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SYNTHESIS OF 2′,5′-O-PROTECTED RIBONUCLEOSIDES

J. Smrt and A. Holy
Institute of Organic Chemistry and Biochemistry
Czechoslovak Academy of Science, Prague

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The naturally occuring internucleotidic bond can be formed either by condensation of a protected nucleoside-5′phosphate with a nucleoside derivative, bearing a free $C^{3'}$-hydroxyl function, or by condensation of a protected nucleoside-3′phosphate with a nucleoside derivative, bearing a free $C^{5'}$-hydroxyl function. The former approach is generally used in the deoxyribonucleotide field (1) where the nucleoside derivative bearing a free $C^{3'}$-hydroxyl group can be readily prepared. In the ribonucleotide field, the latter approach is used exclusivelly, (2,3) because no suitable 2′,5′-protected ribonucleosides had been described. Recently, the preparation of such nucleoside derivatives and their use in internucleotidic bond syntheses was recorded (4,5). Although the general utility of these compounds is not yet in view, their use for the synthesis of internucleotidic bond in cases where the phosphate component is accessible in the 5′-form only can be envisaged.

It was therefore considered desirable to investigate the accessibility of 2′,5′-protected ribonucleosides. A new method for the preparation 2′,5′-protected uridine and N-acetylcytidine has been now developed using enzymic dephosphorylation of 3′-phosphate derivatives* be means of alkaline phosphatase**.

The calcium salt of 2′-O-tetrahydropyranyl-5′-O-acetyl uridine-3′ phosphate (6) (I; 1 mmol) in 0.05 M Tris-buffer pH 8 (50 ml) was incubated with the enzyme (50 mg) at 37° overnight. The precipitated calcium phosphate was centrifuged off and the supernatant, after adjusting to pH 8, evaporated.

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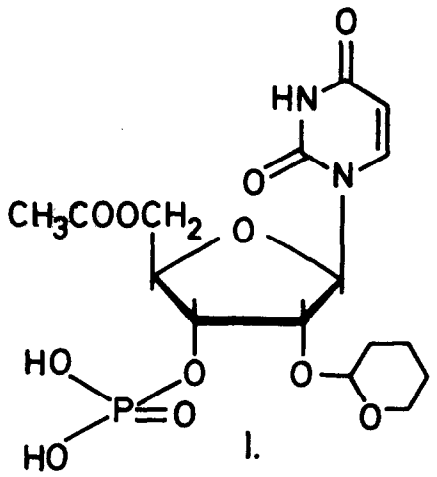
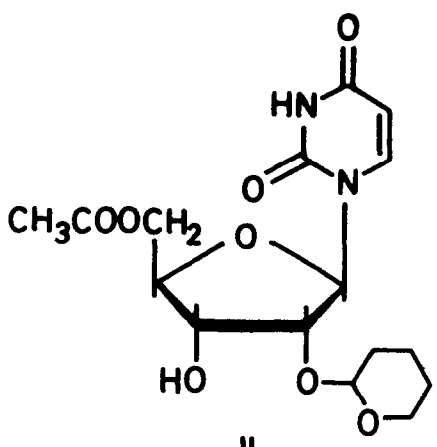
*This possibility was mentioned in our earlier paper (6).

**Phosphatase alkaline (calf intestine) Calbiochem was used.



I.



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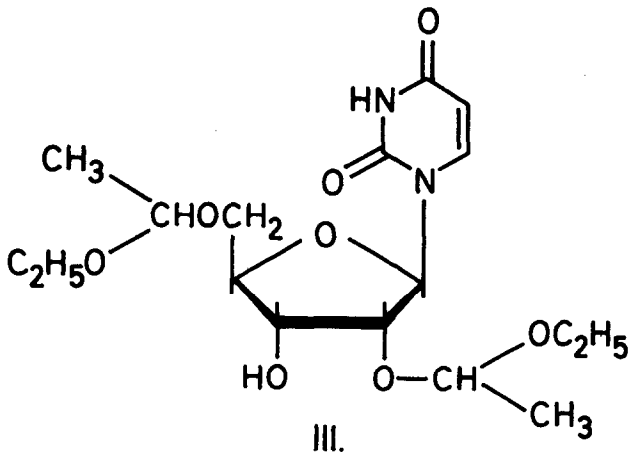
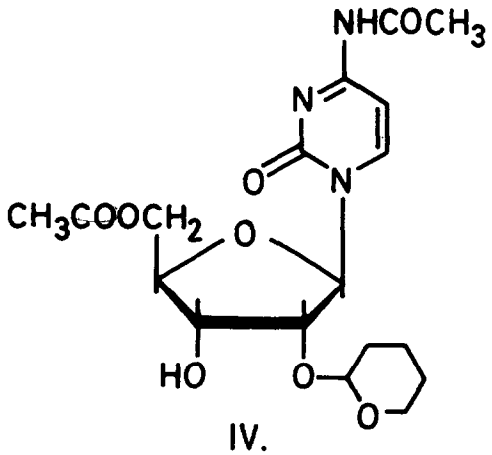


III.



IV.

The residue was chromatographed on six sheets of Whatman 3 MM paper in butanol - water (85 : 15). The broad band (R_F 0.7) was eluted with 70% ethanol. After evaporation and drying in high vacuum 2'-O-tetrahydropyranyl--5'-O-acetyluridine (II; 303 mg; 82%) was obtained. R_F (Butanol-water) 0.69. For $C_{16}H_{22}N_2O_8$ calculated: 51.80% C, 5.99% H, 7.56% N; found: 51.58% C, 6.32% H, 7.21% N.

The suitability in internucleotidic bond synthesis of compound II was next established. Reaction of II (0.1 mmol) with 2′, 3′-O-ethoxymethylene uridine-5′ phosphate* (0.05 mmol) and dicyclohexylcarbodiimide (0.1 g) in anhydrous pyridine for 3 days and the usual manner work-up (3) gave 2′-O-tetrahydropyranyl-(3′→5′)-2′, 3′-O-ethoxymethylene uridine (7) in 53% yield. Removal of the acid-labile protecting groups (20% acetic acid, 50°C) afforded uridylyl-(3′→5′)-uridine. The product contained 1.7% ribonuclease resistant material.

The same procedure, starting from 2′, 5′-di-O-(1-ethoxyethyl)-uridine-3′ phosphate (8), was used to the synthesis of 2′,5′-di-O-(1-ethoxyethyl)-uridine (III; R_F butanol-water 0.80). A protected derivatives of cytidine, N^4, $O^{5'}$--diacetyl-2′-O-tetrahydropyranylcytidine (IV) was obtained by the dephosphorylation of N^4, $O^{5'}$-diacetyl-2′-O-tetrahydropyranyl cytidine-3′-phosphate (9) (yield 82%). The product had λ_{max} 249, 298 um, λ_{min} 227, 273 (ethanol).

*This compound was prepared by the action of ethylorthoformiate on uridine--5′ phosphate in dimethylformamide solution.

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