THE SPECIFIC SYNTHESIS OF $C_{3'}$-$C_{5'}$-LINKED URIDINE OLIGONUCLEOTIDES

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In developing approaches to the specific synthesis of the naturally occurring $C_{3'}$-$C_{5'}$ inter-ribonucleotidic linkage the underlying aim has been to prepare ribonucleoside-3' phosphate derivatives in which the adjacent 2'-hydroxyl group is protected by a group which can be removed subsequently under adequately mild conditions. The key intermediates used in the previously described approach (M. Smith, Rammler, Goldberg and Khorana, 1962; Rammler and Khorana, 1962) were the 2'-O-tetrahydropyranylribonucleoside-3' phosphates, the tetrahydropyranyl group being removed by an acidic treatment. Although specific syntheses of a number of $C_{3'}$-$C_{5'}$ linked ribo-dinucleotides were thus accomplished, caution was necessary in the removal of the tetrahydropyranyl group because prolonged acidic treatment caused detectable isomerization of the $C_{3'}$-$C_{5'}$ to $C_{2'}$-$C_{5'}$ inter-ribonucleotidic linkage. In an alternative approach to the above problem, the use of an alkali-labile group for the protection of the 2'-hydroxyl function was next investigated. The particular merit in the use of an alkali-labile group for the purpose was inferred from the previous observations which showed that while under acidic conditions some isomerization ($C_{3'}$-$C_{5'} \rightleftharpoons C_{2'}$-$C_{5'}$) of the inter-ribonucleotidic linkage accompanies the hydrolytic reaction, under alkaline conditions no such isomerization can be detected (Brown et al., 1956; Smith et al., 1962). In developing this approach, the preparation of 2',5'-di-O-acetyluridine-3' phosphate and 2'-O-acetyluridine-3' phosphate was recorded (Rammler and Khorana, Biochem. Biophys. Research Comm. 1962) and in model experiments

methyl uridine-3' phosphate was prepared from these intermediates. The methyl ester thus obtained was completely degraded by pancreatic ribonuclease. The present communication records the successful application of this approach to the first synthesis of uridine oligonucleotides containing exclusively the $C_{3'}$-$C_{5'}$ inter-nucleotidic linkages.

2'-O-Acetyluridine-3' phosphate was prepared by two routes. In the first route, the starting material was 5'-O-tetrahydropyranyluridine-3' phosphate and it was prepared as follows. Treatment of 2',5'-di-O-tetrahydropyranyluridine-3' phosphate (Ramm1er and Khorana, J. Am. Chem. Soc., 1962) with 50% acetic acid at 0° for 3 hr. gave as the main products a mixture of 2'-O-tetrahydropyranyluridine-3' phosphate and 5'-O-tetrahydropyranyluridine-3' phosphate. The latter, which predominated (75% of the mixture), was purified by selective reaction of 2'-O-tetrahydropyranyluridine-3' phosphate with dimethoxytrityl chloride (Smith, Ramm1er, Goldberg and Khorana, 1962) and separation on a DEAE-cellulose column. 5'-O-Tetrahydropyranyluridine-3' phosphate (0.28 mmole) was treated with a large excess of acetic anhydride (20 ml.) in a mixture of water (20 ml.) and pyridine (30 ml.) in the cold for 30 min. The mixture of resulting products, 2'-O-acetyl-5'-O-tetrahydropyranyluridine-3' phosphate and 5'-O-tetrahydropyranyluridine-2',3' cyclic phosphate, was kept at pH 2.8 (pH controlled by addition of Dowex-50 acidic resin) for 7.5 hr. at room temp. The main product now was 2'-O-acetyluridine-3' phosphate, the other products being uridine-2'(or 3') phosphate and uridine-2',3' cyclic phosphate. The desired product was purified by partition chromatography on a cellulose column using the solvent system ethyl alcohol-1M ammonia acetate (pH 6.5) (5-2, v/v). In the second route to 2'-O-acetyluridine-3' phosphate, the starting material was 5'-O-di-p-methoxytrityluridine-3' phosphate[1], which was prepared by direct treatment of uridine-3' phosphate with di-

(1) Unpublished experiments by Dr. Y. Lapidot of this laboratory showed that treatment of adenosine-3' phosphate with di-p-methoxytrityl chloride in pyridine under controlled conditions gave 5'-O-di-p-methoxytrityladenosine-3' phosphate as the major product. The latter

p-methoxytrityl chloride in dry pyridine followed by separation on a DEAE-cellulose column (Smith, Rammler, Goldberg and Khorana, 1962). Acetylation of the dimethoxytrityl derivative as described above for 5'-O-tetrahydropyranyluridine-3' phosphate followed by lowering the pH to about 2.5 for 7 min. gave 2'-O-acetyluridine-3' phosphate and uridine-2',3' cyclic phosphate as the products. The desired product was again purified by partition chromatography. In the first experiment on inter-nucleotide bond synthesis, 2'-O-acetyluridine-3' phosphate was reacted in anhydrous pyridine with two equivalents of 2',3'-di-O-benzoyluridine in the presence of an excess of dicyclohexylcarbodiimide (DCC). Although under these conditions the major product was uridine-3',5' cyclic phosphate (I)(cf. unpublished results of M. Smith in this laboratory) and the formation of small amounts of polymeric material was noted, uridylyl-uridine (6%) isolated was completely degraded by pancreatic ribonuclease. The inter-nucleotide bond synthesized was thus exclusively of the $C_{3'}$-$C_{5'}$ type. For polymerization, 2'-O-acetyluridine-3' phosphate (0.066 mmole) was reacted in anhydrous pyridine (0.1 ml.) with an excess of DCC (175 mg.) for 5 days (Khorana and Vizsolyi, 1961). After a work-up including treatment with 9N ammonium hydroxide at room temp. for 6 hr., the products were separated by a combination of ion exchange chromatography on a DEAE-cellulose column, preparative paper chromatography and, wherever necessary, preparative paper-electrophoresis. In general, the pattern of results was similar to that obtained in the previous studies on the deoxyribonucleotide polymerizations (Khorana, 1961) except that due, presumably, to the low concentration of the nucleotide the polymerization did not go as far. The products fully identified were uridine-3',5' cyclic phosphate (I), the cyclo-, di-, and tri-nucleotides represented by the general structure (III)

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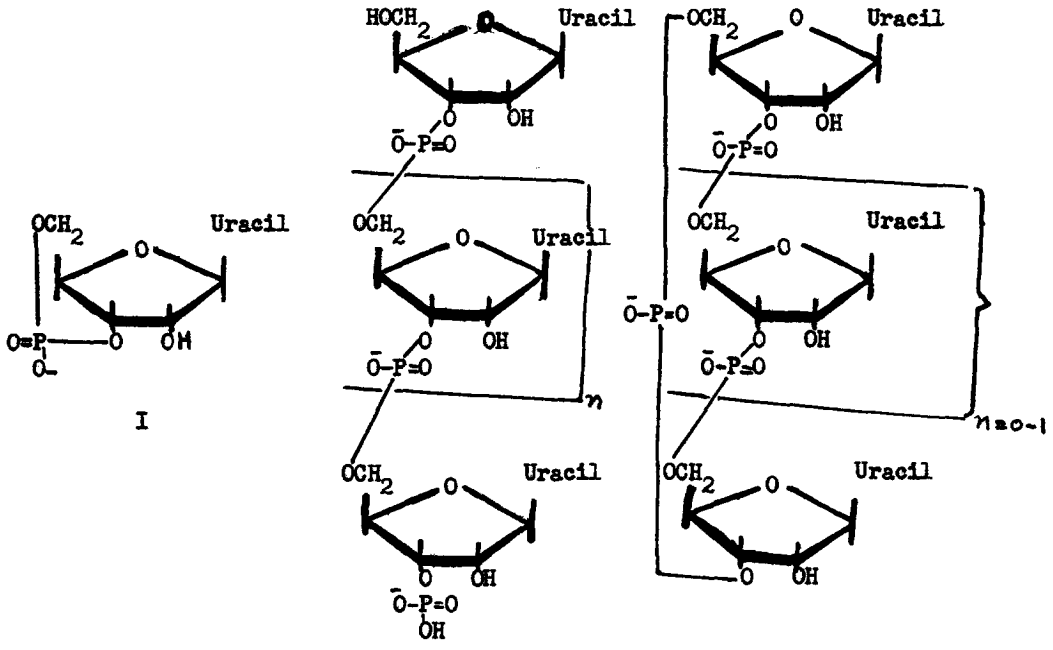
was characterized by ultraviolet absorption spectrum, by its quantitative conversion to the corresponding 2',3'-cyclic phosphate on treatment with dicyclohexyl carbodiimide in aqueous pyridine and by reconversion to pure adenosine-3' phosphate after very mild acidic treatment.



and the linear di- and tri-nucleotides (II). Smaller amounts of the higher homologs, which remain to be identified, were also present. The characterization of the oligonucleotides (II) included degradation by pancreatic ribonuclease, dephosphorylation by bacterial alkaline phosphomonoesterase followed by degradation with pancreatic ribonuclease and determination of the ratio of the two products, uridine and uridine-3' phosphate. It should be emphasized that in all experiments involving pancreatic ribonuclease complete degradation of the internucleotide bonds occurred.

Previously chemical methods for polymerization of ribonucleotides have afforded complex mixtures of oligonucleotides containing random $C_{2'}$-$C_{5'}$ and $C_{3'}$-$C_{5'}$ inter-nucleotide bonds (Smith et al., 1958; Michelson, 1959; Schramm et al., 1962). The development now reported constitutes the first specific synthesis of ribo-oligonucleotides containing $C_{3'}$-$C_{5'}$ linkages. The procedures used are simple and offer promise of being general. Extension of this work to the synthesis of larger polymers and of mixed oligonucleotides is in progress.

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