A REMARK ON THE PREPARATION OF PROTECTED GUANOSINE 3'-PHOSPHATE BY MEANS OF A MIXTURE OF RIBONUCLEASES T1 AND T2*

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$N^2,O^{5'}$-Diacetylguanosine 2',3'-cyclic phosphate (*II*), prepared from guanosine 2',3'-cyclic phosphate by the action of acetic anhydride in the presence of tetraethylammonium hydroxide is not degraded either by ribonuclease T1 or ribonuclease T2.

The general approach developed in this Laboratory for the preparation of specifically substituted ribonucleoside 3'-phosphates, consists in acetylation of the corresponding ribonucleoside 2',3'-cyclic phosphate and the subsequent enzymatical conversion to an acetylated ribonucleoside 3'-phosphate bearing a free 2'-hydroxylic function. This procedure was first applied to the pyrimidine nucleotides[1,2] with the use of pancreatic ribonuclease and then extended to a guanosine derivative[3] using ribonuclease T1 and to both the purine nucleotides[4] using a mixture of ribonucleases T1 and T2, contained in the extract from Taka diastase (Sanzyme R, Calbiochem, USA). According to one of informations of the latter paper[4], ribonuclease T2 may be regarded as completely unspecific, degrading not only N^6-acetyladenosine 2',3'-cyclic phosphate but also N^2-acetylguanosine 2',3'-cyclic phosphate. This information proved correct in the case of the adenosine derivative only.

As shown by repeated attempts to reproduce the preparation of $N^2,O^{5'}$-diacetylguanosine 3'-phosphate according to the reported procedure[4], a considerable amount of the cyclic phosphate did not undergo any degradation even when a great excess of enzymes was used. Consequently, the acetylation of guanosine 2',3'-cyclic phosphate with acetic anhydride in pyridine must afford two substances, one of which is degraded by enzymes while the other one is resistant. The acetylation of the guanosine amino group with acetic anhydride in pyridine is known to proceed sluggishly if at all[5], while the acetylation of the corresponding nucleotide is relatively fast since it is assisted by the intramolecular transfer of the acetyl group from the acetyl phosphate mixed anhydride[6]. Since, however, Holý[7] did not observe either the formation

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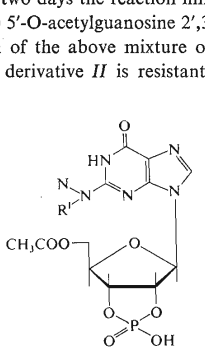
of a mixed anhydride of 2',3'-cyclic phosphate with acetic acid (acetyl phosphodiester) in the reaction of guanosine 2',3'-cyclic phosphate with acetic anhydride in the presence of triethylamine, or a N-acetylation, it is hardly possible to assume that the acetylation could be accelerated by a transfer of the acetyl group, analogous to the transfer from acetylphosphomonoesters.

In fact, the chromatographic control (in 6 : 1 ethanol–1M ammonium acetate) of the acetylation of guanosine 2',3'-cyclic phosphate with acetic anhydride in pyridine has shown that even after two days the reaction mixture contains two acetyl derivatives. The slower (R_F 0·78) 5'-O-acetylguanosine 2',3'-cyclic phosphate (*I*) undergoes degradation by the action of the above mixture of ribonucleases, while the faster (R_F 0·88) $N^2,O^{5'}$-diacetyl derivative *II* is resistant. To prove the structure of the



I, $R^1 = H$
II, $R^1 = COCH_3$

resistant product, authentic $N^2,O^{5'}$-diacetylguanosine 2',3'-cyclic phosphate was prepared by acetylation of guanosine 2',3'-cyclic phosphate with acetic anhydride in the presence of tetraethylammonium hydroxide[5]. The product of this preparation was identical with the chromatographically faster product of the acetylation in pyridine, also with respect to the complete resistance towards the mixture of ribonucleases T1 and T2.

In conclusion, *a*) N^2-acetylguanosine 2',3'-cyclic phosphate is not degraded by ribonuclease T2 and *b*) the considerations on $N^2,O^{5'}$-diacetylguanosine derivatives in the paper discussed[4] obviously refer to the $O^{5'}$-monoacetyl derivatives.

EXPERIMENTAL

$N^2,O^{5'}$-Diacetylguanosine 2',3'-Cyclic Phosphate (*II*)

To a solution of guanosine 2',3'-cyclic phosphate dicyclohexylguanidinium salt[8] (2 mmol) in an 1 : 3 dimethylformamide–pyridine mixture (30 ml) there is added 25% aqueous tetraethyl-

ammonium hydroxide (5 ml), the whole is evaporated, and the residue is coevaporated three times with pyridine. The final residue is kept in a mixture of pyridine (10 ml) and acetic anhydride (6 ml) for 20 h at room temperature. Acetic acid (1 ml) is then added, the mixture evaporated, the residue coevaporated twice with pyridine, and finally dissolved in 50% aqueous pyridine (10 ml). The resulting solution is passed through a column of pyridinium Dowex 50 ion exchange resin (25 ml) and the column is eluted with 50% aqueous pyridine (100 ml). The eluates are evaporated under an occasional addition of pyridine to the distillant. The residue is coevaporated twice with pyridine and finally dissolved in pyridine (10 ml). The solution is added dropwise with stirring into ether (200 ml), the precipitate collected with suction, washed with ether, and dried under diminished pressure. Yield, 920 mg of the pyridinium salt of compound *II*. UV spectrum (spot eluate from paper chromatography; pH 2): λ_{max} 259 nm, λ_{min} 228 nm, $A_{250/260}$ 0·88, $A_{280/260}$ 0·70, $A_{290/260}$ 0·56.

Attempted Degradation with Taka Diastase Extract

To a solution containing the pyridinium salt of compound *II* (90 mg), water (1·6 ml), dimethylformamide (0·8 ml), and 1M ammonium acetate there is added the freshly prepared dialysate (2 ml) of the Sanzyme R (Calbiochem, USA) extract. The mixture was incubated at 37°C; even after 3 days, no phosphomonoester was present, as shown by paper electrophoresis. On the other hand, $N^6,O^{5'}$-diacetyladenosine 2',3'-cyclic phosphate was quantitatively degraded under analogous conditions with the same enzyme preparation.

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