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Syntheses of Potential Antimetabolites. XVII.[1] New Type of Cyclo- and Keto-sugar-nucleosides. 7-(5-O-Trityl-2,3-dideoxy-2-oxo-α-D-pentofuranosyl)- and 5′-O-Trityl-3′,6-anhydro-7-α-D-arabinofuranosylhypoxanthine

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A new type of ketosugar-nucleoside, 7-(5-O-trityl-2,3-dideoxy-2-oxo-α-D-pentofuranosyl)hypoxanthine (VIII) and a new type of purinecyclonucleoside, 5′-O-trityl-3′,6-anhydro-7-α-D-arabinofuranosylhypoxanthine (VI) were synthesized from 5′-O-trityl-2′,3′-di-O-mesyl-α-D-arabinofuranosylhypoxanthine (IV).

The synthesis, chemical behavior and conformational properties of pyrimidine and purine cyclonucleosides have been amply documented.[3–5] In particular, the versatility of purine cyclonucleosides as intermediates for chemical transformations has been established by extensive work of Ikehara and his coworkers.[6] However, to our knowledge, no purine cyclonucleoside involving linkage through C-6 of purine has been described in the literature and the chemistry of this type of cyclonucleoside remains unexplored. Conceivably, cyclonucleosides of this type will be useful as potential intermediates for the synthesis of the pseudovitamin B_{12} nucleoside, 7-α-D-ribofuranosyladenine.[7]

The present paper deals with reactions of 7-(5-O-trityl-2,3-di-O-mesyl-α-D-arabinofuranosyl)hypoxanthine with ethanolic sodium ethoxide or sodium benzoate-dimethyl formamide (DMF) leading to 5′-O-trityl-3′,6-anhydro-7-α-D-arabinofuranosylhypoxanthine (VI) or 7-(5-O-trityl-2,3-dideoxy-2-oxo-α-D-pentofuranosyl)hypoxanthine (VIII), respectively. We wish first to report on the synthesis of the new purine cyclonucleoside (VI).

A blocked nucleoside (I) was prepared in reproducible yield (45%) by slight modifications of an earlier procedure.[8,9] Free nucleoside (II) was obtained by deblocking I according to a reported procedure.[8] Tritylation of 7-α-D-arabinofuranosylhypoxanthine (II) afforded the corresponding 5′-O-tritylated product in 46.5% yield (based on I), which in turn was mesylated by a conventional method to give 5′-O-trityl-2′,3′-O-dimesylnucleoside (IV) in 76.5% yield. Treatment of the latter with two equivalents of 0.1N ethanolic sodium ethoxide at refluxing temperature for 2 hr, followed by silica gel column chromatography gave a crystalline nucleoside (VI). Its ultraviolet (UV) maximum did not shift in either acidic and basic media. Infrared (IR) (KBr) showed the absence of amide (1690 cm^{-1}) and sulfonate (1275 cm^{-1}) groups which had been present in IV. Combustion values were compatible with $C_{29}H_{24}O_4N_4$, and in addition the latter composition was determined directly by high resolution mass spectrometry

1) Part XVI: Y. Mizuno, Y. Watanabe, K. Ikeda, and J.A. McCloskey, *Heterocycles*, (Sendai), **2**, 439 (1974), where a part of this work has been reported in a preliminary form.
2) *a) Kita-12, Nishi-6, Kitaku, Sapporo 060, Japan; b) Houston, Texas 77025, U.S.A.*
3) J.J. Fox and I. Wempen, *Advances in Carbohydrate Chem.*, **14**, 283 (1959).
4) M. Ikehara, *Accounts of Chemical Research*, **2**, 47 (1969).
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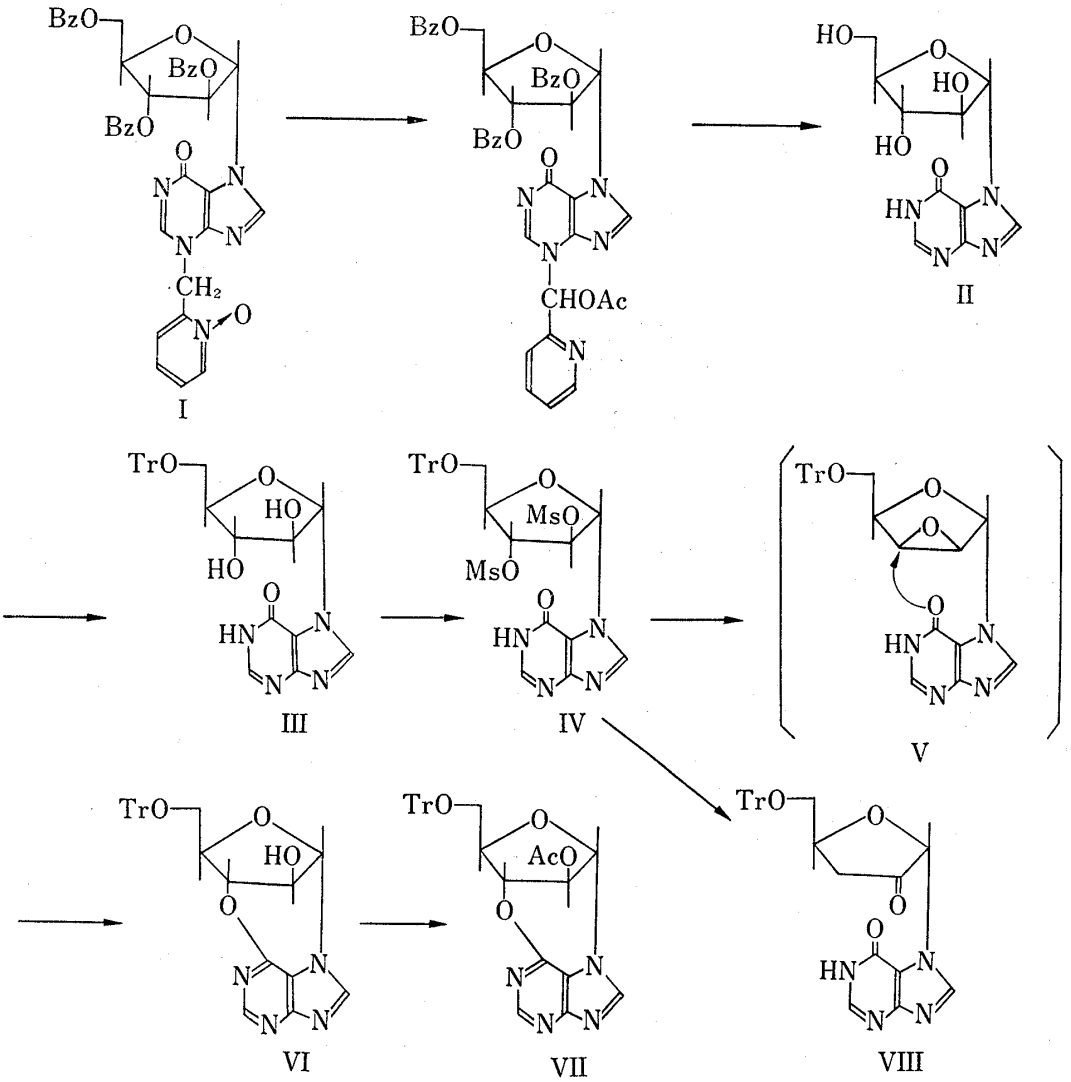

Fig. 1

(found: 492.1794; required; 492.1798). The mass spectrum of VI (Fig. 2) was compared to that of III and in addition to confirmation of the molecular weight gave evidence for the cyclonucleoside structure. In accordance with the expected difference between "normal" and cyclonucleosides,[10-12] VI produced a more intense molecular ion peak (*m/e* 492) than

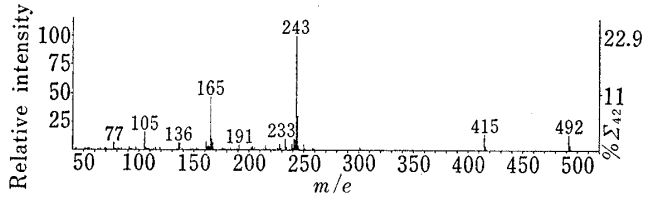

Fig. 2. Mass Spectrum of Compound VI

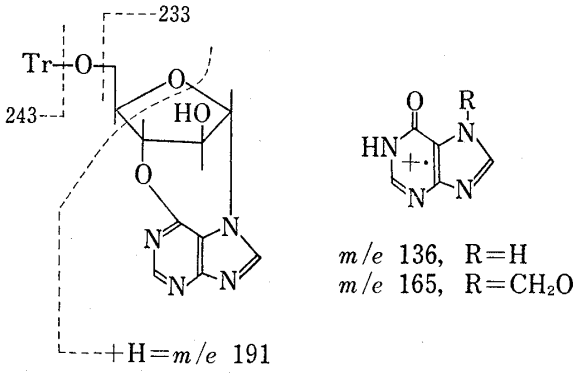

Fig. 3

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III, which produced none. Likewise, other base-containing fragment ions arising mainly from glycosidic bond cleavage were suppressed in VI: base+H[13] (*m/e* 136; 51% rel. int. in III) and sugar+H (absent in IV; *m/e* 374. 2% in III). The prominent ion *m/e* 165 was found at high resolving power to consist of 13% rel. int. base+CH_2O,[13] the remainder being $C_{13}H_9^+$ from trityl moiety. In III the base+CH_2O species was more abundant (*v* 23% rel. int.) as expected from its mechanism of formation.[14] In addition, the ion *m/e* 191 which is characteristic of cyclonucleosides[10–12,15–18] does not appear in the mass spectrum of III. Other significant ions in Fig. 2 are *m/e* 415 (M-phenyl), 77 (phenyl), 105 (C_6H_5CO), and as shown above, *m/e* 233 and 243. As first pointed out by Ikehara,[10] differentiation of O-2′ and O-3′ linked cyclonucleosides by mass spectrometry is difficult,[11] and so in the present case this choice was made on the basis of nuclear magnetic resonance (NMR) spectra of VI and the acetylated nucleoside (VII).

NMR of VI: aside from downfield signals due to 17 aromatic protons, signals due to seven remaining protons appeared at 6.50 (H-1′), 5.5—6.3 (2′-OH), 5.02 (H-3′), 4.68 (H-2′), 4.12 (H-4′), and 3.55 (H-5′). Double resonance experiments showed that H-4′ was spin-coupled with H-3′ and hence adjacent to H-3′ at 5.02. Irradiation at the frequency of H-3′ (5.02) collapsed the broad signal at 4.12 to a triplet. Irradiation at the frequency of H-1′ at 6.50 caused no change in signals at 5.02 (H-3′) and 4.68 (H-2′) because $J_{1'2'}=0$ Hz. NMR signals of the acetylation product (VII) appeared at 6.32 (s, 1H; H-1′), 5.50 (s, 1H; H-2′), 5.23 (d, $J_{3'4'}=2.0$ Hz, 3′-H). The acetylation caused a remarkable downfield shift (*ca.* 0.8 ppm) of the signal due to H-2′, while signals due to H-5′ and H-4′ remained completely unchanged. It is therefore reasonable to assume that the acetyl group was introduced at the 2′-hydroxyl group and hence the anhydro bond in VI and VII was formed between 3′-O- and C-6 of purine. Coupling constants calculated by the use of dihedral angles estimated from a molecular model of VI (Fig. 4) are also consistent with the observed values ($J_{1'2'}=0$ Hz, $\theta=90°$ and $J_{3'4'}=2.0$ Hz, $\theta=115°$).

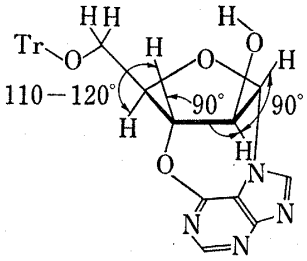

Fig. 4

This nucleoside (VI) was found to be unusual in being quite stable toward a variety of nucleophiles as well as aqueous acid and base. Alkaline hydrolysis with 1N KOH in ethanol failed to cleave both the anhydro- and N-glycosyl bond, and VI was recovered nearly quantitatively. Neither did hydrazine in ethanol cleave both bonds. Treatment of VI by refluxing in 1N hydrochloric acid gave rise to the detritylated product.

Presumably, VI was formed *via* 2′,3′-epoxide of the lyxo configuration. There are several precedents in which an epoxide was formed from vicinal *trans* di-O-mesylate of the carbohydrate.[19] Molecular model showed that C-3′ is more vulnerable than C-2′ to approach by the CO group at the 6-position of the purine base.

We next move on the synthesis of another new type of nucleoside (VIII). Treatment of IV with sodium benzoate in DMF (at 110—115° for 1 hr) and subsequent work-up (including preparative thin-layer chromatography (TLC)) afforded a crystalline product (VIII), albeit in low yield (12.7%). Combustion values of VIII were found to be compatible with $C_{29}H_{24}O_5N_4$. UV absorption maxima appeared at 255—259 mμ (broad, in EtOH), typical of 7-

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14) S.J. Shaw, D.M. Desiderio, K. Tsuboyama, and J.A. McCloskey, *J. Am. Chem. Soc.*, **92**, 2510 (1970).
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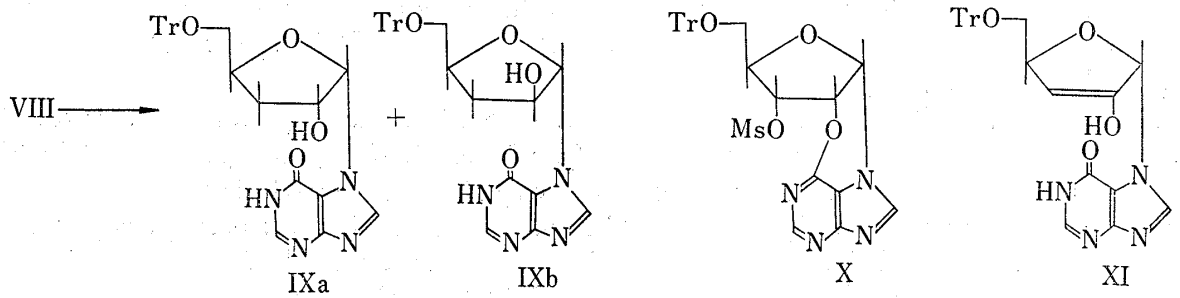
substituted hypoxanthine[20] and bathochromically shifted by *ca.* 5 mμ at pH 13. IR (KBr) spectrum of the product showed the presence of two carbonyl groups (1773 and 1692 cm^{-1} [21]). Reduction of VIII with sodium borohydride[22] was accompanied by the disappearance of one (1773 cm^{-1}) of the carbonyl groups in the IR spectrum. Since in the NMR spectrum (DMSO-d_6) one-proton signal due to anomeric proton (H-1′) appeared as a singlet the product was tentatively assigned the 2′-oxo structure (VIII) rather than the alternative (3′-oxo) structure. The data obtained from the decoupling experiment (NMR) and mass spectrometry substantiated this assignment.



The reaction leading to the formation of VIII is envisioned as proceeding *via* presumed intermediates 2′,6-O-anhydro-7-(5′-O-trityl-3′-O-mesyl-α-D-ribofuranosyl) hypoxanthine(X) and (XI). The proposed intermediates suggest that it might be possible to prepare the 2′,6-anhydro compound, provided subsequent cleavage of the anhydro bond could be blocked.

Experimental

General Procedure——UV spectra were determined on a Hitachi Spectrophotometer Model 3T and IR spectra were run on a Jasco Infrared Spectrophotometer DS-701. NMR spectra were determined on a Hitachi Spectrometer Model R-24. The chemical shifts were reported in parts per million downfield from tetramethylsilane as the internal standard. Symbols s, m, t, b, bs, and bd stand for singlet, multiplet, triplet, broad singlet, and broad doublet. Low resolution mass spectra were determined using a LKB 9000 instrument, under the following conditions: ionizing energy 70 eV, ion source temperature 250°, sample introduction by direct probe. Exact mass measurements were derived from photographically recorded high resolution mass spectra determined with a CEC 21-110B instrument. Silica gel TLC or column chromatography was performed using solvent A: $CHCl_3$–EtOH (25:1); solvent B: $CHCl_3$–EtOH (7:1); solvent C: $CHCl_3$–EtOH (4:1).

3-(2-Picolyl-1-oxide)-7-(2,3,5-tri-O-benzoyl-α-D-arabinofuranosyl)hypoxathine (I)——This blocked nucleoside was prepared by slight modification of an earlier procedure. A suspension of 3-(2-picolyl-1-oxide)-hypoxanthine (4 g, 16.4 mmoles) in nitromethane (200 ml) was azeotropically dried by distilling off 100 ml of the solvent. There was then added with vigorous stirring a mixture of 2,3,5-tri-O-benzoyl-D-arabinofuranosyl chloride (8.66 g, 18.0 mmole), calcium sulfate (8 g), and mercury cyanide (4.14 g, 16.4 mmole). The mixture was continuously stirred for 3 hr. The dark colored solution was cooled and filtered and the nsoluble material was washed with chloroform (200 ml). The combined filtrate and washing were successively washed with 30% KI solution (500 ml) and water (200 ml × 2). The organic layer was dried over Na_2SO_4 and filtered and concentrated to dryness *in vacuo*. A solution of the residue in chloroform (10 ml) was applied to a silica gel column (silica gel, 400 g). The column was washed with solvent A. The fractions having *Rf* 0.42 in TLC (solvent B) were pooled and concentrated to leave a colorless foam. Yield, 5.7 g (50.4%). UV λ_{max}^{EtOH} mμ: 230, 266; λ_{max}^{pH1} mμ: 230, 261; λ_{max}^{pH10}mμ: 265. NMR ($CDCl_3$): 8.63 (s, 1H, 8–H), 7.8—8.4 and 7.1—7.7 (m, 20H, aromatic protons including 2–H), 6.88 (d, $J_{1'2'}$=3.5 Hz, 1′–H), 6.33 (t, $J_{1'2'}$=3.5 Hz, $J_{2'3'}$=4 Hz, 2′–H), 5.90 (t, $J_{2'3'}$=4 Hz, $J_{3'4'}$=4 Hz, 3′–H), 5.54 (s, 2H, CH_2 of 2-picolyl), 5.18 (t, $J_{3'4'}$=4 Hz, $J_{4'5'}$=

20) L.F. Bergmann, M. Kleiner, Z. Neinman, and M. Rashi, *Israel J. Chem.*, **2**, 185 (1964).
21) Another is amide carbonyl.
22) From the reaction mixture, a pair of isomers (*Rf* 0.22 and 0.33, silica gel preparative TLC; solvent system $CHCl_3$–EtOH 7:1) were isolated in 1.4:1 molar ratio. The UV absorption spectra of each product (IXa and IXb) were the same as those of II. Other spectral data (NMR and IR) were also compatible with the structure assigned. Because of the minute amount of each sample, however, the configuration of the hydroxyl groups was not determined.

4.5 Hz, 4′–H), 4.71 (d, $J_{4'5'}$=4.5 Hz, 2H, 5′–CH_2). *Anal.* Calcd. for $C_{37}H_{29}O_9N_5 1/2H_2O$: C, 63.69; H, 4.33; N, 10.00. Found: C, 63.96; H, 4.23; N, 9.91.

7-(5-O-Trityl-α-D-arabinofuranosyl)hypoxanthine (III)——A solution of I (5.8 g, 8.4 mmole) in acetic anhydride (30 ml) was allowed to stand at 45° for 25 hr. Evaporation of the solvent left a colorless foam. The remaining acetic anhydride (or acetic acid) was almost completely removed by codistillation with ethanol (300 ml×3). The final residue was dissolved in 1 N methanolic sodium methoxide (12 ml). The solution was refluxed for 5 hr. The cooled solution was neutralized with Dowex 50W resin(H+form), and concentrated to dryness. The residue was dissolved in H_2O (150 ml). The aq. solution was treated three times with chloroform (150 ml×3). The aq. layer was separated and concentrated to dryness. Yield of II, 2.46 g. The free nucleoside was treated with trityl chloride (3.3 g, 11.8 mmole) in pyridine (50 ml) for 5 days at room temperature. Evaporation of the solvent and subsequent codistillation with ethanol left a faint colored foam which was washed with H_2O (50 ml) to remove pyridine hydrochloride. The insoluble material (III, crude) was collected by filtration, dissolved in chloroform (100 ml), and dried over Na_2SO_4. The salt was filtered off. The filtrate was concentrated to three-fourths and then applied to a silica gel column (silica gel, 60 g). The column was washed with $CHCl_3$–EtOH (10:1). Fractions containing III (*Rf* 0.33, TLC, solvent C) were pooled and concentrated to leave colorless foam. Crystallization from EtOH–$CHCl_3$ gave an analytical sample, mp 222—224°, yield, 2.0 g (46.5%, based on I). UV λ_{max}^{EtOH} mμ: 255—259; λ_{max}^{pH1} mμ: 255; λ_{max}^{pH13} mμ: 262.5. NMR (DMSO-d_6): 12.35 (b, 1H, NH), 8.41 (s, 1H, 8–H), 7.96 (s, 1H, 2–H), 7.1—7.6 (m, 15H, trityl), 6.29 (d, $J_{1'2'}$=4 Hz, 1′–H), 5.0—6.0 (b, 2H, 2′ and 3′–OH), 4.30—4.65 (b, 2H, 2′ and 3′–H), 4.13 (t, 1H, 4′–H), 3.30 (b, 2H, 5′–CH_2). IR (KBr): 1685 (amido). *Anal.* Calcd. for $C_{29}H_{26}O_5N_4 1/3H_2O$: C, 67.43; H, 5.14; N, 10.85. Found: C, 67.30; H, 5.11; N, 10.72. Mass Spectrum (10 most intenze peaks) *m/e*: 105 (16% relative intensity), 136 (51), 165 (72), 166 (19), 167 (19), 239 (15), 241 (23), 242 (24), 243 (100), 244 (46).

7-(5-O-Trityl-2,3-di-O-mesyl-α-D-arabinofuranosyl)hypoxanthine (IV)——A solution of III (520 mg, 1.02 mmole) in pyridine (10 ml) was treated for one day with a solution of mesyl chloride (260 mg, 2.27 mmole) in pyridine (2 ml) at 0° in a stoppered bottle. The solution was then allowed to return to ambient temperature and kept at the same temperature for another day. Evaporation of the solvent and subsequent codistillations with ethanol (10 ml×2) left colorless foam which was washed with H_2O (10 ml) to remove pyridine hydrochloride. The insoluble material was collected by filtration and then dissolved in chloroform (100 ml) containing ethanol (2 ml). The dried (over Na_2SO_4) solution was concentrated to dryness. The residue was dissolved in chloroform (50 ml) and the chloroform solution was concentrated to 10 ml. The solution was allowed to stand at room temperature until crystals deposited. The crystalline product was collected by filtration, yield, 370 mg. Recrystallization from $CHCl_3$–EtOH afforded an analytical sample, mp 185—187°, yield, 280 mg (41.2%). The combined mother liquor were concentrated to dryness and the residue was purified by silica gel column chromatography (silica gel, 20 g, solvent system A). Fractions containing IV (*Rf* 0.48, TLC, solvent B) were pooled and concentrated to dryness. Crystallization of the residue afforded further crop of IV, mp 185—187°, combined yield, 520 mg (76.5%). UV λ_{max}^{EtOH} mμ: 255—259; λ_{max}^{pH1} mμ: 256; λ_{max}^{pH13} mμ: 264. NMR (DMSO-d_6): 12.6 (b, 1H, NH), 8.61 (s, 1H, 8–H), 8.11 (s, 1H, 2–H), 7.40 (m, 15H, phenyl), 6.63 (d, $J_{1'2'}$=4.2 Hz, 1H, 1′–H), 6.08(t, $J_{1'2'}$=4.2, $J_{2'3'}$=4.5 Hz, 1H, 2′–H), 5.58(t, $J_{2'3'}$=4.5 Hz, $J_{3'4'}$=4.5 Hz, 1H, 3′–H), 4.95 (b, 1H, 4′–H), 3.38 (s, 2H, 5′–CH_2), 3.28 (s, 3H, mesyl), 3.19 (s, 3H, mesyl). *Anal.* Calcd. for $C_{31}H_{30}O_9N_4S_2$: C, 55.84; H, 4.53; N, 8.40; S, 9.62. Found: C, 55.56; H, 4.50; N, 8.46; S, 9.58.

3′,6-Anhydro-7-(5-O-trityl-α-D-arabinofuranosyl)hypoxanthine (VI)——To a solution of IV (500 mg, 0.75 mmole) in ethanol (50 ml) was added 0.1 N ethanolic sodium ethoxide (15 ml, 1.5 mmole). The resulting solution was refluxed for 2 hr. The cooled solution was neutralized with Dowex 50W resin (H^+ form). The resin was filtered off and the filtrate was concentrated to dryness. The residue was dissolved in chloroform (2 ml) and applied to a silica gel column (silica gel, 15 g). The column was washed with $CHCl_3$–EtOH (25:1). Fractions containing VI were pooled and concentrated to dryness (150 mg, 40.6%). Crystallization from aq. ethanol afforded an analytical sample, mp 218—220°. UV λ_{max}^{EtOH} mμ: 262; λ_{max}^{pH1} mμ: 262; λ_{max}^{pH13} mμ: 263—266. IR (KBr): 1610, 1565, 1095, 703; NMR ($CDCl_3$): 8.64 (s, 1H, 8–H); 8.32 (s, 1H, 2–H), 7.1—7.6 (m, 15H, phenyl), 6.50 (s, 1H, 1′–H), 5.5—6.3 (b, 1H, 2′–OH), 5.02 (d, $J_{3'4'}$=2 Hz, 3′–H), 4.68 (s, 1H, 2′–H), 4.12 (bs, 1H, 4′–H), 3.55 (bd, 2H, 5′–CH_2). *Anal.* Calcd. for $C_{29}H_{24}O_4N_4 1/3H_2O$: C, 69.86; H, 4.91; N, 11.24. Found: C, 69.91; H, 4.86; N, 10.99.

3′,6-Anhydro-7-(5-O-trityl-2-O-acetyl-α-D-arabinofuranosyl)hypoxanthine (VII)——To a solution of VI (120 mg, 0.24 mmole) in pyridine (5 ml) was added with stirring acetic anhydride (5 drops) at 0°. The solution was allowed to stand at room temperature for 12 hr. After work-up, the crude product obtained was purified by preparative TLC (solvent B, *Rf* 0.66). Yield, 40 mg. NMR ($CDCl_3$): 8.60 (s, 1H, 8–H), 8.30 (s, 1H, 2–H), 7.1—7.6 (m, 15H, phenyl), 6.32 (s, 1H, 1′–H), 5.23 (d, $J_{3'4'}$=2 Hz, 3′–H), 5.50 (s, 1H, 2′–H), 4.12 (bs, 1H, 4′–H), 3.55 (bd, 2H, 5′–CH_2), 2.04 (s, 3H, CH_3CO). UV spectra in EtOH were found to be the same as those of VI.

7-(5-O-Trityl-2,3-dideoxy-2-oxo-α-D-pentofuranosyl)hypoxanthine (VIII)——To a solution of IV (500 mg, 0.75 mmole) in DMF (100 ml) was added sodium benzoate (1.08 g, 7.5 mmoles). The solution was heated with stirring at 110° for 1 hr. The cooled, dark-colored solution was filtered. The insoluble material was washed with chloroform. The combined filtrate and washing were concentrated to dryness. The residue

was purified by preparative TLC (silica gel, $CHCl_3$–EtOH 10:1). After work-up, crystallization from $CHCl_3$–EtOH afforded an analytical sample, mp 207—208°, yield, 47 mg (12.7%). UV λ_{max}^{EtOH} mμ: 255—259; λ_{max}^{pH1} mμ: 255—257; λ_{max}^{pH13} mμ: 260—265. NMR (DMSO-d_6): 12.38 (b, 1H, NH), 8.5 (s, 1H, 8–H), 8.02 (b, 1H, 2–H), 7.1—7.6 (bs, 15H, phenyl), 6.41 (s, 1H, 1′–H), 4.76—5.20 (b, 1H, 4′–H), 3.31 (b, 2H, 5′–CH_2), 2.65—3.15 (m, 2H, 3′–CH_2). IR (KBr): 1773 (CO), 1692 (amido), 705 (phenyl), *Anal.* Calcd. for $C_{29}H_{24}O_4N_4$: C, 70.72; H, 4.91; N, 11.61. Found: C, 70.40; H, 4.88; N, 11.50. Mass Spectrum (10 most intense peaks) *m/e*: 77 (20% relative intensity), 105 (52), 136 (38), 165 (68), 166 (21), 167 (24), 241 (17), 243 (100), 244 (53), 279 (16).

Reduction of VIII with Sodium Borohydride——To a solution of VIII (40 mg, 0.081 mmole) in ethanol (30 ml) was added 0.1% sodium borohydride solution (3 ml). The resulting solution was magnetically stirred at room temperature for 2 hr. The reaction was quenched by addition of acetone (3 ml). Evaporation of the solvent left a colorless foam which was dissolved in chloroform (1 ml). The residue obtained by evaporation of the solvent was subjected to TLC (silica gel, solvent B). Two main bands were detected under a UV lamp. Each band was separately pooled and after the usual work-up, 22 mg (54.8%, *Rf* 0.30) and 15 mg (42.3%, *Rf* 0.22) were obtained. UV of each sample was the same as that of II. λ_{max}^{EtOH} mμ: 255—259; λ_{max}^{pH1} mμ: 255—257; λ_{max}^{pH13} mμ: 260—265. IR (KBr) showed the absence of absorption band at 1773 cm^{-1} which had been present in VIII.

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