[Chem. Pharm. Bull. 15(6) 894~896 (1967)]

UDC 547.963.07

Takuma Sasaki and Yoshihisa Mizuno: Selective Acylation of the Amino or the Primary Hydroxyl Group of Cytidine.*1

(*Faculty of Pharmaceutical Sciences, Hokkaido University**2)

(Received November 8, 1966)

For the chemical syntheses of nucleotides and oligonucleotides involving cytidine, both amino and the hydroxyl groups other than that involved directly in phosphorylation should be blocked with appropriate "protecting groups" such as acyl groups.

Selective acylation of the amino group of cytidine may be achieved with acetic anhydride in the presence of tri-*n*-butylamine,[1] thiolacetic acid,[2] O-acetylhydroxamic acid derivatives,[3] acetic anhydride in methanol[4] or benzoylimidazole.[5]

All the procedures are quite tedious and apart from those of Fox, *et al.*[4] and Montagu, *et al.*,[2] the above methods require the special acylating agents.

In this regard, we have divised a simple procedure for acylation of the amino group of cytidine.

In this paper, selective acylation of 5′-hydroxyl group of the nucleoside will also be briefly dealt with.

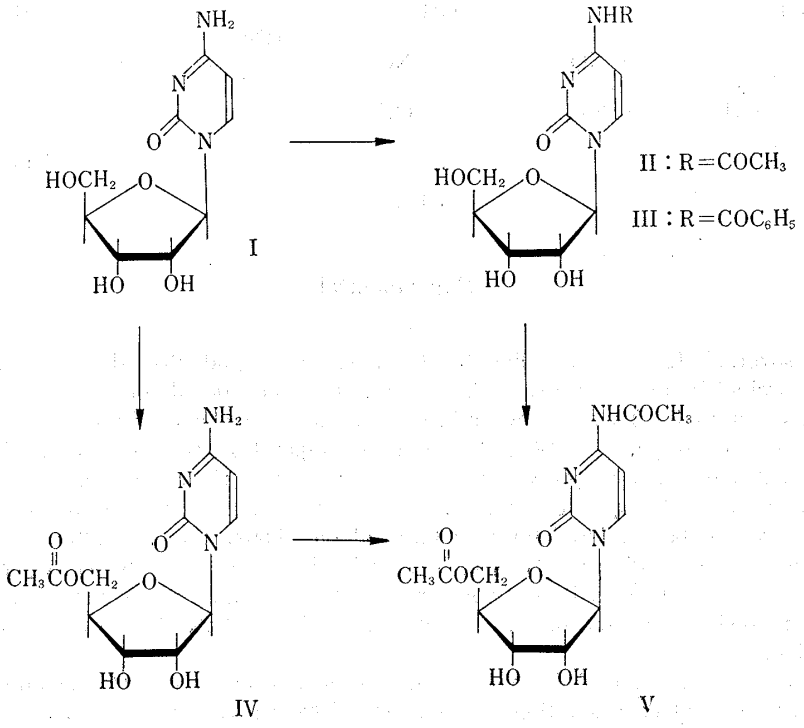

Chart 1.

*1 A preliminary report of part of this work was read at the 23th National Meeting of the Pharmaceutical Society of Japan, Sendai, October 1966: Abstract, p. 55.

*2 Nishi-5-chome, Kita-12-jo, Sapporo (佐々木琢磨, 水野義久).

1) A. M. Michelson: J. Chem. Soc., **1959**, 3655.
2) M. van Montagu, J. Stock: Arch. Intern. Physiol. et Biochim., **73**, 158 (1965).
3) Y. Mizuno, T. Itoh, H. Tagawa: Chem. & Ind. (London), **1965**, 1498.
4) J. J. Fox, K. A. Watanabe: Angew. Chem., **78**, 589 (1966).
5) F. Cramer, W. Saeuger, K. Scheit, J. Tennigkeit: Ann., **679**, 156 (1964).

Our procedure consists in treatment of cytidine (I) with acetic anhydride in pyridine. Thus, acylation of (I) with an equimolar amount of acetic anhydride at refluxing temperature in dry pyridine afforded predominantly N^4-acetylcytidine[3] (II, 66% yield), indicating that acylation under these conditions took place mainly on N^4-amino group rather than hydroxyl groups.

On the other hand, treatment of (I) with refluxing glacial acetic acid in the absence of bases afforded a predominant amount of 5′-O-acetylcytidine (IV, 57%), cytidine (26%) being recovered unchanged.

Compound (IV) was converted into N^4,5′-O-diacetylcytidine (V) with acetic anhydride-pyridine which was also obtained by treatment of (II) with refluxing acetic anhydride.

The above structure assignments for (IV) and (V) rest upon the fact that they were all oxidized with metaperiodate under a standard condition and both their ultraviolet absorption spectra and the combustion values were also in good agreement with such formulations.

N^4-Benzoylcytidine (III) was previously prepared by selective O-debenzoylation of N^4,2′,3′,5′-O-tetrabenzoylcytidine.[6] However, the nucleoside (III) could be obtained in 92% yield from cytidine by one-step procedure (treatment of (I) with excess of benzoic anhydride in pyridine and dimethylformamide).

Our tentative explanation for selective acylation described above is as follows : N^4-Amino group of cytidine in pyridine solution is the best nucleophile among their amino and hydroxyl groups to be selectively acetylated with an equimolar amount of acetic anhydride, whereas amino group of cytidine in glacial acetic acid (under such conditions, cytidine exists probably in N-protonated form) becomes weaker nucleophile than hydroxyl groups.

Thus, in acidic media 5′-O-hydroxyl group which is the least hindered among hydroxyl groups was predominantly acylated. Attempts at economical conversions of N^4-acetylcytidine (II), 5′-O-acetylcytidine (IV), N^4-benzoylcytidine (III) and N^4,5′-O-diacetylcytidine described above into cytidine derivative having free 3′-hydroxyl group (a key intermediate of oligonucleotide synthesis) being investigated in our laboratory.

Experimental

Unless othrwise stated, melting points were corrected. Paper chromatography was carried out on Toyo Roshi No. 51 filter paper by ascending technique with a solvent of *n*-BuOH-H_2O (86:14). Column chromatography also used same solvent. UV absorbing spots on paper chromatogram were detected under the ultraviolet lamp and the compounds containing *cis*-glycol were detected by periodate spray reagent. Ultraviolet absorption spectra were taken with an Hitachi EPS-2U automatic recording spectrophotometer. Infrared spectra were taken with a JASCO DS-301 spectrophotometer.

N^4-Acetylcytidine (II)——To a suspension of cytidine (12.15 g., 50 mmoles) in 1.1 L. of pyridine was added 5 ml. of Ac_2O. The nucleoside was then completely dissolved. The resulting solution was refluxed for 2 hr. The solvent was removed *in vacuo* to leave white solid. Recrystallization from EtOH afforded pure (II); yield, 9.5 g. (66.6%), m.p. and mixed m.p. with an authentic sample[3] 202.5~204.5°, UV $\lambda_{max}^{H_2O}$ mμ: 247, 296. IR cm^{-1} : $\nu_{C=O}$ 1730. *Anal.* Calcd. for $C_{11}H_{15}O_6N_3$: C, 46.31; H, 5.30; N, 14.73. Found : C, 46.31; H, 5.51; N, 14.65.

5′-O-Acetylcytidine (IV)——A solution of cytidine (I, 1.21 g., 5 mmoles) in 200 ml. of glacial AcOH was refluxed for 3 hr. The cooled solution was concentrated to dryness *in vacuo*. The residue was dissolved in 10 ml. of solvent and applied on a cellulose powder column (weight of cellulose powder, 100 g., column size, 2.8×60 cm.).

The column was washed with the same solvent. Ten ml. of eluate was collected as one fraction. Fractions 5~10 contained a nucleoside (Rf 0.37); yield 31 mg. (10%), fractions 11~18 contained two nucleo-

6) D. H. Rammler, H. G. Khorana : J. Am. Chem. Soc., **84**, 3112 (1962).

sides (Rf 0.20 and 0.37)*3; fractions 19~21 contained the nucleoside (IV, Rf 0.20); yield, 566 mg. (39.7%). This sample (Rf 0.20) consumed metaperiodate under a standard condition. UV $\lambda_{max}^{H_2O}$ mμ: 270, 230. IR cm^{-1}: $\nu_{C=O}$ 1720. Though this sample was analytically pure, attempts to crystallize failed. *Anal.* Calcd. for $C_{11}H_{15}O_6N_3$: C, 46.31; H, 5.30; N, 14.73. Found: C, 45.98; H, 5.26; N, 14.58.

Product Distribution of Acetylation of (I) with Glacial AcOH——Cytidine (I, 243 mg., 1 mmole) was treated with glacial AcOH (10 ml.) for 3 hr. at refluxing temperature. The solvent was removed *in vacuo* to leave brown syrup. Chromatography of the reaction mixture showed the presence of 5'-O-acetyl-(Rf 0.20, 57%), and 2' (or 3'), 5'-O-diacetylcytidine (Rf 0.37, 17%) in addition to unreacted cytidine (0.08, 26%). Even after the solution was refluxed for another 3 hr., the product distribution did not change.

N^4,5'-O-Diacetylcytidine (V) from 5'-O-Acetylcytidine (IV)——To a solution of 5'-O-acetylcytidine (IV, 285 mg., 1 mmole) in 50 ml. of pyridine was added 0.1 ml. of Ac_2O. The solution was refluxed for 2 hr. The solvent was removed *in vacuo* to leave gummy residue. Crystallization from EtOH afforded 274 mg., 83.8% yield of N^4,5'-O-diacetylcytidine, m.p. 127~130°. This sample consumed metaperiodate under a standard condition. UV $\lambda_{max}^{H_2O}$ mμ: 248, 296. IR cm^{-1}: $\nu_{C=O}$ 1720, 1730. *Anal.* Calcd. for $C_{13}H_{17}O_7N_3$: C, 47.71; H, 5.23; N, 12.84. Found: C, 47.52; H, 5.35; N, 12.80.

N^4,5'-O-Diacetylcytidine (V) from N^4-Acetylcytidine (II)——To a suspension of N^4-acetylcytidine (II, 2.26 g., 7.9 mmoles) in 150 ml. of dry pyridine was added 0.8 ml. of Ac_2O (*ca.*, 8 mmoles). The suspension was refluxed for 2 hr. The solvent was removed *in vacuo* to leave gummy residue. Crystallization from EtOH afforded a pure sample; yield, 2.12 g. (82%), m.p. 125~129°. UV $\lambda_{max}^{H_2O}$ mμ: 248, 296. IR cm^{-1}: $\nu_{C=O}$ 1720, 1730. Mixed m.p. with the authentic sample described above did not show depression. *Anal.* Calcd. for $C_{13}H_{17}O_7N_3$: C, 47.71; H, 5.23; N, 12.84. Found: C, 47.43; H, 5.49; N, 12.70.

N^4-Benzoylcytidine (III)——To a solution of cytidine (2.43 g., 10 mmoles) in pyridine (70 ml.) and dimethylformamide (30 ml.) was added benzoic anhydride (3.39 g., 15 mmoles). The reaction mixture was stirred for overnight at room temperature, after which it was poured into water and stirred. The solvent was removed *in vacuo* to leave white solid. The solid material was extreacted with ether (four 40 ml. portions) to remove BzOH. The yield of residue was 3.2 g. (92%), m.p. 218°. The analytical sample was prepared by crystallization from EtOH; m.p. 219~220°. UV λ_{max}^{MeOH} mμ: 305, 260. IR cm^{-1}: $\nu_{C=O}$ 1730. *Anal.* Calcd. for $C_{16}H_{17}O_6N_3$: C, 55.04; H, 4.94; N, 12.1. Found: C, 55.05; H, 4.95; N, 12.20.

The present authors are grateful to Dr. T. Ueda of this laboratory for his interest and encouragement. Thanks are also due to Mrs. T. Tohma and Miss A. Maeda of the analytical laboratory of this Faculty for elemental analyses. We also thank to Research Laboratories, Takeda Chemical Industries, Ltd. for supplying us with cytidine.

*3 Nucleoside (Rf 0.37) was assumed to be 2'(or 3'),5'-O-diacetylcytidine, based on its absorption maxima and the fact that the spot (0.37) showed a negative reaction for a metaperiodate spray reagent and the fact that Rf-values in the same solvent of 2'(3'),5'-O-diacetyl-, 2',3',5'-O-triacetyl-, and N^4,2',3',5'-O-tetraacetylcytidines are 0.37, 0.48 and 0.73, respectively.

[Chem. Pharm. Bull. 15(6) 896~898 (1967)]

UDC 577.16B : 543.544.25

Toshio Imanari and Zenzo Tamura: Gas Chromatography of Compounds in Vitamin B_6 Group.

(*Faculty of Pharmaceutical Sciences, University of Tokyo*[*1])

(Received December 22, 1967)

A highly sensitive determination method for the compounds in vitamin B_6 group has been requested because of the extremely small quanties of them present in biological fluids. The fluorometric method and bioassay are usually available, but they are troublesome and tedious, and have some defects. The application of gas chromatography should provide a rapid and simple method. In addition, the microdetermination may

*1 Bunkyo-ku, Hongo, Tokyo (今成登志男, 田村善蔵).