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STUDIES ON THE SYNTHESIS OF THE NATURAL INTERNUCLEOTIDE BOND BY THE USE OF CYCLONUCLEOSIDES*

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The present syntheses of the 3'-5' internucleotide bond are based upon the protection of amino and all but one hydroxyl group to prevent these other groups from entering into undesired reactions with the activated phosphate[1]. An alternative approach, the activation of the 5' alcoholic carbon for the SN2 reaction with a 3' phosphate anion, has not been utilized. This reaction may not require any time-consuming protecting procedures and would more importantly, in our opinion, give a specific synthesis of the natural isomer.

Acting on this principle, Žemlička and Smrt recently prepared uridylyl-2'(3'):5'-(2',3'-isopropylidene) uridine by the reaction of unprotected uridine-3' phosphate with 2',3'-isopropylidene-O^2,5'-cyclouridine[2]. They observed a 1:1 ratio of the two isomers and hence a complete lack of specificity.

This publication has prompted us to report on research we have been pursuing for some time independently along similar lines, but with additional results which contribute to the understanding of the mechanism of this reaction.

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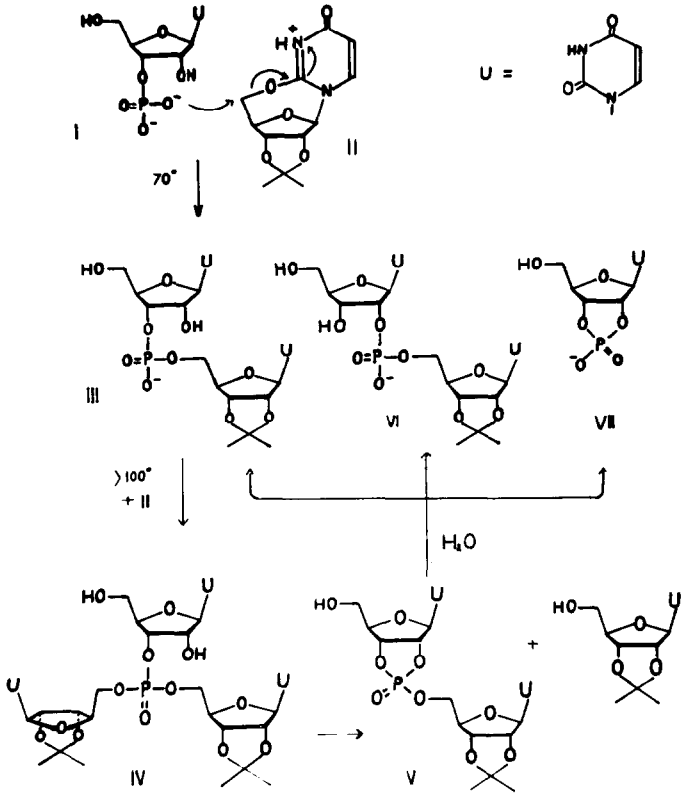
From the reaction of uridine-5' phosphate and thymidine-3' phosphate with an excess of 2', 3'-isopropylidene-O^2, 5'-cyclouridine (II) at or over 100°, we isolated, in addition to the expected diesters, triesters of phosphoric acid in good yield. Trinucleoside-phosphates have not been described before. The structure of triuridine-5' phosphate was sufficiently proved as follows. The molar ratio of the pyrimidine UV absorption to phosphorus was found to be 3:1. Paper chromatographic mobility in water saturated n-butanol relative to uridine was 1.5. The compound did not migrate on electrophoresis at pH 6. It is degraded in dilute alkali at elevated temperatures to a mixture of mono- and diester, according to an electrophoretic analysis. No hydrolysis occurred on incubation with snake venom diesterase. The diesters obtained were similarly analyzed.

We preferred to utilize the nucleotides as monoanions instead of dianions (only the latter were used by the Czechoslovak authors) as the cleavage of the isourea ether type system is facilitated by acid catalysis[3]. Alkalinity, and hence rapid hydrolysis, is also avoided in this procedure. A mixture of acetonitrile and dimethylacetamide was the solvent.

The formation of the triester obviously requires a higher thermal activation than that of the diester. At 70°, using a trifold excess of cyclonucleoside, only traces of the triester were present after a week, but also a low yield (20%) of diester was obtained.

In contrast to this, but according to expectations, no triester of uridine-3' phosphate (I) could be isolated due to the great instability of such triesters[4]. The labile trinucleoside-phosphate (IV) could be easily transformed to the triester V, which upon hydrolysis would give,

The reaction of uridine-3' phosphate
with 2', 3'-isopropylidene-O^2, 5'-cyclouridine



as observed by Žemlička and Smrt, a mixture of the two isomeric dinucleoside phosphates (*III* and *VI*) and some uridine-2', 3' cyclic phosphate (VII). The formation of a labile triester may also be responsible for the isomerization of pure uridylyl-3':5'-uridine on treatment with 2', 3'-isopropylidene-O^2, 5'-cyclocytidine and for the formation of uridylyl-2'(3'):5'-(2', 3'-isopropylidene) cytidine[2].

It may be concluded from our experiments that the specific synthesis of the natural internucleotide linkage via cyclonucleosides is feasible if (1) the formation of the triester is successfully avoided and (2) if the system is neutral.

Taking into account these conclusions we have synthesized uridylyl-3':5'-(2',3'-isopropylidene) uridine (III) in over 95% purity as follows: 0.1 mM tri-n-octylammonium 3'-uridylate and a trifold excess of 2',3'-isopropylidene-O^2,5'-cyclouridine were sealed in vacuo in 2 ml acetonitrile-dimethylacetamide mixture and kept at 70^0 for one week. The reaction products were separated by chromatography on paper in isopropanol-ammonia-water (7:1:2). One half of the total phosphorus was recovered from the monoester fraction. Uridylyl-3':5'-(2',3'-isopropylidene) uridine (III, in 25% yield) could be easily separated from another diester-like fraction which consisted of a mixture of uridine-2',3' cyclic phosphate and a yet unidentified compound. III was almost entirely hydrolyzed by RNase to uridine-3' phosphate and isopropylideneuridine. The yield of the main product is not quite satisfactory at this preliminary stage, but it can certainly be improved.

Besides its theoretical significance, this new approach offers a possible route for the rapid, simple preparation of a large variety of nucleotides of biological interest both in the ribo and deoxyribo series.

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