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THE REACTION OF $O^2,5'$-CYCLOURIDINE AND $O^2,5'$-CYCLOCYTIDINE DERIVATIVES WITH NUCLEOTIDES. A NEW APPROACH TO THE SYNTHESIS OF THE $3'\rightarrow5'$ INTERNUCLEOTIDIC BOND

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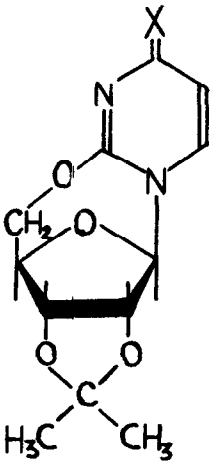
Reactive cyclic nucleoside derivatives (1) have been shown to be versatile intermediates, especially in the pyrimidine series, for the preparation of nucleosides with modified sugar or heterocyclic moieties. In this connection their relatively high reactivity toward a number of nucleophilic agents (e.g. azide (2), alkoxide (3), halide (4) or sulphide (5) ions) has been exploited. The reaction of cyclonucleosides with some weaker nucleophiles (e.g. phosphate or phosphate esters anions), however, has not yet been investigated.

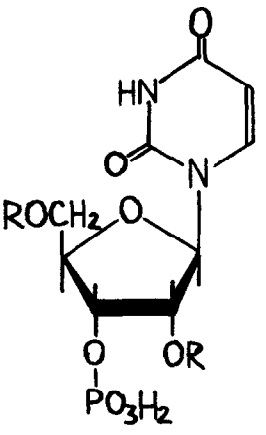
We have now found that 2',3'-O-isopropylidene-O^2,5'-cyclouridine Ia (3) reacts with uridine 3'-phosphate IIa (as the bis-tri-n-octylammonium salt) at 100°C in dioxane solution to give uridylyl $2'(3')\rightarrow5'$-(2',3'-O-isopropylidene)uridine IIIa. Only a minor amount of uridine 2',3'-cyclic phosphate is also formed. Similarly, 2',5'-di-O-acetyluridine 3'-phosphate IIb (6) reacts with



I

Ia: X = O

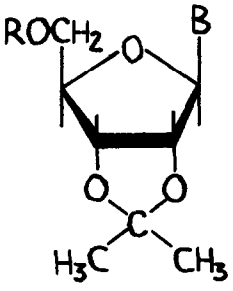
Ib: X = $NH_2^{(+)}$ $CH_3SO_3^{(-)}$



II

IIa: R = H

IIb: R = CH_3CO



III

IIIa: B = uracil residue
R = uridine 2'(3')-phosphoryl residue

IIIb: B = cytosine residue
R = uridine 3'-phosphoryl residue

the cyclonucleoside Ia with the formation of the diester IIIa (after removal of acetyl groups). The reaction of O^2,5'-cyclocytidine derivative Ib[x] with the phosphomonoester IIb proceeds more readily, owing to a higher reactivity of Ib, yielding (after deblocking) uridylyl 3'→5'-(2',3'-O-isopropylidene)cytidine IIIb.

From the results summarized in Table 1 we can conclude that the addition of an excess of a sterically hindered base (tri-n-octylamine) affects the yield of the diester IIIb only slightly. On the other hand, a large surplus of a strong base (triethylamine) lowers the yield, as shown for the case of IIIb. A similar drop in yield has also been observed in an experiment with excess of the nucleotide

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x) This compound was described (as the p-toluenesulphonate salt) earlier (7). A more convenient method has now been elaborated for the preparation of Ib involving treatment of N-acetyl-2',3'-O-isopropylidenecytidine (8,9) (m.p. 117-120°C (acetonitrile), λ_{max} 213-214, 247-248, 298-299 mμ, λ_{min} 227, 274-275 mμ (ethanol)) with methanesulphonyl chloride in pyridine to give N-acetyl-2',3'-O-isopropylidene-5'-O-methanesulphonylcytidine (m.p. 177-180°C (ethanol), λ_{max} 214, 248, 295 mμ, λ_{min} 227, 275 mμ (ethanol)). Deacetylation with methanolic ammonia saturated at 0°C for 1 h at room temperature and refluxing of the resulting 2',3'-O-isopropylidene-5'-O-methanesulphonylcytidine in acetone solution for 9 h afforded the cyclonucleoside Ib (m.p. 246-249°C, λ_{max} 210, 257-258 mμ, λ_{min} 220 mμ (methanol)).

component. Determination of the content of the 2′→5′ isomers in the products IIIa,b has shown that under the conditions used the isomerisation of the 3′→5′ internucleotidic bond occurs to some extent even with a 2′-blocked nucleotide component (phosphomonoester IIb). This appears to establish the partial removal of the 2′-O-acetyl group during the reaction. Experiments with other protecting groups are now in progress. When reaction of Ib were carried out by heating in dimethylformamide- or dimethyl sulphoxide-containing media side reactions were observed.

We have further found that phosphodiesters also react with compounds of type Ia,b. Thus, on heating uridylyl 3′→5′-uridine (10,11) (as triethylammonium salt, 5 μmoles) with the cyclonucleoside Ib (10 μmoles) in dimethylformamide (0.1 ml) in a sealed tube at 100°C for 1 h an interesting exchange of the nucleoside residues takes place: after chromatography of the reaction mixture in solvent system A (see Table 1) a considerable amount of the diester IIIb (containing 24% of the 2′→5′ isomer) was isolated. The extent of 3′→5′ to 2′→5′ isomerisation of the internucleotidic linkage in the starting material was found to be approximately 17%.

The scope and limitations of this reaction from the point of view of the synthesis of compounds containing natural 3′→5′ internucleotidic bonds along with some mechanistic aspects of the present work are being examined. The results will be published in the Collection of Czechoslovak Chemical Communications in due course.

TABLE 1

Nucleotide component[a] (mmole)	Cyclo-nucleoside (mmole)	Base (mmole)	Reaction time[b] (hours)	Solvent[c]	Product (yield[d] %)	Content of 2'→5'isomer in product[e] (%)
IIa (0.1)	Ia (0.3)	tri-n-octylamine (0.2)	12	A	IIIa[f] (71)	50
IIb (0.1)	Ia (0.2)	-	12	A	IIIa (79)	20
IIb (0.025)	Ib (0.05)	-	2	B (1:2)	IIIb (79)	7[g]
IIb (0.025)	Ib (0.1)	triethylamine (1.0)	3/4[h]	B (1:3)	IIIb (38)	-[i]
IIb	Ib	-	1/2[h]	B (1:3)	IIIb (25)	-[i]

[a] Bis-tri-n-octylammonium salts.

[b] The reaction temperature was 100°C in all cases.

[c] A = dioxane, B = dioxane - dimethylformamide mixture.

[d] The yields were estimated spectrophotometrically after chromatography of the reaction mixture on Whatman No 1 or 3 MM paper in isopropyl alcohol - ammonia - water (7:1:2) (solvent system A) and are based on the phosphomonoester recovered. Acetyl groups were removed prior to chromatography

by treatment with methanolic ammonia (saturated at 0°C) overnight at room temperature. The products IIIa,b were characterised by chromatography in system A, by electrophoresis at pH 7,5 and by degradation with pancreatic ribonuclease.

[e] Estimated spectrophotometrically after the degradation of the compounds IIIa,b with pancreatic ribonuclease and chromatography of the products in solvent system A.

[f] In addition to IIIa, 8% of uridine 2',3'-cyclic phosphate was also obtained.

[g] The enzymic degradation was performed with IIIb obtained after a reaction time of 1 h (yield of IIIb 69%).

[h] No increase in yield was observed during further heating.

[i] The extent of 3'→5' to 2'→5' isomerisation was not determined.

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