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A STUDY ON ACETYLATION OF NUCLEOTIDES BY MEANS OF ACETIC ANHYDRIDE IN PYRIDINE

Takashi KURIHARA and Tsujiaki HATA*

Laboratory of Chemistry for Natural Products, Faculty of Science

Tokyo Institute of Technology

Ookayama, Meguro-ku, Tokyo 152

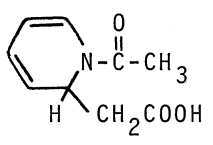
A side-reaction in the course of acetylation of nucleotides has been studied.

A number of experiments on the acetylation of alcohols by means of acetic anhydride have appeared in the literatures. In the synthesis of oligonucleotides especially this reaction was used, usually for the protection of the sugar moiety of nucleotides. During our study on acetylation of N^4-benzoylcytidine 5'-phosphate (pC^{Bz}), we found recently an additional spot on paper chromatogram[1] with Rf value 0.63. Such a phenomenon has been reported by Khorana in the case of thymidine derivative.[2]

In this communication, we describe the structure and the formation mechanism of the by-product.

The by-product was formed by acetylation of pC^{Bz} with acetic anhydride in pyridine according to the ordinary procedure[3] and it was extracted with ether or chloroform. The ultraviolet absorption characteristic of the substance at pH 7 was λ_{max} 297 nm[2] and showed it to be non-nucleotidic. In its behavior on paper electrophoresis, it seemed to be an acidic substance. On the other hand, this substance could not be detected when the reaction of acetic anhydride with pyridine was carried out in the absence of pC^{Bz} or in the persence of N^4-benzoylcytidine (C^{Bz}).

Since it is evident that phosphate is essential for the formation of the by-product, the reaction of acetic anhydride with pyridine was tried in the presence of phenyl phosphate. In this experiment, the same product was formed and isolated as white crystals (mp 118-120°C) which were identified as N-acetyl-2-carboxymethyl-2 H-pyridine, a dihydropyridine derivative (1): Mass, m/e 181(M^+), 138, 122, 93, 81; UV(MeOH), λ_{max} 297 nm (ε 24,900), λ_{min} 252 nm; IR (KBr), ν_{max} 1735, 1625, 1410, and 1365 cm^{-1}; NMR ($CDCl_3$), 8.90 (s, 1H), 6.50 (d), 6.00 (m), 2.50 (d, 2H), and 2.16 (s, 3H); Found: C, 59.34; H, 6.19; N, 7.54. Calcd for $C_9H_{11}NO_3$: C, 59.66; H, 6.67; N, 7.73.

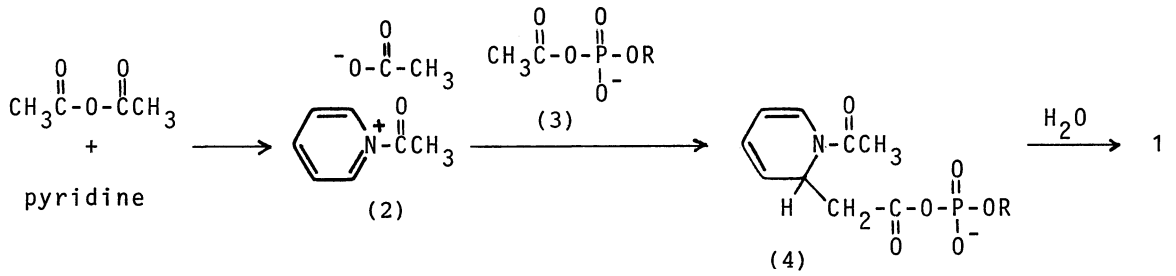


(1)

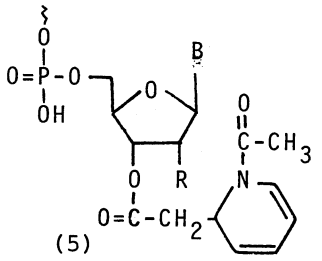
Diphenyl phosphate and diphenyldithiophosphonic acid were also used in place of phenyl phosphate: Acetic anhydride (2 mmole) was added to a solution of diphenyl phosphate (1 mmole) in dry pyridine (2 ml) and the solution was stirred at room temperature for 28 hr. The resulting mixture was quenched with water at 0°C. After removal of the solvent, a crude material (1) was obtained. This was purified with silica gel column chromatography.

In a similar manner, 1 was obtained in 36% yield when diphenyldithiophosphonic acid (1 mmole) was employed in the above experiment.

From these facts, the reaction seems to proceed through N-acetylpyridinium acetate (2) which in turn reacts with a mixed anhydride (3) formed from phosphate and acetic anhydride to afford a final intermediate (4). This is further hydrolyzed to 1 when the reaction mixture is treated with water.[4)]



From a practical point of view, the procedure of removing 1 by washing with ether must be added to the well-known ordinary procedure in preparation of the acetate derivatives of nucleotides. Otherwise, a wrong product showing the structure (5) could be formed by the coupling reaction of 1 and nucleotide in the synthesis of oligonucleotides.



Acknowledgement

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References and Notes

1) Chromatography was carried out on cellulose (Avicel) thin-layer. Solvent system used was: isopropyl alcohol-concentrated ammonia-water (7:1:2 v/v).
2) H.G.Khorana and J.P.Vizsolyi, J.Amer.Chem.Soc., 83, 675(1961): Khorana reported the ultraviolet absorption characteristic at pH 7 was λ_{max} 295 nm.
3) P.T.Gilham and H.G.Khorana, ibid., 80, 6219(1958).
4) When the reaction mixture was treated with methanol, methyl ester of 1 was obtained in good yield.

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