Notes

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Pyrimidine Nucleosides. III.[1] Reaction of Cytidine or N^4-Acetylcytidine with Partially Hydrolyzed Phosphorus Oxychloride

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O^2,2′-Cyclocytidine (II) is an intermediate in the chemical preparation of some cytidine analogues. Among them, arabinosylcytosine (III) has received considerable attention as an antiviral[3] and carcinostatic[4] agent. O^2,2′-Cyclocytidine was synthesized by Walwick,[5] Doerr[6] and Ruyle.[7] Since their methods have some drawbacks, we attempted to find a more convenient method to obtain II from cytidine. Furukawa and Honjo[8] reported that uridine gave 3′,5′-di-O-acetyl-O^2,2′-cyclouridine when it was heated with partially hydrolyzed phosphorus oxychloride in ethyl acetate. We first adapted their method to a direct preparation of acylated cytosine cyclonucleoside, using cytidine instead of uridine under the same conditions as that of Furukawa and Honjo.[9] Cytidine, however, did not give any acylated cytosine cyclonucleosides, but cyclocytidine with no protection was obtained instead. A suspension of cytidine in ethyl acetate was heated for 2 hours with partially hydrolyzed phosphorus oxychloride (see Experimental). Shift of the absorption maximum (265 mμ) at pH<1 of the resulting solution was observed.[10] The cyclized nucleoside was isolated using ion exchange

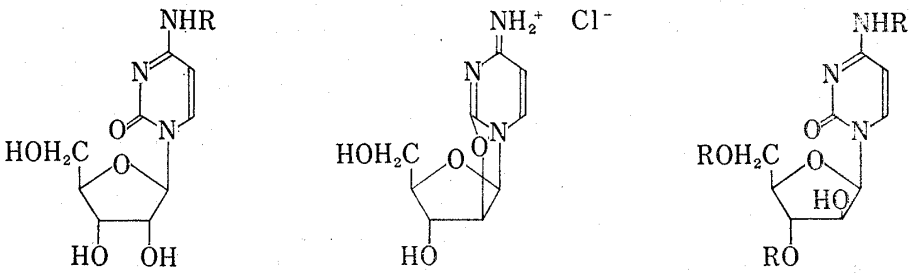

Chart 1

1) Part II: T. Kanai, M. Ichino and T. Kojima, *Chem. Pharm. Bull.* (Tokyo), **17**, 650 (1969).
2) Location: *Higashihama-11277, Saiki, Oita.*
3) H.E. Renis, C.A. Hollowell and G.E. Underwood, *J. Med. Chem.*, **10**, 777 (1967), and footnotes therein.
4) R.J. Rapac, *J. Not, Cancer Inst.* **40**, 997 (1968) and footnotes therein.
5) E.R. Walwick, W.K. Roberts and C.A. Dekker, *Proc. Chem. Soc.*, **1959**, 84.
6) I.L. Doerr and J.J. Fox, *J. Org. Chem.*, **31**, 1465 (1967).
7) W.V. Ruyle and T.Y. Shen, *J. Med. Chem.*, **78**, 331 (1967).
8) Y. Furukawa and M. Honjo, *Chem. Pharm. Bull.* (Tokyo), **16**, 2286 (1968).
9) Furukawa and Honjo had reported that no cyclized product was obtained when cytidine was heated with partially hydrolyzed phosphorus oxychloride in ethyl acetate as in the case of uridine in their experiments.
10) Formation of II from cytidine results in shift of absorption maximum, because the maxima of cytidine and II are 271 mμ and 262 mμ, respectively.

resin (Diaion SK-1B, acid form) in colorless needles and was found identical with $O^2,2'$-cyclocytidine hydrochloride (II) by comparison with published data[5] for the melting point, ultraviolet (UV) absorption maximum and optical rotation as well as the data of elemental analysis. II was thus prepared from cytidine by a single step in 45—65% yield. II was easily converted to arabinosylcytosine (III) by alkaline hydrolysis.

Arabinosylcytosine (III) was more conveniently prepared in a high yield, when an isolation step of II from the reaction mixture was omitted. The cyclized cytidine adsorbed on Diaion SK-1B resin (acid form) as above was eluted with chilled 2N KOH, giving III. Thus, prisms of III was directly obtained in 62.9% yield from cytidine.

The same treatment of N^4-acetylcytidine[11] as that of cytidine deseribed above did not afford the expected N^4-acetylcyclocytidine or N^4-acetylarabinosylcytosine. The product was characterized as $N^4,3',5'$-tri-O-acetylarabinosylcytosine (V)[12] from its melting point, UV spectrum and combustion value. Alkaline hydrolysis of V liberated arabinosylcytosine.[5] Aqueous phase separated from reaction mixture (see Experimental) contained one more component, which was not yet characterized, other than V, cytidine or arabinosylcytosine. Thus, V was probably formed *via* intermediate $N^4,3',5'$-tri-O-acetyl-$O^2,2'$-cyclocytidine. This interesting results suggested that the basicity of amino group of cytosine moiety was probably associated with acetylation of hydroxyl group of cyclonucleosides under such a condition.

The procedure described in this paper can be conveniently applied to the preparation of gram or kilogram quantities of $O^2,2'$-cyclocytidine and arabinosylcytosine.

Experimental[13]

$O^2,2'$-Cyclocytidine Hydrochloride (II)——A suspension of cytidine (10.0 g) in ethyl acetate (1.0 liter) was refluxed for 2 hours with partially hydrolyzed phosphorus oxychloride (76.5 ml, $POCl_3/H_2O=1$ mole/mole) and the reaction mixture was poured into ice–water (1.0 liter) with stirring. The stirring of this suspension was continued for 3 hours at room temperature. After the ethyl acetate was removed by evaporation, the acidic solution was subjected to the column of Diaion SK-1B (acid form, 700 ml). After washing with water, and product was eluted with 0.5M pyridinium formate buffer (pH 4.5, 3 liter). The eluate was evaporated to dryness *in vacuo* and small amount of pyridine was removed by repeated coevaporation with water. The residue was dissolved in water (200 ml) and passed through the column of Diaion SA-11B (chloride form, 200 ml). The resin was washed well with water (1.5 liter). Combined resulting solution and washings was concentrated to small volume to afford colorless needles. Recrystallization was accomplished by dissolving the needles in minimum amount of water and allowing to stand in a refrigerater. Yield was 5.80 g (53.90%). mp 251—255° (decomp.). UV $\lambda_{max}^{pH\,2-7}$ mμ: 232, 262 (ε 9.500, 10.600). Optical rotation: $[\alpha]_D^{24}$ −21.7° (*c*, 2.0, H_2O). The compound was stable to periodate. *Anal.* Calcd. for $C_9H_{12}O_4N_3Cl$: C, 41.31; H, 4.62; N, 16.06. Found: C, 41.43; H, 4.50; N, 15.84.

$N^4,3',5'$-Tri-O-acetylarabinosylcytosine (V)——To a suspension of N^4-acetylcytidine (IV) (20.0 g) in ethyl acetate (2.0 liter) was added partially hydrolyzed phosphorus oxychloride (153.0 ml, $POCl_3/H_2O$ =1 mole/mole) and the mixture was refluxed for 1 hour. The cooled reaction mixture was poured into sodium bicarbonate solution and unreacted partially hydrolyzed phosphorus oxychloride was completely hydrolyzed under cooling with ice (420 g of $NaHCO_3$ in 3 liter of water). Ethyl acetate phase was separated from the neutralized solution followed by washing with water and was concentrated *in vacuo* to give colorless needles. The same compound (needles) was also obtained from the chilled aqueous phase. Yield was 6.20 g (23.3%). mp 217—220°. UV λ_{max}^{MeOH} mμ: 248, 300 (ε 6.300, 5.900). Paper chromatogram: *Rf*(A) 0.82. *Anal.* Calcd. for $C_{15}H_{19}O_8N_3$: C, 48.78; H, 5.19; N, 11.38. Found: C, 48.66; H, 5.33; N, 11.60.

1-β-D-Arabinosylcytosine (III)——Method A (from V): $N^4,3',5'$-Tri-O-acetylarabinosylcytosine (V) (1 g) was dissolved in methanol (50 ml) saturated with ammonia and the solution was allowed to stand for 24 hours at room temperature. The reaction mixture was concentrated to dryness *in vacuo* to give white prisms. Pure material was obtained by recrystallization from 50% ethanol. Yield was 0.59 g (90.0%). mp 215—217°. Optical rotation: $[\alpha]_D^{24}$ +158° (*c*, 0.5, H_2O). UV $\lambda_{max}^{pH\,1}$ mμ: 280 (ε 13.300), $\lambda_{max}^{pH\,12}$ mμ: 230,

11) K.A. Watanabe and J.J. Fox, *Angew. Chem.*, **78**, 501 (1966).
12) H.P.M. Fromageot and C.B. Reese, *Tetrahedron Letters*, **1966**, 3499.
13) All melting points were uncorrected. *Rf* (A) stands for the *Rf* value in the solvent A (*n*-BuOH–H_2O; 86:14).

274 (ε 8.300, 9.800). *Anal.* Calcd. for $C_9H_{13}O_5N_3$: C, 44.45; H, 5.39; N, 17.28. Found: C, 44.39; H, 5.57; N, 17.05.

Method B (from II)——To a solution of O^2,2′-cyclocytidine hydrochloride (II) (1 g) in water (40 ml) was added 2N KOH (3.8 ml) and the solution was allowed to stand for 4 hours at room temperature with stirring. The reaction mixture was treated with Diaion SK-1B (ammonium form, 16 ml) in batch and the resin was removed by filtration. The filtrate was concentrated to dryness *in vacuo* to give white prisms (0.76 g) in 82.0% yield. Recrystallized compound was identical with arabinosylcytosine obtained by the method A by all criteria.

Method C (from I)——A suspension of cytidine (35.0 g) in ethyl acetate (3.5 liter) was refluxed with partially hydrolyzed phosphorus oxychloride (316 ml, $POCl_3/H_2O=1$ mole/mole) for 2 hours. The reaction mixture was cooled and poured into ice water (3.0 liter) with stirring to hydrolyze completely phosphorus oxychloride. The acidic solution, after ethyl acetate was removed by evaporation from it, was subjected to the column of Diaion SK-1B (acid form, 2.45 liter) followed by washing with water (16.6 liter) and elution with chilled 2N KOH solution (4.0 liter). In order to make the elution effective the alkaline solution was circulated through the column. The eluate, after removing potassium ion by Diaion SK-1B (ammonium form, 8.5 liter), was passed through the column of Diaion SA-11B (borate form, 0.42 liter) to remove unreacted cytidine and the resin was washed with water. The effluent containing III and washings were combined and it was concentrated to a small volume to give white prisms (22.0 g).

Recrystallization from minimum amount of water gave pure material which was identical with arabinosylcytosine obtained by the method A.

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Proteolytic Enzymes. V.[1] Further Application of *p*-Nitrophenyl *p*-Amidinobenzoate as a Titrant of Trypsin[2]

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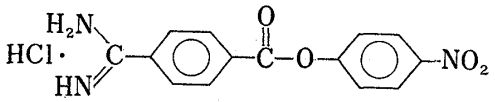
The purity of an enzyme preparation and hence the operational normality of an enzyme solution cannot be fully determined by catalytic rate assay alone. For this purpose "titration" methods using a specific reagent which reacts stoichiometrically with enzyme active sites have been described.[4] *p*-Nitrophenyl *p*-amidinobenzoate hydrochloride (NPAB) **1** was proposed to be a good reagent for active site titration of trypsin (EC 3.4.4.4)[5] in connection with our studies of synthetic trypsin inhibitors.[6] Only fifty to



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2) Presented in part at the Meeting of the Japanese Biochemical Society, Hokkaido Branch, July 1969, Sapporo: Abstracts of papers, *Seikagaku*, **41**, 686 (1969).
3) Location: *a) Kita-12, Nishi-6, Sapporo*; *b)* Present address: *Faculty of Science, Osaka University, Toyonaka, Osaka*; *c) Toyonaka, Osaka.*
4) A. Williams, *Quart. Rev.*, **23**, 1 (1969); G.R. Schonbaum, B. Zerner and M.L. Bender, *J. Biol. Chem.*, **236**, 2930 (1961); M.L. Bender, J.V. Killheffer and R.W. Roeske, *Biochem. Biophys. Res. Comm.*, **19**, 161 (1965); M.L. Bender, M.L. Begué-Cantón, R.L. Blakely, L.J. Brubacher, J. Feder, C.R. Gunter, F.J. Kézdy, J.V. Killheffer, T.H. Marshall, C.G. Miller, R.W. Roeske and J.K. Stoops, *J. Am. Chem. Soc.*, **88**, 5890(1966); D.T. Elmore and J.J. Smith, *Biochem. J.*, **103**, 36p(1967); *idem, ibid.*, **103**, 37p(1967); *idem, ibid.*, **107**, 97 (1968); *idem, ibid* , **107**, 103 (1968); T. Chase, Jr. and E. Shaw, *ibid.*, **29**, 508 (1967); *idem, Biochemistry*, **8**, 2212 (1969); R.J. Baughan and F.H. Westheimer, *Anal. Biochem.*, **29**, 305 (1969).
5) K. Tanizawa, S. Ishii and Y. Kanaoka, *Biochem. Biophys. Res. Comm.*, **32**, 893 (1968).
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