPSNT/ [3], p-toluenesulfonyl tetrazole (TsTet) [2], Tet ("Merck"), DMAP ("Bergkamen", Berlin) were used for this work. ^{31}P NMR spectra were taken with a Bruker HX-90 pulse spectrometer operating at 36.43 MHz, Fourier transform being performed with a B-NC 12 computer using 10 mm reaction tubes.

^{31}P NMR spectra of the reaction mixture of 5'-O-tritylthymidine 3'-(p--chlorophenyl)-phosphate /$(Tr)Tp(C_6H_4Cl)$/ (Ia) with 3'-O-acetylthymidine /T(Ac)/ and TPS show the rapid disappearance of the starting diester Ia signal (5.7ppm) and the accumulation of those of the intermediate IIa (19.6, 19.9 and 20.2ppm) (Fig.1a). Then the intensities of the IIa signals begin to decrease and the

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** The symbol d is omitted in this work, because only deoxynucleosides and their derivatives are used.

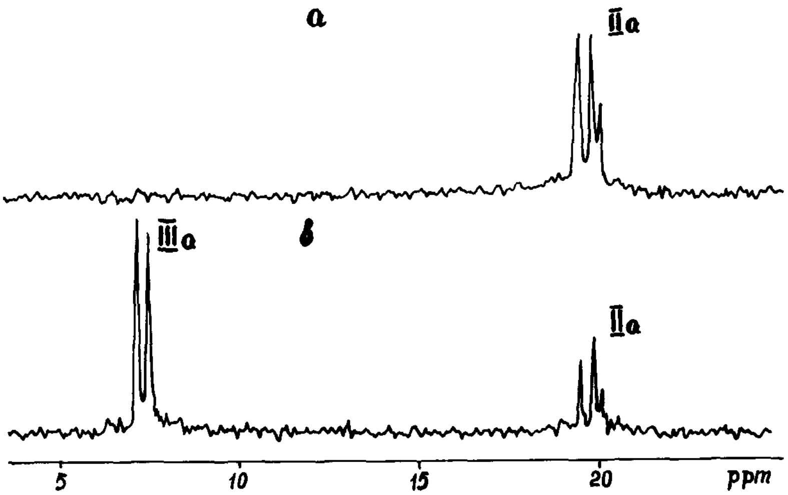


Fig.1. ^{31}P NMR spectra of the reaction mixture of 0.1 M (Tr)Tp(C_6H_4Cl) with 0.1 M T(Ac) and 0.1 M TPS at 30°C in pyridine: a) after 6 min, b)after 4h 46 min.

final product signals appear at 7.3 and 7.6 ppm (Fig.1b); these have been assigned to triester IIIa by means of the chemical shifts values, TLC, electrophoresis and paper chromatography data after the separation and deprotection. The kinetic curves of the process are plotted in Fig.2a.

Chemical shifts values of the intermediate II correspond to a compound with an anhydride bond. Thus a derivative with the same ^{31}P NMR spectrum was obtained by treatment of Ia with 10 eqv. of polystyrenesulfonyl chloride in pyridine and removal of the polymer by filtration. Therefore, intermediate IIa cannot be considered as a mixed anhydride derived from phosphodiester and sulfonic acid. On adding water to the solution of IIa the starting diester is formed, and treatment of IIa with cyclohexylamine results in the equal amounts of Ia and IVa (-4.0 ppm). The identity of IVa was confirmed as the cyclohexylamide of the phosphodiester by means of TLC, electrophoresis and ^{31}P and ^{1}H NMR data. The data obtained permits the identification of IIa as a pyrophosphate tetraester, which is in agreement with other work [8]. The several signals in spectra of IIa and IIIa are due to the existence of diastereoisomers [9].

The couplings of 5'-phosphodiesters Ib and Ic with 3'-hydroxy group of nucleoside in the presence of TsTet, TPSNT, TPS + Tet, TPS + NT, TPS + MeIm, TPS + DMAP were also studied. For 3'- and 5'-diesters the rapid disappearance of starting diester I, and the appearance of the intermediate II and final triester III were followed by ^{31}P NMR. Structures of intermediates IIb and

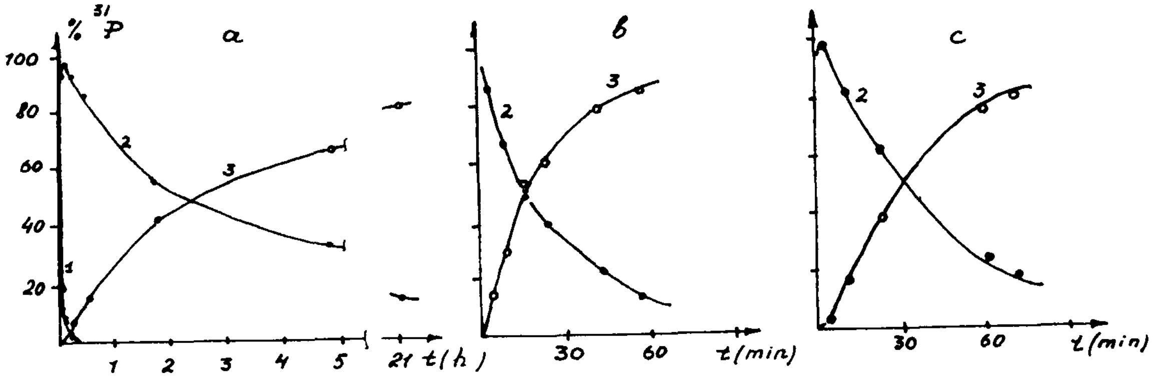


Fig.2. The kinetic curves of the reaction in pyridine: a) 0.1M (Tr)Tp(C_6H_4Cl) with 0.1M T(Ac) and 0.3M TPS at 30°C, b) 0.1M (Tr)Tp(C_6H_4Cl) with 0.1M T(Ac) and 0.3M TsTet at 2°C, c) 0.1M (C_6H_4Cl)pT(Lev) with 0.1M $(CNEt)_2$pT and 0.3M TPSNT at 20°C. I - (Tr)Tp(C_6H_4Cl), 2 - pyrophosphate II, 3 - triester III.

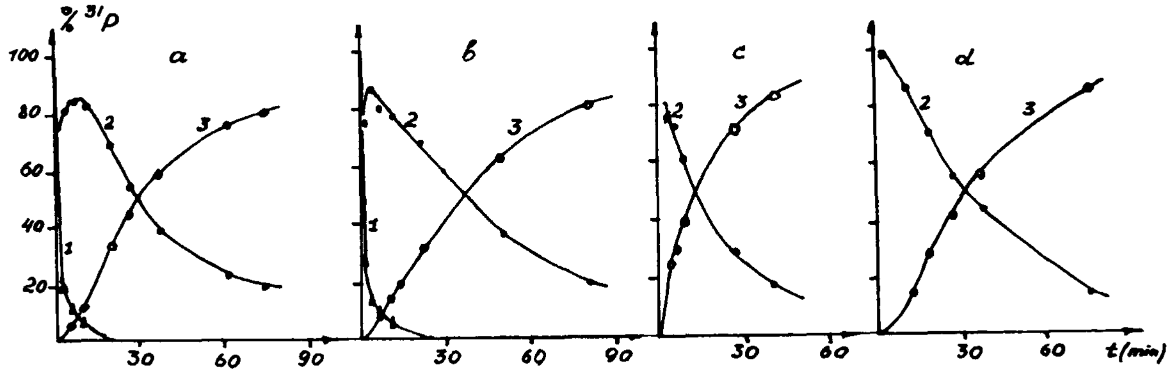
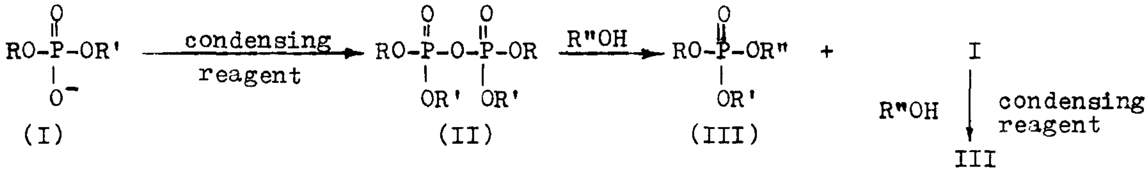
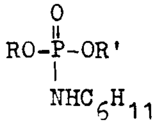


Fig.3. The kinetic curves of the reaction of 0.1M (C_6H_4Cl)pT(Lev) with 0.1M $(CNEt)_2$pT and 0.3M TPS at 20°C in pyridine in the presence of: a) 0.3M NT, b) 0.3M Tet, c) 0.3M MeIm, d) 0.3M DMAP. 1 - (C_6H_4Cl)pT(Lev), 2 - pyrophosphate IIc, 3 - triester IIIc.

IIc were confirmed by analogy with IIa. Figs.2 and 3 show that all the reactions under investigation proceed via pyrophosphate tetraester II intermediates





$$\mathrm{RO\text{-}\overset{O}{\overset{\|}{P}}\text{-}OR'}\ \mathrm{(NHC_6H_{11})}$$

(IV)

a) R = (Tr)T, R' = C_6H_4Cl, R" = T(Ac)
b) R = C_6H_4Cl, R'= T(Ac), R" = (Tr)T
c) R = C_6H_4Cl, R' = T(Lev), R" = $(CNEt)_2pT$

Thus, pyrophosphate II is not to be considered as a by-product as has been proposed earlier for condensation with $ArSO_2Cl$[4]. Figs.2 and 3 show that the step I → II proceeds very rapidly whereas the conversion II → III is slower (especially when TPS is used). The phosphodiester I formed from II is not observed in ^{31}P NMR spectra due to the rapid reaction with condensing reagent. A difference between triester formation rates in these reactions may be explained by the presence of different nucleophilic catalysts, which react with II to form active derivatives [4,5 - 7,10].

Pyrophosphate II does not react with the OH-component in the absence of nucleophilic catalysts [10].

The data obtained imply that the OH-component enters into reaction with active intermediates formed from I and II only after the starting diester I has disappeared from the reaction mixture, because I is more powerful nucleophile in comparison with ROH.

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