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Nucleosides and Nucleotides. XIII. Synthesis of Thiopurine Nucleosides from Adenosine and Guanosine Derivatives by the Sulfhydrolysis¹⁾

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Synthesis of thiopurine nucleosides (and nucleotides) by the application of amino-thiono exchange reaction (sulfhydrolysis) was described.

1-Methyl-6-thioinosine and 2',3'-O-isopropylidene-5'-deoxy-3,5'-cyclo-2-thioxanthosine were obtained from 1-methyladenosine and cycloguanosine, respectively. Sulfhydrolysis of a 3,5'-cycloadenosine gave a 5-amino-N⁵,5'-cycloimidazole-4-thiocarboxamide riboside in high yield.

Synthesis of 6-thioinosine and 6-thioxanthosine by sulfhydrolysis of N⁶-methoxyadenosine and crotonoside, respectively, was also performed.

The synthetic and biological studies on sulfur-containing purine nucleosides have been promoted after the findings that 6-thiopurine showed a significant antitumor activity.³⁾

The first synthesis of thiopurine nucleosides, 6-thioinosine and 6-thioguanosine, described by Fox and his coworkers⁴⁾ was accomplished by the thiation of protected inosine and guanosine with phosphorus pentasulfide, in which the amide function in aglycon moiety was converted to the thioamide. Similar thiation procedure has successfully been applied to pyrimidine nucleosides such as uridine and thymidine.⁵⁾

We have described in our previous reports⁶⁾ that the treatment of cytidine and its analogs with liquid hydrogen sulfide in aqueous pyridine gave 4-thiouridine and its analogs by a facile replacement reaction (sulfhydrolysis) of the amino group with thiono group in cytosine moiety.

The present paper describes the extension of this sulfhydrolysis reaction to aminopurine nucleosides. As will be described in the later part attempts of amino-thiono exchange reaction with adenosine (VIIa) and guanosine met with little success. However, the ring-N-alkylated adenosines underwent smooth sulfhydrolysis.

1-Methyladenosine hydroiodide⁷⁾ (I) was treated in a solution of liquid hydrogen sulfide-pyridine-water at 60° for 46 hr. After removal of H₂S, solvent and elemental sulfur, ultra-violet (UV) absorption measurement of the residue revealed that the displacement reaction proceeded quantitatively to afford 1-methyl-6-thioinosine (II). The structure was identified on the basis of elemental analysis and UV spectra. In alkaline solution the spectral change corresponding to the ring opening reaction was observed from which 1-β-D-ribofuranosyl-5-amino-N-methylimidazole-4-thiocarboxamide (III) was isolated as has been reported in the case of 1-benzyl-6-thioinosine.⁸⁾

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The sulfhydrolysis of 2',3'-*O*-isopropylidene-5'-deoxy-N³,5'-cycloadenosine tosylate⁹⁾ (IV), an N³-alkylated adenosine derivative, at 50° for 6 hr afforded the thionucleoside derivative in good yield. The UV spectrum of the product, however, was somewhat similar to that of III rather than that of 3-methyl-6-thiohypoxanthine.¹⁰⁾ Furthermore, the treatment of the product with mild alkaline solution afforded a compound which revealed very close UV spectrum to that of III. On the basis of its UV spectral behavior together with elemental analysis and mass spectrum (*m/e* 324, molecular peak) the product from the cycloadenosine (IV) was identified as 1-(2,3-*O*-isopropylidene-5'-deoxy-β-D-ribofuranosyl)-5-formamide-N⁵,5'-cycloimidazole-4-thiocarboxamide (V). The spectral change in a mild alkaline solution may be due to the loss of formyl group to give 1-(2,3-*O*-isopropylidene-5'-deoxy-β-D-ribofuranosyl)-5-amino-N⁵,5'-cycloimidazole-4-thiocarboxamide (VI).

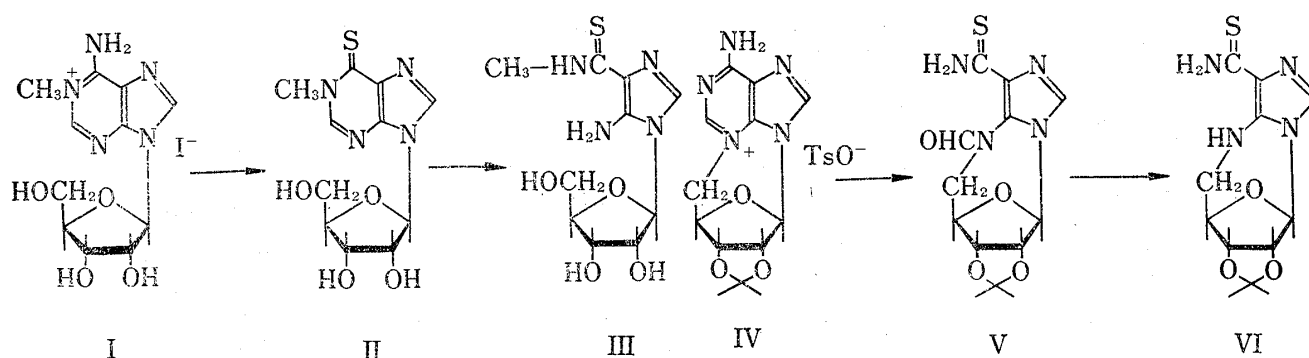


Chart 1

It can be assumed that methoxyamino group is better leaving group than the amino group in 6-substituted purine nucleosides since the methoxy group has electron attracting character. Therefore, N⁶-methoxyadenosine (Xa) was synthesized from adenosine (I) following the procedure reported in the synthesis of N⁶-methoxyadenine.¹¹⁾

Treatment of adenosine-1-oxide (VIIIa)¹²⁾ with methyl iodide at room temperature for 4 hr afforded N¹-methoxyadenosine hydroiodide (IXa) in 89% yield. After removal of hydroiodide by the use of ion exchange resin, a solution of N¹-methoxyadenosine in water was refluxed for 3 hr to form N⁶-methoxyadenosine¹³⁾ (Xa) which was identified on the basis of the elemental analysis, UV spectra and mass spectra.

As expected, sulfhydrolysis of Xa at 60° for 45 hr afforded 6-thioinosine (XIa) in high yield.

It is to be noted that the synthetic method of XIa described above is excellent since this method should be successfully extended for the synthesis of various 6-thioinosine nucleotides. In fact, 6-thioinosine 5'-phosphate¹⁴⁾ (XIb) was synthesized from adenosine 5'-phosphate (Ia) via N⁶-methoxyadenosine 5'-phosphate (Xb) in 30% overall yield.¹⁵⁾

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The amino-thiono exchange reaction was able to apply successfully to the derivative of guanosine.

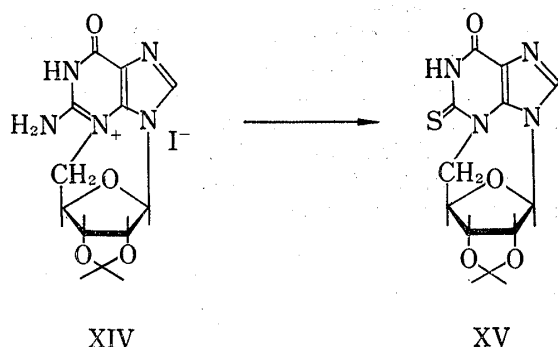


Chart 3

Treatment of 2',3'-O-isopropylidene-5'-deoxy-N³,5'-cycloguanosine iodide²⁰⁾ (XIV) with H₂S in pyridine-water at 50° for 168 hr afforded a thionucleoside (70%) which was identified as 2',3'-O-isopropylidene-5'-deoxy-N³,5'-cyclo-2-thioxanthosine (XV) on the basis of UV spectra ($\lambda_{\text{max}}^{\text{H}_2\text{O}}$ nm: 234, 294) and elemental analysis.

2-Amino group of guanosine and 1-methylguanosine were inactive in sulphydrolysis at 60° for 60–70 hr and starting material was recovered. The difference of the reactivity of sulphydrolysis of 2-amino group between 1- and 3-alkylated guanosines may be a reflection of tautomeric difference in guanine ring. Similar results are obtained in the sulphydrolysis of ring-N-alkylated isocytosines.²¹⁾

In conclusion, the present method for the preparation of thiopurine nucleosides (nucleotides) is especially effective for the synthesis of N-alkylated thiopurine nucleosides which are not readily accessible by the direct alkylation of thiopurine nucleosides.

Experimental

1-Methyl-6-thioinosine (II)—To a frozen solution of 1-methyladenosine hydroiodide⁷⁾ (I) (1 g) in 7 ml of H₂O at –70° in a stainless steel container was added liquid hydrogen sulfide (15 ml)–pyridine (5 ml) solution and sealed. After the reaction mixture was kept at 60° for 65 hr H₂S was vaporized, the solvent was evaporated *in vacuo* and the residue was dissolved in water. The aqueous solution was applied to a column of DEAE-cellulose (column size; 2.7 × 16 cm, bicarbonate form) and eluted with H₂O. The eluates containing III were collected, concentrated to a syrup which was redissolved with a small amount of H₂O and evaporated. The residue was dissolved in a minimum amount of H₂O and kept to stand for overnight in a refrigerator. The separated colorless crystals were collected by filtration, washed with ice-water and dried to give 415 mg of II (57%), mp 217–218°. The more crystals (100 mg) were obtained from the mother liquor. *Anal.* Calcd. for C₁₁H₁₄O₄N₄S: C, 44.30; H, 4.70; N, 18.79; S, 10.67. Found: C, 44.39; H, 4.88; N, 18.74; S, 10.67. UV $\lambda_{\text{max}}^{\text{H}_2\text{O}}$ nm: 230, 320.

1-β-D-Ribofuranosyl-5-amino-N-methylimidazole-4-thiocarboxamide (III)—The solution of 1-methyl-6-thioinosine (II) (200 mg) in 10 ml of EtOH, 1 ml of H₂O and 1.2 ml of 1 N –NaOH was refluxed for 2 hr. The UV spectra changed to $\lambda_{\text{max}}^{\text{H}_2\text{O}, 0.1\text{N-NaOH}}$ nm: 214, 268, 320; $\lambda_{\text{max}}^{0.1\text{N-HCl}}$ nm: 278, 314. After neutralization of the solution with Dowex-50 (H⁺) and filtered, the filtrate was concentrated to dryness. The residue was taken up in a small amount of hot EtOH and kept at room temperature to afford yellowish crystals which was collected by filtration and washed with cold EtOH and dried to give 62 mg of III. From the mother liquor 50 mg of brownish crystals were obtained, mp 174–175°. *Anal.* Calcd. for C₁₀H₁₆O₄N₄S: C, 41.65; H, 5.59; N, 19.43; S, 11.12. Found: C, 41.53; H, 5.61; N, 19.17; S, 10.87.

1-(2,3-O-Isopropylidene-5-deoxy-β-D-ribofuranosyl)-5-formamide-N⁵,5'-cycloimidazole-4-thiocarboxamide (V)—To a frozen solution of cycloadenosine (IV) (200 mg) in H₂O (4 ml) and pyridine (1 ml) at –70° in a stainless steel container was added the liquid H₂S (15 ml)–pyridine (5 ml) solution, sealed and kept at 50° for 6 hr. After H₂S was vaporized and the solvent was removed *in vacuo* the residue was dissolved in hot EtOH–H₂O and filtered. This procedure was repeated twice and the residue was crystallized from EtOH–acetone to afford 89 mg of V (64%), mp 206° (sinter), 252° (decomp.). Mass Spectrum *m/e*: 324 (M⁺). *Anal.* Calcd. for C₁₃H₁₆O₄N₄S: C, 48.15; H, 4.97; N, 17.28; S, 9.86. Found: C, 47.95; H, 5.05; N, 17.00; S, 9.82. UV $\lambda_{\text{max}}^{\text{H}_2\text{O}, 0.01\text{N-HCl}}$ nm: 269, 310; $\lambda_{\text{max}}^{0.01\text{N-NaOH}}$ nm: 275, 328, 370 (sh).

1-(2,3-O-Isopropylidene-5-deoxy-β-D-ribofuranosyl)-5-amino-N⁵,5'-cycloimidazole-4-thiocarboxamide (VI)—A solution of V (100 mg) in pyridine (10 ml) and 50% EtOH–H₂O (20 ml) was refluxed for 5.5 hr.

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After the solvent was removed *in vacuo* the residue was dissolved in hot water and the solvent was evaporated to dryness. The residue was crystallized from water to afford 56 mg of VI (61%), mp 200.5° (sinter), 236.5—237.3°. *Anal.* Calcd. for $C_{12}H_{16}O_5N_4S$: C, 48.64; H, 5.41; N, 18.92; S, 10.68. Found: C, 48.55; H, 5.43; N, 19.06; S, 10.68. UV $\lambda_{\max}^{0.01N-HCl}$ nm: 280, 324; $\lambda_{\max}^{0.01N-NaOH}$ nm: 276, 330.

1-Methoxyadenosine Hydroiodide (IXa)—To a suspension of adenosine-1-oxide (VIIIa) (850 mg) in dimethylformamide (50 ml) was added 1 ml of methyl iodide and stirred for 4 hr at room temperature. The solvent was evaporated *in vacuo* below 40° and the residue was dissolved in a small amount of EtOH (*ca.* 5 ml) without heating followed by addition of ether (100 ml) to precipitate the product. After standing for several hours at room temperature the product was collected by filtration and dried to afford 1.13 g (89%) of IXa. NMR (D_2O): 3.87 (1H, s, $H_{5'a}$), 4.02 (1H, s, $H_{5'b}$), 4.32 (3H, s, CH_3), 6.17 (1H, d, $J=5$ Hz, H_1'), 8.61 (1H, s, H_8), 8.97 (1H, s, H_2). UV $\lambda_{\max}^{H_2O}$ nm: 258; $\lambda_{\max}^{0.01N-HCl}$ nm: 258; $\lambda_{\max}^{0.01N-NaOH}$ nm: 256, 264(sh), 300(sh). This was used without further purification.

6-Methoxyadenosine (Xa)—1-Methoxyadenosine hydroiodide ((IXa) (500 mg) was dissolved in water and the solution was applied to a column of DEAE-cellulose (10 ml, bicarbonate form). The elution was performed with H_2O . Fractions exhibiting UV absorption corresponding to that of 1-methoxyadenosine were combined and refluxed for 3 hr. After cooling, the reaction mixture was concentrated to dryness and the residue was crystallized from EtOH (if necessary, decolorized with active carbon) to afford Xa (211 mg). Analytical sample was obtained after recrystallization from EtOH- H_2O , mp 194—195°. *Anal.* Calcd. for $C_{11}H_{15}O_5N_5 \cdot 1/3H_2O$: C, 43.53; H, 5.17; N, 23.08. Found: C, 43.31; H, 5.34; N, 23.17. Mass Spectrum *m/e*: 297 (M^+), 267 ($M-CH_3O+1^+$), 208 ($M-89^+$). UV $\lambda_{\max}^{H_2O}$ nm: 268; $\lambda_{\max}^{0.01N-HCl}$ nm: 266; $\lambda_{\max}^{0.01N-NaOH}$ nm: 282.

6-Thioinosine (XIa)—To a solution of Xa (200 mg) in H_2O (5 ml) and pyridine (1 ml) was added a solution of liquid H_2S (10 ml)—pyridine (5 ml) in a stainless steel container and kept at 50° for 57 hr. After the solvent was evaporated *in vacuo* the residue was dissolved and insoluble material was removed by the filtration. This procedure was repeated twice. The final solution was concentrated and the residue was crystallized from water affording XIa (150 mg) (77%), mp 205.5—209.5°. *Anal.* Calcd. for $C_{10}H_{12}O_4N_4S$: C, 42.25; H, 4.26; N, 19.71; S, 11.21. Found: C, 41.98; H, 4.35; N, 19.44; S, 11.04. UV $\lambda_{\max}^{H_2O}$ nm: 321.

6-Thioinosine 5'-Phosphate (Ib) from Adenosine 5'-Phosphate (Ib)—To a suspension of Ib (694 mg, 2 mmole) in H_2O (20 ml) was added 5 N-NaOH to adjust pH 7. After the solution was cooled to 1—2° 0.2 M-perphthalic acid-ether solution (40 ml) was added and stirred vigorously while keeping pH at 7 at 0—1°. After 3 hr, additional perphthalic acid-ether solution (10 ml) was added and the mixture was kept for 14 hr with vigorous stirring. The reaction mixture was adjusted to pH 1.0 and subjected with ether extraction. After the aqueous layer was decolorized with active carbon the mixture was neutralized and concentrated *in vacuo* to afford 62500 O.D. unit (at 232 nm) of the N-oxide (VIIIb).²² The residue was dissolved in H_2O (10 ml) and to this was added CH_3I (0.12 ml, 2 mmole) and $AgClO_4$ (410 mg, 2 mmole). After the mixture was stirred at room temperature for 10 min, an additional CH_3I (0.1 ml) was added and the mixture was stirred for 20 min followed by the addition of H_2O (40 ml) and filtered. H_2S gas was bubbled through the filtrate to remove Ag ion and filtered off. To the filtrate was added 1 M-triethylammonium bicarbonate (pH 8.0) (5 ml) and the mixture was refluxed for 2 hr. The reaction mixture was concentrated to a volume of 5 ml *in vacuo*. The concentrated solution was added H_2O (5 ml) and pyridine (5 ml). After the mixture was frozen to -70° in a stainless steel container, liquid H_2S (25 ml)—pyridine (5 ml) solution was added, sealed and heated at 50° for 3 days. After H_2S and solvent were removed, the residue was subjected to a column chromatography of DEAE-cellulose (column size; 2.3×40 cm, bicarbonate form). The elution was performed by the linear gradient of triethylammonium bicarbonate buffer (pH 8.0) (2 liters of H_2O to 2 liters of 0.2 M triethylammonium bicarbonate; one fraction, 20 ml). The appropriate fractions (tube No. 61—180) were combined and concentrated to afford XIb¹⁴ (16850 O.D. unit, 320 nm) (*ca.* 30%), *Rf* (EtOH-1 M NH_4OAc (pH 7.0), 5:2, v/v)=0.04 (containing small amount of byproduct, *Rf*=0.13) (VIIb, *Rf*=0.08). UV $\lambda_{\max}^{H_2O}$ nm: 320; $\lambda_{\max}^{pH12}$ nm: 309.

Crotonoside (XII)—A solution of adenosine-1-oxide (VIIIa) (708 mg) in 50% EtOH- H_2O (500 ml) was irradiated by the use of Ushio-100W Hg lamp (pyrex filter) for 7 hr while keeping pH at 10 with 2.8 N NH_4OH . After the reaction mixture was kept at 37° for 24 hr at pH 10, the solvent was removed *in vacuo* and the residue was dissolved in a small amount of 50% EtOH- H_2O . To the solution was added CH_3CN to afford precipitation and the precipitate was collected by filtration and washed with CH_3CN and ether. The powder was recrystallized 2—3 times from hot water to afford XII (190 mg, 27%), mp 243—245° (decomp.).¹⁷ The spectral properties are identical with those reported.¹⁷

6-Thioxanthosine (XIII)—To a solution of XII (160 mg) in H_2O (5 ml) and pyridine (1 ml) was added liquid H_2S (15 ml)—pyridine (5 ml) solution in a stainless steel container and kept at 60° for 45 hr. After the solvent was removed the residue was dissolved in water and evaporated to dryness. The residue was dissolved in 0.01 N HCl and kept at 5° overnight. The crystals separated were collected and recrystallized from water to afford XIII¹⁹ (80 mg, 48%), mp 170—200°. *Anal.* Calcd. for $C_{10}H_{12}O_5N_4S \cdot 2/3H_2O$: C, 38.45; H, 4.27;

22) M.A. Stevens, H.W. Smith, and G.B. Brown, *J. Am. Chem. Soc.*, **81**, 1734 (1959).

N, 17.95; S, 10.25. Found: C, 38.24; H, 4.04; N, 18.04; S, 10.15. UV $\lambda_{\max}^{\text{H}_2\text{O}}$ nm: 252, 340; $\lambda_{\max}^{0.1\text{N-HCl}}$ nm: 254, 328; $\lambda_{\max}^{0.1\text{N-NaOH}}$ nm: 249, 316, 325 (sh); $\lambda_{\max}^{1\text{N-NaOH}}$ nm: 250, 329, 341.

Sulphydrolysis of Adenosine 5'-Phosphate (VIIb)—To a solution of VIIb (200 mg) in pyridine (1 ml) and H_2O (5 ml) was added liquid H_2S (15 ml)—pyridine (5 ml) in a stainless steel container. After the reaction mixture was heated at 65° for 4 days and additional heating at 100° for a day, H_2S gas and solvent were removed and residue was subjected to DEAE-cellulose column chromatography (column size, 2.3×50 cm). After the reaction mixture was adsorbed the column was washed with 200 ml of water and the elution was performed by linear concentration gradient of triethylammonium bicarbonate buffer (pH 8.0) (1 liter of 0.02 M triethylammonium bicarbonate to 1 liter of 0.1 M triethylammonium bicarbonate; one fraction, 20 ml) to give four peaks (I—IV). Each peak was collected and concentrated to dryness. The products were identified by the spectral and chromatographic comparisons with respective authentic samples and their properties and yield are summarized in Table.

Peak	Tube No.	$\lambda_{\max}^{\text{H}_2\text{O}}$ nm	O.D. unit (max) (%)	$R_f^a)$	Migration ^{b)} (cm)	Structure
I	8—18	258	1722(26.0)	0.47	1.0	VIIa
II	37—50	322	879(9.1)	0.36	8.5	XIa
III	82—102	257	2072(25.3)	0.07	13.3	VIIb
IV	116—123	322	941(12.3)	0.05	17.4	XIb

a) paper chromatography, solvent system: isopropanol—conc. NH_4OH — H_2O (7:1:2, v/v)

b) Paper electrophoresis, buffer: 0.05M triethylammonium bicarbonate (pH 8.0), 700 volts, 1.5 hr.

2',3'-O-Isopropylidene-5'-deoxy-3,5'-cyclo-2-thioxanthosine (XV)—To a solution of the cycloguanosine²⁰⁾ (XIV) (200 mg) in H_2O (5 ml) and pyridine (1 ml) was added liquid H_2S (16 ml)—pyridine (4 ml) solution and kept at 50° for 168 hr in a stainless steel container. After the solvent was removed *in vacuo* the residue was dissolved in hot water and the insoluble material was filtered off. This procedure was repeated twice. When the filtrate was cooled the crystals (XV) were separated which was collected by filtration (75 mg). The mother liquor was acidified with hydrochloric acid and kept at 5° . More crystals (29 mg) were obtained. Total yield was 70%. The analytically pure sample was obtained by recrystallization from water, mp $188-192.5^\circ$. Anal. Calcd. for $\text{C}_{13}\text{H}_{14}\text{O}_4\text{N}_4\text{S} \cdot 1/3\text{H}_2\text{O}$: C, 47.52; H, 4.47; N, 17.16; S, 9.75. Found: C, 47.57; H, 4.41; N, 17.22; S, 9.89. UV $\lambda_{\max}^{\text{H}_2\text{O}}$ nm: 234, 294; $\lambda_{\max}^{0.01\text{N-HCl}}$ nm: 248, 297. R_f ($n\text{-BuOH-H}_2\text{O}$, 86:14, v/v)=0.43.

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