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Alteration of 5-Chloropyrimidine Nucleosides in Alkaline Media

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5-Chlorouridine (III), 5-chlorocytidine (IV), and 5-chloro-1-β-D-arabinofuranosylcytosine (VIII) were obtained in a yield of 61%, 61%, and 11.2% respectively by reaction of pyrimidine nucleosides with N-chlorosuccinimide. 2,2′-Anhydro-5-chloro-1-β-D-arabinofuranosyl cytosine (XI) was prepared in a yield of 53% by reaction of IV with Vilsmeier-Haack reagent. 5-Chloropyrimidine nucleosides (III, 5-chloro-1-β-D-arabinofuranosyluracil (VII), IV, and VIII) were found to degrade in aqueous alkali (1 N NaOH) at 50° *via* deamination or 6,2′ (or 5′)-anhydro open-chain ureido compounds. IV was more readily degraded in 0.3 N KOH at 37° than non-chlorinated (II). An unique reaction was that the treatment of XI with 0.1 N NaOH at room temperature afforded 2,2′-anhydro-5-hydroxy-1-β-D-arabinofuranosylcytosine (XII) without splitting the anhydro bond.

The increasing attention being paid to the biological importance of 5-chloropyrimidine nucleosides has necessitated a re-investigation of the existing synthetic methods and a search for chemical properties of them for characterization and preparation of other 5-substituted pyrimidine nucleosides. 5-Chloropyrimidine nucleosides occur in salmon sperm deoxyribonucleic acid (DNA)[2] and are found in DNA or ribonucleic acid (RNA) treated with a disinfectant of drinking water, sodium hypochlorite or hypochlorous acid.[3] Poly(5ClC)·poly(I) complex has been shown to be more resistant to ribonuclease than poly(C)·poly(I) complex.[4] Some of the chlorinated pyrimidine nucleosides have been found to inhibit the growth of microorganisms.[5] Nevertheless, little is known about the properites of 5-chloropyrimidine nucleosides. This time several 5-chloropyrimidine nucleosides were prepared and the properties of them towards alkali were investigated.

5-Chloropyrimidine nucleosides have been prepared by reaction of pyrimidine nucleosides using chlorine and ultraviolet (UV) irradiation[5a,b] or N-chlorosuccinimide (NCS),[6] but the yields of the chlorinated nucleosides are only moderate. This time, uridine (UR, I), cytidine (CR, II), and 1-β-D-arabinofuranosylcytosine (CA, VI) were treated with a small excess of NCS in acetic acid at 105° for 1.5 hr to afford 5-chloro-UR (III) (yield, 61%), 5-chloro-CR (IV) (yield, 61%), and 5-chloro-CA (VIII) (yield, 11.2%) respectively. 2,2′-Anhydro-5-chloro-1-β-D-arabinofuranosylcytosine (5-chloro-anhydro-CA, XI) hydrochloride was prepared in a yield of 53% by reaction IV with phosphorus oxychloride-N,N-dimethylformamide complex

1) Location: *51-Komiya-cho, Hachiouji, Tokyo.*
2) A.W. Lis, R.K. McLaughlin, D.I. McLaughlin, G.D. Davis, and W.R. Anderson, *J. Amer. Chem. Soc.*, **95**, 5789 (1973).
3) H. Hayatsu, S-K. Pan, and T. Ukita, *Chem. Pharm. Bull.* (Tokyo), **19**, 2189 (1971); W. Patton, V. Bacon, A.M. Duffield, B. Halpern, Y. Hoyano, W. Pereira, and J. Lederberg, *Biochem. Biophys. Res. Commun.*, **48**, 880 (1972); Y. Hoyano, V. Bacon, R.E. Summons, W.E. Pereira, B. Halpern, and A.M. Duffield, *ibid.*, **53**, 1195 (1973).
4) M.A.W. Eaton and D.W. Hutchinson, *Biochemistry*, **11**, 3162 (1972).
5) *a*) T.K. Fukuhara and D.W. Visser, *J. Biol. Chem.*, **190**, 95 (1951); *b*) *Idem*, *J. Amer. Chem. Soc.*, **77**, 2393 (1955); *c*) D.M. Frisch and D.M. Visser, *ibid.*, **81**, 1756 (1959).
6) British Patent 1167605 (1969).

Table I. 5-Chloropyrimidine Nucleoside

Compound	mp	$[\alpha]_D^{24°}$	*Rf* in solvent 1	*Rf* in solvent 2	UV spectrum, mμ ($\varepsilon \times 10^{-3}$) $\lambda_{max}^{(pH\ 1)}$	$\lambda_{max}^{(H_2O)}$	$\lambda_{max}^{(pH\ 13)}$
5-Chloro-UR (III)	220—223° decomp.[a]	+2.7° (*c*; 0.57, H_2O)	0.39	0.34	278(8.70)	278(8.80)	276(5.90)
5-Chloro-UA (VII)	228—234° decomp.	+79.3° (*c*; 0.25, H_2O)	0.51	0.76	278(8.87)	278(9.34)	278(7.43)
5-Chloro-CR (IV)	203—205°[b]	+27.6° (*c*; 0.36, H_2O)	0.28	0.39	217.5(11.40) 298(10.90)	217.5(12.00) 287(7.50)	288(8.00)
5-Chloro-CA (VIII)	218—223° decomp.[c]	+117.6° (*c*; 0.45, H_2O)	0.34	0.77	217.5(11.20) 299(10.70)	218(12.20) 287(7.90)	290(8.20)
5-Chloro-anhydro-CA (XI)·HCl	237—241° decomp.	−69.7° (*c*; 0.35, H_2O)	0.07	0.75	232(8.00) 277(9.26)	232(8.00) 277(9.05)	

NMR spectrum, δ (ppm) in d_6-DMSO	Formula	Analysis (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
8.45 (1H,singlet, H_6) 5.80 (1H, doublet, H_1')	$C_9H_{11}O_6N_2Cl$	38.79	3.97	10.05	38.85	4.01	10.05
7.90 (1H, singlet, H_6) 5.86 (1H, doublet, H_1')	$C_9H_{11}O_6N_2Cl$	38.79	3.97	10.05	38.93	4.10	10.26
8.34 (1H, singlet, H_6) 5.73 (1H, doublet, H_1')	$C_9H_{12}O_5N_3Cl$	38.93	4.36	15.13	38.93	4.33	15.28
7.86 (1H, singlet, H_6) 6.03 (1H, doublet, H_1')	$C_9H_{12}O_5N_3Cl$	38.93	4.36	15.13	39.05	4.31	15.55
8.97 (1H, singlet, H_6) 6.63 (1H, doublet, H_1') 5.53 (1H, doublet,H_2') $J_{1',2'}=6$ cps	$C_9H_{10}O_4N_3Cl\cdot HCl$	36.50	3.74	14.19	36.69	3.72	14.45

a) lit.[5a] 217—217.5° *b*) lit.[5b] 202—202.5°, lit.[6] 200—202° *c*) lit.[6] 211—214°

(Vilsmeier-Haack reagent) according to the previously reported methods.[7] Physicochemical properties of these chlorinated pyrimidine nucleosides are presented in Table I.

5-Chloropyrimidine nucleosides (III, VII, IV, and VIII) and non-chlorinated I, V, II, and VI were treated with 1N NaOH at 50°. UV spectra of the treated mixtures are presented in Fig. 1. Although the spectrum of UR (I) did not change, maximum absorbances of 1-β-D-arabinofuranosyluracil (UA, V), CR (II), and CA (VI) decreased to 55%, 75%, and 51% of the initial ones respectively after treatment for 22 hr. UV loss of V and VI that have a 2'-hydroxyl group in the *cis*-position relative to the chromophore has been previously observed, and the formation of 6,2'-anhydro open-chain ureido compound (XV or XVI) has been suggested.[8,9] Decrease of absorbance of CR (II) shown in Fig. 1 seemed due to the formation of I by deamination, and that of CA (VI) seemed due to the formation of XVI or XV.

Treatment of 5-chloro-UR (III) for 22 hr showed about 10% decrease of absorbance at 278 mμ. This decrease was probably due to the formation of 6,5'-anhydro open-chain ureido

7) K. Kikugawa and M. Ichino, *Tetrahedron Letters*, **1970**, 867; *idem*, *J. Org. Chem.*, **37**, 284 (1972); J.J. Fox, E.A. Falco, I. Wempen, D. Pomeroy, M.D. Dawling, and J.H. Burchenal, *Cancer Res.*, **32**, 2269 (1972).
8) J.J. Fox, N.C. Miller, and R.J. Cushley, *Tetrahedron Letters*, **1966**, 4297.
9) R.E. Notari, M.L. Chin, and R. Wittebort, *J. Pharm. Sci.*, **61**, 1189 (1972).

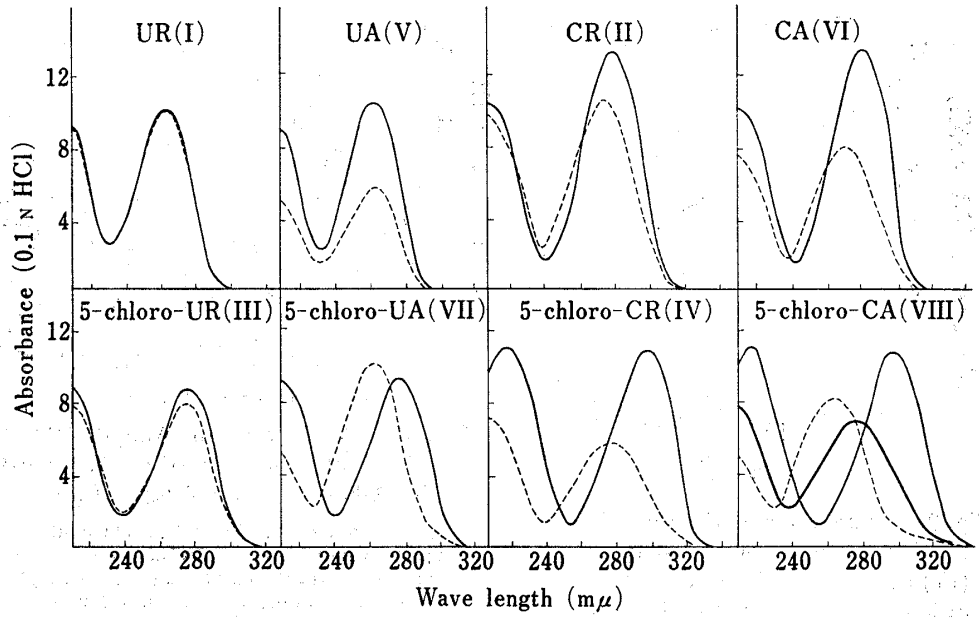


Fig. 1. UV Spectra of 5-Chlorinated and Non-chlorinated Pyrimidine Nucleosides treated with 1N NaOH at 50° for 0 hr (——), 2 hr (▬▬), and 22 hr (- - - - -)

compound (XIX) as was suggested in case of 5-fluoro-UR[10] and not to the formation of 5-hydroxy-UR[11] observed in case of alkaline treatment of 5-bromo-UR.[12] Treatment of 5-chloro-UA (VII) for 22 hr showed a gradual decrease of absorbance at 278 mμ accompanying a new absorption maximum at 265 mμ (ε: 10000), which indicated an almost quantitative formation of the known 1-(β-D-arabinofuranosyl)-2-oxo-4-imidazoline-4-carboxylic acid (XIII)[13] through 6,2′-anhydro open-chain ureido compound (XVII) analogously in case of 5-fluoro- and 5-bromo-UA.[8,13] Treatment of 5-chloro-CR (IV) during the period of 22 hr showed 67% decrease of absorbance at 298 mμ accompanying a new absorption maximum at 278 mμ (ε: 5800). Paper chromatography of the reaction mixture showed only one UV spot corresponding to III, which was isolated in a crystalline form. Large decrease of absorbance, however, suggested the formation of 6,5′-anhydro open-chain ureido compound (XIX or XX) besides III. Treatment of 5-chloro-CA (VIII) for 2 hr showed 58% decrease of absorbance at 298 mμ accompanying a new absorption maximum at 280 mμ (ε: 7000), and that for 22 hr showed 89% loss of absorbance accompanying another new absorption maximum at 266 mμ (ε: 8100). VII and XIII were isolated from the reaction mixture at 2 hr and 22 hr respectively. Similar observation has been noted in case of 5-fluoro-CA.[8]

5-Chloropyrimidine nucleosides (III, VII, IV, and VIII) were found to degrade in aqueous alkali, *via* deamination or 6,2′ (or 5′) anhydro open-chain ureido compounds as in case of 5-fluoropyrimidine nucleoside degradation,[8,10,12,13] and all these transformation of 5-chloropyrimidine nucleosides were effected by 2′- or 5′-hydroxyl function of their sugar moieties for 5-chlorocytosine (XIV) was quite intact under the same conditions.

When 5-chloro-CR (IV) was treated with 0.3N KOH at 37° for 20 hr (conditions for the complete hydrolysis of RNA), 42% loss of absorbance was observed, whereas only 6% loss of absorbance of CR (II) was observed (Fig. 2). The results indicated that a great care must be taken in treatment of RNA or DNA with alkali that might contain 5-chloropyrimidine nucleosides.

10) B.A. Otter, E.A. Falco, and J.J. Fox, *J. Org. Chem.*, **34**, 1390 (1969).
11) III treated for 22 hr showed λ_{max} (1 N NaOH) 277 mμ (ε: 6200), which was different from that of 5-hydroxy-UR: λ_{max} (pH 14) 303 mμ (ε: 7100) (Ref. 12).
12) B.A. Otter, E.A. Falco, and J.J. Fox, *J. Org. Chem.*, **34**, 2636 (1969).
13) B.A. Otter, E.A. Falco, and J.J. Fox, *J. Org. Chem.*, **33**, 3593 (1968).

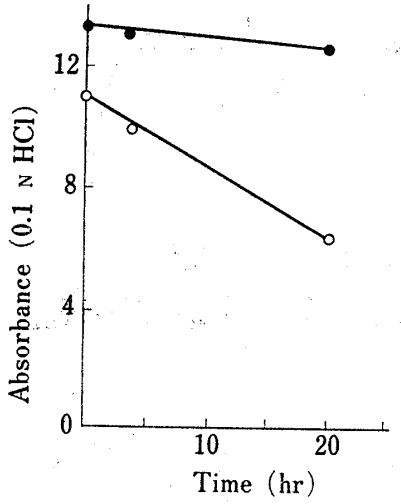


Fig. 2. Time Course of Decrease of Absorbance of 5-Chloro-CR (IV) at 298 mμ (○) and CR (II) at 280 mμ (●) treated with 0.3 N KOH at 37°

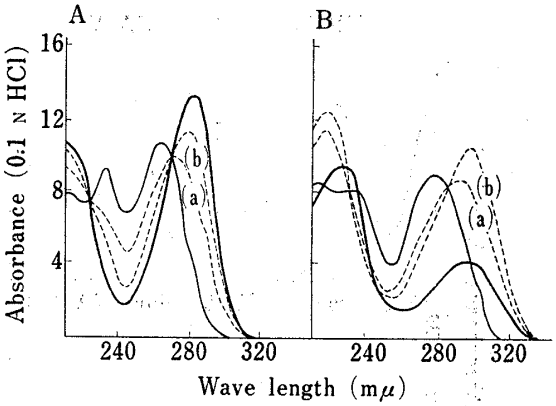


Fig. 3. UV Spectra of Anhydro-CA (X) and 5-Chloro-anhydro-CA (XI) treated with Alkali at Room Temperature

A: anhydro-CA (X), B: 5-chloro-anhydro-CA (XI). Spectra were taken in 0.1N HCl after treatment with none (——), at pH 10 for 10 min (---(a)---), at pH 10 for 30 min (---(b)---), and with 0.1N NaOH for 10 min (——).

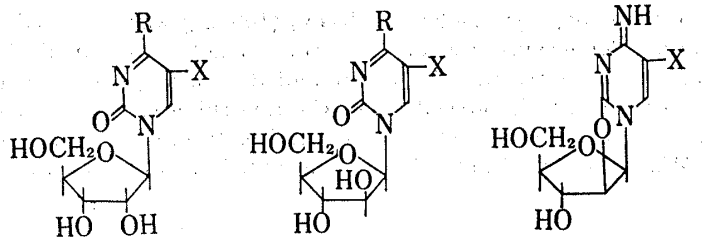
Transformation of 5-chloro-anhydro-CA (XI) towards milder alkali at room temperature was investigated. Non-chlorinated anhydro-CA (X) has been known to be readily converted into VI by aqueous alkali.[14] X tested as a control was quantitatively converted into VI by treatment with 0.1N NaOH at room temperature for 10 min, and was gradually converted into VI by treatment at pH 10 and at room temperature for 5 min (yield of VI: 27%), 10 min (44%), 30 min (78.5%), and 60 min (93.5%) (Fig. 3A). On the contrary, transformations of 5-chloro-anhydro-CA (XI) with NaOH and at pH 10 were quite different. Thus, treatment of XI with 0.1N NaOH at room temperature for 10 min gave large decrease of absorbance at 278 mμ accompanying new absorption maxima at 294 mμ (0.1N HCl) and 305 mμ (0.1N NaOH), indicating an unexpected reaction occurred. Under these conditions 5-chloro-CA (VIII) which might be formed by splitting the anhydro bond was quite intact. XI was gradually converted into 5-chloro-CA (VIII) by treatment at pH 10 and at room temperature for 5 min (yield of VIII: 50%), 10 min (73%), and more than 30 min (100%) (Fig. 3B). VIII was isolated in a yield of 48% from the reaction mixture of XI at pH 10. At pH 10, as well as anhydro-CA (X), 5-chloro-anhydro-CA (XI) was converted into the arabinosyl derivative by splitting the anhydro bond. Yung, *et al.*[15] reported 5-halogenation of anhydro-UA stabilized the anhydro bond, but observations described here showed that the 5-halogenation reversed the stability of the anhydro bond of anhydro-CA (X).

Paper chromatography of 5-chloro-anhydro-CA (XI) treated with NaOH showed two UV spots having *Rf*'s of 0.34 (corresponding to VIII) and 0.06 (corresponding to XII) and an Ehrlich's reagent positive spot at the origin. The product (XII) was isolated in a yield of 54% by means of preparative paper chromatography and was identified with the known 5-hydroxy-anhydro-CA.[16] The properties of 5-hydroxy-anhydro-CA have been well characterized, and it was rather stable in 0.1N NaOH at room temperature.[16] The products of XI treated with NaOH at room temperature were thus found to be 5-chloro-CA (VIII), 5-hydroxy-anhydro-CA (XII), and non-UV absorbing substance(s).

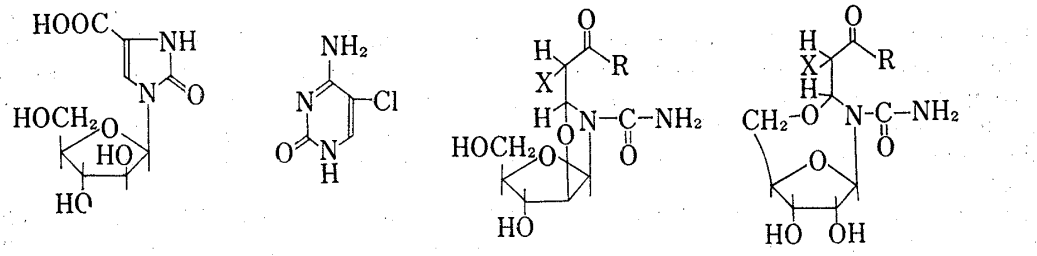
Hydroxy substitution of chlorine atom occurred in the treatment of 5-chloro-anhydro-CA (XI) with NaOH without the splitting of the anhydro bond. The substitution of chlorine

14) I.L. Doerr and J.J. Fox, *J. Org. Chem.*, 32, 1462 (1967).
15) N.C. Yung, J.H. Burchenal, R. Fecher, R. Duschinsky, and J.J. Fox, *J. Amer. Chem. Soc.*, 83, 4060 (1961).
16) K. Kikugawa, *J. Pharm. Sci.*, "in press."

atom in XI is unique for other 5-chloropyrimidine nucleosides (II, VII, IV, and VIII) suffered no such substitution with NaOH but deamination and/or loss of UV absorption owing to 6,2′(or 5′)-anhydro open-chain ureido compound formation. The reaction might be useful for the preparation of other 5-substituted pyrimidine nucleosides.



I : R=OH, X=H
II : R=NH_2, X=H
III : R=OH, X=Cl
IV : R=NH_2, X=Cl
V : R=OH, X=H
VI : R=NH_2, X=H
VII : R=OH, X=Cl
VIII : R=NH_2, X=Cl
IX : R=NH_2, X=OH
X : X=H
XI : X=Cl
XII : X=OH



XIII XIV
XV : R=OH, X=H
XVI : R=NH_2, X=H
XVII : R=OH, X=Cl
XVIII : R=NH_2, X=Cl
XIX : R=OH, X=Cl
XX : R=NH_2, X=Cl

Chart

Experimental[17]

5-Chloro-UR (III)

Method A——From UR(I): UR(I) (1.10 g, 0.45 mmole) was suspended in 10.0 ml of distilled glacial acetic acid and to the mixture was added 0.65 g (0.50 mmole) of NCS. The mixture was heated at 105° for 1.5 hr under anhydrous conditions. Paper chromatography (solvent 1) of the mixture revealed mono spot (*Rf* 0.39) corresponding to III. The cooled mixture was diluted with 100 ml of H_2O, adjusted to pH

17) Melting points were determined with Buchi melting point apparatus and uncorrected. UV spectra were taken with Hitachi Recording Spectrophotometer EPS-3T. Nuclear magnetic resonance (NMR) spectra were taken with Varian T-60 spectrometer with tetramethylsilane as an internal standard, and the measurements were greatly acknowledged to Mr. T. Kawashima and his coworkers of the laboratories. Optical rotation and infrared (IR) spectra were measured with JASCO automatic polarimeter DIP-SL and Hitachi 285 Grating Infrared Spectrophotometer respectively. Paper chromatography was carried out on Toyo Roshi No. 51A or Whatman 3MM (for preparative) with solvent 1: *n*-BuOH–H_2O (84: 16, v/v) and solvent 2: 5 M NH_4OAc–0.5 M ethylenediamine tetraacetic acid Na_2-saturated $Na_2B_4O_7$–EtOH (60:1.5:240:660, v/v). Paper electrophoresis was done on Toyo Roshi No. 51A with 0.05 M phosphate buffer (pH 7.5) at 1000 V/25 cm for 1 hr. Cellulose column was prepared with cellulose powder (100—200 mesh, Toyo Roshi). TOD was total optical density. UA(V) (ref. 18), CA (VI) (ref. 7), and 5-chlorocytosine (XIV) (ref. 19) were prepared according to the reported methods.

18) D.M. Brown, A.R. Todd, and S. Varadarajan, *J. Chem. Soc.*, **1956**, 2388.

19) I. Wempen and J.J. Fox, *J. Med. Chem.*, **6**, 688 (1963).

1.0 with concentrated HCl, and adsorbed to a column of active carbon (20 g). The column washed well with H_2O was eluted with 500 ml of EtOH–10% NH_4OH (1: 1), and the eluate was evaporated *in vacuo* to a small volume. Insoluble material was filtered off and the filtrate was again evaporated to dryness. The residue was crystallized from 10 ml of *n*-BuOH–H_2O (84: 16). Needles of III was obtained in a yield of 61.0% (700 mg), mp 209—210°, decomp. Recrystallization from EtOH gave pure columns, mp 220—223°, decomp. Physicochemical data are listed in Table I.

Method B——From 5-Chloro-CR(IV): 5-Chloro-CR(IV) (100 mg) was dissolved in 3.0 ml of 1 N NaOH and warmed at 50° for 1 day. The mixture was passed through a column of 10 ml of Dowex 50×4(H^+) and washed until free of UV absorbing material. The eluate and the washings were combined and evaporated *in vacuo* to dryness. The residue, which showed mono UV absorbing spot corresponding to III on paper chromatogram (solvent 1 and 2), was crystallized and recrystallized from EtOH to afford III in a yield of 50%, mp 220—223°, decomp. Mixed fusion test of this material with that obtained by method A showed that both were identical.

5-Chloro-CR(IV)

CR(II) (2.0 g, 8.23 mmoles) was suspended in 18.2 ml of distilled glacial acetic acid and to the mixture was added 1.80 g (13.5 mmoles) of NCS. The mixture was heated at 105° for 1.5 hr under anhydrous conditions. Paper chromatography (solvent 1) of the mixture revealed one major (*Rf* 0.28) and another minor (*Rf* 0.39) spots. The cooled mixture was diluted with 100 ml of H_2O, adjusted to pH 1.0 with concentrated HCl, and adsorbed to a column of active carbon (40.0 g). The column washed well with H_2O was eluted with 1500 ml of EtOH–10% NH_4OH (1: 1), and the eluate was evaporated *in vacuo* to dryness. The residue was purified through a cellulose column (1.8×65 cm) with solvent 1. Fractions (170—300 ml) were pooled and evaporated *in vacuo* to dryness, and the residue was crystallized from EtOH to afford 1.40 g (yield, 61%) of IV, mp 194—200°. Recrystallization from EtOH gave pure needles, mp 203—205°. Physicochemical data are listed in Table I.

5-Chloro-UA(VII)

5-Chloro-CA(VIII) (100 mg) was dissolved in 3.0 ml of 1 N NaOH and warmed at 50° for 2 hr. The mixture was immediately passed through a column of 10 ml of Dowex 50×4(H^+) and washed until free of UV absorbing material. The eluate and the washings were combined and evaporated *in vacuo* to a small volume. Needles separated was obtained in a yield of 45% by filtration, mp 228—234°, decomp. Recrystallization from H_2O gave pure material. Physicochemical data are presented in Table I.

5-Chloro-CA(VIII)

Method A——From CA(VI): CA(VI) (1.10 g, 4.5 mmoles) was suspended in 10.0 ml of glacial acetic acid and to the mixture was added 0.65 g (5.0 mmoles) of NCS. The mixture was heated at 105° for 1.5 hr. Paper chromatography (solvent 1) of the mixture showed one spot (*Rf* 0.34) besides the spot (*Rf* 0.17) corresponding to the starting material. Purification and isolation of the product were performed as in the synthesis of 5-chloro-CR(IV). Needles of pure VIII were obtained from EtOH in a yield of 11.2% (145 mg), mp 218—223°, decomp. Physicochemical data are listed in Table I.

Method B——From 5-Chloro-anhydro-CA(XI): 5-Chloro-anhydro-CA(XI) formate (200 mg) was dissolved in 10 ml of H_2O and to the solution was added 10% NH_4OH to maintain the pH of the solution at 10.0 during the period of 1 hr. The mixture was evaporated *in vacuo* to dryness and dissolved in 10 ml of 0.1 N HCl to absorb to a column of 30 ml of Dowex 50×4(H^+). The column washed well with H_2O was eluted with 100 ml of 3 N NH_4OH. The eluate was evaporated *in vacuo* below 20°, and the residue was crystallized from EtOH to afford 86 mg (yield, 48%) of VIII, mp 206—211° decomp. Recrystallization from EtOH gave pure material, mp 215—218° decomp., and it was identical with the sample obtained by method A by mixed fusion test and comparison of IR(KBr) spectra.

5-Chloro-anhydro-CA(XI)

Phosphorus oxychloride (6.0 g) was added to 20.0 ml of N,N-dimethylformamide and the mixture was stored at room temperature for 30 min. A solution of 5-chloro-CR(IV) (1.008 g, 3.6 mmoles) in 4.0 ml of N,N-dimethylformamide was added to the mixture with stirring. After 3 hr at room temperature the mixture was poured into 100 ml of ice-water, which was further stored for 3 hr. Storing the aqueous mixture converted the initial UV absorption maximum (0.1 N HCl) at 338 mμ to 282 mμ. $TOD^{pH\ 1}_{280\ m\mu}$ was 31200. The aqueous mixture was put onto a column of 120 ml of Dowex 50×4 (pyridinium form) and the column was washed with H_2O until the washings became neutral. The column was eluted with 0.1 M pyridinium formate (pH 4.8) to separate two fractions; F_1 (1200—1400 ml, $TOD^{pH\ 1}_{280\ m\mu}$=8750) and F_2 (1800—3400 ml, $TOD^{pH\ 1}_{280\ m\mu}$=19500). After the fraction F_2 was adjusted to pH 4.0 with formic acid, it was evaporated and coevaporated with EtOH *in vacuo* to dryness. The residue was crystallized from EtOH to afford 5-chloro-anhydro-CA (XI) formate in a yield of 53.0% (579.7 mg), mp 181—183°, decomp. UV: λ^{H^+,H_2O}_{max}233 and 278 mμ. XI·formate (200 mg) was quantitatively converted into XI·hydrochloride by use of 10 ml of Dowex 1×2 (Cl^-) resin. XI·hydrochloride was crystallized and recrystallized from EtOH–H_2O in leaflet forms, mp 237—241°, decomp. Physicochemical data are presented in Table I.

1-(β-D-Arabinofuranosyl)-2-oxo-4-imidazoline-4-carboxylic Acid (XIII)

5-Chloro-CA(VIII) (100 mg) was dissolved in 10.0 ml of 1 N NaOH and warmed at 50° for 22 hr. The solution was passed through a column of 10 ml of Dowex 50×4(H^+) and washed with H_2O until free of UV

absorbing material. The effluent and the washings were combined and evaporated to dryness. The residue was crystallized from a small volume of H_2O to afford XIII in a yield of 48%, mp 105—121°, eff. Recrystallization from H_2O gave fine needles melting at 189—194°, decomp. with shrinking at 110—128°. UV: $\lambda_{max}^{H^+}$ 266, $\lambda_{min}^{H^+}$ 229.5, $\lambda_{max}^{H_2O}$ 255, $\lambda_{min}^{H_2O}$ 227, $\lambda_{max}^{OH^-}$ 258, and $\lambda_{min}^{OH^-}$ 232.5 mμ. *Rf*, 0.01 (solvent 1) and 0.52 (solvent 2). Paper electrophoresis showed the compound migrated towards anode. Beilstein test on this sample was negative. [lit.[13] mp 118—120° resolidified, 196—198°, decomp. UV: $\lambda_{max}^{pH\,1}$ 263 ($\varepsilon \times 10^{-3}$: 11.38), $\lambda_{min}^{pH\,1}$ 226 (1.82), $\lambda_{max}^{pH\,7}$ 252 (9.62), $\lambda_{min}^{pH\,7}$ 224 (3.66) mμ]

5-Hydroxy-anhydro-CA(XII)

5-Chloro-anhydro-CA(XI)·formate (400 mg) was dissolved in 10 ml of 0.3 N NaOH and the solution was stirred at room temperature for 30 min. The mixture was immediately submitted to preparative paper chromatography (solvent 1). Two UV absorbing bands (*Rf* 0.34 and 0.06) and one Ehrlich positive band at the origin were observed. The band (*Rf* 0.06) was cut out and extracted with 500 ml of H_2O. The extract was adjusted to pH 1 with concentrated HCl, adsorbed to a column of 10 ml of Dowex 50×4(H^+). The column washed well with H_2O was eluted with 100 ml of 3 N NH_4OH, and the eluate was evaporated *in vacuo* to dryness. The residue was crystallized and recrystallized from EtOH–H_2O to afford granules of XII, 170 mg (yield, 54%), mp 275—281°, decomp. UV: $\lambda_{max}^{pH\,1}$ 284 ($\varepsilon \times 10^{-3}$: 9.98), 215 (12.5), $\lambda_{min}^{pH\,1}$ 251.5 (3.3), $\lambda_{max}^{pH\,7}$ 316 (8.2), 258 (4.97), 218.5 (17.7), $\lambda_{min}^{pH\,7}$ 279.5 (2.94), 245 (4.1) mμ. *Anal.* Calcd. for $C_9H_{11}O_5N_3$: C, 44.81; H, 4.59; N, 17.42. Found: C, 44.91; H, 4.33; N, 17.40. *Rf* 0.06 (solvent 1) and 0.57 (solvent 2).

Mixed fusion test and the comparison of IR(KBr) spectra of the sample with the authentic 5-hydroxy-anhydro-CA[16] confirmed the structure of the compound.

Alkaline Transformation of Pyrimidine Nucleosides

Each nucleoside (10.0 mg) of UR(I), UA(V), CR(II), CA(VI), 5-chloro-UR(III), 5-chloro-UA(VII), 5-chloro-CR(IV), and 5-chloro-CA(VIII) was dissolved in 1.50 ml of 1 N NaOH or 0.3 N KOH and warmed at 50° or at 37°. UV spectra were measured after an aliquot (20 μl) was diluted with 10.0 ml of 0.1 N HCl (Fig. 1 and 2).

Alkaline Transformation of Anhydro-CA(X) and 5-Chloro-anhydro-CA(XI)

Each (2.00 mg) of anhydro-CA(X)·hydrochloride, 5-chloro-anhydro-CA(XI)·hydrochloride, and 5-chloro-CA(VIII) was dissolved in 4.00 ml of 0.1 N NaOH, 0.05 M glycine–NaOH buffer (pH 10.0), and H_2O, and kept at room temperature (—22°). An aliquot (100 μl) of each of the mixture was diluted with 10.0 ml of 0.1 N HCl or 0.1 N NaOH and UV spectrum was recorded as soon as possible. The results are shown in Fig. 3.

X was quantitatively converted into VI by treatment with 0.1 N NaOH at room temperature for 10 min when calculated from the $\varepsilon_{282}^{0.1N\ HCl}$ (13400) value of VI.[7] X was gradually converted into VI by treatment at pH 10 and the formation of VI was 93.5% at room temperature for 60 min calculated from the $\varepsilon_{295}^{0.1N\ HCl}$ value (Fig. 3A).

UV spectrum of XI treated with 0.1 N NaOH at room temperature for 10 min and 75 min showed $\lambda_{max}^{0.1N\ HCl}$ 294 ($\varepsilon \times 10^{-3}$: 4.2), $\lambda_{max}^{0.1N\ NaOH}$ 305 (5.9), and $\lambda_{max}^{0.1N\ HCl}$ 294 (4.0), $\lambda_{max}^{0.1N\ NaOH}$ 294 mμ (4.9) respectively, which indicated that hydrolyzed products of XI were not only 5-chloro-CA(VIII). XI was gradually converted into VIII by treatment at pH 10 and the formation of VIII was quantitative at the period of 30 min calculated from the $\varepsilon_{299}^{0.1N\ HCl}$ (10700) value of VIII (Table I). Amount of VIII formed from XI was also calculated from $\varepsilon_{315}^{0.1N\ HCl}$ value (Fig. 3B).

VIII was found quite intact by treatment with 0.1 N NaOH or at pH 10 for more than 75 min.