CHEMICAL & PHARMACEUTICAL BULLETIN

Vol. 15 No. 7 July 1967

[Chem. Pharm. Bull. 15(7) 909~914 (1967)]

UDC 547.964.07 : 547.857.07

110. Mineo Saneyoshi and Goro Chihara : Synthetic Nucleosides and Nucleotides. I. On Synthesis and Properties of Several Thiocyanato Derivatives of Purines and Their Ribonucleosides.*1

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Several thiocyanato derivatives of purines and their ribonuclesides as potential antineoplastic agents, were prepared by the reaction of mercaptopurines and their ribonucleosides with cyanogen bromide.

The reaction of these thiocyanato derivatives were examined with acid, alkali, sodium methoxide and Raney Ni.

(Received April 15, 1966)

Since some unnatural purines and their nucleosides have been tentatively applied to the clinic for neoplastic diseases, a considerable number of potential antimetabolites of the natural purines have been synthesized and studied on their chemical properties and biological activities. Especially, antitumor activities of 6-purinethiol (6-MP)[1,2] and 2-amino-6-purinethiol (6-TG)[3,4] are well known, and the 9-β-D-ribofuranosyl derivative of 6-purinethiol possesses the highest therapeutic index of any purine derivatives ever studied for antitumor activities against experimental tumors.[5]

The 6-alkyl or aryl derivatives of these purines and their nucleosides were less effective on the experimental tumor, which were synthesized and studied by Robins[6] and Montgomery.[7]

With regard to thiocyanato derivatives of purines some effect has been reported of 6-thiocyanatopurine on Adenocarcinoma 755 and several fungi,[8] but their systematic studies have not been done so far.

This paper describes synthesis of several thiocyanato derivatives of purines and their ribonucleosides and their chemical properties to obtain more active substances against malignant tumor than those ever studied.

The preparative method, which was employed for synthesis of 6-thiocyanatopurine by Hitchings,[9] that is, thiocyanation of 6-chloropurine with potassium thiocyanate in

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*1 A part of this paper was presented at the Kanto Local Meeting of the Pharmaceutical Society of Japan, Tokyo, Sept. 1965.

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1) J. A. Montgomery : Cancer Research, **19**, 447 (1959).
2) F. M. Schabel Jr., J. A. Montgomery, H. E. Skipper, W. R. Laster Jr., J. R. Thomson : *Ibid.*, **21**, 690 (1961).
3) A. Goldin, J. M. Venditti : *Ibid.*, **21**, 617 (1961).
4) D. P. Griswold, W. R. Laster Jr., M. Y. Show, F. M. Schabel Jr., H. E, Skipper : *Ibid.*, **23**, 271 (1963).
5) R. K. Robins : J. Med. Chem., **7**, 186 (1964).
6) C. W. Noell, R. K. Robins : J. Med. Pharm. Chem., **5**, 558 (1962).
7) J. A. Montgomery, T. P. Johnston, A. Gollagher, C. R. Stringfellow, F. M. Schabel Jr. : *Ibid.*, **3**, 265 (1961).
8) W. T. Bradner, D. A. Clarke : Cancer Research, **18**, 299 (1958).
9) G. B. Elion, I. Goodman, W. Lange, G. H. Hitchings : J. Am. Chem. Soc., **81**, 1898 (1959).

refluxing methanol, was found not to be applicable to those of 2 or 8-derivatives. This must be due to lower reactivities of chlorine atom on 2 or 8-position toward nucleophilic displacement.

Then, we tried thiocyanation of mercaptopurines and their ribonucleosides with the use of cyanogen bromide in aqueous sodium hydroxide. This method has been developed for synthesis of thiocyanato derivatives of pyridazine and pyrimidine by Kinugawa.[10] The preparative method is as follows. Mercaptopurines or their ribonucleosides were treated with cyanogen bromide in the solution containing an equimoler amount of sodium hydroxide at 0°. Thiocyanato derivatives were precipitated and easily isolated from the reaction mixture in excellent yield.

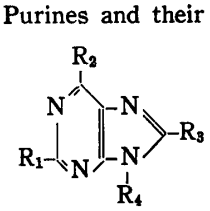
The reaction took place under mild conditions (at low temperatures and in short period, see Experimental).

It is worthy, furthermore, to note that mercaptopurine ribonucleosides can also undergo this thiocyanation reaction without blocking sugar moieties.

The chemical structure of these derivatives were established by the analytical data, the ultraviolet absorption spectra and infrared absorption band at 2160~2180 cm^{-1} (-S-C$^-$≡N).

The newly prepared purines and their ribonucleosides are listed in Table I, including physical constants and analytical data. In Table II and III are shown their ultraviolet absorption maxima, their extinction coefficients and Rf values for paper chromatography. It is to be noted that ultraviolet absorption spectra of these derivatives are almost the same as that of methylthiopurine. The thiocyanato derivatives thus obtained are quite stable in neutral or acidic media. Thus these compounds could be recrystallized even from 10% aqueous hydrogen chloride without degradation.

Table I. Physical Constants and Analytical Results of Thiocyanato Derivatives of Purines and their Ribonucleosides

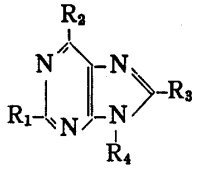


No.	R_1	R_2	R_3	R_4	Formula	m.p. (°C)	Yield (%)	Analysis (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
I	H	SCN	H	H	$C_6H_3N_5S$	225~226 (decomp.)	88	40.66	1.69	39.55	40.26	1.88	38.25
II	H	SCN	H	Rf[a]	$C_{11}H_{11}O_4N_5S$	>240	65	42.72	3.56	22.65	41.99	3.49	22.96
III	NH_2	SCN	H	H	$C_6H_4N_6S$	>240	85	37.50	2.08	43.75	37.46	1.98	43.69
IV	NH_2	SCN	H	Rf	$C_{11}H_{12}O_4N_6$	>240	60	40.74	3.70	25.93	40.67	3.84	26.08
V	SCN	SCN	H	H	$C_7H_2N_6S_2$	>240	81	35.90	0.86	35.90	35.82	1.01	35.98
VI	SCN	OH	H	H	$C_6H_3ON_5S$	>240	88	37.31	1.55	36.27	37.42	1.49	36.33
VII	H	NH_2	SCN	H	$C_6H_4N_6S$	>240	38	37.50	2.08	43.75	36.98	2.49	43.67
VIII	H	NH_2	SCN	Rf	$C_{11}H_{12}O_4N_6S$	>240	33	40.74	3.70	25.93	40.92	3.75	26.01
IX	NH_2	OH	SCN	Rf	$C_{11}H_{12}O_5N_6S$	>240	48	38.82	3.52	24.70	38.81	3.25	24.84
X	OH	OH	SCN	Rf	$C_{11}H_{11}O_6N_5S$	>240	67	38.71	3.22	20.53	39.04	3.34	20.66

a) Rf=β-D-ribofuranose

10) J. Kinugawa, M. Ochiai, K. Yamamoto: Yakugaku Zasshi, **83**, 767 (1963); **83**, 1086 (1963).

Table II. Ultraviolet Absorption Spectra of Thiocyanato Derivatives of Purines and Their Ribonucleosides



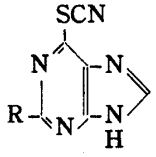
No.	R_1	R_2	R_3	R_4	λ_{max} (mμ) (10^{-3} in parentheses) pH 1	pH 7
I	H	SCN	H	H	277(8.55)	276(8.41)
II	H	SCN	H	Rf	254(5.04) 276(3.21)	254(5.00) 276(3.20)
III	NH_2	SCN	H	H	326(6.74)	321(6.00)[246]
IV	NH_2	SCN	H	Rf	224(11.28)[250] 319(1.61)	226(11.71)[249] 329(4.08)
V	SCN	SCN	H	H	[225]228(21.26) 296(9.32)	229(21.89) 296(9.32)
VI	SCN	OH	H	H	257(8.50)	266(8.30)
VII	H	NH_2	SCN	H	275(14.64)	284(14.73)
VIII	H	NH_2	SCN	Rf	279(18.50)	281(17.20)
IX	NH_2	OH	SCN	Rf	277(11.09) 238(9.01)	[232]270(11.90)
X	OH	OH	SCN	Rf	270(19.90)	[229]269(18.81)

a) Rf=β-D-ribofuranose, [ ]=shoulder

Table III. Rf Values of Thiocyanato Derivatives of Purines and Their Ribonucleosides

Compound	10% Hydrochloric acid	Isopropanol 2 HCl 2 Water 6	Conc. HCl 1 AcOH 1 H_2O 3
I	0.85	0.68	0.81
II	0.94	0.72	0.87
III	0.66	0.54	0.73
IV	0.81	0.70	0.86
V	0.62	0.55	0.72
VI	0.75	0.58	0.48
VII	0.78	0.68	0.83
VIII	0.98	0.78	0.93
IX	0.53	0.33	0.66
X	0.48	0.30	0.64

Table IV. Stability of Thiocyanato Derivatives of Purines



R=H, NH_2, SCN

Solvent	Conc.	Reaction temperature (C°)	Reaction time (hr.)	Hydrolysate
Water	—	100	6	—
HCl	0.1*N*	room temperature	6	—
HCl	0.1*N*	room temperature	20	—
HCl	0.1*N*	60~70	6	—
HCl	1*N*	room temperature	20	—
HCl	1*N*	60~70	6	—
NaOH	1*N*	room temperature	instantly	+
NaOH	0.1*N*	room temperature	1	+
NaOH	0.01*N*	room temperature	3	+
NaOH	0.001*N*	room temperature	10	+
Glycine-sodium hydroxide buffer	pH 11	room temperature	20	+

The details are summarized in Table IV. The table also includes alkaline hydrolysis data of thiocyanato derivatives to their corresponding mercapto derivatives. Ultraviolet absorption of the thiocyanato derivatives shifted toward longer wavelength soon after it was dissolved in 0.1*N* aqueous sodium hydroxide. For instance, 6-thiocyanato-9-β-D-ribofuranosylpurine (6-thiocyanatoinosine) (II) was converted to 6-thioinosine, as shown in Fig. 1. When II was warmed in sodium methoxide-methanol, 6-methylthioinosine and 6-thioinosine were obtained, the former being identical with that obtained directly from 6-thioinosine by Fox's procedure[11] (see Experimental).

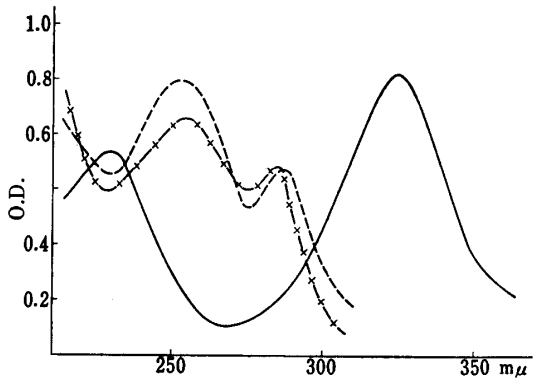
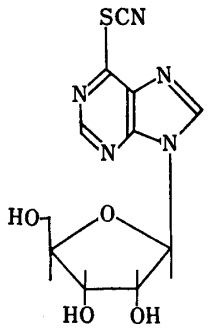


Fig. 1.
Ultraviolet Absorption Spectra of II

—x—x— pH 1
pH 7
——— pH 11



The reaction of these compounds with Raney Ni in aqueous solution gave desulfurization products. 6-Thiocyanatoinosine, for example, gave 9-β-D-ribofuranosylpurine (Nebularine)[11] in 45% yield. The details of reaction conditions for other derivatives are summarized in Table V.

It is concluded that thiocyanato group behaves just as analogously as alkylthio groups in such neutral reaction conditions as desulfurization by Raney Ni, but that in contrast with a considerable stability of alkylthio groups, thiocyanato is easily hydrolized to afford a mercapto derivatives. It can, therefore, be considered that thiocyanato group is in a sense in the masked state of mercapto group.

Table V. Desulfurization of Thiocyanato Derivatives of Purines and Their Ribonucleosides

Compounds	Solvents	Condition	Products
I	Water	6 hr. reflux	Purine
II	$CH_3OCH_2CH_2OH$	6 hr. reflux	Nebularine
II	Water	6 hr. reflux	Nebularine
III	——	——	——
IV	$CH_3OCH_2CH_2OH$	6 hr. reflux	2-Aminopurine riboside
V	Water	12 hr. reflux	Purine
VI	Water	12 hr. reflux	Hypoxanthine
VII	Water	12 hr. reflux	Adenine
VIII	$CH_3OCH_2CH_2OH$	6 hr. reflux	Adenosine
IX	$CH_3OCH_2CH_2OH$	6 hr. reflux	Guanosine
X	$CH_3OCH_2CH_2OH$	6 hr. reflux	Xanthosine

For instance, 6-thiocyanatopurine can be easily converted to its mono-N-oxide by an ordinary N-oxidation procedure with hydrogen peroxide in acetic acid, while unblocked 6-mercaptopurine itself is known to be converted to hypoxanthine *via* 6-purinesulfinic acid under the same reaction condition.[12]

11) J. J. Fox, I. Wempen, A. Hampton, I. L. Doerr : J. Am. Chem. Soc., **80**, 1669 (1958).
12) M. Saneyoshi : unpublished experiment.

Photochemical properties of these derivatives were reported in our another series.[13] Antitumor activities of these compounds will be reported in a forthcoming paper.[14]

Experimental

Paper chromatograms were run by the ascending technique on Toyo Roshi No. 51A in the several solvent systems. Spots of the base and nucleoside were located by visual examination under ultraviolet light.

Starting Materials——

Following 9 mercaptopurines and mercaptopurine ribonucleosides prepared by the original procedure were used as the starting materials for the present study.

6-purinethiol,[15] 2-amino-6-purinethiol,[16] 6-hydroxy-2-purinethiol,[17] 2,6-purinedithiol,[17] 6-amino-8-purinethiol,[18] 9-β-D-ribofuranosyl-6-purinethiol,[11] (6-thioinosine) 2-amino-9-β-D-ribofuranosyl-6-purinethiol,[11] (6-thioguanosine) 6-amino-9-β-D-ribofuranosyl-8-purinethiol,[19] (8-thioadenosine) 2-amino-6-hydroxy-6-β-D-ribofuranosyl-8-purinethiol,[19] (8-thioguanosine).

Direct Bromination of Xanthosine——

Xanthosine dihydrate[20] (3.0 g.) was dissolved in hot methylcellosolve (300 ml.) and kept at 60~80° with stirring. Anhydrous calcium carbonate (8.5 g.) was added into the clear solution, followed by dropwise addition of bromine (0.85 ml.) dissolved in dry dioxane (25 ml.) during 30~60° min. Stirring was continued for 24 hr. at 40~50°. Calcium carbonate was filtered off while hot. The combined filtrate and washings were concentrated to *ca.* 25 ml. Ethanol (300 ml.) was added and cooled, then the orange color precipitates which had been separated, were collected and recrystallized from boiling water. White needles were obtained. 3 g. Yield 60.5% m.p.>200°(decomp.) $\lambda_{max}^{pH\ 1}$ mμ : 253, 278, $\lambda_{max}^{pH\ 11}$ mμ : 238, 271. *Anal.* Calcd. for $C_{10}H_{11}O_6N_4Br \cdot H_2O$: C, 31.51; H, 3.44; N, 14.70. Found : C, 31.61; H, 3.41; N, 14.76.

9-β-D-Ribofuranosyl-2,6-dihydroxy-8-purinethiol (8-thioxanthosine)——

Method A : Thiourea (400 mg.) was added to a suspension of 8-bromoxanthosine monohydrate (2 g.) in 50 ml. of absolute isobutyl alcohol and the mixture was refluxed for 5 hr. The solution was allowed to cool to room temperature and then filtered. The residue was washed with ice-water and then ethanol and recrystallized from boiling water to give 1.4 g. of the product. Yield, 86.9% light yellow prism. m.p.>240°. UV $\lambda_{max}^{pH\ 1}$ mμ : 235, 260, 300; $\lambda_{max}^{pH\ 11}$ 290 mμ. *Anal.* Calcd. for $C_{10}H_{12}O_6N_4S \cdot H_2O$: C, 35.92; H, 4.22; N, 16.76. Found : C, 35.86; H, 3.79; N, 16.93.

Method B : 8-Thioguanosine (1 g.) was suspended in 20 ml. of 2% hydrochloric acid and then, 1g. of sodium nitrite dissolved in 3 ml. of water was added. The mixture was kept at 0°.

The solution was stirred for 5 hr. at this temperature. The reaction mixture was neutralized with saturated sodium hydrogen carbonate solution, then concentrated to dryness, and the residue was dissolved a minimum amount of hot water, after allowing the solution to cool to 10°, the white precipitate was filtered and recrystallized from water to yield 550 mg. (61.4%) of product.

This sample was identical with a specimen obtained by method A.

General Procedure of Cyanation of Mercaptopurines and their Ribonucleosides with Cyanogen Bromide (see Table I)——

Mercaptopurines or their ribonucleosides were converted to their sodium salts by addition of an equimolar amount of sodium hydroxide in water. This solution was mechanically stirred, kept at 0° in ice bath, and an ethanolic solution of equivalent amount of cyanogen bromide was added dropwise with stirring over a period of 10 min. The white precipitate, which soon appeared in the reaction mixture kept 0°, was filtered after 1 hr's stirring and washed with water and ethanol. The product was purified by recrystallization from boiling water or aqueous ethanol. Melting points, yield and elemental analytical data were summarized in Table I. Ultraviolet absorption spectral data showed in Table II.

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*3 This method was application of original bromination procedure of guanosine.[21,22]

13) K. Zenda, M. Saneyoshi, G. Chihara : This Bulletin, **13**, 1108 (1965).
14) M. Saneyoshi, R. Tokuzen, M. Maeda, F. Fukuoka : to be published. This Bulletin, submitted
15) G. B. Elion, G. H. Hitchings : J. Am. Chem. Soc., **77**, 1677 (1955).
16) G. B. Elion, G. H. Hitchings, E. Burgi : *Ibid.*, **74**, 441 (1952).
17) A. G. Beaman : *Ibid.*, **76**, 5633 (1954).
18) R. K. Robins : *Ibid.*, **80**, 6671 (1958).
19) R. E. Holmes, R. K. Robins : *Ibid.*, **86**, 1242 (1964).
20) P. A. Levene : Chem. Ber., **43**, 3163 (1910).
21) A. M. Michelson : "The Chemistry of Nucleosides and Nucleotides," p. 34 (1963). Academic Press, N. Y.
22) M. Ikehara, H. Taba, K. Muneyama : This Bulletin, **13**, 639 (1965).

Reaction of Thiocyanatopurine Derivatives with 0.1*N* Hydrochloric Acid (General Procedure)——

Thiocyanatopurine derivate (0.01 mol.) was added to a 30 ml. of 0.1*N* hydrochloric acid and stored at room temperature for 6 hr., then reaction mixture was neutrlized with sodium hydrogen carbonate. Solidified material was collected by filtration and recrystallized from boiling water. This was proved to be identical with the starting material.

Reaction of 2-Amino-6-thiocyanato-9-β-D-ribofuranosylpurine (6-Thiocyanatoguanosine) with 0.1*N* Sodium Hydroxide——

6-Thiocyanatoguanosine (100 mg.) was added to a 10 ml. of 0.1*N* sodium hydroxide and stored at room temperature for 3 hr., all solid materials were dissolved. After 4 hr., the reaction mixture was acidified with acetic acid to up to pH 4. Crystalline material was precipitated as a white needles on standing in ice box. Collection and subsequent recrystallization from boiling water of the precipitate gave pure 6-thioguanosine (83 mg.), m.p. 221~223°. This sample was identical with a specimen obtained by Fox's procedure.[11]

Reaction of 6-Thiocyanato-9-β-D-ribofuranosylpurine with Sodium Methoxide in Methanol——

A 6-thiocyanato-9-β-D-ribofuranosylpurine (500 mg.) was mixed with methanol solution of 0.1*N* sodium methoxide. The solution was gently warmed on a water bath at 35~40° for 3 hr. under exclusion moisture. Then solvent was evaporated *in vacuo* and 10 ml. of water added then, yellow oily material appeared. Aqueous layer was separated by decantation, then was acidified with acetic acid. Crystalline material was precipitated as a white solid at pH 4. Solid was collected by filtration and recrystallized from hot water. 100 mg. of 6-thioinosine was obtained. m.p. 208~210°. Crystallization of an oily material from aqueous methanol gave 6-methylthioinosine (120 mg.) as micro prism. m.p. 163~165°. Mixed melting points of these samples with an authentic specimen obtained by Fox's procedure[11] showed no depression.

Desulfurization of 6-Thiocyanato-9-β-D-ribofuranosylpurine (6-Thiocyanatoinosine) with Raney Ni——

One gram of 6-thiocyanatoinosine was dissolved in 100 ml. of boiling water and 4 g. of Raney Ni were added with stirring. After 3 hr.'s refluxing, then ultraviolet absorption maximum at 275 mμ had disappeared. The reaction mixture was filtered and catalyst rinsed several times with boiling water. The original filtrate and washings were combined in hot ethanol. The solvent was removed. Addition of ethanol and subsequent removal were repeated several times. The dried syrup was dissolved in hot absolute ethanol. The solution was charcoaled, filtered, concentrated to *ca.* 15 ml. and cooled. 320 mg. of precipitate was obtained. Yield 45% m.p. 174~176°. Recrystallization from ethanol. It was identified with Nebularin by the mixed melting point test with an authentic specimen.[11] Other thiocyanato derivatives were summarized in Table V.

The authors wish to express their hearty gratitudes to Dr. F. Fukuoka, chief of this laboratory for her guidance throughout the course of this study. They also wish to thank Mr. M. Maeda for syntheses of some starting materials and technical assistance, Mr. N. Uehara for IR spectrophotometry, and members of analytical laboratory of Institute of Applied Microbiology, University of Tokyo, for elemental analyses.