A NEW APPROACH TO THE SPECIFIC SYNTHESIS OF THE $C_{3'}$-$C_{5'}$ INTER-RIBONUCLEOTIDE LINKAGE

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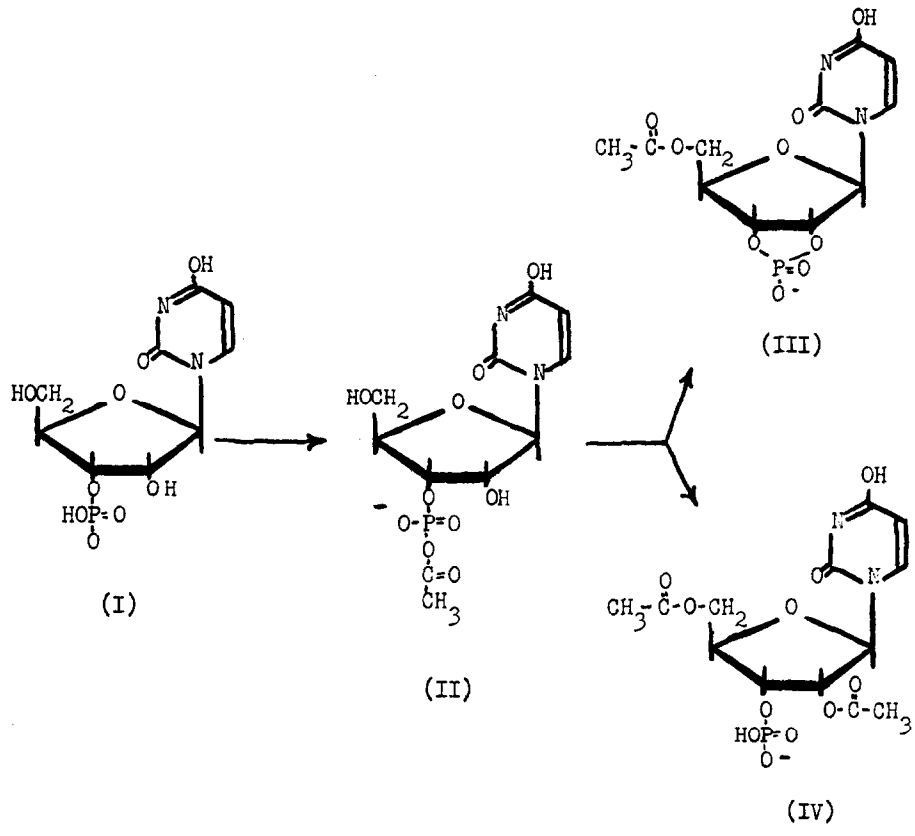
As a part of the program of synthetic work in the polynucleotide field, which has been in progress in this laboratory (Khorana, 1961), attention has been focussed on the development of approaches to the specific synthesis of the naturally occurring $C_{3'}$-$C_{5'}$ inter-ribonucleotide linkage (Smith, Rammler and Khorana, 1962; Rammler and Khorana, 1962). The presence of the 2'-hydroxyl group in the ribonucleosides presents a serious complication and the aim has been to prepare ribonucleoside and ribonucleotide derivatives in which the 2'-hydroxyl group is suitably protected. In principle, the specific synthesis of an inter-nucleotide bond may be effected either by the condensation of a suitably protected ribonucleoside-5' phosphate with a second ribonucleoside bearing a free 3'-hydroxyl function or by the condensation of a suitably protected ribonucleoside-3' phosphate with a ribonucleoside bearing a free 5'-hydroxyl group. A systematic investigation (Rammler and Khorana, 1962) showed the latter approach to be the more feasible and using it, the synthesis of several $C_{3'}$-$C_{5'}$ linked di-ribonucleotides was accomplished. The 2'-hydroxyl function in the ribonucleoside-3' phosphate derivatives used was protected by the tetrahydropyranyl group, the latter being removed at the end of the synthesis by a mildly acidic treatment. However, caution was necessary because prolonged acid treatment resulted in detectable isomerisation of the $C_{3'}$-$C_{5'}$ to the $C_{2'}$-$C_{5'}$ internucleotide linkage. In the present communication we report on the development of an alternative approach in which the

alkali-labile acetyl group is used to protect the 2'-hydroxyl group in ribonucleoside-3' phosphates. This approach appears to be more general and attractive for the elaboration of ribopolynucleotide synthesis.

The reaction of an acylating agent such as acetic anhydride with the anion of a mononucleotide rapidly gives a mixed anhydride (Avison, 1955). In the case of ribonucleoside-3' phosphates (e.g., I) the initially formed anhydride (II) would be expected to give the 2',3'-cyclic phosphate (III) (Brown, Magrath and Todd, 1952).

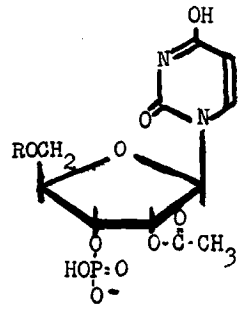


It has now been found that the nature of the products obtained by treatment of a ribonucleoside-3' phosphate with acetic anhydride is influenced greatly by the conditions used. When uridine-3' phosphate is acetylated under acidic conditions, or in sodium acetate buffer, the

product is mainly uridine-2',3' cyclic phosphate. However, when an excess of acetic anhydride in pyridine is used, acetylation of the 2'-hydroxyl group also occurs and consequently a mixture of 5'-O-acetyluridine-2',3' cyclic phosphate and 2',5'-di-O-acetyluridine-3' phosphate is formed. The latter has been isolated pure in 35% yield. The use of an excess of acetyl bromide in pyridine in the above reaction gives 2',5'-di-O-acetyluridine-3' phosphate in 51% yield. Further work is directed towards a more thorough investigation of the acylating agents and the reaction conditions in order to further increase the extent of the acylation at the 2'-hydroxyl group. The present method has also been applied to the preparation of other fully acetylated ribonucleoside-3' phosphates and this simple method is, thus, general for the preparation of useful synthetic intermediates from the readily accessible ribonucleoside-3' phosphates.

2',5'-Di-O-acetyluridine-3' phosphate was treated in anhydrous pyridine with an excess of methyl alcohol and dicyclohexylcarbodiimide. After removal of the acetyl groups with ammonia at room temperature, methyl uridine-3' phosphate was isolated in quantitative yield. This product was completely degraded by pancreatic ribonuclease to uridine-3' phosphate. 2',5'-Di-O-acetylribonucleoside-3' phosphates can thus be used in the specific synthesis of $C_{3'}$-$C_{5'}$ inter-ribonucleotide bonds analogously to the previously described 2',5'-di-O-tetrahydropyranyl ribonucleoside-3' phosphates (Rammler and Khorana, 1962).

The further development of the acetylated ribonucleoside-3' phosphates for use in polymerization and stepwise synthesis would require the preparation of derivatives of the type (V) in which group R can be selectively removed. In the present work, 5'-O-tetrahydropyranyluridine-3' phosphate was prepared from 2',5'-di-O-tetrahydropyranyluridine-3' phosphate. Acetylation of this substance with acetic anhydride in pyridine followed by removal of the tetrahydropyranyl group yielded, after purification by preparative paper chromatography, 2'-O-acetyluridine-3' phosphate



(V)

in 38% yield. This substance was shown to be homogenepus by paper chromatographic and electrophoretic methods. Its reaction with methyl alcohol, using dicyclohexylcarbodiimide in pyridine, gave methyl uridine-3' phosphate in quantitative yield. This product was completely degraded by pancreatic ribonuclease.

The preparation of 2'-O-acetyl-5'-O-tetrahydropyranyluridine-3' phosphate and its use in stepwise synthesis will be reported subsequently. It is clear, however, that 2'-O-acetyluridine-3' phosphate is a suitable starting material for polymerization reactions by the methods already developed for the polymerization of deoxyribomononucleotides. Work along these lines is in active progress.

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