

## Platelet Aggregation Inhibitors. IX. Chemical Transformation of Adenosine into 2-Thioadenosine Derivatives<sup>1)</sup>

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A facile synthesis of 2-thioadenosine (IX) and its derivatives from adenosine (I) was described. Treatment of 5-amino-1- $\beta$ -D-ribofuranosylimidazole-4-carboxamide O-benzyl-oxime (IV), obtained *via* 3 steps from I, with CS<sub>2</sub>-MeOH-pyridine at room temperature gave N<sup>1</sup>-benzyloxy-2-thioadenosine (VII) which was readily transformed into 2-benzylthioadenosine N-oxide (VIII) by heating. Treatment of IV with CS<sub>2</sub>-MeOH-NaOH at 180° in an autoclave gave a mixture of 2-thioadenosine (IX), 2-methylthioadenosine (X) and 2-benzylthioadenosine (XI). Reaction of 5-amino-1- $\beta$ -D-ribofuranosylimidazole-4-carboxamidoxime (XV) obtained from I with CS<sub>2</sub>-MeOH-H<sub>2</sub>O at 120° in an autoclave gave IX in an overall yield of 60% (practically 2 steps). Treatment of XV with CS<sub>2</sub>-MeOH-pyridine at room temperature yielded 2-thioadenosine N-oxide (XVIII) which was subsequently transformed into IX by H<sub>2</sub>S. Adenines (XX) were also transformed into 2-thioadenine or 9-benzyl-2-thioadenine (XXV) by treatment of the intermediate compounds (XXII) of carboxamidoxime-type with CS<sub>2</sub>-MeOH-H<sub>2</sub>O at 120°.

**Keywords**—adenosine N-oxide; 5-amino-1- $\beta$ -D-ribofuranosylimidazole-4-carboxamide O-benzyloxime; N<sup>1</sup>-benzyloxy-2-thioadenosine; 2-thioadenosine N-oxide; 2-benzylthioadenosine N-oxide; 2-thioadenosine; 5-amino-1- $\beta$ -D-ribofuranosylimidazole-4-carboxamidoxime; 2-thioadenine; 9-benzyl-2-thioadenine; carbon disulfide

A naturally occurring nucleoside, adenosine (I), is an effective compound on cardiovascular system as coronary vasodilator<sup>3)</sup> and inhibitor of platelet thrombi,<sup>4)</sup> but the duration of the effects is relatively short because it is deaminated by adenosine deaminase of blood cells.<sup>5)</sup> 2-Substituted adenosines such as 2-halogenoadenosines,<sup>6)</sup> 2-thioadenosine derivatives,<sup>7-9)</sup> 2-aminoadenosine derivatives<sup>8,10)</sup> and 2-alkoxyadenosines<sup>8,10)</sup> are resistant to the action of the adenosine deaminase, exerting stronger cardiovascular effects.

The synthesis of these 2-substituted adenosines has been very troublesome. The processes hitherto known are: (1) condensation of a base and a ribose derivative;<sup>7-11)</sup> (2) conver-

- 1) A preliminary account of this work has been described by H. Suehiro, and K. Kikugawa in the abstract of the 3rd Symposium on Nucleic Acid Chemistry, Sapporo, September, 1975.
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- 3) A.N. Drury and A. Szent-Gyorgyi, *J. Physiol.* (London), **68**, 213 (1929).
- 4) G.V.R. Born, A.J. Honour, and J.R.A. Mitchell, *Nature* (London), **202**, 761 (1964).
- 5) R.D. Bunag, C.R. Douglas, S. Imai, and R.M. Berne, *Circ. Res.*, **15**, 83 (1964); M.A. Packham, N.G. Ardlie, and J.F. Mustard, *Am. J. Physiol.*, **217**, 1009 (1969).
- 6) D.A. Clarke, J. Davoll, F.S. Phillips, and G.B. Brown, *J. Pharmacol. Exp. Ther.*, **106**, 291 (1952); R.H. Thorp and L.B. Cobbin, *Arch. Int. Pharmacodyn. Ther.*, **118**, 95 (1959).
- 7) M.H. Maguire, D.M. Nobbs, R. Einstein, and J.C. Middleton, *J. Med. Chem.*, **14**, 415 (1971).
- 8) J.A. Argus, L.B. Cobbin, R. Einstein, and M.H. Maguire, *Brit. J. Pharmacol.*, **41**, 592 (1971); R. Einstein, J.A. Argus, L.B. Cobbin, and M.H. Maguire, *Eur. J. Pharmacol.*, **19**, 246 (1972); L.B. Cobbin, R. Einstein and M.H. Maguire, *Brit. J. Pharmacol.*, **50**, 25 (1974).
- 9) K. Kikugawa, H. Suehiro, and M. Ichino, *J. Med. Chem.*, **16**, 1381 (1973).
- 10) R. Marumoto, Y. Yoshioka, O. Miyashita, S. Shima, K. Imai, K. Kawazoe, and M. Honjo, *Chem. Pharm. Bull.* (Tokyo), **23**, 759 (1975).
- 11) J. Davoll and B.A. Lowy, *J. Am. Chem. Soc.*, **74**, 1563 (1952); H.J. Schaeffer and H.J. Thomas, *ibid.*, **80**, 3738 (1958); J.A. Montgomery and K. Hewson, *J. Heterocycl. Chem.*, **1**, 213 (1964).

sion from a naturally occurring nucleoside, guanosine<sup>9)</sup> or 5-amino-1- $\beta$ -D-ribofuranosylimidazole-4-carboxamide (AICAR).<sup>10)</sup> All the methods included many steps and troublesome processes and the compounds were obtained in low overall yields. 2-Thioadenosine, a key intermediate for 2-substituted adenosines, has been prepared *via* 5 steps from guanosine in an overall yield of 7%<sup>9)</sup> or *via* 7 steps from AICAR in an overall yield of 10%.<sup>10)</sup>

This paper deals with the facile synthetic procedures for 2-thioadenosine and its derivatives. 2-Thioadenosine (IX) was obtained from adenosine (I) *via* adenosine N-oxide (II) in an overall yield of 60%, which was much improved than that described in a previous paper.<sup>12)</sup>

Adenosine N-oxide (II) has been obtained by treatment of adenosine (I) with  $H_2O_2$ - $CH_3COOH$  by Stevens, *et al.*<sup>13)</sup> or Fujii, *et al.*<sup>14)</sup> The procedures, however, included use of an expensive reagent or tedious passing through an ion-exchanger to remove peroxide, which made the synthesis of II in an industrial scale difficult. This time use of active carbon to

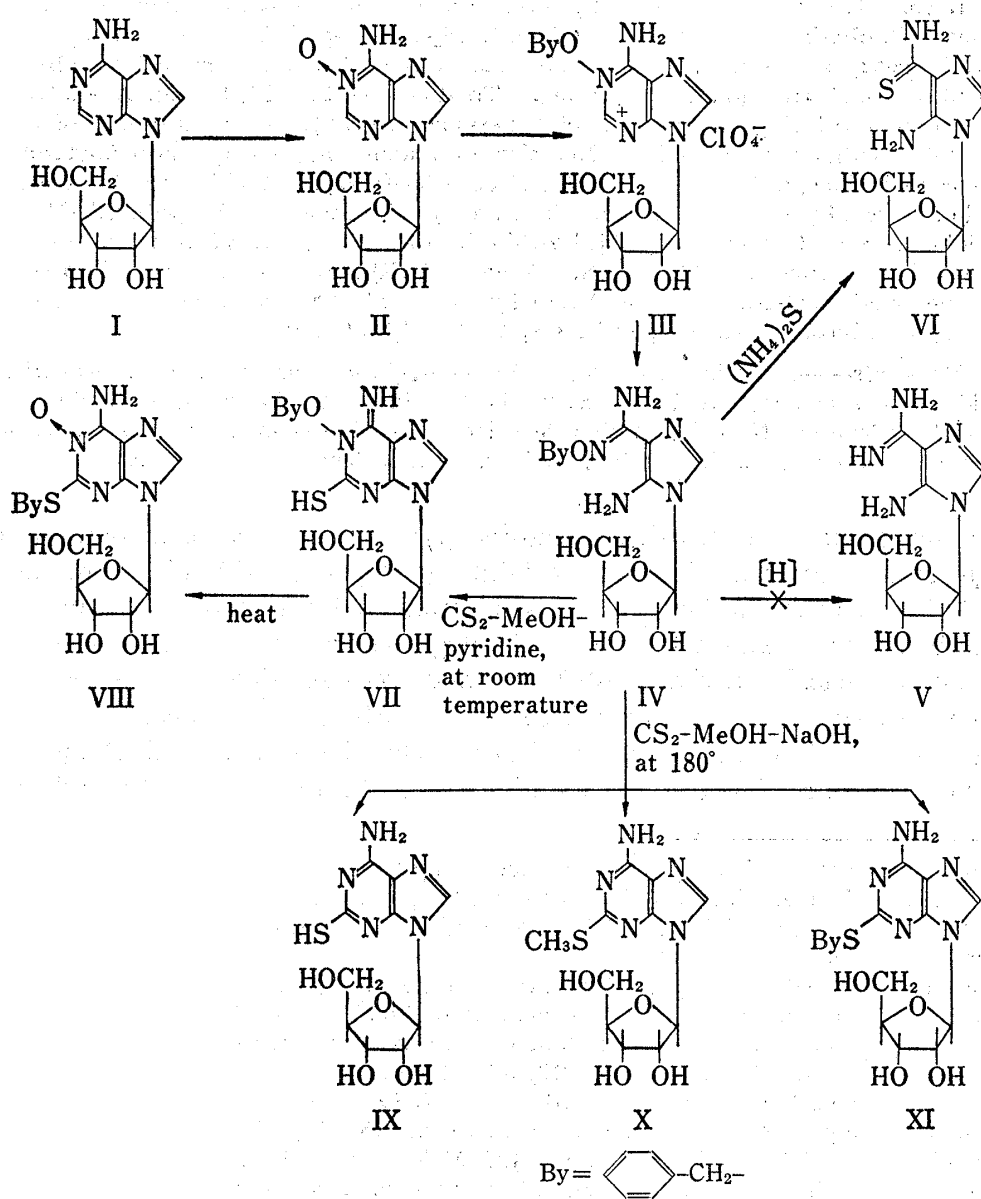


Chart 1

12) K. Kikugawa and H. Suehiro, *J. Carbohydrates·Nucleosides·Nucleotides*, **2**, 159 (1975).

13) M.A. Stevens, D.I. Magrath, H.W. Smith, and G.B. Brown, *J. Am. Chem. Soc.*, **80**, 2755 (1958); M.A. Stevens and G.B. Brown, *ibid.*, **80**, 2759 (1959).

14) T. Fujii, T. Saito, T. Itaya, and K. Yokoyama, *Chem. Pharm. Bull. (Tokyo)*, **21**, 209 (1973).

remove the peroxide was found successful and II was obtained in a yield of more than 80% in a 100 g-scale.

N<sup>1</sup>-Benzyloxyadenosine perchlorate (III)<sup>15,16</sup> obtained from II was converted into 5-amino-1-β-D-ribofuranosylimidazole-4-carboxyamido O-benzyloxime (IV)<sup>16,17</sup> according to the method of Itaya, *et al.*<sup>17</sup> Several attempts to convert IV into the carboxamidine-type compound (V) by catalytic reduction<sup>16</sup> were unsuccessful. Application of (NH<sub>4</sub>)<sub>2</sub>S-induced de-O-benylation<sup>18</sup> to IV gave thio-AICAR (VI)<sup>19</sup> instead of V.

Treatment of IV with CS<sub>2</sub>-MeOH-pyridine at room temperature for several days gave N<sup>1</sup>-benzyloxy-2-thioadenosine (VII). The chemical shift of the benzylic methylene protons in the nuclear magnetic resonance (NMR) spectrum of VII was very similar to that of III and rather different from that of N<sup>6</sup>-benzyloxyadenosine (Table I). The compound (VII) consumed molecular iodine, indicating the presence of free mercaptan group. It was so unstable in a solution as to be readily transformed into 2-benzylthioadenosine N-oxide (VIII). Thus, the compound (VIII) was produced by heating VII in aqueous EtOH. The chemical shift of the benzylic methylene proton of VIII was very close to that of 2-benzylthioadenosine (XI) (Table I) and VIII consumed no molecular iodine. The ultraviolet (UV) spectrum

TABLE I. NMR Spectra of 2-Thioadenosine Derivatives

Compound	Solvent	δ (ppm)					
		Purine (singlet)	Phenyl (multiplet)	Methylene (singlet)	Methyl (singlet)	Sugar	
						H <sub>1'</sub> (doublet)	H <sub>2'</sub> (triplet)
Adenosine (I)	<i>d</i> <sub>6</sub> -DMSO	8.20 8.37	—	—	—	5.93(6)	4.63(6)
Adenosine N-oxide (II)	<i>d</i> <sub>6</sub> -DMSO	8.57 8.63	—	—	—	5.93(6)	4.58(6)
N <sup>1</sup> -Benzyloxy- adenosine (III) <sup>a)</sup>	<i>d</i> <sub>6</sub> -DMSO	8.80 8.93	7.53	5.46	—	5.97(5)	5.53(5)
N <sup>6</sup> -Benzyloxy- adenosine <sup>b)</sup>	<i>d</i> <sub>6</sub> -DMSO	7.70 8.13	7.40	5.05	—	5.80(5)	4.50(5)
N <sup>1</sup> -Benzyloxy- 2-thioadenosine (VII)	<i>d</i> <sub>6</sub> -DMSO	8.26	7.40+ 7.80	5.43	—	5.80(6)	4.50(6)
2-Benzylthio- adenosine (XI)	<i>d</i> <sub>6</sub> -DMSO	8.26	7.33	4.40	—	5.90(5)	4.60(5)
2-Benzylthioadenosine N-oxide (VIII)	<i>d</i> <sub>6</sub> -DMSO	8.47	7.40	4.32	—	6.00(5)	4.60(5)
2-Thioadenosine (IX)	<i>d</i> <sub>6</sub> -DMSO	8.36	—	—	—	5.88(5)	4.50(5)
2-Thiadenosine N-oxide (XVIII)	<i>d</i> <sub>6</sub> -DMSO	8.33	—	—	—	5.81(6)	4.50(6)
2-Methylthio- adenosine (X)	<i>d</i> <sub>5</sub> -Pyridine-D <sub>2</sub> O	8.40	—	—	2.66	6.30(6)	4.83(6)
2-Methylthioadenosine N-oxide (XIX)	<i>d</i> <sub>5</sub> -Pyridine-D <sub>2</sub> O	8.36	—	—	2.58	6.13(5)	4.63(5)

NMR spectra were measured with a Varian T-60 spectrometer with an internal standard, tetramethylsilane or 4,4-dimethyl-4-silapentanesulfonic acid sodium salt. DMSO designates dimethylsulfoxide. The number in parentheses indicates the *J*<sub>1',2'</sub> coupling constant in Hz.

a) Data were also described in Ref. 15.

b) Kindly supplied by Professor T. Fujii (T. Fujii, *et al.*, *Chem. Pharm. Bull.* (Tokyo), **21**, 1676 (1973)).

15) T. Fujii, C.C. Wu, and T. Itaya, *Chem. Pharm. Bull.* (Tokyo), **19**, 1368 (1971).

16) J.A. Montgomery and H.J. Thomas, *J. Med. Chem.*, **15**, 182 (1972).

17) T. Itaya, T. Saito, S. Kawakatsu, and T. Fujii, *Chem. Pharm. Bull.* (Tokyo), **23**, 2643 (1975). The experimental details had been given by Professor T. Fujii of Kanazawa University prior to their publication.

18) E.C. Taylor and J.W. Barton, *J. Org. Chem.*, **24**, 127 (1959).

19) A. Yamazaki, I. Kumashiro, T. Takenishi, and M. Ikehara, *Chem. Pharm. Bull.* (Tokyo), **16**, 2172 (1968).

of VIII was very close to that of 2-methylthioadenosine N-oxide (XIX) described below. The intramolecular or intermolecular rearrangement of the benzyl group observed for VII was readily understandable from the results of Fujii, *et al.*<sup>20)</sup> who described that N<sup>1</sup>-alkoxyadenosines are alkylating agents when they are treated with nucleophiles such as mercaptans and produce adenosine N-oxide (II) and alkyl mercaptans.

Treatment of IV with sodium methyl xanthate (CS<sub>2</sub>-MeOH-NaOH) at 180° for 3 hr in an autoclave afforded three compounds, 2-thioadenosine (IX),<sup>9)</sup> 2-methylthioadenosine (X)<sup>9)</sup> and 2-benzylthioadenosine (XI).<sup>9)</sup> The mechanisms of the production of these compounds might be as follows. Ring closure must have occurred in a sequence of XII→XIII→VII-Na salt accompanying evolution of H<sub>2</sub>S. 2-Thioadenosine (IX) would be yielded by the reductive debenzoylation of VII with H<sub>2</sub>S at an elevated temperature. 2-Benzylthioadenosine (XI)

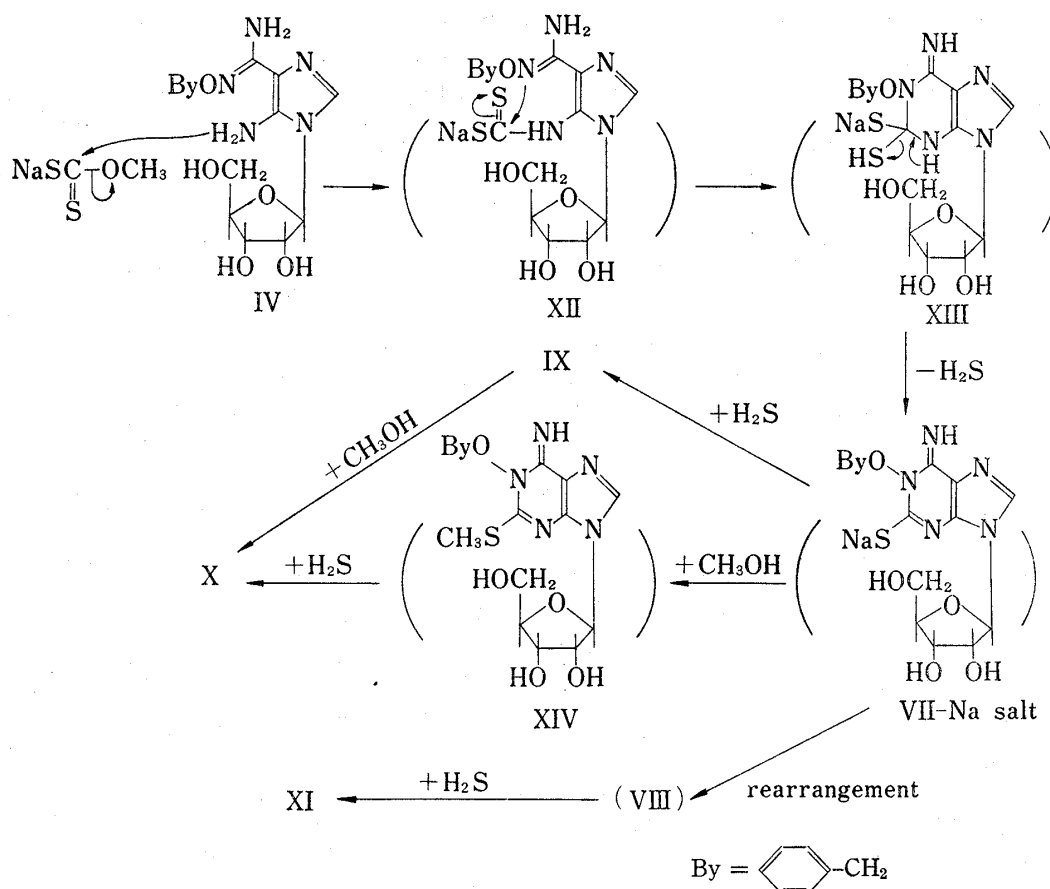


Chart 2

could be yielded by the reduction with H<sub>2</sub>S of 2-benzylthioadenosine N-oxide (VIII) produced from an intramolecular or intermolecular rearrangement of the benzyl group of VII. Methylation of 2-thioadenosine (IX) with methanol in the presence of Na at an elevated temperature to yield 2-methylthioadenosine (X) will be discussed in a separate paper.<sup>21)</sup>

Stevens, *et al.*<sup>22)</sup> have reported that adenosine N-oxide (II) was hydrolyzed into an unstable compound having UV-absorption ( $\lambda_{\text{max}}^{\text{PH } 3}$  nm, 210, 257 and  $\lambda_{\text{max}}^{\text{PH } 13}$  228, 260) and suggested that it might be 5-amino-1- $\beta$ -D-ribofuranosylimidazole-4-carboxamidoxime (XV). The present authors treated II with 5 N NaOH under reflux to obtain a rather stable compound (XV) having UV-absorption ( $\lambda_{\text{max}}^{\text{PH } 1}$  nm, 280 and  $\lambda_{\text{max}}^{\text{PH } 7}$  271 and  $\lambda_{\text{max}}^{\text{PH } 13}$  259). Although the carboxamidoxime-type compound (XV) could not be isolated in a crystalline form, the structure

20) T. Fujii, T. Itaya, and S. Moro, *Chem. Pharm. Bull.* (Tokyo), **20**, 958 (1972).

21) K. Kikugawa, H. Suehiro, and A. Aoki, *Chem. Pharm. Bull.* (Tokyo), "in press".

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

must be correct when compared its spectra with those of the known carboxamidoxime-type compounds described previously.<sup>12)</sup> Purification of this compound as a starting material of the subsequent reactions was performed: neutralization of the reaction mixture with HCl. (Method A in Experimental), neutralization with HCl followed by silica gel column chromatography (Method B), neutralization with Diaion-SK-1B (Method C) and neutralization with Amberlite IRC-50 (Method D).

Treatment of XV with sodium methyl xanthate ( $\text{CS}_2$ -MeOH-NaOH) at  $180^\circ$  in an autoclave gave 2-methylthioadenosine (X). Analogous treatment of XV with  $\text{CS}_2$ -cyclopentanol-

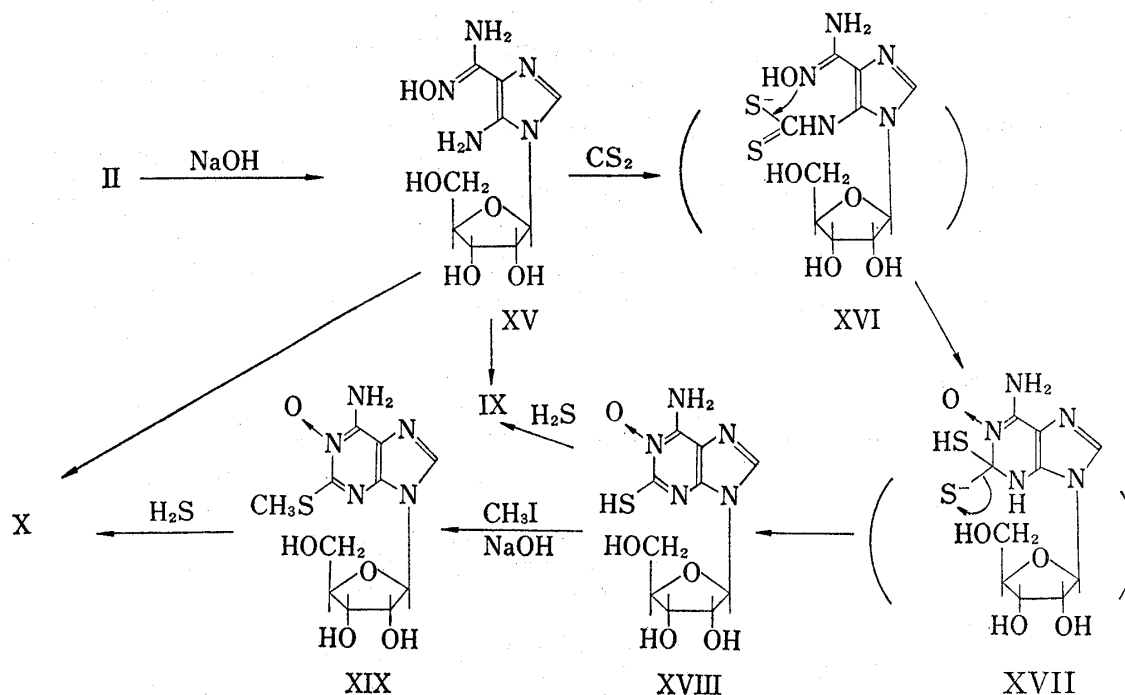


Chart 3

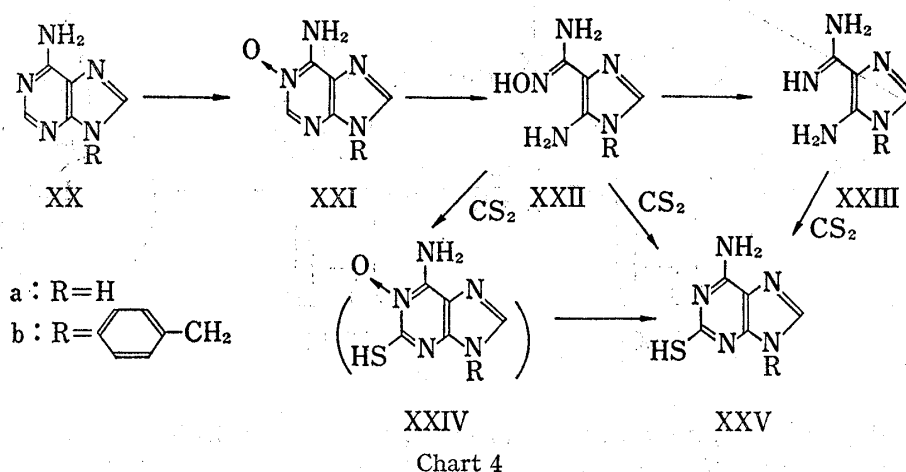
NaOH (or KOH) did not give an aimed product, 2-cyclopentylthioadenosine.<sup>9)</sup> Treatment of XV with  $\text{CS}_2$ -*t*-BuOH-Na gave only a tar. The formation of X from XV must proceed *via* 3 steps of reaction: cyclization, reduction of N-oxide function and methylation by methanol, which were suggestive of the possible synthetic procedure for 2-thioadenosine (IX) under milder conditions.

The reaction of dried XV (XV-B or -C) with  $\text{CS}_2$ -MeOH-pyridine was studied under several conditions. At  $180^\circ$  for 3 hr, 2-methyl thioadenosine (X), 2-thioadenosine (IX) and adenosine (I) were produced. At  $120^\circ$  for 3 hr, IX was produced but some of XV remained unchanged. At reflux neither IX nor X was produced. From the reaction mixture at  $120^\circ$  for 6 hr, IX was obtained in a yield (based on II) of 50.1%.<sup>12)</sup> The procedure,<sup>12)</sup> however, was rather troublesome and found not to be reproducible in a large scale preparation.

Several attempts were made to obtain a more facile and reproducible synthetic procedure for IX. It was found that the presence of water in the reaction mixture was essential for the reproducibility of the preparation of IX in a high yield. Treatment of hydrous XV (XV-A or -D) with  $\text{CS}_2$ -MeOH-pyridine- $\text{H}_2\text{O}$  (10:15:20:5) at  $120^\circ$  for 5 hr yielded 60.0% of IX, with  $\text{CS}_2$ -MeOH- $\text{H}_2\text{O}$  (50:175:25) yield 76.0%, and with  $\text{CS}_2$ - $\text{H}_2\text{O}$  (10:40) (biphasic) yielded 48.0%. The use of a homogeneous reaction mixture containing  $\text{CS}_2$ -MeOH- $\text{H}_2\text{O}$  was found most suitable for preparation of IX in a large scale (100 g and kilogram) preparation. This procedure had an additional advantage that there were no needs to use an expensive pyridine and to remove salt and water completely from the reaction mixture. 2-Thioadenosine (IX) was, thus, obtained in an overall yield of 60% from adenosine (I) *via* adenosine N-oxide (II).

The formation of IX from XV was considered to proceed through cyclization (XV→XVI→XVII→XVIII) and subsequent reduction of XVIII with H<sub>2</sub>S. Treatment of XV with CS<sub>2</sub>-MeOH-pyridine at room temperature gave 2-thioadenosine N-oxide (XVIII), whose UV-absorption spectrum was close to that of 2-thioadenine N-oxide (XXIVa).<sup>23)</sup> The compound (XVIII) was methylated into 2-methylthioadenosine N-oxide (XIX), whose structure was confirmed by the comparison of its UV spectrum with that of 2-methylthioadenine N-oxide<sup>23)</sup> and by the presence of the CH<sub>3</sub>S proton signal in its NMR spectrum (Table I). 2-Thioadenosine N-oxide (XVIII) was converted into IX by treatment with H<sub>2</sub>S-MeOH-pyridine-H<sub>2</sub>O or CS<sub>2</sub>-MeOH-pyridine-H<sub>2</sub>O at 120° for 5 hr. The results indicated that the formation of IX from XV proceeded *via* the reduction of N-oxide function of the intermediate compound (XVIII). 2-Methylthioadenosine N-oxide (XIX) was similarly reduced into 2-methylthioadenosine (X) by treatment with H<sub>2</sub>S at an elevated temperature.

Adenine N-oxide (XXIa) was converted into 4-aminoimidazole-5-carboxamidoxime (XXIIa)·2HCl, which was reduced into 4-aminoimidazole-5-carboxamide (XXIIIa)·2HCl by reaction with hydrogen in the presence of Raney Ni catalyst according to the method of Stevens, *et al.*<sup>13)</sup> Compound (XXIIIa·2HCl) was treated with CS<sub>2</sub>-MeOH-pyridine at room temperature to give 2-thioadenine (XXVa) which was identical with an authentic sample<sup>24)</sup>



prepared according to the procedure of Bendich, *et al.*<sup>25)</sup> Free base of XXIIa was treated with CS<sub>2</sub>-MeOH-H<sub>2</sub>O at 120° giving 2-thioadenine (XXVa). The reaction must proceed through 2-thioadenine N-oxide (XXIVa) and subsequent reduction with H<sub>2</sub>S. 9-Benzyladenine N-oxide (XXIb)<sup>15)</sup> prepared from 9-benzyladenine (XXb)<sup>26)</sup> was converted into 5-amino-1-benzylimidazole-4-carboxamidoxime (XXIIb), which was transformed into 9-benzyl-2-thioadenine (XXVb) by treatment with CS<sub>2</sub>-MeOH-pyridine at 120°.

9-Substituted 2-thioadenines such as 9-cyclopentyl-2-thioadenine<sup>16)</sup> and 2-thioadenosine 3', 5'-cyclic monophosphate<sup>27)</sup> have been prepared from the carboxamide-type compounds *via* 4 or 5 steps in a lower overall yield of about 20%. The present study showed that 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide O-benzylxime (IV), 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamidoxime (XV) and 5-aminoimidazole-4-carboxamidoxime derivatives (XXII) were treated with CS<sub>2</sub> to yield several 2-thioadenosine and 2-thioadenine derivatives in good yields. 2-Thioadenosine (IX) was obtained in 60% yield from I *via* practically 2 steps. The reactions proceeded through cyclization at 2-position and subse-

23) R.M. Creswell and G.B. Brown, *J. Org. Chem.*, **28**, 2560 (1963).

24) K. Kikugawa, H. Suehiro, and A. Aoki, *Chem. Pharm. Bull. (Tokyo)*, **25**, 1811 (1977).

25) A. Bendich, J.F. Tinker, and G.B. Brown, *J. Am. Chem. Soc.*, **70**, 3109 (1948).

26) J.A. Montgomery and H.J. Thomas, *J. Heterocycl. Chem.*, **1**, 113 (1964).

27) R.B. Meyer, Jr., D.A. Shuman, and R.K. Robins, *J. Am. Chem. Soc.*, **96**, 4962 (1974).

quent reduction of N-oxide function with H<sub>2</sub>S evolved. Reduction of the N-oxide function from various purine N-oxides has generally been performed with catalytic hydrogenation in the presence of palladium on charcoal or Raney Ni catalyst.<sup>22,28</sup> It is to be emphasized that the N-oxide function of purines could be readily removed by reduction with H<sub>2</sub>S.

The new 2-substituted adenosine (VII, VIII, XVIII and XIX) obtained here were tested as inhibitors of rabbit platelet aggregation. 2-Thioadenosine N-oxide (XVIII) was found to be a very strong inhibitor. The details of the biological results will be described in a separate paper.<sup>21</sup>

#### Experimental<sup>29</sup>

**Adenosine N-Oxide (II)**—To a suspension of adenosine (I) (100 g, 0.375 mol) in 1.0 l of glacial acetic acid in a glass container was added 100 ml of 30% aqueous H<sub>2</sub>O<sub>2</sub>. The mixture was allowed to stand at 50° overnight. To this was added 20 g of active carbon and the mixture was vigorously stirred at 50° until the peroxide was free from the reaction mixture when detected with KI-starch paper. The active carbon was removed by filtration and the filtrate was evaporated *in vacuo* at 40° to dryness. The residue was coevaporated with 500 ml of H<sub>2</sub>O repeatedly and finally it was dissolved in 1.0 l of H<sub>2</sub>O. The crystalline precipitate was collected by filtration and dried to give 90.0 g (yield 80%) of the monohydrate of II, mp 206° dec. UV:  $\lambda_{\text{max}}^{\text{H}^+}$  (ε) nm, 260 (11100),  $\lambda_{\text{max}}^{\text{H}_2\text{O}}$  233 (38800), 263 (8100),  $\lambda_{\text{max}}^{\text{pH}^7}$  233, 270, 310. Rf 0.09 (solvent 1, Rf of I: 0.27) and 0.50 (solvent 2, Rf of I: 0.53). (lit.<sup>13</sup>) mp 155° and 160° dec. (anhydrous), lit.<sup>14</sup>) mp 219—221° dec. (anhydrous), 224—225° dec. (monohydrate), UV:  $\lambda_{\text{max}}^{\text{H}^+}$  (ε) nm, 257 (11300),  $\lambda_{\text{max}}^{\text{pH}^7}$  232 (39800), 261 (7700), 295 (2100),  $\lambda_{\text{max}}^{\text{pH}^3}$  231 (22900), 268 (7900), 307 (4200).

**N<sup>1</sup>-Benzyloxyadenosine Perchlorate (III) and 5-Amino-1-β-D-ribofuranosylimidazole-4-carboxamide O-Benzyloxime (IV)**—Compounds, III and IV, were prepared according to the methods of Fujii, *et al.*<sup>15</sup>) and Itaya, *et al.*,<sup>17</sup>) respectively.

III was obtained from II in a yield of 83.3%, mp 149—152° dec. UV:  $\lambda_{\text{max}}^{\text{H}^+}$  nm, 260.5,  $\lambda_{\text{max}}^{\text{H}_2\text{O}}$  260.5,  $\lambda_{\text{max}}^{\text{OH}^-}$  252 shoulder, 259, 266 shoulder, 290 shoulder. Rf 0.35 (solvent 1) and 0.63 (solvent 2). NMR spectrum is shown in Table I. (lit.<sup>15</sup>) mp 149—151° dec. UV:  $\lambda_{\text{max}}^{\text{H}^+}$  nm, 258,  $\lambda_{\text{max}}^{\text{pH}^7}$  258,  $\lambda_{\text{max}}^{\text{pH}^3}$  256).

III was boiled in aqueous NaOH and the mixture was extracted with ethyl acetate. The extract was evaporated to dryness to afford IV-extract (yield, 93.5%). It was purified through a silica gel column with elution solvent of CHCl<sub>3</sub>-MeOH (19:1 and 9:1), and IV-column was obtained in a yield of 54%. UV:  $\lambda_{\text{max}}^{\text{H}^+}$  nm, 283,  $\lambda_{\text{max}}^{\text{H}_2\text{O}}$  263,  $\lambda_{\text{max}}^{\text{OH}^-}$  263. Rf 0.66 (solvent 1) and 0.72 (solvent 2). (lit.<sup>17</sup>) UV:  $\lambda_{\text{max}}^{\text{H}^+}$  284 nm).

**Attempts to Convert IV into 5-Amino-1-β-D-ribofuranosylimidazole-4-carboxamide (V)**—(A) Catalytic Reduction in the Presence of Raney Ni: IV-Column (80 mg) in 10 ml of ethanol was shaken in an atmospheric pressure of hydrogen in the presence of 0.6 ml of Raney Ni, W<sub>2</sub>, for 2 days. Paper chromatography (solvent 1 and 2) of the mixture revealed a spot corresponding to IV, aqueous extract of which showed the same UV-absorption profile as IV.<sup>30</sup>

(B) Treatment of IV with (NH<sub>4</sub>)<sub>2</sub>S: A solution of IV-extract (500 mg) in 6 ml of ethanol and 6 ml of concentrated NH<sub>3</sub>-water was saturated with H<sub>2</sub>S under cooling. It was heated at 180° for 3.4 hr in a sealed tube. Reaction mixture revealed 6 spots (Rf 0.81, 0.59, 0.49, 0.36 (major), 0.30 and 0.24) on a paper chromatogram (solvent 1). The mixture was evaporated *in vacuo* to dryness and the residue was purified through a column of cellulose (1.7 × 50 cm) with elution by *n*-BuOH-H<sub>2</sub>O (84:16) and successively through a column of silica gel (20 g) with elution by CHCl<sub>3</sub>-MeOH (49:1). A small amount of the major product, thio-AICAR (VI), was isolated. UV:  $\lambda_{\text{max}}^{\text{H}^+}$  nm, 272, 328,  $\lambda_{\text{max}}^{\text{pH}^7}$  270, 329,  $\lambda_{\text{max}}^{\text{pH}^3}$  270, 329. Rf 0.36 (solvent 1) and 0.56 (solvent 2). (lit.<sup>19</sup>) UV:  $\lambda_{\text{max}}^{\text{H}^+}$  nm, 282, 327,  $\lambda_{\text{max}}^{\text{pH}^7}$  273, 330,  $\lambda_{\text{max}}^{\text{pH}^3}$  271.5, 330).

28) T. Fujii and T. Itaya, *Tetrahedron*, **27**, 351 (1971).

29) Melting points were determined on a Buchi-Tottoli apparatus and are uncorrected. UV spectra were measured with a Hitachi recording spectrophotometer, EPS-3T. IR spectra were measured with a Hitachi 285 Grating Infrared spectrometer. Paper chromatography was carried out with solvent systems (1) *n*-BuOH-H<sub>2</sub>O (84:16) and (2) *n*-BuOH-glacial acetic acid-H<sub>2</sub>O (2:1:1). Thin-layer chromatography was carried out using Merck Kieselgel HF<sub>254</sub> nach Stahl (Type 60). Cellulose column chromatography and silica gel column chromatography were done with cellulose powder (Toyo Roshi Kaisha, Ltd.), 100—200 mesh, and Merck Kieselgel 60 (0.063—0.200 mm), respectively. TOD designates total optical density. Pauly test and ferric chloride test were done according to the method described in reference 13.

30) UV spectrum of V was not described in ref. 16. UV spectra of the same carboxamide-type of compounds such as 5-amino-1-cyclopentylimidazole-4-carboxamide ( $\lambda_{\text{max}}^{\text{pH}^1}$  nm, 283,  $\lambda_{\text{max}}^{\text{pH}^7}$  284,  $\lambda_{\text{max}}^{\text{pH}^3}$  284) (ref. 16) and 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide 3',5'-cyclic monophosphate ( $\lambda_{\text{max}}^{\text{H}^+}$  nm, 280,  $\lambda_{\text{max}}^{\text{pH}^7}$  281,  $\lambda_{\text{max}}^{\text{pH}^1}$  280) (ref. 31) were different from that of IV.

31) R.B. Meyer, Jr., D.A. Shuman, R.K. Robins, J.P. Miller, and L.N. Simon, *J. Med. Chem.*, **16**, 1319 (1973).

**N<sup>1</sup>-Benzyloxy-2-thioadenosine (VII)**—A solution of 4.3 g (11.9 mmol) of IV-extract in 40 ml of methanol, 40 ml of pyridine and 20 ml of carbon disulfide was allowed to stand in dark at room temperature for 10 days. The mixture was evaporated *in vacuo* to dryness, and the residue was crystallized from methanol, 1.6 g (yield 31.7%). Careful recrystallization from ethanol–water (3:7) gave pure sample of VII, mp 130° shrink, 203–206° dec. UV:  $\lambda_{\max}^{\text{H}^+}$  (ε) nm, 296 (17200),  $\lambda_{\max}^{\text{H}_2\text{O}}$  294 (16400),  $\lambda_{\max}^{\text{pH}13}$  248 (14400), 285 (13700), 310 shoulder (11800). NMR spectrum is presented in Table I. *Anal.* Calcd. for C<sub>17</sub>H<sub>19</sub>N<sub>5</sub>O<sub>5</sub>S·H<sub>2</sub>O; C, 48.22; H, 5.00; N, 16.54. Found: C, 48.02; H, 4.54; N, 16.45.

The compound (VII) consumed molecular iodine. It was very unstable in paper chromatography and in thin-layer chromatography to degrade into the compound (VIII). *Rf* 0.13 (VII) and 0.55 (VIII) (solvent 1), and 0.49 (VII) and 0.76 (VIII) (solvent 2). *Rf* on thin-layer chromatogram (solvent CHCl<sub>3</sub>–MeOH, 4:1) 0.5 (VII) and 0.2 (VIII). Treatment of 30 mg of VII in 2.0 ml of ethanol–water (3:7) with 0.5 ml of 0.1 N iodine gave an insoluble compound, mp 239–243° dec. UV:  $\lambda_{\max}^{\text{H}^+}$  nm, 243, 277 shoulder,  $\lambda_{\max}^{\text{H}_2\text{O}}$  243, 277 shoulder,  $\lambda_{\max}^{\text{OH}^-}$  244, 256 shoulder, 280 shoulder, 315 shoulder. *Rf* 0.02 (solvent 1) and 0.32 (solvent 2). *Rf* on thin-layer chromatogram (solvent, CHCl<sub>3</sub>–MeOH, 4:1) 0.00.

**2-Benzylthioadenosine N-Oxide (VIII)**—A solution of 300 mg (0.7 mmol) of N<sup>1</sup>-benzyloxy-2-thioadenosine (VII) in 20 ml of ethanol–water (3:7) was heated at 50° for 4 days. A crystalline precipitate was collected by filtration and recrystallized from ethanol–water (3:7), 133 mg (yield, 44.3%), mp 214–218° dec. UV:  $\lambda_{\max}^{\text{EtOH-H}_2\text{O}(1:9)}$  (ε) nm, 249.5 (39700), 275 shoulder (12900),  $\lambda_{\max}^{\text{EtOH-pH}1 \text{ H}_2\text{O}}$  230 shoulder (20300), 274 (16000),  $\lambda_{\max}^{\text{EtOH-pH}13 \text{ H}_2\text{O}}$  250 (34600), 280 shoulder (11700), 310 shoulder (3300). NMR spectrum is presented in Table I. *Rf* 0.55 (solvent 1) and 0.76 (solvent 2). *Rf* on thin-layer chromatogram (solvent, CHCl<sub>3</sub>–MeOH, 4:1) 0.2. It consumed no iodine. *Anal.* Calcd. for C<sub>17</sub>H<sub>19</sub>N<sub>5</sub>O<sub>5</sub>S·H<sub>2</sub>O; C, 48.22; H, 5.00; N, 16.54. Found: C, 48.55; H, 4.73; N, 16.60.

**Treatment of IV with Sodium Methyl Xanthate (CS<sub>2</sub>–NaOH–MeOH) at an Elevated Temperature**—IV-extract, 900 mg (2.5 mmol), was treated with 10 ml of methanol, 600 mg of NaOH and 1.20 g of CS<sub>2</sub> at 180° for 3 hr in an autoclave. The reaction mixture was evaporated to dryness and the residue was dissolved in 5 ml of methanol. Insoluble material was removed by filtration and the filtrate was evaporated *in vacuo* to dryness. The residue was stirred in a mixture of 25 ml of water and 25 ml of ethyl acetate. The resulting precipitate was collected by filtration and recrystallized from ethanol–water to afford 215 mg (yield 26.7%) of 2-methylthioadenosine (X), mp 225–228.5° eff. UV:  $\lambda_{\max}^{\text{H}^+}$  nm, 270,  $\lambda_{\max}^{\text{H}_2\text{O}}$  235, 275,  $\lambda_{\max}^{\text{OH}^-}$  235, 275. *Rf* 0.42 (solvent 1) and 0.64 (solvent 2). Mixed fusion test of the sample with an authentic sample<sup>9)</sup> confirmed the structure. (lit.<sup>9)</sup> mp 228–229.5°, UV:  $\lambda_{\max}^{\text{H}^+}$  nm, 270,  $\lambda_{\max}^{\text{H}_2\text{O}}$  235, 277,  $\lambda_{\max}^{\text{pH}13}$  277).

The aqueous layer of the filtrate revealed 3 spots on a paper chromatogram (solvent 1): *Rf* 0.27 (corresponding to I), 0.20 and 0.12 (major spot). It was neutralized with 1 N HCl to pH 5 and evaporated. The residue was applied onto a cellulose column (1.7 × 50 cm) which was eluted with 400 ml of *n*-BuOH–H<sub>2</sub>O (84:16). Then the column was equilibrated with *n*-BuOH–glacial acetic acid–H<sub>2</sub>O (2:1:1). Crystalline materials were separated on the top fraction of the column, which was extracted with 1 N NH<sub>4</sub>OH (20–30 ml). The extract was acidified to pH 2 with 6 N HCl and the crystals separated were collected by filtration. They were purified by dissolving in 1 N NH<sub>4</sub>OH and subsequent addition of 6 N HCl. 2-Thioadenosine (IX), 42 mg (yield 5.3%), mp 184–189° dec., was obtained. *Rf* 0.12 (solvent 1). IR spectrum of the sample was identical with that of an authentic sample.<sup>9)</sup>

The organic layer of the filtrate showed 2 spots on a paper chromatogram (solvent 1): *Rf* 0.76 (corresponding to XI) and 0.42 (corresponding to X). The aqueous extract of the faster moving spot showed UV-absorption maxima at 273 (H<sup>+</sup>), 234, 278 (H<sub>2</sub>O), 278 (OH<sup>-</sup>) nm.

**5-Amino-1-β-D-ribofuranosylimidazole-4-carboxamidoxime (XV)**—Method A: Adenosine N-oxide (II), 8.0 g (26.7 mmol), was dissolved in 75 ml (375 mmol) of refluxed 5 N NaOH and the mixture was refluxed for 15 min. It was cooled and adjusted to pH 9.0 with concentrated HCl (about 30 ml). The mixture was evaporated *in vacuo* to a small volume. Sodium chloride separated was removed by filtration and washed with methanol. The filtrate was evaporated to a small volume and NaCl was removed again. The filtrate was evaporated *in vacuo* to dryness to afford a slightly amber-colored gum of crude XV (XV-A).

Method B: The gum of XV-A obtained from 10.0 g of II and 100 ml of 5 N NaOH was applied onto a silica gel column (200 g). The column was eluted with CHCl<sub>3</sub>–MeOH (4:1). The UV-absorbing fraction (TOD<sub>281</sub><sup>pH1</sup> 236000, recovery 78%) was evaporated to dryness to yield an amber-colored gum (XV-B). UV:  $\lambda_{\max}^{\text{H}^+}$  nm, 280,  $\lambda_{\max}^{\text{OH}^-}$  259. *Rf* 0.10 (solvent 1) and 0.52 (solvent 2). Pauly test: pink and FeCl<sub>3</sub> test: blue. The properties of the compound were very close to those of 4-aminoimidazole-5-carboxamidoxime (XXIIa), 5-amino-1-benzylimidazole-4-carboxamidoxime (XXIIb), 5-amino-1-cyclopentylimidazole-4-carboxamidoxime<sup>19)</sup> and 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamidoxime 3',5'-cyclic monophosphate.<sup>21)</sup>

Method C: Adenosine N-oxide (II), 3.2 g (10.7 mmol), was refluxed in 15 ml (75 mmol) of 5 N NaOH for 15 min. The mixture was diluted to 100 ml with H<sub>2</sub>O (TOD<sub>280</sub><sup>pH1</sup> 94000). It was passed through a column of 200 ml of Diaion SK-1B (NH<sub>4</sub><sup>+</sup>), which was eluted with water to obtain 800 ml of the eluate (TOD<sub>280</sub><sup>pH1</sup> 90900, recovery 97%). It was evaporated *in vacuo* below 40° and co-evaporated to dryness with ethanol. The amber-colored residue was dried *in vacuo* over P<sub>2</sub>O<sub>5</sub> (XV-C). *Rf* 0.10 (solvent 1) and 0.52 (solvent 2).

Method D: Adenosine N-oxide (II) (3.2 g) was refluxed in 30 ml of 5 N NaOH for 15 min. It was diluted to 50 ml with water, and rapidly passed through a column of 120 ml of Amberlite IRC-50 (H<sup>+</sup>), which was



subsequently eluted with water to obtain 2000 ml of the eluate ( $\text{TOD}_{260}^{\text{H}} 93000$ , recovery 94.5%). The eluate was evaporated *in vacuo* below 40° to dryness (XV-D).

Attempts to obtain pure crystals of XV were made. XV-C was dissolved in water and adjusted to pH 3 with HCOOH and the solution was passed through a column of Dowex 1 × 2 (Cl<sup>-</sup>). The effluent was evaporated *in vacuo* to dryness and the residue was crystallized from ethanol-methanol to afford a small amount of crystals of XV·HCl. UV:  $\lambda_{\text{max}}^{\text{H}} 280$ ,  $\lambda_{\text{max}}^{\text{H}_2\text{O}} 271$ ,  $\lambda_{\text{max}}^{\text{H}^{18}} 259$ . *Rf* 0.54 (solvent 2). Recrystallization was unsuccessful.

Conversion of II into XV was not observed when II was treated with Et<sub>3</sub>N–MeOH–H<sub>2</sub>O under reflux or with NaOH–MeOH under reflux. Conversion was incomplete in 2.5 N NaOH. The best molar ratio of 5 N NaOH to II for the adequate conversion was 14. XV prepared should be used for the subsequent reaction as soon as possible. Among the four purification procedures, XV-A was most suitable for the starting material of the subsequent reaction in a large scale, because the volume of the solution was so small that XV was not degraded during the evaporation processes.

**Treatment of XV with Sodium Methyl Xanthate (CS<sub>2</sub>–MeOH–NaOH)**—Dried XV-C, corresponding to 740 mg (2.46 mmol) of II, was dissolved in a mixture of 10 ml of methanol, 600 mg of NaOH and 1.2 g of CS<sub>2</sub> and heated at 180° for 3 hr in an autoclave. Paper chromatography of the reaction mixture (solvent 1) showed 2 spots: *Rf* 0.1 (unidentified) and 0.42 (corresponding to X). The mixture was evaporated to dryness and the residue was triturated with water-ethyl acetate. Insoluble material was collected by filtration and recrystallized from water. 2-Methylthioadenosine (X), 120 mg (yield 15% based on II), was obtained.

**Treatment of XV with CS<sub>2</sub>–MeOH–Pyridine**—(A) At 180° for 3 hr: Dried XV-B, corresponding to 400 mg (1.33 mmol) of II, was dissolved in a mixture of 5 ml of MeOH, 5 ml of pyridine and 2.5 ml of CS<sub>2</sub> and heated at 180° for 3 hr in an autoclave. Reaction mixture was evaporated to dryness and the residue was dissolved in a mixture of dimethylformamide–H<sub>2</sub>O (50 ml). Paper chromatography (solvent 1) of an aliquote revealed 3 spots: *Rf* 0.42 (corresponding to X), 0.27 (corresponding to I) and 0.12 (corresponding to IX). The extract of each spot by 0.1 N HCl was measured spectrophotometrically and the yields of each compound were 19.1% (X), 11.6% (I) and 17.9% (IX), respectively, based on II when calculated from the molecular absorbance of each compound.

(B) At 120° for 3 hr: Dried XV-B was treated at 120° for 3 hr in an autoclave. Paper chromatography of the reaction mixture (solvent 1) revealed 3 spots: *Rf* 0.27 (I), 0.12 (IX) and 0.10 (XV). Two spots corresponding to 2-thioadenosine (IX) and the starting material (XV) were found major.

(C) Under Reflux for 3 hr: Dried XV-B was treated under reflux for 3 hr. The mixture was evaporated to dryness and the residue was suspended in 50 ml of MeOH–H<sub>2</sub>O. Paper chromatography (solvent 1) of the supernatant revealed 3 spots: *Rf* 0.27 (I), 0.10 (XV) and 0.04 (unidentified).

**2-Thioadenosine (IX)**—(A) Treatment of XV with CS<sub>2</sub>–MeOH–Pyridine: Dried XV-C, corresponding to 640 mg (2.13 mmol) of II, was treated with a mixture of 8 ml of methanol, 8 ml of pyridine and 4 ml of CS<sub>2</sub> at 120° for 6 hr in an autoclave. 2-Thioadenosine (IX) was isolated in a yield of 50.1% based on II. Details have been described in the previous paper.<sup>12</sup> The method was, however, unpractical and lack of reproducibility in a large scale preparation.

(B) Treatment of XV with CS<sub>2</sub>–MeOH–Pyridine–H<sub>2</sub>O: Hydrous XV-D, corresponding to 1.60 g (5.35 mmol) of II, was dissolved in H<sub>2</sub>O to make the final volume 5.0 ml (pH 9.8) and to this were added 15 ml of methanol, 20 ml of pyridine and 10 ml of CS<sub>2</sub>. The mixture was heated at 120° for 5 hr in an autoclave (10 kg/cm<sup>2</sup>). It was evaporated to dryness and the residue was triturated with 50 ml of acetone. Insoluble material was collected by filtration and dissolved in 12.5 ml of 2.5 N NH<sub>4</sub>OH, and insoluble sulfur was removed by filtration. The filtrate was added with 37.5 ml of a mixture of *n*-BuOH–glacial acetic acid (2:1). Yellow needles of IX precipitated on cooling were collected and dried, 1.01 g (yield 60.0% based on II).

(C) Treatment of XV with CS<sub>2</sub>–MeOH–H<sub>2</sub>O: Hydrous XV-A, corresponding to 8.0 g (26.7 mmol) of II, was dissolved in water to make the final volume 25 ml (pH 9.0) and to this were added 175 ml of methanol and 50 ml of CS<sub>2</sub>. The homogeneous mixture was heated at 120° for 5 hr. From the reaction mixture 6.40 g (yield 76.0% based on II) of 2-thioadenosine (IX) was isolated.

(D) Treatment of XV with CS<sub>2</sub>–H<sub>2</sub>O: Hydrous XV-A, corresponding to 1.60 g (5.35 mmol) of II, was dissolved in water to make the final volume 40 ml (pH 9.0) and to this was added 10 ml of CS<sub>2</sub>. The heterogeneous mixture was heated at 120° for 5 hr in an autoclave. From the reaction mixture 811 mg (yield 48.0% based on II) of IX was isolated.

**2-Thioadenosine N-Oxide (XVIII)**—The compound (XV-D), corresponding to 3.2 g (10.7 mmol) of II, was treated with a mixture of CS<sub>2</sub> (20 ml)–MeOH (40 ml)–pyridine (40 ml) at room temperature for two months under dark. Insoluble materials separated were removed by filtration and the filtrate was evaporated *in vacuo* to dryness. The residue (3.2 g) was dissolved in a mixture of 50 ml of water and 15 ml of 1 N NaOH and it was filtered. The filtrate was made pH 1 by addition of 10% HCl. The needles separated were collected by filtration, 1.54 g (yield 47.0% based on II), mp above 270°. Recrystallization from water gave pure plates of 2-thioadenosine N-oxide (XVIII), mp above 270°. UV:  $\lambda_{\text{max}}^{\text{H}} (\epsilon) 242$  (16400), 292 (18000),  $\lambda_{\text{max}}^{\text{H}_2\text{O}} 240$  (14100), 260 (14800), 293 (18000),  $\lambda_{\text{max}}^{\text{H}^{18}} 259$  (28100), 293 (15900). *Rf* 0.40 (solvent 2). This compound was unstable in solution to convert negatively charged unidentified compound. NMR spectrum is presented in Table I. *Anal.* Calcd. for C<sub>10</sub>H<sub>13</sub>N<sub>5</sub>O<sub>5</sub>S; C, 38.09; H, 4.16; N, 22.21. Found: C, 37.87; H, 4.15; N, 22.09.

**2-Methylthioadenosine N-Oxide (XIX)**—2-Thioadenosine N-oxide (XVIII), 800 mg (2.5 mmol), was dissolved in 8 ml of 1.25 N NaOH and to this was added 530 mg (3.75 mmol) of  $\text{CH}_3\text{I}$ . The mixture was stirred at room temperature for 3 hr. It was then evaporated *in vacuo* to dryness after neutralization with hydrochloric acid. The residue was applied onto a cellulose column ( $2.7 \times 50$  cm) and the column was eluted with *n*-BuOH- $\text{H}_2\text{O}$  (84:16). Fractions containing UV-absorbing material were evaporated to dryness and the residue was crystallized from *n*-BuOH- $\text{H}_2\text{O}$  (84:16). 2-Methylthioadenosine N-oxide (XIX) was obtained, 307 mg (yield 37.3%), mp 202–212° dec. Recrystallization from water gave 240 mg, mp 214.5–217° dec. UV:  $\lambda_{\text{max}}^{\text{pH}^1}$  (e) nm, 228.5 (17700), 271.5 (16000),  $\lambda_{\text{max}}^{\text{H}_2\text{O}}$  248 (35500), 274 (12500),  $\lambda_{\text{max}}^{\text{pH}^{13}}$  248 (32700), 273 shoulder (12300), 310 shoulder (3400). NMR spectrum is presented in Table I. *Rf* 0.18 (solvent 1) and 0.58 (solvent 2). *Anal.* Calcd. for  $\text{C}_{11}\text{H}_{15}\text{N}_5\text{O}_5\text{S}$ : C, 40.11; H, 4.59; N, 21.27. Found: C, 39.85; H, 4.58; N, 20.94.

**Treatment of 2-Thioadenosine N-Oxide (XVIII) with  $\text{H}_2\text{S}$** —2-Thioadenosine N-oxide (XVIII), 100 mg (0.32 mmol), was suspended in a mixture of 1.5 ml of methanol, 2 ml of pyridine and 0.5 ml of water, and the mixture was saturated with  $\text{H}_2\text{S}$  gas under cooling. It was heated at 120° for 5 hr in an autoclave. The reaction mixture was evaporated *in vacuo* and the residue was dissolved in 1 ml of 2.5 N  $\text{NH}_4\text{OH}$  and filtered. To the filtrate was added a mixture of 3 ml of *n*-BuOH-glacial acetic acid (2:1) and 2-thioadenosine (IX) was separated, 33 mg (yield 33%), mp 184–198° dec. UV:  $\lambda_{\text{max}}^{\text{H}^+}$  nm, 235.5, 292,  $\lambda_{\text{max}}^{\text{H}_2\text{O}}$  230.5, 288.5,  $\lambda_{\text{max}}^{\text{OH}^-}$  242, 283. *Rf* 0.12 (solvent 1).

**Treatment of 2-Thioadenosine N-Oxide (XVIII) with  $\text{CS}_2$** —2-Thioadenosine N-oxide (XVIII), 100 mg (0.32 mmol), was suspended in a mixture of 1.5 ml of methanol, 2 ml of pyridine, 1 ml of  $\text{CS}_2$  and 0.5 ml of water. It was heated at 120° for 5 hr in an autoclave. 2-Thioadenosine (IX) was obtained in a yield of 63% (64 mg), mp 182–199° dec. UV:  $\lambda_{\text{max}}^{\text{H}^+}$  nm, 227, 292.5,  $\lambda_{\text{max}}^{\text{H}_2\text{O}}$  231, 288.5,  $\lambda_{\text{max}}^{\text{pH}^{13}}$  242, 283. *Rf* 0.12 (solvent 1).

**Treatment of 2-Methylthioadenosine N-Oxide (XIX) with  $\text{H}_2\text{S}$** —2-Methylthioadenosine N-oxide (XIX), 100 mg (0.30 mmol), was suspended in a mixture of 1.5 ml of methanol, 2 ml of pyridine and 0.5 ml of water, and this was saturated with  $\text{H}_2\text{S}$  under cooling. It was heated at 120° for 5 hr in an autoclave. The reaction mixture was filtered and the filtrate was evaporated *in vacuo* to dryness. The residue was crystallized from water to yield 79 mg (yield 82%) of 2-methylthioadenosine (X), mp 221.5–223.5°. UV:  $\lambda_{\text{max}}^{\text{H}^+}$  nm, 270,  $\lambda_{\text{max}}^{\text{H}_2\text{O}}$  235.5, 277,  $\lambda_{\text{max}}^{\text{OH}^-}$  233 shoulder, 276.5. *Rf* 0.42 (solvent 1) and 0.64 (solvent 2).

**4-Aminoimidazole-5-carboxamidoxime (XXIIa)**—4-Aminoimidazole-5-carboxamidoxime (XXIIa)·2HCl was obtained in a yield of 91% by treatment of adenine N-oxide (XXIa) with 3 N HCl under reflux according to the method of Stevens, *et al.*<sup>13</sup> Recrystallization from methanol gave pure sample, mp 196° dec. UV:  $\lambda_{\text{max}}^{\text{H}^+}$  nm, 217, 278. Pauly test: orange and 1% ferric chloride test: blue. (lit.<sup>13</sup>) mp 175° dec., UV:  $\lambda_{\text{max}}^{\text{pH}^2}$  nm, 222, 277).

The crude XXIIa·2HCl, 2.15 g (10 mmol), in 2 ml of water was treated with 5 ml (20 mmol) of 4 N NaOH. The precipitates thus formed were collected by filtration and recrystallized from water to afford yellow plates of XXIIa, free base, 400 mg, mp 171–181° dec. UV:  $\lambda_{\text{max}}^{\text{pH}^1}$  (e) nm, 222 (7600), 279 (8900),  $\lambda_{\text{max}}^{\text{H}_2\text{O}}$  218 (8800), 261 (8300),  $\lambda_{\text{max}}^{\text{pH}^{13}}$  276 (9300). *Rf* 0.21 (solvent 1) and 0.58 (solvent 2). *Anal.* Calcd. for  $\text{C}_4\text{H}_7\text{N}_5\text{O}$ : C, 34.04; H, 5.00; N, 49.62. Found: C, 33.83; H, 5.07; N, 49.40. This compound was found unstable in pH 13  $\text{H}_2\text{O}$ .

**4-Aminoimidazole-5-carboxamidine (XXIIIa)**—4-Aminoimidazole-5-carboxamidine (XXIIIa)·2HCl was obtained in a yield of 97.2% by reduction of XXIIa·2HCl in hydrogen atmosphere by use of Raney Ni catalyst according to the method of Stevens, *et al.*<sup>13</sup> UV:  $\lambda_{\text{max}}^{\text{H}^+}$  nm, 282,  $\lambda_{\text{max}}^{\text{H}_2\text{O}}$  285,  $\lambda_{\text{max}}^{\text{OH}^-}$  293. Pauly test: orange. (lit.<sup>13</sup>) UV:  $\lambda_{\text{max}}^{\text{pH}^2}$  nm, 282,  $\lambda_{\text{max}}^{\text{pH}^{6.5}}$  285,  $\lambda_{\text{max}}^{\text{pH}^{12}}$  292).

**2-Thioadenine (XXVa)**—(A) From 4-Aminoimidazole-5-carboxamidine (XXIIIa): 4-Aminoimidazole-5-carboxamidine (XXIIIa)·2HCl, 140 mg (0.17 mmol), was dissolved in a mixture of 2.8 ml of methanol, 3.5 ml of pyridine and 1.4 ml of  $\text{CS}_2$ . The mixture was allowed to stand at room temperature for 5 days. The precipitated material was collected by filtration and dried, 149 mg. It was recrystallized from 5%  $\text{H}_2\text{SO}_4$  to afford 120 mg (yield 75%) of 2-thioadenine (XXVa)·0.5 $\text{H}_2\text{SO}_4$ ·0.5 $\text{H}_2\text{O}$ , mp above 300°. UV:  $\lambda_{\text{max}}^{\text{H}^+}$  nm, 239, 285,  $\lambda_{\text{max}}^{\text{H}_2\text{O}}$  230, 282,  $\lambda_{\text{max}}^{\text{OH}^-}$  240, 276. *Rf* 0.19 (solvent 1). The sample was identical with an authentic sample<sup>24,25</sup> in respect to IR (KBr) spectrum.

(B) From 4-Aminoimidazole-5-carboxamidoxime (XXIIa): 4-Aminoimidazole-5-carboxamidoxime (XXIIa), free base, 1.0 g (7.1 mmol), was heated in a mixture of 7 ml of methanol and 15 ml of  $\text{CS}_2$  in an autoclave at 120° for 5 hr. The green-colored heterogeneous reaction mixture was evaporated *in vacuo* to dryness. The residue was dissolved in 50 ml of 2.5 N  $\text{NH}_4\text{OH}$  and filtered. The filtrate was acidified to pH 1 with  $\text{H}_2\text{SO}_4$ . The crystalline precipitates of 2-thioadenine (XXVa)·0.5 $\text{H}_2\text{SO}_4$ ·0.5 $\text{H}_2\text{O}$  were collected by filtration and dried, 800 mg (yield 50.0%). Recrystallization gave pure material.

**5-Amino-1-benzylimidazole-4-carboxamidoxime (XXIIb)**—9-Benzyladenine N-oxide (XXIb)<sup>15</sup> which was prepared from 9-benzyladenine (XXb),<sup>26</sup> 2.0 g (8.3 mmol), was boiled in 40 ml of 3 N HCl for 10 min. The mixture was evaporated *in vacuo* to dryness and the residue was redissolved in 10 ml of water. It was adjusted to pH 12 with 5 N NaOH. The needles appeared were collected by filtration, 5-amino-1-benzylimidazole-4-carboxamidoxime (XXIIb), 1.10 g (yield 57.4%). Recrystallization from ethanol-water gave slightly green-colored needles, mp 193–201° dec. UV:  $\lambda_{\text{max}}^{\text{pH}^1}$  (e) nm, 274 (8600),  $\lambda_{\text{max}}^{\text{H}_2\text{O}}$  233 shoulder (8800),

262 (8900),  $\lambda_{\max}^{\text{pH } 13}$  262 (8900). The compound was found unstable in pH 13  $\text{H}_2\text{O}$ . *Rf* 0.53 (solvent 1) and 0.72 (solvent 2). Ferric chloride test: blue. *Anal.* Calcd. for  $\text{C}_{11}\text{H}_{13}\text{N}_5\text{O}$ : C, 57.13; H, 5.67; N, 30.29. Found: C, 57.23; H, 5.48; N, 29.94.

**9-Benzyl-2-thioadenine (XXVb)**—5-Amino-1-benzylimidazole-4-carboxamidoxime (XXIIb), 500 mg (2.2 mmol), was dissolved in a mixture of 15 ml of methanol, 15 ml of pyridine, 5 ml of water and 6 ml of  $\text{CS}_2$  and the mixture was heated at  $125^\circ$  for 5 hr in an autoclave. The reaction mixture was evaporated *in vacuo* to dryness. The residue was dissolved in 10 ml of hot ethanol and filtered. The filtrate was cooled and the crystalline precipitate separated was collected by filtration. 9-Benzyl-2-thioadenine (XXb) was obtained in a yield of 39% (230 mg). Recrystallization from ethanol-water gave a pure sample, mp  $261\text{--}264^\circ$  dec. UV:  $\lambda_{\max}^{\text{EtOH-pH } 1 \text{ H}_2\text{O}(7:93)}$  ( $\epsilon$ ) nm, 239 (15400), 291 (17000),  $\lambda_{\max}^{\text{EtOH-H}_2\text{O}(7:93)}$  233 (17700), 287 (16700),  $\lambda_{\max}^{\text{EtOH-pH } 13 \text{ H}_2\text{O}(7:93)}$  243 (19300), 280 (13400). *Rf* 0.69 (solvent 1) and 0.79 (solvent 2). *Anal.* Calcd. for  $\text{C}_{12}\text{H}_{11}\text{N}_5\text{S}\cdot 0.5\text{H}_2\text{O}$ : C, 54.12; H, 4.54; N, 26.30. Found: C, 53.77; H, 4.09; N, 26.25.

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