Notes

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Studies on Nucleic Acid Antagonists. XII.[1] Synthesis of Aminosugar Nucleotides

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Since the discovery of puromycin in 1952, many nucleoside antibiotics have been found in nature. The recent discoveries of gougerotin and blasticidin S have stimulated interest in nucleosides containing aminosugar moieties as potential medicinals.[3] This study deals with the syntheses of nucleotides containing aminosugars in their structure.

In 1958, Baer and Fischer[4] reported a novel synthesis of 3–nitro– and 3–amino–3–deoxy-pyranosides by the "periodate–nitromethane" reaction with glycosides. Fox and his co-workers[5,6] adapted this procedure to nucleosides and developed a facile synthesis of (3′–nitro– and (3′–amino–3′–deoxy)pyranosyl nucleosides from the naturally occurring nucleosides; uridine, adenosine, *etc.* Later, Lichtenthaler and co–workers[7] reported the application of this procedure to uridine, adenosine, inosine, xanthosine and glucopyranosylthymine.

We have found that this procedure could also be applied to nucleotides, such as, 5′–CMP and 5′–UMP as shown in Chart 1. Oxidation of 5′–CMP(Na_2) (I) with one mole equivalent of sodium metaperiodate yielded the dialdehyde (II), which, without isolation, was cyclized with an excess of nitromethane and sodium hydroxide. On treatment of the reaction mixture with Amberlite IR–120 (H^+ form), 1–(3′–deoxy–3′–nitro–β–D–glucopyranosyl)cytosine–6′ phosphate (III) was obtained as fine crystals in 46% overall yield. Hydrogenation of III with Raney Nickel catalyst afforded 1–(3′–amino–3′–deoxy–β–D–glucopyranosyl)cytosine–6′ phosphate (IV) as a colorless powder analytically and electrophoretically pure in 73.5% yield. Compound IV was hydrolyzed with a phosphatase[8] in acetate buffer (pH 5.5) at 37° for 16 hr. After application of the reaction mixture to Amberlite IR–120 (H^+ form), and elution with ammonia–water, 1–(3′–amino–3′–deoxy–β–D–glucopyranosyl)cytosine (V) was obtained in 97.5% yield. The aminosugar nucleoside (V) was converted to the corresponding pentaacetate (VI) in 74% yield.

1) The contents of this paper were presented at the Meeting of Pharmaceutical Society of Japan in Sendai (Oct. 22, 1966). Part XI: T. Sugawa, Y. Kuwada, K. Imai, M. Morinaga, K. Kaziwara, and K. Tanaka, *Takeda Annual Report*, **20**, 7 (1961).
2) Location: *Juso, Higashiyodogawa, Osaka.*
3) J.J. Fox, K.A. Watanabe, and A. Bloch, "Progress in Nucleic Acid Research and Molecular Biology," **5**, Academic Press, New York, London, 1966, p. 251. H. Umezawa, "Recent Advances in Chemistry and Biochemistry of Antibiotics," Microbial Chemistry Research Foundation, Tokyo, 1964, p. 67, p. 167.
4) H.H. Baer and H.O.L. Fischer, *Proceeding of National Academy of Science*, **44**, 991 (1958).
5) K.A. Watanabe and J.J. Fox, *Chem. Pharm. Bull.* (Tokyo), **12**, 975 (1964); K.A. Watanabe, J. Beránek, H.A. Friedmann, and J.J. Fox, *J. Org. Chem.*, **30**, 2735 (1965).
6) J. Beránek, H.A. Friedman, K.A. Watanabe, and J.J. Fox, *J. Heterocyclic Chem.*, **2**, 188 (1965).
7) *a*) F.W. Lichtenthaler, H.P. Albrecht, and G. Olfermann, *Angew. Chem.*, **77**, 131 (1965). *b*) F.W. Lichtenthaler and H.P. Albrecht, *Ann. Chem.*, **99**, 575 (1966). *c*) F.W. Lichtenthaler, H.P. Albrecht, G. Olfermann, and J. Yoshimura, *Angew. Chem.*, **77**, 731 (1965).
8) A non–specific phosphatase obtained from the culture filtrate of *Phythophthora infestans* kindly supplied from Microbiological Research Laboratories of this company.

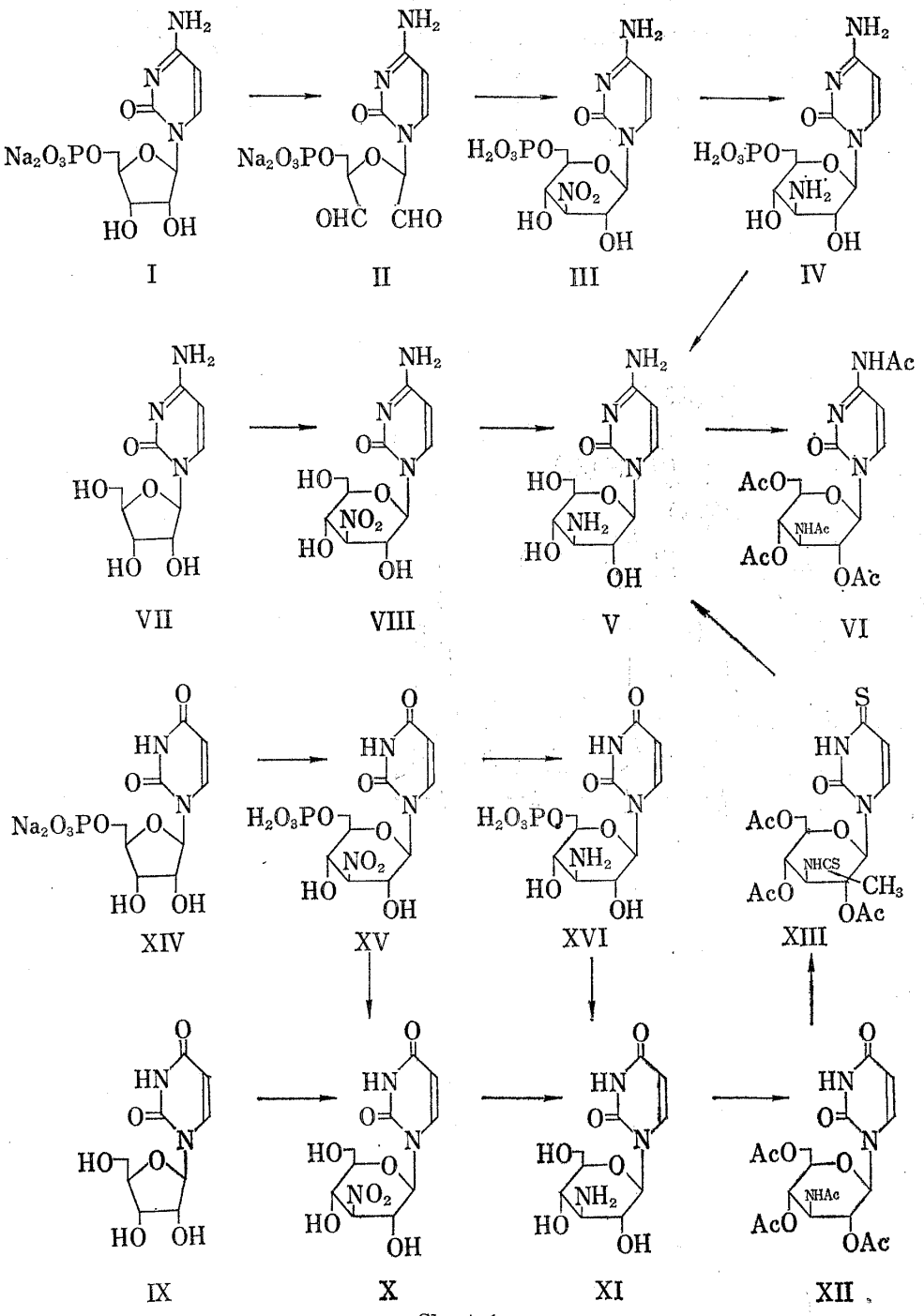

Chart 1

It would be expected that the aminosugar nucleoside (V) and its pentaacetate (VI) might be obtained by adaptation of the periodate–nitromethane procedure directly to cytidine (VII), and indeed from this reaction, the nitrosugar nucleoside (VIII) was obtained as a powder.[9] Hydrogenation of this powder yielded the aminosugar nucleoside (V) as colorless needles in 55% yield calculated from VII. Acetylation of V gave its pentaacetate (VI) as colorless needles in 80% yield.

9) Since completion of this study we have been informed of the successful application of the periodate–nitromethane reaction to cytidine achieved by H.A. Friedman, K.A. Watanabe and J.J. Fox, *J. Org. Chem.*, in press. These workers obtained the manno as well as the gluco isomer (V) from their reaction. J.J. Fox, personal communication.

Previously, Fox and his co-workers[5] have reported the synthesis of V from uridine, and established the gluco configuration of the sugar moiety by chemical and physical means. Compounds V and VI were synthesized from uridine (IX) following Fox's method,[5] and were found to be identical with V and VI respectively which in turn were obtained from IV and from cytidine (VII). Therefore all these compounds are of the gluco configuration.

Furthermore, NMR spectral studies on VI (Fig. 1) along with decoupling data clearly supported the conclusion that the sugar moieties of these nucleosides have gluco configuration. The NMR spectrum of VI is shown in Fig. 1 for an example:

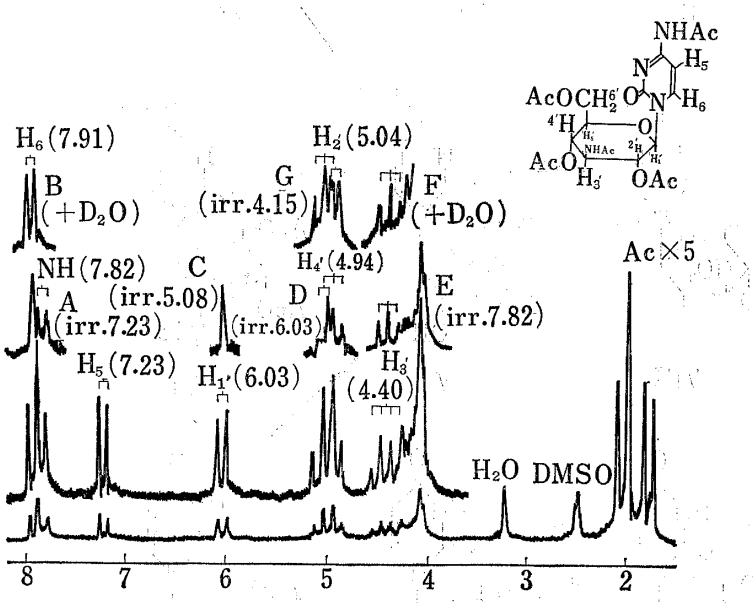

Fig. 1. The NMR Spectrum of VI and Its Decoupling Data in d_6DMSO (δ, ppm).

In the paper by Lichtenthaler, *et al.*,[10] configurations of sugar moiety of the periodate–nitromethane reaction products were determined by examination of the chemical shifts of acetyl resonances of the acetylated nucleosides and by isolation of sugar component. However, as pointed out by Cushley, Watanabe and Fox[11] chemical shifts of acetyl signals are unreliable for determining the configuration of the sugar moiety.

Therefore, we examined each of the protons in VI and the couplings. As is shown in Fig. 1,[12] the H_5 and H_6 protons on the cytosine moiety were easily identified as doublets at δ 7.23 and δ 7.91, respectively. By irradiating the H_5 signal (δ 7.23), the H_6 doublet was converted to a singlet (see Fig. 1, insert A), and could be separated from a doublet of the amide H signal (δ 7.82, J=10.0 cps), which disappeared by addition of deuterium oxide to the solution (see insert B). The doublet at δ 6.03 ($J_{H_1'H_2'}$=9.0 cps) is easily assigned to the anomeric proton. The H_2' proton signal at δ 5.04, which is a quartet due to overlapping with the H_4' triplet, was verified by collapse of the H_1' signal to a singlet by irradiating at δ 5.08 (see insert C). On the other hand, by irradiating the H_1' signal, the H_2' signal became a doublet ($J_{H_2', H_3'}$=9.0 cps) which could be clearly separated from the H_4' triplet (see insert D). The H_3' signal was assigned the quartet pattern at δ 4.40 with J=9.0—10.0 cps, because this was changed to a triplet by irradiation of the amide H or by addition of deuterium oxide to the solution (see insert E and F). The triplet at δ 4.94 (J=9.0 cps) which overlapped the H_2' triplet is assigned to the H_4' proton, because it should have appeared as a more complex pattern if it were derived from the H_5' proton. Irradiation at δ 4.10 which is assumed to be the center of the H_5' signal, changed the H_4' triplet to a doublet ($J_{H_3', H_4'}$=9.0 cps) (see insert G). The H_5' and two of the $H_{6'}$ protons overlapped each other as shown in Fig. 1 and gave a complex pattern from which accurate values for chemical shifts could not be determined.

The magnitude of the coupling constants between vicinal *trans* diaxial hydrogen atoms of six-membered ring compounds with chair conformation is calculated to be about 9.2 cps by Karplus' equation. The large values of the couplings among the protons in the sugar

10) See ref. 7*b*) for example.
11) R.J. Cushley, K.A. Watanabe, and J.J. Fox, *J. Am. Chem. Soc.*, **89**, 394 (1967).
12) NMR spectra were taken with a Varian HA—100 spectrometer with tetramethylsilane as an internal standard.

moiety of VI are consistent with a diaxial orientation for all the protons in the sugar moiety of VI and for the gluco configuration in the C1 conformation.

The periodate–nitromethane reaction was carried out with 5′–UMP in order to obtain the uracil analogues. Although the nitrosugar nucleotide (XV) and aminosugar nucleotide (XVI) were not obtained as crystalline products, XVI showed a single spot when examined by paper electrophoresis and gave good elemental analyses. Compound XV was hydrolyzed enzymatically in acetate buffer to give a uracil nucleoside which was identical with 1–(3′–deoxy–3′–nitro–β–D–glucopyranosyl)uracil (X) obtained previously from uridine.[5] Enzymatic hydrolysis of XVI by using phosphatase and acetylation gave tetraacetate which was also identical with 1–(3′–acetamido–2′,4′,6′–tri–O–acetyl–3′–deoxy–β–D–glucopyranosyl)uracil (XII) prepared from uridine.

Thus, this procedure is applicable to 5′–CMP and 5′–UMP, and should be useful for the conversion of readily–available purine nucleotides to their 3′–amino–3′–deoxy–β–D–hexopyranosylpurine 6′–nucleotides.

Experimental

1-(3′-Deoxy-3′-nitro-β-D-glucopyranosyl)cytosine-6′ Phosphate (III)——To a cold solution of 10.7 g (0.05 mole) of $NaIO_4$ in 50 ml of H_2O, 18.2 g (0.05 mole) of 5′–CMP (Na_2) was added portionwise over a 5 min period at 20—25°. After 15 min, the oxidation was complete as determined by zinc iodide–starch test paper. Nitromethane (10 ml, 0.15 mole) was added and, with continuous stirring and cooling at 5°, 50 ml of N NaOH was added dropwise to the reaction mixture over a 15 min period. After additional 3 hr of stirring, the reaction mixture was allowed to remain in a refrigerator overnight. The product was applied to a warm column (*ca.* 60°) containing 150 ml of Amberlite IR—120 (H^+) resin and eluted with hot water. The effluent was concentrated *in vacuo* and the fine crystals which separated were recrystallized from H_2O. III was obtained as colorless needles in 46% yield (9.2 g), mp 199—200° (decomp.), $[\alpha]_D^{20}$ +49.3° (c=0.175, H_2O), UV: $\lambda_{max}^{0.1N\,HCl}$ 277.5 mμ (ε, 13900). *Anal.* Calcd. for $C_{10}H_{15}O_{10}N_4P\cdot H_2O$: C, 30.00; H, 4.28; N, 14.00; P, 7.74. Found: C, 29.92; H, 4.39; N, 13.72; P, 7.35.

1-(3′-Amino-3′-deoxy-β-D-glucopyranosyl)cytosine-6′ Phosphate (IV)——A solution of 1.0 g (0.025 mole) of III in 50 ml of H_2O was added to a suspension of 2 g of Raney Ni in 50 ml of H_2O and hydrogenated at room temperature and atmospheric pressure. Within 2 hr, 144 ml of hydrogen (at 23°, 79% of the theoretical amount) was consumed, and the uptake virtually ceased. After filtering off the catalyst, H_2S was bubbled to the filtrate to remove the inorganic salt as sulfide. The filtrate was then evaporated *in vacuo* at 45° to give a powder which was purified by reprecipitation from a minimum amount of H_2O with a large volume of EtOH, mp 260° (decomp.). Paper electrophoresis[13] showed a single spot $R_{IV/5'\text{-CMP}}$=0.84. Yield 0.65 g (73.5%), $[\alpha]_D^{22}$ +11.2° (c=1.0, H_2O), UV: $\lambda_{max}^{0.1N\,HCl}$ 277.5 mμ (ε, 14300). *Anal.* Calcd. for $C_{10}H_{17}O_8N_4P\cdot H_2O$: C, 32.44; H, 5.17; N, 15.13; P, 8.37. Found: C, 33.06; H, 5.04; N, 14.77; P, 8.20.

1-(3′-Amino-3′-deoxy-β-D-glucopyranosyl)cytosine (V) and Its Pentaacetate (VI)——From IV: A solution of 1 g of IV in 100 ml of 0.5 M acetate buffer (pH 5.5) was incubated with 15 mg of phosphatase[8] in the presence of a drop of toluene at 37° for 16 hr. After removal of a small amount of insoluble material, the filtrate was applied to 50 ml of Amberlite IR–120 (H^+) column, which, after washing with H_2O, was treated with N NH_4OH. The eluates were evaporated *in vacuo*. Recrystallization from MeOH–H_2O afforded 0.75 g (97.5%) of V as a colorless semicrystalline powder, mp 260—265° (decomp.). IR and NMR spectra were identical with those of V prepared from cytidine and uridine.

Acetylation of 0.5 g of V was carried out by warming with 10 ml of Ac_2O and one drop of conc. H_2SO_4 at 100° for 1 hr. After pouring onto 50 ml of stirred ice–water, the $CHCl_3$ extract gave, after concentration to dryness, a solid which was recrystallized from MeOH to yield 0.65 g (74%) of VI as colorless needles, mp 282—283° (decomp.), $[\alpha]_D^{23}$ +11.8° (c=0.65, MeOH). *Anal.* Calcd. for $C_{20}H_{26}O_{10}N_4$: C, 49.79; H, 5.43; N, 11.61. Found: C, 49.50; H, 5.26; N, 11.46.

From Cytidine (VII): To a solution of 21.3 g (0.1 mole) of $NaIO_4$ in 150 ml of H_2O, was added 24.3 g (0.1 mole) of VII portionwise at 20—25°. After 15 min for completion of the oxidation, 150 ml of MeOH was added and the precipitated inorganic salts were removed. Nitromethane (20 ml, 0.3 mole) was added to the filtrate followed by dropwise addition of 100 ml of 2N NaOH with cooling and stirring for 1 hr. The mixture was kept in a refrigerator overnight, and then applied to 300 ml of Amberlite IR–120 (H^+) column, which was washed with H_2O and then treated with N HCl. The effluent was evaporated to dryness *in vacuo*. Extraction of the residue with MeOH gave 20.0 g (66.5%) of VIII as an yellow powder. This was dissolved

13) Paper electrophoreses were carried out in borate buffer, pH 9.2, 1000 V, 1 hr.

in 100 ml of H_2O and hydrogenated in the presence of 5 g of Raney Ni. Within 7 hr about 5 liter of hydrogen (at 22°, 87% of the theoretical amount) was consumed. After removal of the catalyst, the filtrate was treated with 300 ml of Amberlite IR-120 (H^+) column and eluted with N NH_4OH. The eluate was concentrated to a colorless powder which was recrystallized from MeOH–H_2O to yield 15.0 g (55% yield from cytidine) of V as colorless needles, mp 246—247° (with browning), 254° (eff.), $[\alpha]_D^{25}$ +38.0° (c=0.5, H_2O), UV: $\lambda_{max}^{1N\,HCl}$ 277 mμ (ε, 12800).

The above crystals (V) (0.45 g) were acetylated with 10 ml of Ac_2O and 0.1 g of NaOAc by gentle refluxing for 15 min. The product isolated by the usual procedure was recrystallized from MeOH to afford 0.6 g (80%) of VI as colorless needles, mp 283—285° (decomp.), $[\alpha]_D^{23}$ +12.0° (c=0.65, MeOH), UV: $\lambda_{max}^{0.1N\,HCl}$ 253 mμ (ε, 9300), 280 mμ (ε, 8700). *Anal.* Calcd. for $C_{20}H_{26}O_{10}N_4$: C, 49.79; H, 5.43; N, 11.61. Found: C, 49.71; H, 5.67; N, 11.41.

From Uridine (IX): Compound X→XI→XII→XIII→V→VI as shown in Chart 1 were prepared by following Watanabe and Fox's procedure[5] comparing with their data.[14] X was main product, 70% yield, colorless triangular crystals as hemihydrate, mp 158—159° (sinter), 175° (eff.), $[\alpha]_D^{22}$ +35.2° (c=0.5, MeOH); XI, fluffy crystals, mp 164° (sinter), 197° (eff.), $[\alpha]_D^{20}$ +28.0° (c=1.0, H_2O); XII, colorless needles, mp 249—250°, $[\alpha]_D^{24}$ −8.9° (c=0.75, $CHCl_3$), −0.8° (c=1.0, H_2O); XIII, yellow triangular crystals, mp 233°, $[\alpha]_D^{27}$ −116.5° (c=1.0, $CHCl_3$); V, colorles needles, mp 248° (with browning), 258° (eff.), $[\alpha]_D^{25}$ +35.4° (c=0.5, H_2O); VI, colorless fluffy crystals, mp 282—283° (decomp.), $[\alpha]_D^{23}$ +11.5° (c=1.0, MeOH). *Anal.* Calcd. for $C_{20}H_{26}O_{10}N_4$: C, 49.79; H, 5.43; N, 11.61. Found: C, 49.59; H, 5.40; N, 11.58.

1-(3′-Nitro- and 1-(3′-Amino-3′-deoxy-β-D-glucopyranosyl)uracil-6′ Phosphate (XV and XVI)——These compounds were synthesized by essentially the same procedure as described above for the syntheses of III and IV. 5′-UMP (Na_2) (XIV) (20.4 g, including 20% H_2O) was treated with 10.6 g of $NaIO_4$ in 100 ml of H_2O at 20—25°. After 20 min, 10 ml of nitromethane and then 125 ml of N NaOH were added at room temperature. The product was passed through 350 ml of Amberlite IR-120 (H^+) column. The effluent and washings were evaporated *in vacuo* to a sirup which did not crystallize. The above sirup was hydrogenated with 10 g of Raney Ni at 27° for 8 hr until consumption of hydrogen ceased (1.6 liter, 42% of theoretical amount calcd. from 5′-UMP). After treatment of the filtrate with H_2S and removal of the precipitate, the resulting solution was evaporated *in vacuo* to afford 6.3 g of dark sirup which was purified by reprecipitation with H_2O–MeOH. A colorless powder (XVI) was obtained, 1.3 g (7.3%), mp 215° (eff.), $[\alpha]_D^{22}$ 0° (c=1.0, H_2O), UV: $\lambda_{max}^{H_2O}$ 259 mμ (ε, 9100). *Anal.* Calcd. for $C_{10}H_{16}O_9N_3P\cdot 2H_2O$: N, 10.86; P, 8.00. Found: N, 10.68; P, 8.05. Paper electrophoresis[13] showed a single spot and $R_{XVI/5'\text{-}UMP}$=0.73. Hydrolysis of XVI by using of phosphatase[8] as described above and then acetylation gave tetraacetate (XII) as colorless needles which showed mp 248—249°, $[\alpha]_D^{25}$ −7.4° (c=0.5, $CHCl_3$), −0.7° (c=1.0, H_2O). IR and NMR spectra were superimposed with those of XII obtained from uridine.

X from 5′-UMP (XIV)——The crude nitrosugar nucleotide (XV) obtained from 3.8 g of XIV was dissolved in 300 ml of 0.05 M acetate buffer (pH 5.5) and incubated with 50 mg of phosphatase[8] in the presence of a drop of toluene at 37° for 24 hr. The reaction mixture was placed on a column of 50 ml of Amberlite IR-120 (H^+), washed with H_2O, and the combined eluate and washings were evaporated *in vacuo*. Recrystallization from MeOH–H_2O gave 1.2 g (37%) of X as colorless triangular crystals, mp 162—163° (sinter), 174—175° (eff.), $[\alpha]_D^{25}$ +33.2° (c=0.5, MeOH), UV: $\lambda_{max}^{H_2O}$ 256.5 mμ (ε, 11200). *Anal.* Calcd. for $C_{10}H_{13}O_8N_3\cdot\frac{1}{2}H_2O$: C, 38.46; H, 4.52; N, 13.46. Found: C, 38.76; H, 4.48; N, 13.45. IR and NMR data were identical with those of X prepared from uridine.

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14) Reported by Fox, *et al.* in ref. 5): X, mp 175—176°, $[\alpha]_D^{25}$ +33° (c=0.75, MeOH). (Lichtenthaler reported in ref. 7*b*) as monohydrate, mp 162—164°, $[\alpha]_D^{20}$ +36° (c=0.6, H_2O), which was converted to hemihydrate after drying 110° *in vacuo*.) XI, mp 166—167° (sinter), 179—182° (eff.), $[\alpha]_D^{25}$ +33° (c=0.88, H_2O). XII, mp 253—254°, $[\alpha]_D^{23}$ 0° (c=0.75, $CHCl_3$). XIII, mp 234—235°, $[\alpha]_D^{23}$ −111° (c=0.7, $CHCl_3$). V, mp 248—250° (browning), 260—261° (eff.), $[\alpha]_D^{23}$ +36° (c=0.94, H_2O).