

### Summary

In order to determine whether Acetylcholine (ACh) receptor is identical with Cholinesterase (ChE) or not at the pharmacological or functional level, the mechanism of affinity for the active site on ACh receptor surface is considered with the pharmacological and biochemical method using a useful tool, organophosphoryl choline. These experiments gave following results :

1) By Magnus method using the rectus abdominis of frog, dose-inhibition curve of amyltrimethylammonium salt (ATMA) and dose-inhibition curve of hydrogen ion against it were observed. From the results, one molecule of ATMA combines with an ACh receptor in the process of the contractile response like the case of ACh, and the site of action of hydrogen ion is located on the anionic site of ACh receptor.

2) The apparent pKa value of the anionic site of ACh receptor is about 5.92 and very close to that of ChE.

3) The two combination constants of organophosphorylcholine with the esteratic site of ACh receptor and of ChE were about  $5.53 \times 10^{-4}$  and  $4.63 \sim 8.27 \times 10^{-3}$ . Therefore, there is the difference between ACh receptor and ChE regarding the esteratic site.

4) From the correlation between anti-ACh and anti-ChE activity of some organophosphorylcholine derivatives, it was indicated that the former increased in the order of  $-O-\overset{|}{\underset{|}{P}}=O$ ,  $-O-\overset{|}{\underset{|}{P}}=S$ , and  $-S-\overset{|}{\underset{|}{P}}=S$ , while the latter was the reverse order of them.

5) From the results above mentioned, it may be concluded that ACh receptor is different from ChE molecule.

(Received September 29, 1964)

[Chem. Pharm. Bull.]  
13(1) 7 ~ 16 (1965)

UDC 612.398.145

### 2. Kin-ichi Imai and Mikio Honjo : Synthesis of 5-Substituted Pyrimidine Nucleosides.\*<sup>1</sup>

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Until recently, several aldehyde hydrazone derivatives have been reported from different laboratories to be significantly effective as growth retarding agents against some strains of transplantable mammary tumors.<sup>1,2)</sup> It has been also reported that a number of 5-substituted pyrimidines (*e.g.*, 5-fluorouracil<sup>3)</sup>) are strong antimetabolites for biosynthesis of nucleic acids. In our Research Laboratories, Tanaka and his co-workers<sup>4)</sup> synthesized various hydrazone derivatives of *p*-(2,4,6-triamino-5-pyrimidinylazo)benzaldehyde, which were also shown to exhibit similar antitumor activities. Whereas synthesis of 5-substituted aldehyde hydrazone derivatives of the pyrimidines have

\*<sup>1</sup> Presented at the Kinki Branch Annual Meeting of the Pharmaceutical Society of Japan (Osaka, November, 1963).

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1) B.L. Freedlander, F.A. French : *Cancer Res.*, **18**, 360, 1286 (1958).

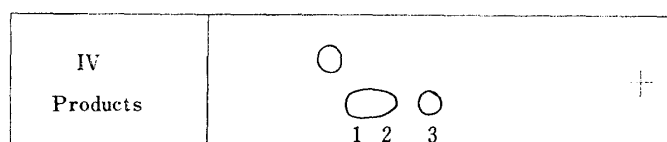
2) R.H. Wiley, R.L. Clevenger : *J. Med. Pharm. Chem.*, **5**, 1367 (1962).

3) C. Heidelberger : "Biological Approaches to Cancer Chemotherapy," 47 (1961). Academic Press, London and New York.

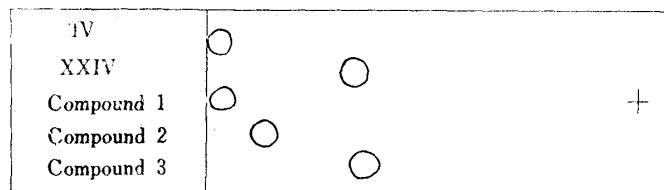
4) K. Tanaka, T. Sugawa, Y. Kuwada, K. Imai, M. Morinaga, J. Watanabe, T. Komeda, T. Usui, H. Yokotani, H. Ito, S. Hemmi, M. Kato, H. Mima, K. Kaziwara : *Ann. Rept. Takeda Res. Lab.*, **22**, 192 (1963).

been reported by Wiley, *et al.*<sup>5)</sup> who have studied on their antitumor activities, no investigation has been done on the chemical syntheses of 5-aldehyde hydrazone of ribosides of pyrimidines. On the basis of these informations, we have attempted in the present studies to synthesize some pyrimidine nucleosides substituted at position 5 with aldehyde hydrazone group.

For the present investigation, the work of Cline, *et al.*<sup>6)</sup> is pertinent who found that catalytic oxidation of uridine-5-methanol resulted in the formation of two products separable by paper chromatography, one of which was assumed to be uridine-5-carboxaldehyde (I), based on its positive color reaction specific for the aldehyde group with *o*-dianisidine. We repeated their work to isolate and characterize these compounds. Thus, we have carried out the oxidation of uridine-5-methanol (IV) in 50% acetic acid in the presence of appropriate amount of platinum oxide as catalyst at room temperature. Paper electrophoresis of the reaction mixture, using a borate buffer (pH 9.2) effected separation of three components (compounds (1, 2, and 3)), which could be detected under the ultraviolet light. Both the compounds (1 and 3) were shown to be positive for the reaction with *o*-dianisidine<sup>7)</sup> on the paper chromatogram. In the preparative isolation of these compounds, the resulting oxidation products were applied on a column



Buffer 1: 0.05M Borate buffer, pH 9.2, 22 v./cm., 30 min. (IV): Uridine-5-methanol



Buffer 2: 0.05M Citrate buffer, pH 3.7, 22 v./cm., 30 min. (IV): Uridine-5-methanol. (XXIV): 1-( $\beta$ -D-Ribofuranosyluronic acid)uracil

Fig. 1. Paper Electrophoresis of Products

The compound (1) was a major product of catalytic oxidation of uridine-5-methanol and gave a positive color reaction with *o*-dianisidine. Its yield was usually 70~80% of the reaction products. Column chromatographic separation, followed by evaporation of the combined effluent fractions containing 1 gave a crystalline residue, which after recrystallization from aqueous ethanol gave a novel aldehyde derivative as needles, m.p. 178~179° (decomp.). Its elementary analysis was in good agreement with  $C_{10}H_{12}O_7N_2$ , the chemical formula of uridine-5-carboxaldehyde, the formation of which has been previously supposed by Cline, *et al.*<sup>6)</sup> from the positive color reaction with *o*-dianisidine on paper. Another evidence for the above results was given by the formation of uridine-5-methanol (IV) after reduction of I with sodium borohydride. Reaction of I with several carbonyl reagents, such as thiosemicarbazide, isonicotinic acid hydrazide, and *p*-bromophenylhydrazine led to the formation of the corresponding crystalline hydrazone derivatives (VII~X). Furthermore, since Tanaka, *et al.*<sup>4)</sup> have observed that a guanylhydrazone of 5-phenylazopyrimidine had a remarkable antitumor activity, we

5) R. H. Wiley, A. B. Canon, K. F. Hussung: *J. Med. Chem.*, **6**, 333 (1963).

6) R. E. Cline, R. M. Fink, K. Fink: *J. Am. Chem. Soc.*, **81**, 2521 (1959).

7) F. Feigl: "Spot Test in Organic Analysis," 225 (1961). Maruzen Asian Edition.

have prepared similarly a guanylylhydrazone sulfate (X) of I by heating the solution of I in aqueous ethanol with aminoguanidine sulfate.

The compound (2) corresponding to about 10% of the total yield of the reaction products was obtained after concentration of the effluent fractions. It was recrystallized from ethanol to yield needles containing 1 mole of ethanol ( $C_{10}N_{12}O_8N_2 \cdot C_2H_5OH$ ), and shown to be negative for the color reaction with *o*-dianisidine. Evolution of carbon dioxide from the solution of sodium bicarbonate on addition of this compound as well as the low  $pK_a$  value of 4.2 indicated that this is an acidic substance having a carboxyl group in the molecule. Further, this compound apparently differs from I in its unsusceptibility to reduction with sodium borohydride. Recently, Moss, *et al.*<sup>8)</sup> have reported the synthesis of 1-( $\beta$ -D-ribofuranosyluronic acid)uracil (XXIV) by means of catalytic oxidation of uridine in solution buffered at pH 8.8 and shown that the  $pK_a$  value of XXIV was 3.07. As can be seen from Figs. 2 and 3, the ultraviolet absorption spectra of the newly synthesized compound was distinctly different from either those of I or those of XXIV. A higher  $pK_a$  value of the compound (2) and its slower electrophoretic mobility at pH 3.7, as compared with those of XXIV suggested that a carboxyl group is located at position 5 of the pyrimidine moiety. From these results, it is quite probable that the compound (2) is uridine-5-carboxylic acid (II), a novel isomer of orotidine. In biological test using a bacterium, *Lactobacillus bulgaricus* 09 that is known to require orotic acid as a growth factor, II was found to be inactive not only in replacing or antagonizing orotic acid, but also did not show any stimulating effect upon the growth of the microorganism.\*<sup>3</sup> Further oxidation of II with platinum oxide in a solution of an equivalent amount of sodium bicarbonate yielded 1-( $\beta$ -D-ribofuranosyluronic acid)uracil-5-carboxylic acid (VI), which could also be prepared directly from uridine-5-methanol (IV).

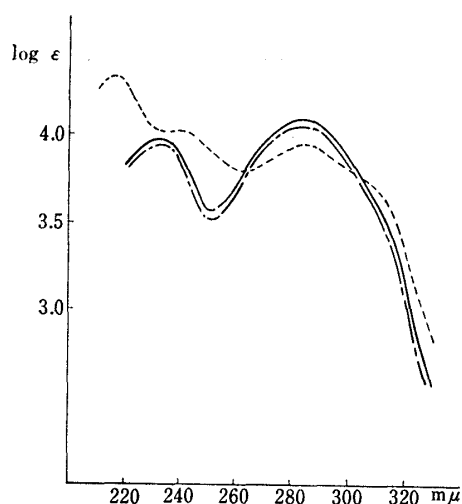


Fig. 2. Ultraviolet Absorption Spectra of Uridine-5-carboxaldehyde (I)

— 0.01N HCl  
 - - - 0.01N NaOH  
 - · - · - H<sub>2</sub>O

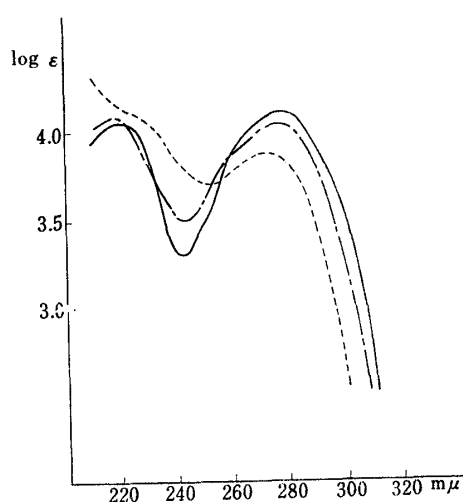


Fig. 3. Ultraviolet Absorption Spectra of Uridine-5-carboxylic Acid (II)

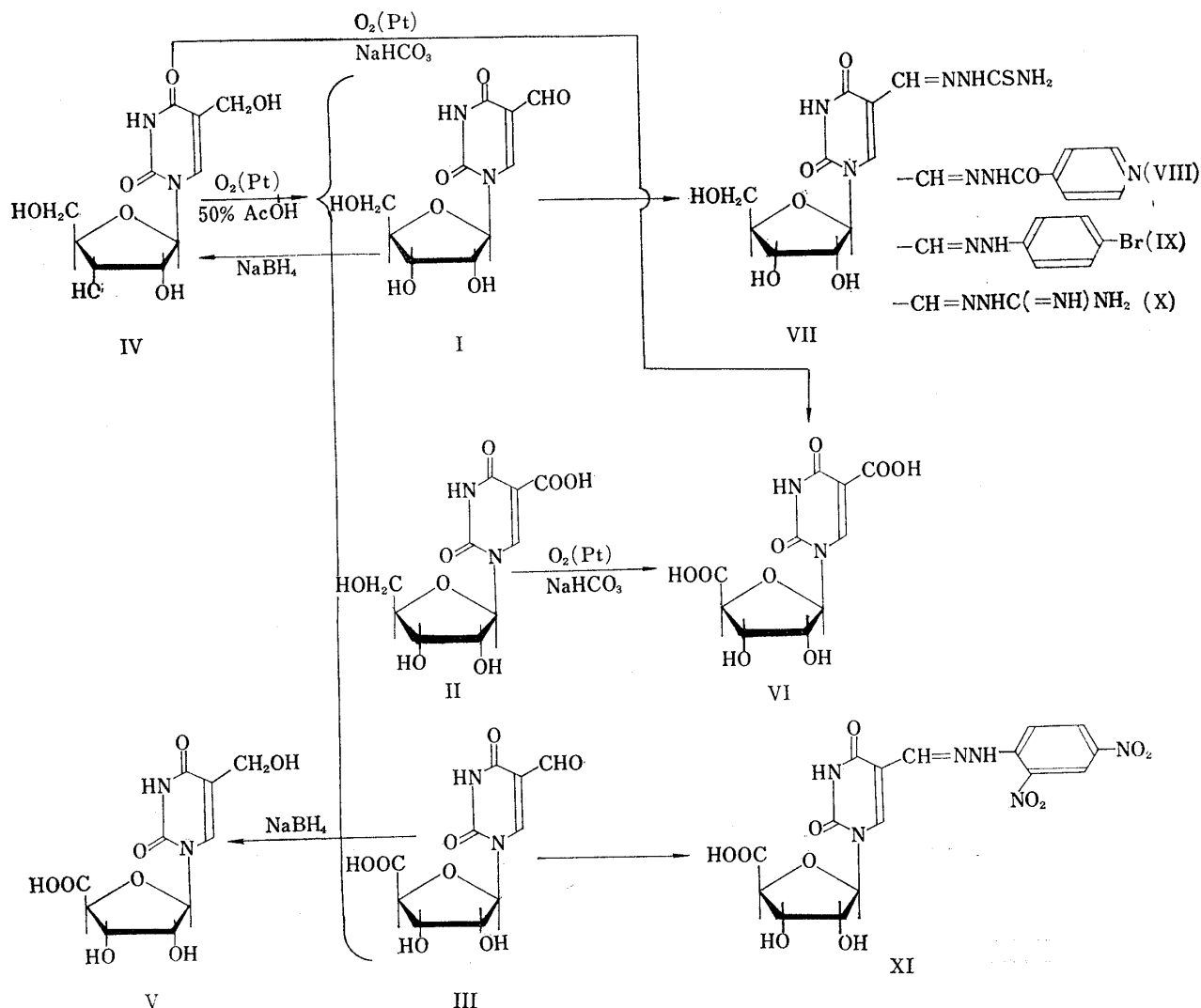
— 0.01N HCl  
 - - - 0.01N NaOH  
 - · - · - H<sub>2</sub>O

The compound (3) obtained in yield of 10~20% of the reaction products had a similar electrophoretic behavior to that of XXIV, thus suggesting that the ribose moiety was oxidized. The residue left after concentration of the combined effluent fractions containing 3 gave a positive color reaction with *o*-dianisidine and its ultraviolet absorption

\*<sup>3</sup> E. Ohmura, S. Hemmi : Unpublished results.

8) G. P. Moss, C. B. Reese, K. Schofield, R. Shapiro, L. Todd : J. Chem. Soc., 1963, 1149.

spectra were similar to those of I. These results indicated that this novel compound is an uronic acid derivative with an aldehyde group at position 5 of the pyrimidine moiety, namely 1-( $\beta$ -D-ribofuranosyluronic acid)uracil-5-carboxaldehyde (III). It gave a crystalline 2,4-dinitrophenylhydrazone (XI),  $C_{16}H_{14}O_{11}N_6 \cdot H_2O$ . In order to confirm the location of the aldehyde group, III was reduced with sodium borohydride to give a compound showing a negative color reaction for aldehyde group. If an aldehyde group were present at position 5' of the ribose moiety and a carboxyl group being at position 5 of the pyrimidine, II should be obtained on reduction of the compound (3) (III). However, in view of the close similarity of ultraviolet absorption spectra of the compound to those of IV and its similarity in the electrophoretic mobility to that of XXIV, it is obvious that the possibility of the formation of II could be eliminated and that reduction of the aldehyde group at position 5 of the pyrimidine moiety of III took place to yield 1-( $\beta$ -D-ribofuranosyluronic acid)uracil-5-methanol (V). The whole process of the synthesis of these compounds was formulated in Chart 1.



In 1963, Moss, *et al.*<sup>8)</sup> have reported the catalytic oxidation of nucleosides. On the basis of the finding that the primary hydroxyl group of the ribose moiety in the nucleosides was preferentially oxidized, several pyrimidine nucleosides were examined in order to elucidate the difference in the influence of various group substituted at position 4 or

5 of the pyrimidine ring on such oxidation reaction. All nucleosides tested in this experiments were oxidized in a solution containing an equivalent amount of sodium bicarbonate at 50~60°. Uracil derivatives, such as 2'-deoxyuridine (XII), 5-bromo-2'-deoxyuridine (XIV), and 5-iodo-2'-deoxyuridine (XVI) were readily oxidized, giving rise to the corresponding uronic acid derivatives, XIII, XV, and XVII. The ease of the oxidation of these uridine derivatives was found to be dependent upon the substituted groups, the decreasing order being I>Br>H. In the case of the oxidation of XII and XIV, small amount of the free bases were found, which were probably due to the breakdown of the resulting uronic acid derivatives during the oxidation. While 5-iodo-2'-deoxycytidine (XVIII) was oxidized to the corresponding uronic acid derivative (XIX), the rate of the oxidation of the cytosine derivative was much slower than those of similar uridine derivative (XVI). Heating of the solution of XIX caused the decomposition of a large part of XIX, resulting in simultaneous release of 5-iodocytosine. These results showed that substitution of the pyrimidine nucleosides at position 5 with halogen atoms had marked influence upon the rate of the oxidation and that the uracil derivatives were much more susceptible than the cytosine compound to the oxidation reaction. The oxidative process of these derivatives was illustrated in Chart 2.

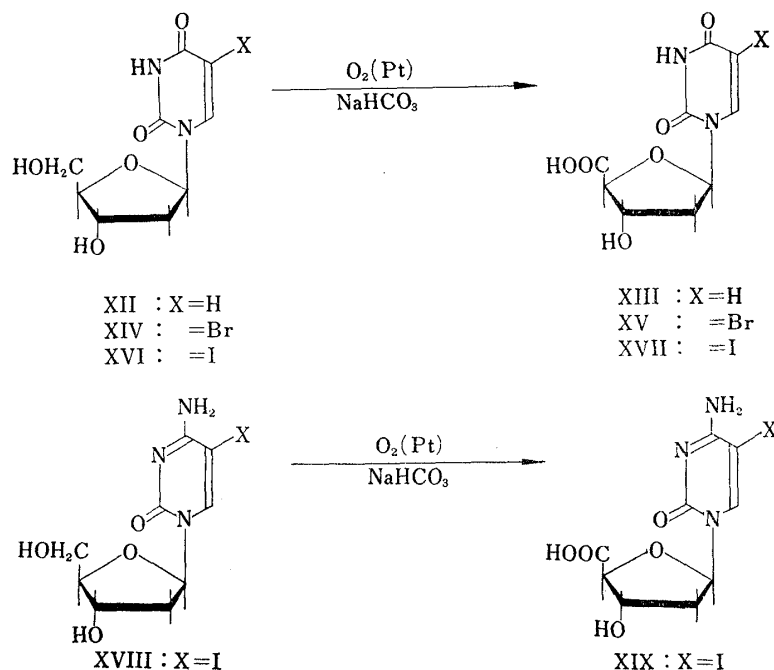


Chart 2.

As regards catalytic oxidation of 5-substituted pyrimidine nucleosides, 1- $\beta$ -D-arabinofuranosylcytosine (XX) was oxidized to give three compounds, major one of which was identified as 1-( $\beta$ -D-arabinofuranosyluronic acid)cytosine (XXI). Deamination of XXI was conducted by heating an aqueous solution of the compound, giving the corresponding uracil derivative (XXIII), which was subsequently shown to be identical with the oxidation product of 1- $\beta$ -D-arabinofuranosyluracil (XXII). On the other hand, oxidation of XXII led to the formation of a by-product in 10% yield, which was oxidized presumably in the other part of the molecule. Furthermore, the arabinosides of uracil derivatives were proved to be readily oxidized, when compared with the case of cytosine derivatives. The relationship of these compounds in the oxidation reaction was summarized in Chart 3.

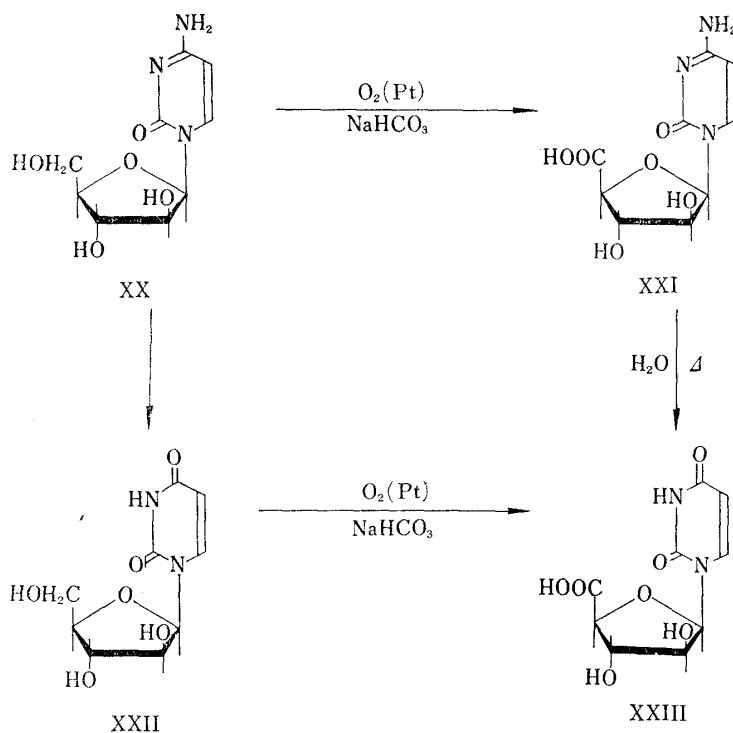


Chart 3.

Examination of all these compounds for biological activities are under investigation through various screening tests.

#### Experimental\*4

**Analytical Methods**—Paper partition chromatography (PPC) was carried out by a conventional ascending method on Whatman No. 1 paper in the following solvent system; A, BuOH-AcOH-H<sub>2</sub>O (4:1:5 v/v); B, BuOH-AcOH-H<sub>2</sub>O (5:3:2 v/v); C, BuOH-EtOH-H<sub>2</sub>O (50:15:35 v/v); D, iso-PrOH-28% NH<sub>4</sub>OH-H<sub>2</sub>O (7:1:2 v/v). Thin-layer chromatography (TLC) was performed on a glass plate (5×16 cm.) coated with cellulose powder (screened through 300 mesh sieve) of Toyo Roshi Co. containing 5% calcium sulfate as a stationary phase. Usually the layer was developed for 40 min., using BuOH saturated with H<sub>2</sub>O as solvent system.

Paper electrophoresis (PE) was run on Whatman No. 1 paper at a constant voltage of 22 V/cm. for 30~60 min. in the following buffers; 1, 0.05M borate buffer, pH 9.2; 2, 0.05M citrate buffer, pH 3.7; 3, 0.1M acetate buffer, pH 3.7.

Location of the spots on paper or layer was detected by UV absorption or fluorescence under UV light.

**Oxidation of Uridine-5-methanol<sup>(6)</sup> (IV)**—Platinum oxide (2.25 g.) was suspended in 50% AcOH (224 ml.), shaken under H<sub>2</sub> and after exchange of H<sub>2</sub> with O<sub>2</sub>, the mixture continued shaking for 30 min. To this was added IV (2.24 g., 8.2 mM) and the mixture was shaken under O<sub>2</sub> for 31.5 hr. After removal of the catalyst by filtration, the filtrate was concentrated *in vacuo* under N<sub>2</sub> below 40° to give syrupy residue, which was dissolved in 1,300 ml. of 0.02M AcOH (T. O. D.<sub>280</sub><sup>\*5</sup> 88,300). This was separated by a column chromatography on Dowex-1 X-8 (100~200 mesh, acetate form, 325 ml.).

TABLE I.

Fraction	Solvent	Volume (ml.)	T. O. D. <sub>280</sub>
1	0.02M AcOH	1,800	57,600
2	0.5M AcOH	1,000	7,500
3	3.0M AcOH	1,500	18,300

\*4 All melting points are uncorrected.

\*5 Total optical density at 280 mμ.

The recovery of the reaction products eluted with AcOH of different concentrations was described in Table I.

**Uridine-5-carboxaldehyde (I)**—After chromatographic separation, the combined effluent fractions containing the compound (1) (850 ml., T. O. D. <sub>280</sub> 27,200) was concentrated *in vacuo* to dryness under N<sub>2</sub> below 40°. The resulting syrupy residue was extracted with EtOH (100 ml.) and the extract was concentrated to 5 ml., giving crystals, m.p. 178~179° (decomp.), (483 mg., yield 46%). It gave yellowish brown color with *o*-dianisidine.<sup>7)</sup> *Anal.* Calcd. for C<sub>10</sub>H<sub>12</sub>O<sub>7</sub>N<sub>2</sub>: C, 44.12; H, 4.44; N, 10.30. Found: C, 43.92; H, 4.69; N, 10.00. UV:  $\lambda_{\max}^{0.01N\text{HCl}}$  282 m $\mu$  ( $\epsilon$  12.2  $\times$  10<sup>3</sup>), 232 m $\mu$  ( $\epsilon$  9.35  $\times$  10<sup>3</sup>),  $\lambda_{\min}^{0.01N\text{HCl}}$  250 m $\mu$ ;  $\lambda_{\max}^{\text{H}_2\text{O}}$  282 m $\mu$  ( $\epsilon$  12.1  $\times$  10<sup>3</sup>), 232 m $\mu$  ( $\epsilon$  9.13  $\times$  10<sup>3</sup>),  $\lambda_{\min}^{\text{H}_2\text{O}}$  250 m $\mu$ ;  $\lambda_{\max}^{0.01N\text{NaOH}}$  283 m $\mu$  ( $\epsilon$  8.88  $\times$  10<sup>3</sup>), 215 m $\mu$ ,  $\lambda_{\min}^{0.01N\text{NaOH}}$  260 m $\mu$ . A single UV absorbing spot was detected which had R<sub>F</sub>\*<sup>6</sup> 1.3 by PE in the buffer 1 and R<sub>f</sub> 0.31 by TLC.

**Uridine-5-carboxylic Acid (II)**—The combined effluent fractions of the compound (2) (1,000 ml., T. O. D. <sub>280</sub> 7,500) was evaporated to dryness *in vacuo* to give a colorless powder (190 mg.), which after recrystallization from 95% EtOH (3 ml.), gave colorless needles, m.p. 118°, pK<sub>a</sub> 9.5, 4.2 (determined potentiometrically). *Anal.* Calcd. for C<sub>10</sub>H<sub>12</sub>O<sub>8</sub>N<sub>2</sub>·C<sub>2</sub>H<sub>5</sub>OH: C, 43.11; H, 5.43; N, 8.38; OC<sub>2</sub>H<sub>5</sub>, 13.48. Found: C, 42.91; H, 5.26; N, 8.36; OC<sub>2</sub>H<sub>5</sub>, 12.37. UV:  $\lambda_{\max}^{0.01N\text{HCl}}$  279 m $\mu$  ( $\epsilon$  12.9  $\times$  10<sup>3</sup>), 220 m $\mu$ ,  $\lambda_{\min}^{0.01N\text{HCl}}$  242 m $\mu$ ;  $\lambda_{\max}^{\text{H}_2\text{O}}$  275 m $\mu$  ( $\epsilon$  11.1  $\times$  10<sup>3</sup>), 218 m $\mu$ ,  $\lambda_{\min}^{\text{H}_2\text{O}}$  242 m $\mu$ ;  $\lambda_{\max}^{0.01N\text{NaOH}}$  271 m $\mu$  ( $\epsilon$  7.89  $\times$  10<sup>3</sup>),  $\lambda_{\min}^{0.01N\text{NaOH}}$  250 m $\mu$ . It gave a single UV absorbing spot with R<sub>XXIV</sub> 0.26 by PE in the buffer 2 and R<sub>f</sub> 0.19 by PPC with the solvent B.

**1-( $\beta$ -D-Ribofuranosyluronic Acid)uracil-5-carboxaldehyde (III)**—Concentration of the combined effluent fractions containing the compound (3) (1,500 ml., T. O. D. <sub>280</sub> 8,300) under N<sub>2</sub> below 40° yielded colorless residue, which gave a positive color reaction with *o*-dianisidine. UV:  $\lambda_{\max}^{0.01N\text{HCl}}$  280 m $\mu$ ,  $\lambda_{\min}^{0.01N\text{HCl}}$  250 m $\mu$ ;  $\lambda_{\max}^{0.01N\text{NaOH}}$  281 m $\mu$ ,  $\lambda_{\min}^{0.01N\text{NaOH}}$  260 m $\mu$ . In PE, it was located as a single spot detectable under UV light and had R<sub>I</sub> 1.3 and R<sub>XXIV</sub> 1.1 with the buffers 1 and 2. The concentrated residue (T. O. D. <sub>280</sub> 3,470) was dissolved in H<sub>2</sub>O (5 ml.) and to this was added 2,4-dinitrophenylhydrazine reagent (3 ml.) comprising 2,4-dinitrophenylhydrazine (80 mg.), conc. H<sub>2</sub>SO<sub>4</sub> (0.4 ml.), H<sub>2</sub>O (0.6 ml.), and 95% EtOH (2 ml.), resulting in the instant formation of orange colored precipitate. Crystallization from H<sub>2</sub>O gave orange crystals of 1-( $\beta$ -D-ribofuranosyluronic acid)uracil-5-carboxaldehyde 2,4-dinitrophenylhydrazone (XI), m.p. >250°. *Anal.* Calcd. for C<sub>16</sub>H<sub>14</sub>O<sub>11</sub>N<sub>6</sub>·H<sub>2</sub>O: C, 39.68; H, 3.33; N, 17.36. Found: C, 39.43; H, 3.47; N, 17.10. Analysis of this substance by PPC and PE showed the presence of a single UV absorbing spot having the following respective R<sub>III</sub> and R<sub>f</sub> values: R<sub>III</sub> 0.51 and 0.17 with the buffer 1 and 2, and R<sub>f</sub>'s 0.36 and 0.31 with the solvents A and C.

**Uridine-5-carboxaldehyde Thiosemicarbazone (VII)**—To a solution of I (2.4 mM, calcd. for O. D.) in 50% AcOH (3 ml.) was added thiosemicarbazide (270 mg., 3.0 mM) in 50% AcOH (8 ml.) and held at 70° for 30 min. The crystalline precipitate formed was recrystallized from H<sub>2</sub>O to give pale yellow crystals, m.p. 248~249° (decomp.), (635 mg., yield 75%). *Anal.* Calcd. for C<sub>11</sub>H<sub>15</sub>O<sub>6</sub>N<sub>5</sub>·½H<sub>2</sub>O: C, 37.29; H, 4.55; N, 19.77; S, 9.06. Found: C, 37.26; H, 4.40; N, 19.35; S, 8.91.

**Uridine-5-carboxaldehyde Isonicotinoylhydrazone (VIII)**—A mixture of I (3.1 mM, calcd. from O. D.) and isonicotinic acid hydrazide (425 mg., 3.3 mM), each in 3 ml. of 50% AcOH was kept at 80° for 10 min. and then left at room temperature until crystalline residue formed. After standing in the cold overnight, it was recrystallized from 50% EtOH to yield pale yellow needles, m.p. 168° (800 mg., yield 63%). *Anal.* Calcd. for C<sub>16</sub>H<sub>17</sub>O<sub>7</sub>N<sub>5</sub>·½H<sub>2</sub>O: C, 45.95; H, 4.82; N, 16.74. Found: C, 45.77; H, 4.92; N, 16.54. It gave a single fluorescent spot with R<sub>f</sub> 0.35 under UV light by TLC.

**Uridine-5-carboxaldehyde *p*-Bromophenylhydrazone (IX)**—Synthesis of IX was made by the reaction of I (3.4 mM calcd. for O. D.) in 50% AcOH (7 ml.) with *p*-bromophenylhydrazine (700 mg., 3.7 mM) in 50% EtOH (6 ml.). The mixture was held at 70° for 20 min. and allowed to stand at room temperature to yield yellow crystalline residue, which after standing in the cold overnight, was recrystallized from 50% EtOH to give yellow needles, m.p. 245~246° (decomp.), (1.126 g., yield 78%). *Anal.* Calcd. for C<sub>16</sub>H<sub>17</sub>O<sub>6</sub>N<sub>4</sub>Br: C, 43.56; H, 3.88; N, 12.70. Found: C, 43.71; H, 4.23; N, 12.32. Examination of this compound by TLC showed a single fluorescent spot having R<sub>f</sub> 0.44 under UV light.

**Uridine-5-carboxaldehyde Guanylhydrazone Sulfate (X)**—A solution of I (562 mg., 2.1 mM) in EtOH (16 ml.) and aminoguanidine sulfate (280 mg., 2.1 mM) in H<sub>2</sub>O (8 ml.) was heated under reflux for 2 hr. On cooling to room temperature, crystalline residue was obtained which after recrystallization from 50% EtOH gave colorless crystals, m.p. 220° (527 mg., yield 62%). *Anal.* Calcd. for C<sub>11</sub>H<sub>16</sub>O<sub>6</sub>N<sub>6</sub>·½H<sub>2</sub>SO<sub>4</sub>·2H<sub>2</sub>O: C, 31.96; H, 5.12; N, 20.33; S, 3.88. Found: C, 31.91; H, 5.24; N, 20.30; S, 3.74. It was found to remain immobile on the starting line when analyzed by TLC.

**Reduction of Uridine-5-carboxaldehyde (I)**—To an aqueous solution (5 ml.) of I (2.2 mg., 8  $\mu$ M) was added NaBH<sub>4</sub> (10 mg.) and the mixture was held at room temperature for 30 min. After decomposition of an excess of NaBH<sub>4</sub> with AcOH, the reaction mixture was stirred mechanically with Amberlite IR-120 (hydrogen form), filtered off and evaporated to dryness *in vacuo*. Distillation of the dried residue with MeOH effected removal of the resulting boric acid as methyl borate. The residue thus obtained was dis-

\*<sup>6</sup> Ratio of the migration distance of the sample to that of IV.

solved in small quantity of H<sub>2</sub>O and analyzed by PPC and TLC. It migrated as a single spot toward the anode and gave a negative color reaction with *o*-dianisidine. The results of these analyses shown in Table II, together with the data of spectrophotometric assay indicated the formation of uridine-5-methanol (IV). UV:  $\lambda_{\max}^{0.01N\text{HCl}}$  264 m $\mu$ ,  $\lambda_{\min}^{0.01N\text{HCl}}$  232~235 m $\mu$ .

TABLE II.

Nucleosides	R <sub>XXIV</sub>		Rf System A
	Buffer 1	Buffer 2	
IV	0.63		0.25
I	0.83		0.30
I reduced with NaBH <sub>4</sub>	0.63		0.25
II	0.92	0.26	0.26
II reduced with NaBH <sub>4</sub>	0.92	0.26	0.26
III	1.1	1.1	
III reduced with NaBH <sub>4</sub> (V)	0.92	0.96	

**Reduction of Uridine-5-carboxylic Acid (II)**—The compound (II) (2.7 mg., 8  $\mu$ M) was reduced as described above. The R<sub>f</sub> values and electrophoretic mobilities of the product was summarized in Table II. UV:  $\lambda_{\max}^{0.01N\text{HCl}}$  278 m $\mu$ ,  $\lambda_{\min}^{0.01N\text{HCl}}$  246 m $\mu$ .

**Reduction of 1-( $\beta$ -D-Ribofuranosyluronic Acid)uracil-5-carboxaldehyde (III)**—To an aqueous solution (20 ml.) containing III (1.5 mM, calcd. from O. D.) was added NaBH<sub>4</sub> (560 mg.) and the reduction was performed with the same manner as above to give a white powder which exhibited no color reaction with *o*-dianisidine. UV:  $\lambda_{\max}^{0.01N\text{HCl}}$  263 m $\mu$ ,  $\lambda_{\min}^{0.01N\text{HCl}}$  234.5 m $\mu$ ;  $\lambda_{\max}^{\text{H}_2\text{O}}$  263 m $\mu$ ,  $\lambda_{\min}^{\text{H}_2\text{O}}$  237 m $\mu$ ;  $\lambda_{\max}^{0.01N\text{NaOH}}$  264 m $\mu$ ,  $\lambda_{\min}^{0.01N\text{NaOH}}$  245 m $\mu$ . Spectrophotometric determination and analyses by PE of the compound showed the formation of 1-( $\beta$ -D-ribofuranosyluronic acid)uracil-5-methanol (V) (Table II).

**Catalytic Oxidation of Nucleosides, A Standard Procedure**—The following condition was used as a standard procedure for catalytic oxidation of the nucleosides unless otherwise specified. An appropriate amount of PtO<sub>2</sub> was suspended in H<sub>2</sub>O in a shaking flask and shaken mechanically under H<sub>2</sub>. After H<sub>2</sub> was exchanged with O<sub>2</sub>, the suspension continued shaking for 30 min. To this was added a nucleoside to be oxidized and an equivalent quantity of NaHCO<sub>3</sub>. The mixture was shaken continuously at 50~60° under O<sub>2</sub>. In the case of the oxidation of 1- $\beta$ -D-arabinofuranosylcytosine (XX), the reaction was carried out at 80° under O<sub>2</sub> in a closed indented flask with mechanical stirring.

**1-( $\beta$ -D-Ribofuranosyluronic Acid)uracil-5-carboxylic Acid (VI)**—i) Oxidation of uridine-5-methanol (IV). Oxidation of IV (500 mg., 1.8 mM) in H<sub>2</sub>O (50 ml.) was conducted as described above with NaHCO<sub>3</sub> (300 mg., 3.7 mM) in the presence of PtO<sub>2</sub> (500 mg.) for 24 hr. A portion of the reaction mixture (T. O. D. 274 7,450) was filtered off and then stirred with Amberlite IR-120 (6 ml., hydrogen form). After removal of the resin by filtration, evaporation of the filtrate below 40° yielded a syrupy residue, which was dissolved in EtOH. On standing at room temperature, colorless needles were obtained, m.p. 218° (decomp.). *Anal.* Calcd. for C<sub>10</sub>H<sub>10</sub>O<sub>9</sub>N<sub>2</sub>: C, 39.74; H, 3.34; N, 9.27. Found: C, 39.52; H, 3.34; N, 9.19. UV:  $\lambda_{\max}^{0.01N\text{HCl}}$  276 m $\mu$  ( $\epsilon$  12.7  $\times$  10<sup>3</sup>),  $\lambda_{\min}^{0.01N\text{HCl}}$  241 m $\mu$ ;  $\lambda_{\max}^{\text{H}_2\text{O}}$  275 m $\mu$  ( $\epsilon$  11.5  $\times$  10<sup>3</sup>),  $\lambda_{\min}^{\text{H}_2\text{O}}$  242 m $\mu$ ;  $\lambda_{\max}^{0.01N\text{NaOH}}$  271 m $\mu$  ( $\epsilon$  7.8  $\times$  10<sup>3</sup>),  $\lambda_{\min}^{0.01N\text{NaOH}}$  250 m $\mu$ . Analysis of this compound by PE gave a single UV absorbing spot having R<sub>II</sub> values 1.3 and 2.1 with the buffers 1 and 2 respectively.

ii) Oxidation of Uridine-5-carboxylic acid (II). An aqueous solution of II (10 mg., 30  $\mu$ M) was reacted under the standard condition in the presence of PtO<sub>2</sub> (20 mg.). The product thus obtained was confirmed to be identical with IV when examined by PE and spectrophotometric determination. PPC with the solvent B indicated the presence of a single UV absorbing spot having R<sub>f</sub> of 0.19.

**1-(2'-Deoxy- $\beta$ -D-erythro-pentofuranosyluronic Acid)uracil (2'-Deoxyuridine-5'-carboxylic Acid) (XIII)**—A similar oxidation of 2'-deoxyuridine (XII) was performed in a reaction mixture comprising XII (939 mg., 4.1 mM), NaHCO<sub>3</sub> (380 mg., 4.5 mM), and PtO<sub>2</sub> (750 mg.) in H<sub>2</sub>O (100 ml.). Analysis of the reaction product by PE revealed that about 95% of XII was oxidized to XIII. After filtration, the resulting filtrate was concentrated *in vacuo* to 50 ml. and treated with Amberlite IR-120 (15 ml., hydrogen form). Further concentration of the solution yielded colorless crystalline residue (720 mg., 73% yield). It was recrystallized from H<sub>2</sub>O to give colorless plates, m.p. 222~223° (decomp.). *Anal.* Calcd. for C<sub>9</sub>H<sub>10</sub>O<sub>8</sub>N<sub>2</sub>: C, 44.63; H, 4.16; N, 11.57. Found: C, 44.43; H, 4.08; N, 11.40. UV:  $\lambda_{\max}^{0.01N\text{HCl}}$  260 m $\mu$  ( $\epsilon$  10.6  $\times$  10<sup>3</sup>),  $\lambda_{\min}^{0.01N\text{HCl}}$  230 m $\mu$ ;  $\lambda_{\max}^{\text{H}_2\text{O}}$  261 m $\mu$  ( $\epsilon$  11.0  $\times$  10<sup>3</sup>),  $\lambda_{\min}^{\text{H}_2\text{O}}$  231 m $\mu$ ;  $\lambda_{\max}^{0.01N\text{NaOH}}$  261 m $\mu$  ( $\epsilon$  8.3  $\times$  10<sup>3</sup>),  $\lambda_{\min}^{0.01N\text{NaOH}}$  241 m $\mu$ . It was located on paper as a single UV absorbing spot with R<sub>XII</sub> 2.4 after PE with the buffer 1.



**1-(2'-Deoxy- $\beta$ -D-erythro-pentofuranosyluronic Acid)-5-bromouracil(5-Bromo-2'-deoxyuridine-5'-carboxylic Acid) (XV)**—5-Bromo-2'-deoxyuridine (XIV) (614 mg., 2.0 mM) in H<sub>2</sub>O (50 ml.) was catalytically oxidized in the presence of PtO<sub>2</sub> (503 mg.) and NaHCO<sub>3</sub> (178 mg., 2.1 mM) for 8 hr. In the analysis by PE with the buffer 1, a large part of XIV was found to be converted to XV after oxidation. After filtration and deionization with Amberlite IR-120 (10 ml., hydrogen form), it was adjusted at pH 6 with NH<sub>4</sub>OH and placed on a column (2.0 × 5.5 cm.) of Dowex-1, X-8 (100~200 mesh, chloride form). The column was first washed with H<sub>2</sub>O until the substance with an absorption at 280 m $\mu$  were eliminated and eluted with 0.01N HCl (total volume, 1,140 ml.). Subsequently, the effluent fractions were combined, adsorbed on a small column of activated charcoal (4 g.) and eluted with 95% EtOH. Removal of the solvent by evaporation left a crystalline residues, which was recrystallized from H<sub>2</sub>O to give colorless needles, m.p. 185° (decomp.), (282 mg., 44% yield). *Anal.* Calcd. for C<sub>9</sub>H<sub>9</sub>O<sub>6</sub>N<sub>2</sub>Br: C, 33.67; H, 2.83; N, 8.73; Br, 24.89. Found: C, 33.43; H, 2.61; N, 8.74; Br, 24.37. UV:  $\lambda_{\max}^{0.01N\text{HCl}}$  280 m $\mu$  ( $\epsilon$  9.8 × 10<sup>3</sup>),  $\lambda_{\min}^{0.01N\text{HCl}}$  244 m $\mu$ ;  $\lambda_{\max}^{\text{H}_2\text{O}}$  280 m $\mu$  ( $\epsilon$  9.9 × 10<sup>3</sup>),  $\lambda_{\min}^{\text{H}_2\text{O}}$  244 m $\mu$ ;  $\lambda_{\max}^{0.01N\text{NaOH}}$  276 m $\mu$  ( $\epsilon$  7.3 × 10<sup>3</sup>),  $\lambda_{\min}^{0.01N\text{NaOH}}$  252 m $\mu$ . It traveled as a single UV absorbing spot in the following respective R<sub>XIV</sub> and R<sub>f</sub> values; R<sub>XIV</sub> 1.9 and 4.0 by PE with the buffers 1 and 3, and R<sub>f</sub> 0.33 by PPC using the solvent D.

**1-(2'-Deoxy- $\beta$ -D-erythro-pentofuranosyluronic Acid)-5-iodouracil (5-Iodo-2'-deoxyuridine-5-carboxylic Acid) (XVII)**—A solution of 5-iodo-2'-deoxyuridine (XVI) (1 g., 2.8 mM) in H<sub>2</sub>O (120 ml.) was oxidized in the presence of PtO<sub>2</sub> (800 mg.) and NaHCO<sub>3</sub> (252 mg., 3.0 mM) for 7.5 hr. Analysis of the reaction mixture by PE with the buffer 1 confirmed the completion of the oxidation of XVI. After removal of the catalyst by filtration, the filtrate was deionized by stirring mechanically with Amberlite IR-120 (12 ml., hydrogen form). After removal of the resin by filtration, the filtrate was concentrated *in vacuo* to 40 ml., giving rise to colorless crystals, which after recrystallization from H<sub>2</sub>O gave colorless needles, m.p. 187~189° (decomp.), (756 mg., yield 73%). *Anal.* Calcd. for C<sub>9</sub>H<sub>9</sub>O<sub>6</sub>N<sub>2</sub>I: C, 29.37; H, 2.46; N, 7.61; I, 34.47. Found: C, 29.35; H, 2.53; N, 7.33; I, 34.31. UV:  $\lambda_{\max}^{0.01N\text{HCl}}$  287 m $\mu$  ( $\epsilon$  7.7 × 10<sup>3</sup>),  $\lambda_{\min}^{0.01N\text{HCl}}$  247 m $\mu$ ;  $\lambda_{\max}^{\text{H}_2\text{O}}$  287 m $\mu$  ( $\epsilon$  8.0 × 10<sup>3</sup>),  $\lambda_{\min}^{\text{H}_2\text{O}}$  247 m $\mu$ ;  $\lambda_{\max}^{0.01N\text{NaOH}}$  279 m $\mu$  ( $\epsilon$  5.9 × 10<sup>3</sup>),  $\lambda_{\min}^{0.01N\text{NaOH}}$  253 m $\mu$ . PE using the buffer 1 indicated the presence of a single UV absorbing spot with R<sub>XVI</sub> of 1.9.

**1-(2'-Deoxy- $\beta$ -D-erythro-pentofuranosyluronic Acid)-5-iodocytosine (5-Iodo-2'-deoxycytidine-5-carboxylic Acid) (XIX)**—A solution of 5-Iodo-2'-deoxycytidine (XVIII) (1 g., 2.8 mM) in H<sub>2</sub>O (120 ml.) was oxidized in the presence of PtO<sub>2</sub> (1 g.) and NaHCO<sub>3</sub> (252 mg., 3.0 mM) for 16 hr. Examination by PE of the reaction product showed that a large part XVIII was oxidized. After removal of the catalyst by filtration, the filtrate was concentrated *in vacuo* to 26 ml. and adjusted at pH 3 with NH<sub>2</sub>SO<sub>4</sub> to yield colorless crystals (567 mg., yield 55%). It gave colorless needles after recrystallization from H<sub>2</sub>O, m.p. 192~194° (decomp.). *Anal.* Calcd. for C<sub>9</sub>H<sub>10</sub>O<sub>5</sub>N<sub>3</sub>I: C, 29.45; H, 2.75; N, 11.45. Found: C, 29.58; H, 2.71; N, 11.67. UV:  $\lambda_{\max}^{0.01N\text{HCl}}$  306 m $\mu$  ( $\epsilon$  8.1 × 10<sup>3</sup>),  $\lambda_{\min}^{0.01N\text{HCl}}$  261 m $\mu$ ;  $\lambda_{\max}^{\text{H}_2\text{O}}$  294 m $\mu$  ( $\epsilon$  6.3 × 10<sup>3</sup>),  $\lambda_{\min}^{\text{H}_2\text{O}}$  264 m $\mu$ ;  $\lambda_{\max}^{0.01N\text{NaOH}}$  294 m $\mu$  ( $\epsilon$  6.3 × 10<sup>3</sup>),  $\lambda_{\min}^{0.01N\text{NaOH}}$  264 m $\mu$ . The purity of this compound was confirmed by the location of a single spot detectable under UV light after PE. It also showed a single spot with R<sub>f</sub> 0.12 by TLC. The mother liquor was found to contain a considerable amount of 5-iodocytosine.

**1-( $\beta$ -D-Arabinofuranosyluronic Acid)uracil (XXIII)**—An aqueous solution (6 ml.) of 1- $\beta$ -D-arabinofuranosyluracil (XXII) (10 mg., 41  $\mu$ M) was oxidized in the presence of PtO<sub>2</sub> (20 mg.) and NaHCO<sub>3</sub> (4.2 mg., 50  $\mu$ M) for 2.5 hr. Analysis of the reaction mixture by PE with the buffer 1 showed the presence of two UV absorbing spots; the slower-moving one had R<sub>XXII</sub> 2.6 (90% yield calcd. from O. D.). UV:  $\lambda_{\max}^{0.01N\text{HCl}}$  261 m $\mu$ ,  $\lambda_{\min}^{0.01N\text{HCl}}$  232 m $\mu$ ;  $\lambda_{\max}^{0.01N\text{NaOH}}$  261 m $\mu$ ,  $\lambda_{\min}^{0.01N\text{NaOH}}$  242 m $\mu$ . The faster-moving one had R<sub>XXII</sub> 3.3 (10% yield calcd. from O. D.).

**1-( $\beta$ -D-Arabinofuranosyluronic Acid)cytosine (XXI)**—1- $\beta$ -D-Arabinofuranosylcytosine hydrochloride (XX) (560 mg., 2.0 mM) in H<sub>2</sub>O (100 ml.) was oxidized in the presence of PtO<sub>2</sub> (800 mg.) and NaHCO<sub>3</sub> (370 mg., 4.4 mM) for 24 hr. After removal of the catalyst by filtration, the filtrate was concentrated *in vacuo* to 15 ml. and adjusted at pH 4 with NH<sub>2</sub>SO<sub>4</sub> to give colorless crystalline residue. After recrystallization from H<sub>2</sub>O, it gave colorless crystals, m.p. 235~238° (decomp.). *Anal.* Calcd. for C<sub>9</sub>H<sub>11</sub>O<sub>6</sub>N<sub>3</sub>: C, 42.03; H, 4.31; N, 16.34. Found: C, 42.08; H, 4.24; N, 16.14. UV:  $\lambda_{\max}^{0.01N\text{HCl}}$  279 m $\mu$  ( $\epsilon$  13.6 × 10<sup>3</sup>),  $\lambda_{\min}^{0.01N\text{HCl}}$  240 m $\mu$ ;  $\lambda_{\max}^{\text{H}_2\text{O}}$  271 m $\mu$  ( $\epsilon$  9.9 × 10<sup>3</sup>),  $\lambda_{\min}^{\text{H}_2\text{O}}$  249 m $\mu$ ;  $\lambda_{\max}^{0.01N\text{NaOH}}$  271 m $\mu$  ( $\epsilon$  9.9 × 10<sup>3</sup>),  $\lambda_{\min}^{0.01N\text{NaOH}}$  249 m $\mu$ . PE in the buffer 1 indicated that it migrated as a single UV absorbing spot. A considerable amount of XXIII was recovered from the mother liquor.

We express our gratitude to Dr. S. Tatsuoka, director of the Research Laboratories for his permission to publish this paper and to Dr. K. Tanaka for his valuable advice and encouragement throughout this work. Thanks are also due to Mr. M. Kan for elementary analysis, to Miss T. Hiratsuka for physico-chemical determinations and to Messrs. Y. Furukawa, Y. Yoshioka, T. Hirata, and Y. Sato for their technical assistance.

### Summary

Uridine-5-carboxaldehyde (I), uridine-5-carboxylic acid (II), and 1-( $\beta$ -D-ribofuranosyluronic acid)uracil-5-carboxaldehyde (III) were isolated and characterized from the reaction products obtained after catalytic oxidation of uridine-5-methanol (IV). Five aldehyde hydrazone derivatives of I and III were synthesized. Catalytic oxidation of several pyrimidine nucleosides gave the corresponding uronic acid derivatives. The ease of the oxidation of these compounds was found different, depending upon the base moieties.

(Received April 14, 1964)

[Chem. Pharm. Bull.]  
13(1) 16 ~ 21 (1965)

UDC 612.398.145

### 3. Yoshiyasu Furukawa, Yoshiko Mizuno, Yasushi Sanno, and Mikio Honjo: Synthesis of Cytidine 5'-Diphosphate- and Deoxycytidine 5'-Diphosphate-L-serine.\*<sup>1</sup>

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CDP-\*<sup>3</sup> (or dCDP-) choline and CDP- (or dCDP-) ethanolamine participate in the biosynthesis of lecithine and phosphatidylethanolamine.<sup>1,2)</sup> By analogy, CDP- or dCDP-serine seems to have some bearings on the biosynthesis of phosphatidylserine. However, the occurrence of these compounds in nature has not yet been recorded, and on the contrary, another biosynthetic pathway of phosphatidylserine has been postulated.<sup>3,4)</sup> We, therefore, attempted the chemical synthesis of these compounds.

First, a mixture of CMP and O-phosphoryl-L-serine<sup>5)</sup> (I) was treated with DCC in aqueous pyridine or a solution of the dicyclohexylguanidinium salt of CMP-NH<sub>2</sub> (II)<sup>6)</sup> in *o*-chlorophenol was allowed to react with I. The examination of the reaction mixtures by ion exchange chromatography and paper electrophoresis (PEP), however, showed no formation of the desired CDP-serine. The reason seemed to be due to the interference with the reaction by the free amino group of I. Therefore, O-phosphoryl-N-carbobenzyloxy-L-serine (III) was synthesized and used instead of I. Compound (I) was treated with carbobenzyloxychloride by the usual method in the presence of sodium hydroxide. The crude reaction mixture was applied to the column of Dowex-50 (H<sup>+</sup> form), which on elution with water afforded III and I in succession. Compound (III) gave a positive

\*<sup>1</sup> Published at the 83rd Annual Meeting of the Pharmaceutical Society of Japan (Nov. 2nd, 1963, Tokyo). Afterwards, Michelson, *et al.* have synthesized CDP-serine by another method, but no detailed description is available (Bull. soc. chim. biol., 45, 1353 (1963)).

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\*<sup>3</sup> CDP, cytidine 5'-diphosphate; dCDP, deoxycytidine 5'-diphosphate; CMP, cytidine 5'-phosphate; CMP-NH<sub>2</sub>, cytidine 5'-phosphoramidate; DCC, dicyclohexylcarbodiimide; DCCP, P<sup>1</sup>,P<sup>2</sup>-dicytidine 5'-pyrophosphate; UMP, uridine 5'-phosphate; dCMP-NH<sub>2</sub>, deoxycytidine 5'-phosphoramidate; dCMP, deoxycytidine 5'-phosphate.

1) E. P. Kennedy, S. B. Weiss: J. Biol. Chem., 222, 193 (1956).

2) E. P. Kennedy, L. F. Borckenhagen, S. W. Smith: J. Biol. Chem., 234, 1998 (1959).

3) G. Hübscher, R. R. Dils, W. F. R. Pover: Biochim. Biophys. Acta, 36, 518 (1959).

4) J. N. Kanfer, E. P. Kennedy: J. Biol. Chem., 237, pc 270 (1962).

5) G. Fölsch, O. Mellander: Acta Chem. Scand., 11, 1234 (1957).

6) R. W. Chambers, P. Shapiro, V. Kurkov: J. Am. Chem. Soc., 82, 970 (1960).