[Chem. Pharm. Bull. 21(5)1143—1146(1973)]

UDC 547.963.32.04

Synthesis of 2′- and 3′-Deoxyinosines

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(Received October 30, 1972)

Research[2] on the purine cyclonucleosides having 8,2′-, 8,3′- or 8,5′-anhydro linkage has progressed rapidly in recent years, and the purine cyclonucleosides as well as the pyrimidine cyclonucleosides have been shown to be useful intermediates in the synthesis of deoxyribonucleosides and biologically active nucleosides. For instance, naturally occurring purine deoxyribonucleosides, such as 2′-deoxyadenosine[3] and 2′-deoxyguanosine[4] have been synthesized *via* 8,2′-S-cyclonucleoside. More recently, 9-β-D-arabinofuranosyladenine[5] and 3′-deoxyadenosine[3b] (the antibiotic Cordycepin) having antitumor activity have been prepared from adenosine.

It has been shown earlier by Dr. Yamada[6] in these laboratories that the direct thiation of suitably protected purine nucleoside with sulfur in N,N-dimethylformamide leads to 8-mercaptopurine nucleoside derivative. By this method, 8-mercaptoinosine (I) was readily obtained in good yield from inosine. The present work describes the preparation of 2′-deoxyinosine (IX) and 3′-deoxyinosine (X) as one of the best utilization of I.

According to the improved method[7] originated from that of Hampton,[8] I was treated with acetone in the presence of 2,2-dimethoxypropane and dry hydrochloride acid, but the expected 2′,3′-O-isopropylidene-8-mercaptoinosine (II) was formed in very poor yield. A successful preparation of II was achieved by reaction with acetone in the presence of 2,2-dimethoxypropane and perchloric acid.[9] The yield of II was 81%. Acetylation of II with acetic anhydride in pyridine afforded 5′-O-acetyl derivative (III), which in turn was deblocked in 85% formic acid to yield 5′-O-acetyl-8-mercaptoinosine (IV). Compound (IV) reacted with *p*-toluenesulfonyl chloride in pyridine to give a mixture of 2′-(V) and 3′-O-tosyl derivative (VI). Difficulty was encountered in the separation of both compounds but, after ammoniacal treatment, the mixture was satisfactorily separated to VI and 8,2′-anhydro-8-mercapto-9-β-D-arabinofuranosyladenine (VII)[10]: on ammoniacal treatment of V and VI at 0°, the former was easily cyclized to 2′,8-cyclonucleoside (VII), while VI remained unchanged. Then, VI was extracted with chloroform and V was readly isolated from water layer. In cyclization of VI, a more drastic condition was required. When VI was allowed to react with ammonia in an autoclave at 80° for 5 hrs, 8,3′-anhydro-8-mercapto-9-β-D-xylofuranosyladenine (VIII)

1) Location: *a*) *Suzuki-cho, Kawasaki*; *b*) *Toneyama, Toyonaka, Osaka.*
2) M. Ikehara, *Accounts of Chemical Research*, **2**, 47 (1969).
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8) A. Hampton, *J. Am. Chem. Soc.*, **83**, 3643 (1961).
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10) This compound[11] was also prepared by deamination of 8,2′-anhydro-8-mercapto-9-β-D-arabinofuranosyladenine with barium nitrite in acetic acid.
11) M. Ikehara and M. Muraoka, *Chem. Pharm. Bull.* (Tokyo), **20**, 550 (1972).

was obtained in a yield of 5%. On the other hand, sodium ethoxide in place of ammonia gave VIII in 40% yield.

Support for the structure of VII and VIII was given by the nuclear magnetic resonance (NMR) spectra in DMSO-d_6. Compound (VII) showed that H_1' proton had a doublet signal at 6.55 ppm and coupling constant with $J_{1'-2'}$ 6.5 Hz. This is reasonably assigned as 8,2'-cyclonucleoside.[2] The structure of VII was also supported by desulfurization with Raney nickel to give IX, whose chemical and physical properties were identical with those of an

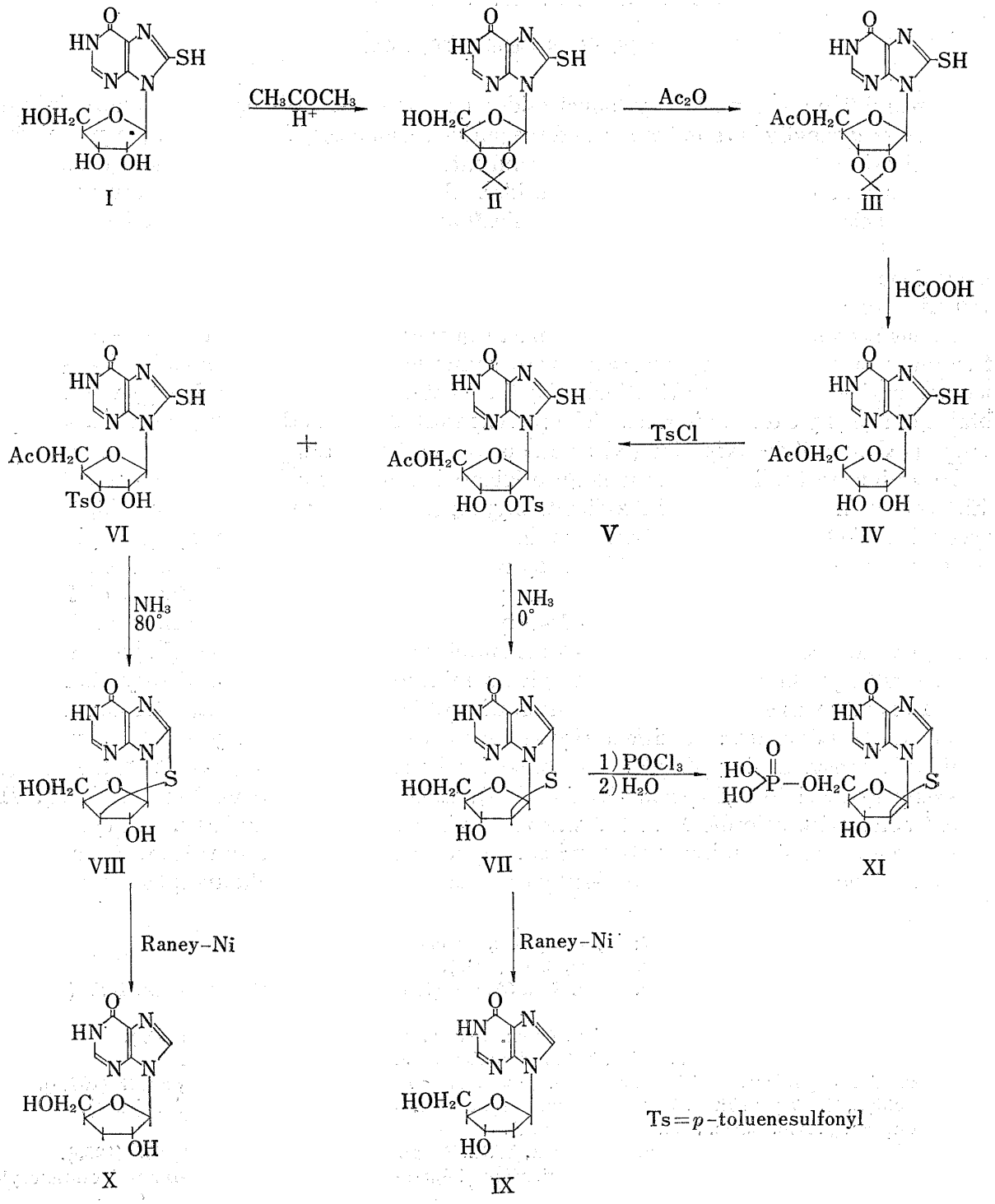

Chart 1

authentic sample. In the case of VIII, anomeric protons appeared at 5.80 ppm and $J_{1'-2'}$ was 0 Hz, indicating that this compound should be 8,3'-cyclonucleoside. Also, desulfurization with Raney nickel gave the expected X.

Our interest[12] in flavoring activity-structure relationship has led to undertake the preparation of 8,2'-anhydro-8-mercapto-9-β-D-arabinofuranosylhypoxanthine-5'-phosphate (XI) as an analog of inosine-5'-phosphate. Compound (VII) was phosphorylated with phosphoryl chloride in trimethylphosphate to yield XI accompanied with a small amount of 3',5'-diphosphate of VII. The compound (XI) thus obtained was found to be tasteless and it is interesting to note that a remarkable similarity was observed in no flavoring activity of XI and 8-substituted 6-hydroxypurine ribotide such as 2-ethylthio-8-methylinosine-5'-phosphate[13] and 8-hydroxyguanosine-5'-phosphate.[14]

Experimental[15]

2',3'-O-Isopropylidene-8-mercaptoinosine (II)——To a mixture of acetone (600 ml), 2,2-dimethoxypropane (8 ml), and 70% perchloric acid (8 ml), 8-mercaptoinosine[6] (I) (3 g, 0.01 mole) was added with stirring at room temperature. Within 10 min, the mixture became clear. After 2 hr, *ca.* 30 ml of pyridine was added in order to neutralize the above solution. At this point, the yellow color disappeaed to give colorless solution. On evaporation of the solvent *in vacuo*, a crystalline compound precipitated gradually. The mixture was diluted with 10% of sodium bicarbonate, concentrated to about 15 ml, and extracted with chloroform (2×35 ml) to remove pyridine and 2,2-dimethoxypropane. The pH of the aqueous layer was adjusted to 4.5 with acetic acid. On cooling, the crystalline compound precipitated, which was collected by filtration, dried at room temperature, and recrystallized from ethanol to afford 2.75 g (81%) of the product; mp 198—200° (decomp.); *Rf*: 0.80 (solvent A); 0.79 (solvent B); UV λ_{max}^{pH1} mμ(ε): 216 (12800), 294 (19900); λ_{max}^{pH7}mμ(ε): 216 (9700), 293.5 (15800); λ_{max}^{pH13} mμ(ε): 291.5 (18800). *Anal.* Calcd. for $C_{13}H_{16}O_5N_4S$–1/2H_2O: C, 44.69; H, 4.90; N, 16.04. Found: C, 44.33; H, 5.21; N, 15.67.

2',3'-O-Isopropylidene-5'-O-acetyl-8-mercaptoinosine (III)——Compound II (13.3 g) was dissolved in a mixture of pyridine (133 ml) and acetic anhydride (107 ml), and the solution was allowed to stand at room temperature overnight. After the solvent was removed *in vacuo*, ethanol was added and the mixture was concentrated. The treatment was repeated several times to decompose acetic anhydride completely. The resulting precipitate was filtered and recrystallized from aqueous ethanol. The product was dried and weighed 12.6 g (84%). mp 164—166° (decomp.); *Rf*: 0.89 (solvent A); 0.90 (solvent B); UV λ_{max}^{pH1} mμ(ε): 216 (12600), 294 (21300); λ_{max}^{pH7} mμ(ε): 216 (12200), 293.5 (22700); λ_{max}^{pH13} mμ(ε): 291.5 (18100). *Anal.* Calcd. for $C_{15}H_{18}O_6N_4S$: C, 47.12; H, 4.75; N, 14.66. Found: C, 47.29; H, 4.94; N, 14.72.

5'-O-Acetyl-8-mercaptoinosine (IV)——To a stirred mixture of water (180 ml) and 85% of formic acid (300 ml), compound III (15 g) was added. The clear solution was left at room temperature for 3 hr and concentrated *in vacuo* at 35°. Ethanol was added and the solvent was removed *in vacuo*. This procedure was repeated several times until the smell of formic acid disappeared. The residue was crystallized from water to give a needless of 12.3 g (91%). mp 208—210° (decomp.); *Rf* 0.68 (solvent A); 0.54 (solvent B); UV λ_{max}^{pH1} mμ(ε): 216 (13300), 294 (22300); λ_{max}^{pH7} mμ(ε): 216 (10400), 293.5 (17500); λ_{max}^{pH13} mμ(ε): 291.5 (21000). *Anal.* Calcd. for $C_{12}H_{14}O_6N_4S$: C, 42.11; H, 4.12; N, 16.37. Found: C, 42.21; H, 4.28; N, 16.14.

8,2'-Anhydro-8-mercapto-9-β-D-arabinofuranosylhypoxanthine (VII)——To a stirred solution of compound IV (6 g, 17.53 mmoles) in pyridine (150 ml) being cooled at 0°, *p*-toluenesulfonyl chloride (7.14 g, 37.5 mmoles) was added. The solution was stored in a refrigerator for 2 days. Water (40 ml) was added with cooling and, after 20 min, aqueous sodium bicarbonate solution was added to neutralize the solution.

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13) S. Suzaki, A. Yamazaki, and I. Kumashiro, Abstracts of Papers, Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, 1968, p. 155.

14) M. Ikehara, I. Tazawa, and T. Fukui, *Chem. Pharm. Bull.* (Tokyo), **17**, 1019 (1969).

15) All melting points are uncorrected. Ultraviolet absorption spectra were taken with a Hitachi EPS-2 automatic recording spectrophotometer. The NMR spectra were measured with a Varian A-60 using tetramethylsilane as an internal standard. Paper chromatography was carried out on Toyo Filter Paper No. 51 by the ascending technique. Solvent systems were A, *n*-PrOH–NH_3 (28%)–H_2O (20: 12: 3, v/v); B, *n*-BuOH–AcOH–H_2O (4: 1: 1, v/v); C, iso AmOH–AcOH–5%·Na_2HPO_4 (3: 3: 2, v/v).

The resulting 2′- and 3′-tosyl derivatives were extracted 3 times with 250-ml portions of chloroform. The combined chloroform extracts were dried over anhydrous sodium sulfate and the solvent was removed *in vacuo* to give a gummy product, which was dissolved in 100 ml of methanol saturated with ammonia at 0°. The solution was kept in a refrigerator overnight. After removal of the solvent *in vacuo*, 300 ml of water was added with stirring. The insoluble 3′-tosyl derivative was filtered and used in next reaction without purification. Concentration of the filtrate *in vacuo* gave a crystalline product. The product was recrystallized from water with charcoal and dried *in vacuo* over phosphorus pentoxide to give a pure sample. Yield was 0.89 g (18% based on IV); mp 225—226° (decomp.); $[\alpha]_D^{30}$ −152° (c=1.00, pyridine); *Rf* 0.60 (solvent A); 0.28 (solvent B); UV λ_{max}^{pH1} mμ(ε): 267 (15000); λ_{max}^{pH7} mμ(ε): 266.5 (16000); λ_{max}^{pH13} mμ(ε): 272 (16600). *Anal.* Calcd. for $C_{10}H_{10}O_4N_4S \cdot 1/3H_2O$: C, 41.61; H, 3.70; N, 19.44. Found: C, 42.07; H, 3.99; N, 19.70.

8,3′-Anhydro-8-mercapto-9-β-D-xylofuranosylhypoxanthine (VIII)——A gummy 3′-tosyl derivative, described for the synthesis of 8,2′-anhydro derivative VII, was added to 50 ml of methanol saturated with ammonia at 0°, and the mixture was heated in an autoclave at 80° for 5 hr. The solvent was removed *in vacuo*, an insoluble material was filtered, and the filtrate was evaporated to give a crystalline precipitate. A pure compound was obtained by recrystallization from water. Yield was 0.25 g (5.1% based on IV). mp>300° *Rf* 0.66 (solvent A); 0.35 (solvent B); UV λ_{max}^{pH1} mμ(ε): 272.5 (17800); λ_{max}^{pH7} mμ(ε): 271 (17600); λ_{max}^{pH13} mμ(ε): 282 (18800). *Anal.* Calcd. for $C_{10}H_{10}O_4N_4S$: C, 42.56; H, 3.57; N, 19.85. Found: C, 43.06; H, 3.57; N, 20.18.

2′-Deoxyinosine (IX)——To a solution of VII (1.2 g) in 150 ml of water was added Raney nickel[16] (8 ml) with stirring. The solution was heated to reflux for 6 hr. The nickel was filtered off and washed with a small amount of boiling water. The filtrate and washing were combined and evaporated *in vacuo*. The resulting precipitate was crystallized from water to give 0.64 g (60%) of a product, which was identical with an authentic sample in all respects; mp>250°; $[\alpha]_D^{30}$ −21° (c=1.00, H_2O); *Rf* 0.67 (solvent A); 0.33 (solvent B). *Anal.* Calcd. for $C_{10}H_{12}O_4N_4$: C, 47.62; H, 4.80; N, 22.22. Found: C, 47.61; H, 4.87; N, 22.27.

3′-Deoxyinosine (X)——Compound (VIII) (600 mg) was dissolved in 250 ml of a boiling water. Raney nickel[16] (7 ml) was added, and the mixture was heated to reflux for 3 hr. Raney nickel was filtered and the filtrate was concentrated to dryness. The residue was triturated in a small amount of water, collected by filtration, and crystallized from a solution of water and ethanol (1 : 4) to give 215 mg (40%) of a pure sample. The sample was dried at 60° for 6 hr. mp 197—199°, $[\alpha]_D^{30}$ −87.9° (c=1.00, pyridine); *Rf* 0.68 (solvent A); 0.35 (solvent B); UV λ_{max}^{pH1} mμ(ε): 251 (10900); λ_{max}^{pH7} mμ(ε): 250.5 (11300); λ_{max}^{pH13} mμ(ε): 255.5 (13300). *Anal.* Calcd. for $C_{10}H_{12}O_4N_4 \cdot 2/3H_2O$: C, 45.45; H, 5.09; N, 21.20. Found: C, 44.98; H, 5.26; N, 21.37.

8,2′-Anhydro-8-mercapto-9-β-D-arabinofuranosylhypoxanthine-5′-phosphate (XI)——To a stirred solution of phosphoryl chloride (1.48 ml, 16 mmoles) in 8 ml of trimethyl phosphate[17] cooled to −10°, the compound VII (1.12 g, 4 mmoles) and water (0.11 ml, 6.08 mmoles) were added. The solution was stirred at 0——5° for 3 hr. The mixture was then poured into 40 ml of ice water and the pH was adjusted to 2 with 6N sodium hydroxide. On paper chromatogram (solvent C), two spots were detected besides the starting material (*Rf* 0.45), one of which was mainly the desired 5′-phosphate (XI) (*Rf* 0.31) and the other was assumed to be 3′,5′-diphosphate (*Rf* 0.17). The pH was again adjusted to 2 and the solution was passed through a column (2.5×60 cm) of a decolorizing resin.[18] When the column was washed with water, 3′,5′-diphosphate only was absorbed and VIII and XI were eluted. After the eluate was concentrated to 20 ml, the solution was passed through a column of Dowex 1-X1 ($HCOO^-$ form, 80 ml). VIII was eluted with water and XI with 0.1N formic acid. An aliquot of the latter eluate showed one spot on a paper chromatogram in solvent C. The eluate was then concentrated *in vacuo* at 30°. Aqueous ethanol was added, and the solvent was evaporated *in vacuo*. This procedure was repeated several times until the smell of formic acid disappeared. The residue was dissolved in water (10 ml) and the pH of the solution was adjusted to 8 with 6N sodium hydroxide. After the solution was heated with charcoal, the same volume of ethanol was added to give a precipitate, which was dissolved by heating. The solution was allowed to stand at room temperature, giving crystals. The sample was dried at 60° over phosphorus pentoxide for 6 hr. Yield was 148 mg (8.5%). mp 119—121°; *Rf* 0.28 (solvent A); 0.03 (solvent B); 0.30 (solvent C); the migrating distance in paper electrophoresis (10% acetic acid buffer, 800 V, 2 hr): 7.9 cm; UV λ_{max}^{pH1} mμ(ε): 267 (14100); λ_{max}^{pH7} mμ(ε): 275 (10300); λ_{max}^{pH13} mμ(ε): 269—272 (15700). *Anal.* Calcd. for $C_{10}H_9O_7N_4SPNa_2 \cdot 3H_2O$: C, 26.09; H, 3.28; N, 12.17. Found: C, 26.56; H, 2.96; N, 11.68.

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