described for the preparation of **10**. Obtained was **11** (16.8 g, 60%), bp 130° (1 mm). *Anal.* $C_{14}H_{24}N_2O_4$) C, H, N.

1-Methyl-3-*n*-butoxymethyl-5-ethyl-5-phenylbarbituric Acid (12). LiH (0.8 g, 0.1 mol) was added to an ice-cold, stirred solution of 1-methyl-5-ethyl-5-phenylbarbituric acid (24.2 g, 0.1 mol) in DMF (250 ml). After 90 min $ClCH_2OC_4H_9$ (12.3 g, 0.1 mol) was added dropwise over a period of 30 min. The solution was stirred for 2 hr and then poured into ice–H_2O (600 g). The solid precipitate was filtered, washed with H_2O, and crystallized from aqueous EtOH to give **12** (27.3 g, 82%), mp 55.5–56.5°. *Anal.* ($C_{18}H_{24}N_2O_4$) C, H, N.

1-Benzyl-3-methoxymethyl-5-ethyl-5-phenylbarbituric Acid (13). LiH (0.176 g, 0.022 mol) was added to a solution of **2** (6.44 g, 0.02 mol) in DMF (25 ml). After 30 min $ClCH_2OCH_3$ (1.77 g, 0.022 mol) was added dropwise. The solution was stirred for 2 hr and then poured into ice–H_2O (100 g). The solid precipitate was filtered, washed with H_2O, and crystallized from Et_2O to provide **13** (4.9 g, 67%), mp 78–79°. *Anal.* ($C_{21}H_{22}N_2O_4$) C, H, N.

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Platelet Aggregation Inhibitors. 6.[1] 2-Thioadenosine Derivatives

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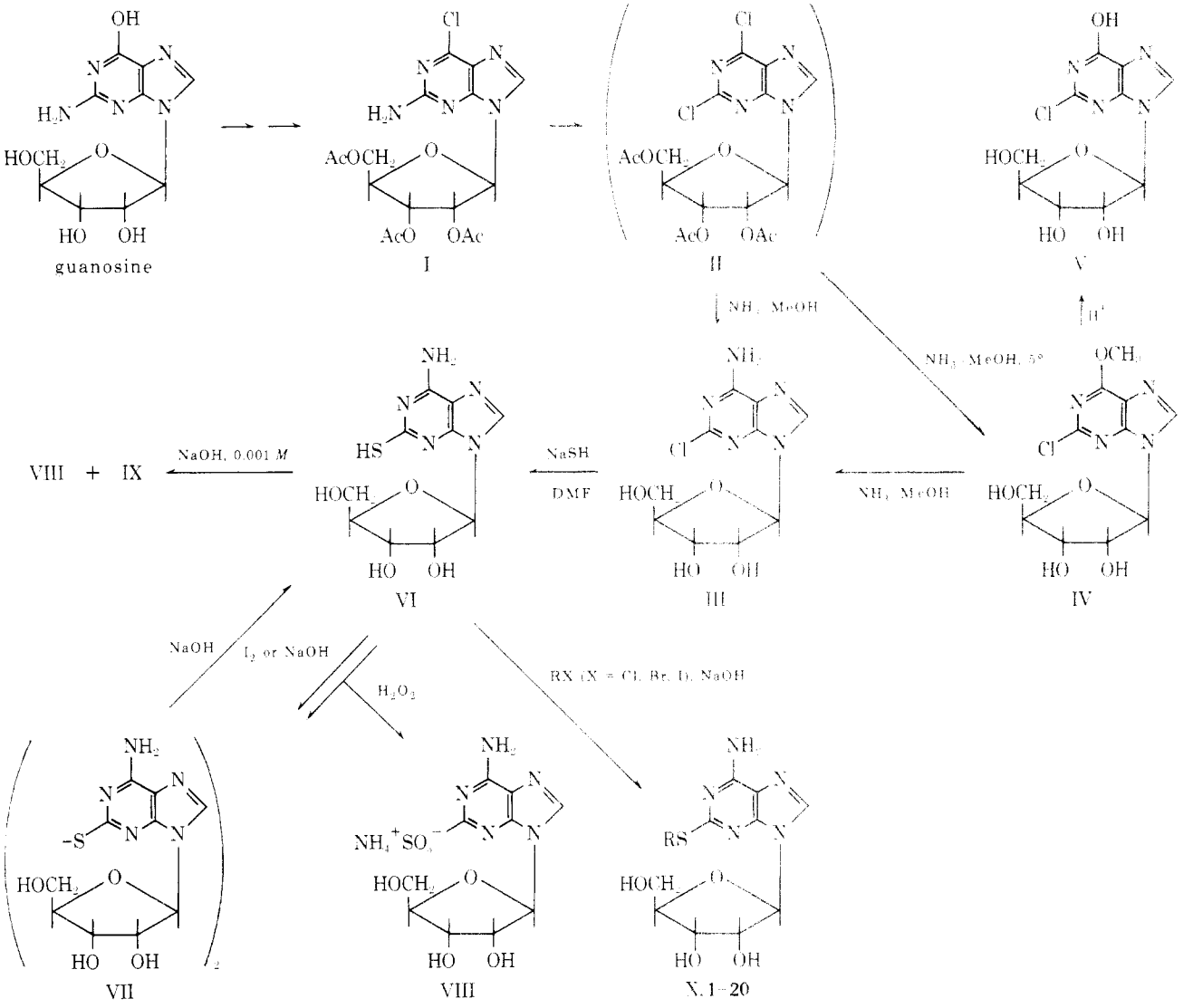
2-Thioadenosine (VI) was prepared in an overall yield of 7.6% from guanosine *via* 2′,3′,5′-tri-*O*-acetyl-6-chloroguanosine (I), 2,6-dichloro-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)purine (II), and 2-chloroadenosine (III). Oxidations of VI with iodine and hydrogen peroxide gave disulfide VII and sulfonate VIII, respectively. VI was readily oxidized to disulfide VII or unidentified substance(s) in alkali media without special oxidants. Various S-substituted 2-thioadenosines (X, 1–20) were prepared in 20–80% yields by reaction of VI with the requisite halide in the presence of alkali. Whereas VI, VII, and IX were inactive as inhibitors of platelet aggregation, most of the compounds X were strong inhibitors of platelet aggregation. Thus, 2-ethyl- (**2**), 2-*n*-amyl- (**3**), 2-isoamyl- (**4**), 2-*n*-hexyl- (**5**), 2-*n*-heptyl- (**6**), 2-*n*-octyl- (**7**), 2-*n*-nonyl- (**8**), 2-*n*-decyl- (**9**), 2-(2-ethyl-*n*-hexyl)- (**10**), 2-cyclopentyl- (**11**), 2-cyclohexyl- (**12**), 2-cyclohexylmethyl- (**13**), 2-benzyl- (**14**), 2-(*p*-chlorobenzyl)- (**15**), 2-(*p*-nitrobenzyl)- (**16**), 2-allyl- (**17**), 2-(*trans*-crotyl)- (**18**), and 2-(β-methallyl)thioadenosine (**19**) inhibited 50–100% of ADP- and collagen-induced rabbit platelet aggregation at 10^{-4} *M*. The inhibitory activity of **11** and **12** was the most powerful showing 50–90% inhibition at 10^{-5} *M* and comparable to that of III. Some of the compounds (**14** and **17**) were also active when tested against ADP-induced human platelet aggregation. These active compounds were characterized by long-lasting activity as they were not degraded by adenosine deaminase bound to platelets with incubation at 37° for 120 min.

Predominance of platelets in the white clot of blood in arteries has attracted attention of their primary importance in arterial occlusion or thrombosis.[2] Standard anticoagulant therapy is of little effect against arterial thrombosis and it is suggested that the inhibitors of platelet aggregation might be useful agents that prevent arterial thrombosis.[3] Several compounds that inhibit adenosine 5′-diphosphate (ADP)- and/or collagen-induced platelet aggregation have been investigated,[4,5] but few of them have been evaluated as antithrombotic agents because of their undesirable side effects or toxicity.

Adenosine, a structural analog of ADP, is a powerful inhibitor of platelet aggregation,[6] but it has intense effects on the cardiovascular system and is readily inactivated by contact with erythrocytes[7] or platelets.[8,9] Among the derivatives of adenosine, 2-chloroadenosine[10] has more potent and long-lasting inhibitory action, but with rather serious undesirable side effects.[11] Our previous studies have shown that certain N^6-substituted adenosines are also active inhibitors with long-lasting activity.[12-14] 2-Methylthioadenosine[15] and its 5′-monophosphate ester[16] are known as less active inhibitors; nevertheless, the ester has been recently evaluated by Michael, *et al.*,[16] to be an antithrombotic agent owing to its nontoxicity. This time, in order to obtain further information on the structure–activity relationships in the series of 2-methylthioadenosine derivatives, some additional 2-thioadenosine derivatives were prepared and examined for the inhibitory effect of platelet aggregation. This study revealed that activity is distributed broadly throughout the series.

Synthesis. 2-Alkylthioadenosines and their derivatives have been synthesized by (1) condensation of 2-alkylthioadenines with appropriate ribose derivatives,[17-20] (2) amination of the condensation products of 2-alkylthio-6-chloropurines[19] or 2,6-dimethylthiopurine[21] with ribose derivatives, or (3) reaction of alkylmercaptans with 2-chloroadenosine.[19,22] Several 2-alkylthioadenosines such as 2-methyl-,[17-19,22] 2-ethyl-,[19] 2-*n*- (and -iso-) propyl-,[19] and 2-*n*- (and -iso-) butylthioadenosines[19] have so far been synthesized according to either of the above procedures. However, the procedures are not readily accessible routes for the synthesis of a variety of 2-thioadenosine derivatives. In procedures 1 and 2, a purine having a given thio substituent at the 2 position has to be prepared in every case prior to condensation, and in procedure 3 the synthesis is restricted by availability of the required mercaptan. This time attempts were made to prepare a variety of S-substituted 2-thioadenosines X by reaction of 2-thioadenosine (VI) obtained from 2-chloroadenosine (III) with readily available halides (Scheme I).

Scheme I



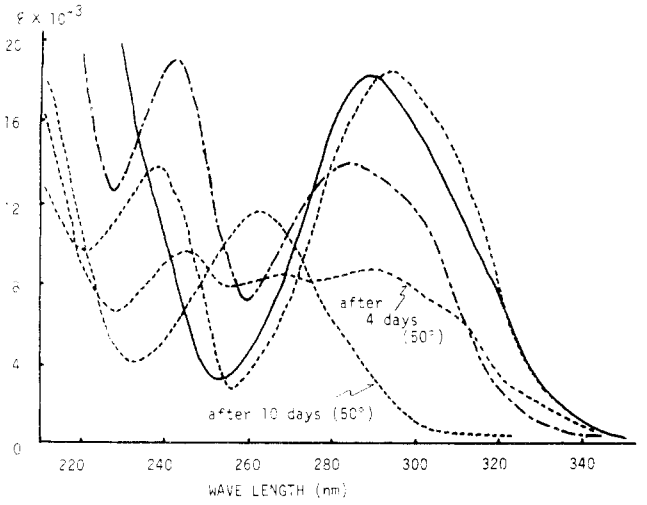


Figure 1. Uv spectra of 2-thioadenosine (VI) dissolved at 0.001 *M* in the presence of 2 equiv of NaOH. Spectra were taken in pH 1, HCl (- - - - -); pH 3.2, 0.1 *M* glycine-HCl buffer (———); and pH 12.8, NaOH (— — —).

2-Chloroadenosine (III), which has been prepared *via* tedious steps including condensation of 2-chloroadenine, 2,8-dichloroadenine, and 2,6-dichloropurine with ribose derivatives,[17,18,22-24] was synthesized conveniently from guanosine *via* 2′,3′,5′-tri-*O*-acetyl-6-chloroguanosine (I)[25] and 2,6-dichloro-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-purine (II)[26] according to the method of Gerster, *et al.*, and subsequently of Montgomery and Hewson.[24] Treatment of the crude syrup of II with NH_3–MeOH at room temperature for 7 days afforded III in a yield of 29% from I. An overall yield of III from guanosine was 10.5%. When II was treated with NH_3–MeOH at 5° overnight 2-chloro-6-methoxy-9-β-D-ribofuranosylpurine (IV) was obtained besides III. IV, which was readily converted into 2-chloroinosine (V) by acid, was turned into III upon heating with NH_3–MeOH. Thus, the formation of III from II seemed to proceed *via* IV.

2-Thioadenosine (VI) was obtained by treatment of III with NaSH in dimethylformamide in a yield of 72%. Oxidation of VI with iodine gave 2-thioadenosine disulfide (VII), and hydrogen peroxide oxidation of VI afforded adenosine 2-sulfonate (VIII) and VII. 2-Thioadenosine (VI) was readily oxidized in aqueous alkaline media without special oxidants. Thus, ultraviolet absorption maximum of VI (293 nm at pH 1) shifted to 263 nm during the preservation at 0.001 *M* concentration and 20–50° for 10 days in the presence of alkali (Figure 1). The products which could not be isolated were a mixture of sulfinate or sulfenate derivative IX and VIII analyzed by paper chromatography and electrophoresis. At 0.1 *M* concentration VI was gradually converted into VII in the presence of an equimo-

lar amount of alkali. VI was completely transformed to disulfide VII during paper electrophoresis at pH 7.5. When 2-thioinosine,[27] a structural analog of VI, was treated under the same conditions, it was readily degraded at 0.001 *M* concentration while it was quite stable at 0.1 *M* concentration. When disulfide VII was dissolved in an aqueous alkali under heated conditions, VI was obtained in a yield of 72% accompanying other unidentified product(s).

Treatment of 2-thioadenosine (VI) with MeI and EtI in the presence of aqueous alkali gave known 2-methylthioadenosine (X, 1) and 2-ethylthioadenosine (X, 2), respectively. Accordingly, treatment of VI with a 1–10 equimolar amount of several kinds of halides in the presence of a 1–3 equimolar amount of alkali afforded 2-alkylthioadenosines (X, 3–10), 2-cycloalkylthioadenosines (X, 11–13), 2-benzylthioadenosines (X, 14–16), 2-allylthioadenosines (X, 17–19), and 2-dialkylaminoalkylthioadenosine (X, 20). The structures and physical data of X are summarized in Table I.

Pharmacological Results and Discussion. 2-Thioadenosine (VI) and its derivatives VII, VIII, and X were tested *in vitro* as inhibitors of ADP- and collagen-induced rabbit platelet aggregation according to the method of Born and Cross.[28] Platelet-rich citrated plasma (PRCP) was pretreated with a test compound at 37° for 3 min. The inhibitory activity of every compound was estimated by the extent of the decrease in the optical density of PRCP after the addition of ADP or collagen. Compounds insoluble in saline were tested with solutions of dimethyl sulfoxide (DMSO). Although DMSO had slight inhibitory effect against both ADP- and collagen-induced aggregation,[14,29] adenosine dissolved in the solvent showed considerable inhibition as in saline and it was a useful solvent (see paragraph at the end of paper regarding supplementary material). Inhibition per cent and relative potency (Rad) of every compound to adenosine when it was treated for 3 min prior to addition of aggregation agents are listed in Table II. Compounds that showed a Rad value greater than 0.5 at 10^{-4} *M* were considered active inhibitors.

2-Thioadenosine (VI), disulfide (VII), and sulfonate (VIII) were inactive whereas most of the S-substituted 2-thioadenosines (X) were found active. All the compounds (X) except 1 and 20 inhibited 50–100% of ADP- and collagen-induced platelet aggregation at 10^{-4} *M*.

Among the alkyl-substituted derivatives 1–10, compounds 2–10 showed inhibition with a Rad value greater than 0.7 at 10^{-4} *M* against both ADP- and collagen-induced aggregation. The inhibitory activity of compounds 2–10 was much stronger than that of 2-methylthioadenosine (1)[15,16] which was almost inactive (Rad = 0.2) in our assay system. 2-Ethylthioadenosine (2) has been previously tested by Maguire, *et al.*,[19] and reported to be an inactive compound against sheep platelet aggregation. In the present study, however, 2 exhibited considerable inhibitory effect. The larger alkyl-substituted 2-thioadenosines such as 2-*n*-amyl- (3), 2-isoamyl- (4), 2-*n*-hexyl- (5), 2-*n*-heptyl- (6), and 2-*n*-octylthioadenosine (7) showed stronger inhibitory effect than adenosine (Rad ≤ 1.0) or 2-chloroadenosine (III). The activity of compounds 2–10 was concentration dependent and these were much less active than adenosine at 10^{-5} *M* with the only one exception that compound 10 bearing a branched β-carbon atom in the substituent showed considerable inhibition at the low concentration.

Cycloalkyl- or cycloalkylmethyl-substituted derivatives 11–13 were also as active as adenosine at 10^{-4} *M*. 2-Cyclopentyl- (11) and 2-cyclohexylthioadenosine (12) both having branched α-carbon atoms in the substituents inhibited 50–90% of aggregation at 10^{-5} *M* whereas 2-cyclohexylmethylthioadenosine (13) having a branched β-carbon atom in the substituent was not so active at 10^{-5} *M*. Comparisons on the inhibitory activity of 11 with that of 3 and 12 with 5 were made. It is interesting that *n*-alkyl-substituted derivatives 3 and 5 were almost inactive at 10^{-5} *M* while cycloalkyl-substituted derivatives 11 and 12 were active even at the concentration lower than 10^{-5} *M* against both ADP- and collagen-induced aggregation. Representative profiles are presented in Figure 2 (see also paragraph at the end of paper regarding supplementary material). Thus, 11 and 12 were the most powerful inhibitors among the S-substituted 2-thioadenosines (X), and the inhibitory activity was comparable to that of III.

2-Benzylthioadenosine (14) and its derivatives 15 and 16 and 2-allylthioadenosine (17) and its derivatives 18 and 19 showed inhibition with Rad around 1.0 at 10^{-4} *M* but Rad less than 0.5 at 10^{-5} *M*. The inhibitory effect of 2-diethylaminoethylthioadenosine (20) was different from that of other S-substituted 2-thioadenosines; it was inhibitory against collagen-induced aggregation but much less against ADP-induced aggregation.

Inhibitory activity of S-substituted 2-thioadenosines against ADP-induced aggregation increased in the following order: HS–, SO_3^{--}, CH_3S- < CH_3CH_2S- < $-CH_2CH_2S-$, $-C_6H_4-CH_2S-$, $-CH{=}CCH_2S-$ < >CHS–, and the tendency was same in the case of inhibition of collagen-induced aggregation. The larger the substituent was and the more the substituent was hindered sterically, the greater the degree of inhibitory potency of the compound increased. Bulkiness and hydrophobic character of the substituent would be required for the effective inhibition.

Some of the compounds (X) were also tested as inhibitors of washed rabbit platelet aggregation. 2-*n*-Amyl- (3), 2-benzyl- (14), and 2-allylthioadenosine (17) inhibited both ADP- and collagen-induced aggregation of washed platelet suspension in the presence of Ca^{2+} and bovine serum albumin at 10^{-4} *M* (Table III). Compounds 14 and 17 were as active as adenosine when compared at a single concentration (10^{-4} *M*) as inhibitors of ADP-induced human platelet aggregation (Figure 3). Deaggregation potency of rabbit platelet aggregates by 3, 11, and 14 was investigated. They strongly deaggregated ADP-induced aggregates but could not deaggregate irreversible aggregates mediated by collagen (see paragraph at the end of paper regarding supplementary material).

The active compounds were incubated with rabbit PRCP at 37° for the longer intervals before the addition of ADP (Figure 4). At 10^{-4} *M* (Figure 4A), the per cent inhibition of adenosine increased for 10–40 min of incubation to 65% which completely decreased thereafter, while that of 2-chloroadenosine (III) increased for 10–40 min of incubation to 90% which was maintained for up to 120 min. The results agreed with those of other investigators.[8,10,28,30] The pattern of inhibitory action of the active compounds 3, 4, 11, 14, 15, 16, and 17 was very similar to that of III and the per cent inhibition increased for 10–40 min of incubation to around 90% which was maintained for up to 120 min. At 10^{-5} *M* (Figure 4B), 11 showed about 80% inhibition for 10–120 min of incubation as III. The activity of 3, 14, and 17 which was almost inactive at the 3-min interval of incubation increased to 30–40% as the incubation time proceeded whereas that of adenosine was completely lost with prolonged incubation. The results indicated that these compounds were strong inhibitors of platelet aggregation characterized by long-lasting activity.

Rozenberg and Holmsen[9] concluded that loss of the in-

Table I. S-Substituted 2-Thioadenosines X

No.	Substituent	R	Halide	Molar ratio[a]	Rxn conditions[b]	Purifcn solvent[c]	Yield, %
1	2-Methyl[e]	CH_3–	MeI	I	A	1	60.5
2	2-Ethyl[f]	C_2H_5–	EtI	I	A	1	80.3
3	2-*n*-Amyl	$CH_3(CH_2)_4$–	*n*-$C_5H_{11}Br$	II	B	1	78.1
4	2-Isoamyl	$(CH_3)_2CH(CH_2)_2$–	*i*-$C_5H_{11}Br$	II	C	2	64.2
5	2-*n*-Hexyl	$CH_3(CH_2)_5$–	*n*-$C_6H_{13}Br$	II	C	2	52.7
6	2-*n*-Heptyl	$CH_3(CH_2)_6$–	*n*-$C_7H_{15}Br$	II	C	2	30.8
7	2-*n*-Octyl	$CH_3(CH_2)_7$–	*n*-$C_8H_{17}Br$	II	C	2	58.6
8	2-*n*-Nonyl	$CH_3(CH_2)_8$–	*n*-$C_9H_{19}Br$	II	C	2	78.5
9	2-*n*-Decyl	$CH_3(CH_2)_9$–	*n*-$C_{10}H_{21}Br$	II	C	2	52.3
10	2-(2-Ethyl-*n*-hexyl)	$CH_3(CH_2)_3CH(C_2H_5)CH_2$–	2-Et-*n*-$C_6H_{12}Br$	II	C	2	76.5
11	2-Cyclopentyl	c-C_5H_9–	c-C_5H_9Cl	II	D	3	75.5
12	2-Cyclohexyl	c-C_6H_{11}–	c-$C_6H_{11}Br$	II	D	2	41.4
13	2-Cyclohexylmethyl	c-$C_6H_{11}CH_2$–	c-$C_6H_{11}CH_2Br$	II	D	2	60.0
14	2-Benzyl	$C_6H_5CH_2$–	$C_6H_5CH_2Br$	I	A	2	72.8
15	2-(*p*-Chlorobenzyl)	*p*-$ClC_6H_5CH_2$–	*p*-$ClC_6H_4CH_2Cl$	I	A	3	38.6
16	2-(*p*-Nitrobenzyl)	*p*-$NO_2C_6H_5CH_2$–	*p*-$NO_2C_6H_4CH_2Br$	I	A	2	36.1
17	2-Allyl[h]	CH_2=$CHCH_2$–	CH_2=$CHCH_2Br$	I	A	3	75.6
18	2-(*trans*-Crotyl)[h]	CH_3CH=$CHCH_2$–	*trans*-CH_3CH=$CHCH_2Cl$	I	A	1	62.0
19	2-(β-Methallyl)[h]	CH_2=$C(CH_3)CH_2$–	CH_2=CCH_3CH_2Cl	I	C	1	69.8
20	2-Diethylaminoethyl	$(C_2H_5)_2NC_2H_4$–	$Et_2NC_2H_4Cl \cdot HCl$	III	A	3	26.5

[a]Molar ratio (VI–0.25 *N* NaOH–halide): I, 1:1:1; II, 1:3.3:10; III, 1:2.4. [b]Reactions conditions: A, at room temperature overnight; B, at 50° for 2 days; C, with EtOH at room temperature overnight; D, with EtOH at 50° for 2 days. [c]Purification

Table II. Inhibition of Rabbit Platelet Aggregation by 2-Thioadenosine Derivatives

No.	Compound	Solvent[a]	Inhibition of ADP-induced aggregation[b] 10^{-4} *M* %	Rad	10^{-5} *M* %	Rad	Inhibition of collagen-induced aggregation[b,c] 10^{-4} *M* %	Rad	10^{-5} *M* %	Rad
VI	2-Thioadenosine	2	10	0.2			36	0.4		
VII	2-Thioadenosine disulfide	3	6	0.1			24	0.3		
VIII	Adenosine 2-sulfonate	1	12	0.2			0	0		
X, 1	2-Methylthioadenosine	2	12	0.2			25	0.3		
2	2-Ethylthioadenosine	2	60	0.7	0	0	69	0.8	16	0.3
3	2-*n*-Amylthioadenosine	2	78	1.2	34	0.4	89	1.5	14	0.4
4	2-Isoamylthioadenosine	2	70	1.1	38	0.5	98	1.6	0	0
5	2-*n*-Hexylthioadenosine	3	80	1.6	7	0.2	94	1.0	20	0.4
6	2-*n*-Heptylthioadenosine	3	80	1.6	0	0	94	1.0	15	0.2
7	2-*n*-Octylthioadenosine	3	67	1.3	11	0.2	93	1.0	20	0.3
8	2-*n*-Nonylthioadenosine	3	37	0.7	0	0	91	1.0	15	0.2
9	2-*n*-Decylthioadenosine	3	35	0.7	0	0	86	0.9	9	0.1
10	2-(2-Ethyl-*n*-hexyl)thioadenosine	3	90	1.0	51	0.6	92	1.0	78	0.9
11	2-Cyclopentylthioadenosine	2	100	1.4	67	0.8	90	1.2	55	0.8
12	2-Cyclohexylthioadenosine	3	100	1.1	79	0.9	93	1.0	87	1.0
13	2-Cyclohexylmethylthioadenosine	3	99	1.2	17	0.4	96	1.1	56	0.7
14	2-Benzylthioadenosine	2	85	1.0	36	0.4	92	1.1	26	0.4
15	2-(*p*-Chlorobenzyl)thioadenosine	2	86	1.1	0	0	88	1.1	31	0.4
16	2-(*p*-Nitrobenzyl)thioadenosine	2	88	1.1	0	0	95	1.4	30	0.4
17	2-Allylthioadenosine	2	77	0.9	23	0.3	62	0.8	0	0
18	2-(*trans*-Crotyl)thioadenosine	2	58	0.7	29	0.4	80	1.0	30	0.4
19	2-(β-Methallyl)thioadenosine	2	84	1.1	32	0.5	97	1.1	28	0.5
20	2-Diethylaminoethylthioadenosine	1	33	0.5			86	1.1		
III	2-Chloroadenosine	2	93	1.1	68	0.9	93	1.0	87	1.0
	Adenosine	1	50–70	1.0			80–90	1.0		
		2	50–95	1.0	50–90	1.0	60–90	1.0	30–80	1.0
		3	50–90	1.0	30–90	1.0	80–95	1.0	70–90	1.0

[a]Compounds dissolved in the following solvents at 10^{-4} or 10^{-5} *M* (final concentration) were incubated with buffered PRCP (1 ml) at 37° for 3 min before addition of ADP or collagen: (1) saline, 10 μl; (2) saline, 100 μl; (3) DMSO, 10 μl. [b]Inhibition percentages were not absolute and representative ones are listed in the table. Rad, average values of 2–6 experiments of inhibition of every compound to adenosine compared under the same conditions was a direct measure of potency of inhibition, and the range of Rad was ±0.1. [c]Collagen; optical density was 0.5, 1.0, or 2.0.

hibitory effect of adenosine is due to its disappearance from the plasma by conversion in plasma to inosine and hypoxanthine and incorporation into platelets. Our previous results[14] showed that the activity of adenosine is not lost and not even a trace of inosine could be detected when adenosine was incubated with platelet-poor plasma at 37° for 120 min. The results agreed with those by Packlam, *et al.*,[8] that the loss of activity of adenosine was partly due to the enzyme bound to native platelets and differed from the hypotheses proposed by Born[10] and Holmsen and Rozenberg[31] who concluded that the degrading activity was present in plasma. Adenosine, III, and compounds 3, 11, 14, and 17 were incubated with washed platelet suspension at 37° for 120 min, and the supernatant fluid was examined spectrophotometrically. The results (Table IV) showed that adenosine had completely disappeared and had been converted into inosine or hypoxanthine, while III, 3, 11, 14, and 17 remained intact in the supernatant fluid. It might be concluded that the long-lasting activity of III and S-substituted 2-thioadenosines was due to the resistance of these compounds to adenosine deaminase bound to platelet membrane and to incorporation into platelets.

In summary, various S-substituted 2-thioadenosines were found active inhibitors of platelet aggregation characterized by long-lasting activity and some of them were

Mp, °C	Paper chromatography[d] $R_{f(1)}$	$R_{f(2)}$	Uv, λ max, nm (ε × 10^{-3}) pH 1	H_2O	pH 13	Formula
228–229.5	0.40	0.68	270 (17.3)	235 (22.3), 277 (15.6)	234.5 (22.3), 277 (15.4)	$C_{11}H_{16}O_4N_6S \cdot 0.5H_2O$
209–210	0.56	0.77	272 (16.5)	236 (20.8), 277 (14.8)	236 (20.5), 278 (14.6)	$C_{12}H_{17}O_4N_6S \cdot 0.25H_2O$
180–181	0.77	0.88	272 (16.1)	237 (21.0), 278 (14.6)	236 (22.0), 279 (14.7)	$C_{15}H_{23}O_4N_6S$
179–181	0.82	0.92	272 (15.9)	237 (20.6), 278 (14.7)	237 (20.2), 278 (14.2)	$C_{15}H_{23}O_4N_6S$
166–169	0.82	0.88	272 (15.3)	237 (18.7), 278 (13.4)	237 (18.3), 279 (13.3)	$C_{16}H_{25}O_4N_6S \cdot 0.25C_2H_5OH$
157–161	0.80	0.86		238 (23.7),[g] 280 (15.3)[g]		$C_{17}H_{27}O_4N_6S \cdot 0.5C_2H_5OH$
155–158	0.83	0.86		238 (23.1),[g] 280 (15.0)[g]		$C_{18}H_{29}O_4N_6S$
146–151	0.86	0.90		238 (21.7),[g] 279.5 (13.9)[g]		$C_{19}H_{31}O_4N_6S$
147–151	0.88	0.89		238 (24.1),[g] 279.5 (15.5)[g]		$C_{20}H_{33}O_4N_6S \cdot 0.5C_2H_5OH$
151.5–153.5	0.87	0.90		238.5 (23.7),[g] 280 (15.2)[g]		$C_{19}H_{29}O_4N_6S$
223–224.5	0.75	0.86	273 (16.4)	237 (20.4), 278 (14.9)	236 (21.4), 279 (15.0)	$C_{16}H_{21}O_4N_6S$
225–227.5	0.77	0.86	273 (16.2)	237 (20.8), 278 (13.6)	237 (20.2), 279 (13.4)	$C_{16}H_{23}O_4N_6S \cdot 0.25C_2H_5OH$
226.5–228.5	0.82	0.91	273 (16.0)	237 (21.1), 278 (14.6)	236 (21.9), 279 (14.6)	$C_{17}H_{25}O_4N_6S$
192.5–195	0.70	0.80	272.5 (16.4)	235 (23.9), 278 (15.9)	234 (24.6), 278 (15.9)	$C_{17}H_{19}O_4N_6S \cdot 0.5C_2H_5OH \cdot 0.5H_2O$
208.5–209.5	0.81	0.96	272.5 (16.4)	233.5 (28.8), 278 (16.4)	278 (15.9)	$C_{17}H_{18}O_4N_6SCl \cdot 0.33C_2H_5OH$
130.5–132	0.69	0.85	272 (21.8)	233 (24.7), 277 (23.8)	233 (23.3), 278 (22.3)	$C_{17}H_{15}O_6N_6S \cdot 0.5C_2H_5OH \cdot 0.5H_2O$
161–163	0.65	0.93	272 (16.1)	235 (21.4), 278 (15.0)	234 (21.7), 278 (14.9)	$C_{13}H_{17}O_4N_6S \cdot 0.75C_2H_5OH$
95–98	0.69	0.85	272.5 (16.0)	236 (20.8), 277.5 (15.0)	236 (20.9), 277.5 (15.1)	$C_{14}H_{19}O_4N_6S \cdot 0.5H_2O$
119–123	0.71	0.86	272.5 (15.7)	235 (20.1), 278 (14.8)	235.5 (20.0), 278 (14.8)	$C_{14}H_{19}O_4N_6S \cdot 0.5H_2O$
217–218 dec	0.09	0.66	270.5 (15.4)	232 (24.2), 275 (15.8)	235 (21.5), 278 (15.4)	$C_{16}H_{25}O_4N_6S \cdot HCl$

solvent: 1, H_2O; 2, EtOH–H_2O; 3, EtOH. [d]See Experimental Section. [e]Lit. mp 227°,[17] 222–223°,[18] 226–227°,[19] and 220° dec.[22] [f]Lit. mp 211–212°.[19] [g]Spectra were taken in EtOH instead of H_2O. [h]Presence of allyl group was confirmed by $KMnO_4$ oxidation.

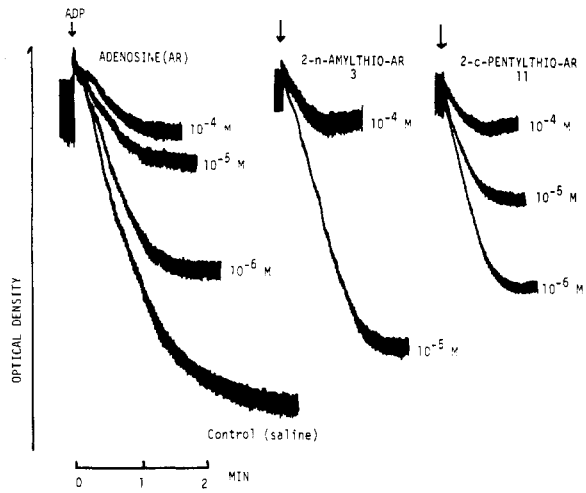


Figure 2. Inhibitory profiles of rabbit platelet aggregation by S-substituted 2-thioadenosines. Buffered PRCP (1.0 ml) was treated with a solution of each of the test compounds in 100 μl of saline at 37° for 3 min and was then challenged with ADP (indicated by arrows).

as active as 2-chloroadenosine. Since Maguire, *et al.*,[19] have recently reported that the coronary vasodilator potency of 2-alkylthioadenosines increased with increasing length of the alkyl chain by comparison of four compounds including 2-methyl-, 2-ethyl-, 2-*n*-propyl-, and 2-isopropylthioadenosine, the coronary vasodilator activity of the compounds newly synthesized in the present study is now under investigation.

Experimental Section†

2-Chloroadenosine (III) and 2-Chloro-6-methoxy-9-β-D-ribofuranosylpurine (IV) from Guanosine. 2′,3′,5′-Tri-*O*-acetylguanosine[32] obtained in a yield of 83% (10-g scale preparation) or 54% (100-g scale preparation) from guanosine was converted into 2′,3′,5′-tri-*O*-acetyl-6-chloroguanosine (I)[25] in a yield of 44% (10-g scale preparation) or 21% (100-g scale preparation).

I (10.0 g) was treated with HCl and $NaNO_2$ to obtain about 3 g of a syrup of 2,6-dichloro-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)purine (II) according to the method of Gerster and Robins.[26] The syrup of II was treated with 60 ml of NH_3–MeOH in a sealed tube at room temperature for 7 days according to the method of Montgomery and Hewson[24] and the mixture was evaporated to dryness. After repeated coevaporation with EtOH, the residue was crystallized from 20 ml of *n*-BuOH–H_2O (92.5:7.5, v/v). Fine needles of 2-chloroadenosine (III) were collected by filtration: 2.02 g (29% based on I); mp 161–162° dec, 130° shrink (lit. 135°,[17] 142–143° dec,[18] 142° dec,[22] 147–148°,[23] 143–145°[24]), uv λ max (pH 1) 266 nm (ε × 10^{-3} 14.6), (pH 7) 265 (15.0), and (pH 13) 266 (15.0) [lit.[24] (pH 1) 265 (14.6), (pH 7) 264 (15.2), and (pH 13) 265 (15.3)]; $[\alpha]^{25}D$ −69° (*c* 0.33, H_2O) [lit.[18] $[\alpha]^{12}D$ −49.8° (*c* 1.0,

† Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. Melting points were determined on a Büchi-Tottoli apparatus and are uncorrected. Uv spectra, nmr spectra (TMS, internal standard), and optical rotation were measured with a Hitachi recording spectrophotometer EPS-3T, Varian T-60 spectrometer, and JASCO automatic polarimeter DIP-SL, respectively. Paper chromatography was performed using Tōyō Roshi No. 51A paper in an ascending technique with solvent systems (1) *n*-BuOH–H_2O (84:16, v/v), (2) *n*-BuOH–AcOH–H_2O (2:1:1, v/v), and (3) pH 10 NH_4OH–H_2O. The spots were represented by R_f with the number in parentheses corresponding to the number of the solvent. Paper electrophoresis was carried out in 0.05 *M* phosphate buffer (pH 7.5) at 1000 V/10 cm for 1 hr and the mobility was represented by the relative value to that of 5′-AMP.

Table III. Inhibition of Washed Rabbit Platelet Aggregation by S-Substituted 2-Thioadenosines[a]

Compound	% inhibition ADP-induced	Collagen-induced
2-*n*-Amylthioadenosine (**3**)	88	89
2-Benzylthioadenosine (**14**)	79	88
2-Allylthioadenosine (**17**)	75	85
Adenosine	75	86

[a]Washed platelet suspension was treated with each of the test samples (10^{-4} *M* final concentration) at 37° for 3 min and was then challenged with ADP or collagen (optical density, 0.06).

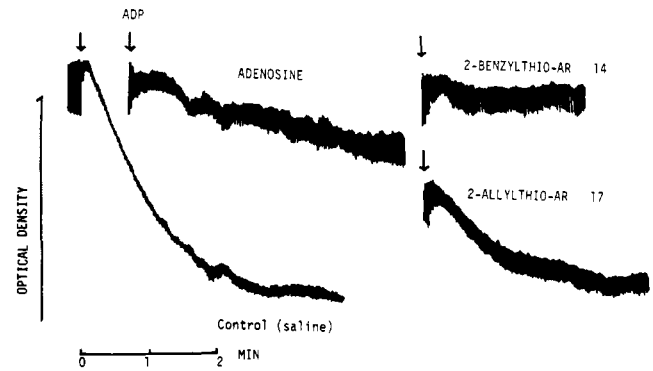


Figure 3. Inhibitory profiles of human platelet aggregation by S-substituted 2-thioadenosines. PRCP (1.0 ml) was treated with each of the test compounds in saline (10^{-4} *M* final concentration) at 37° for 3 min and was then challenged with ADP (indicated by arrows).

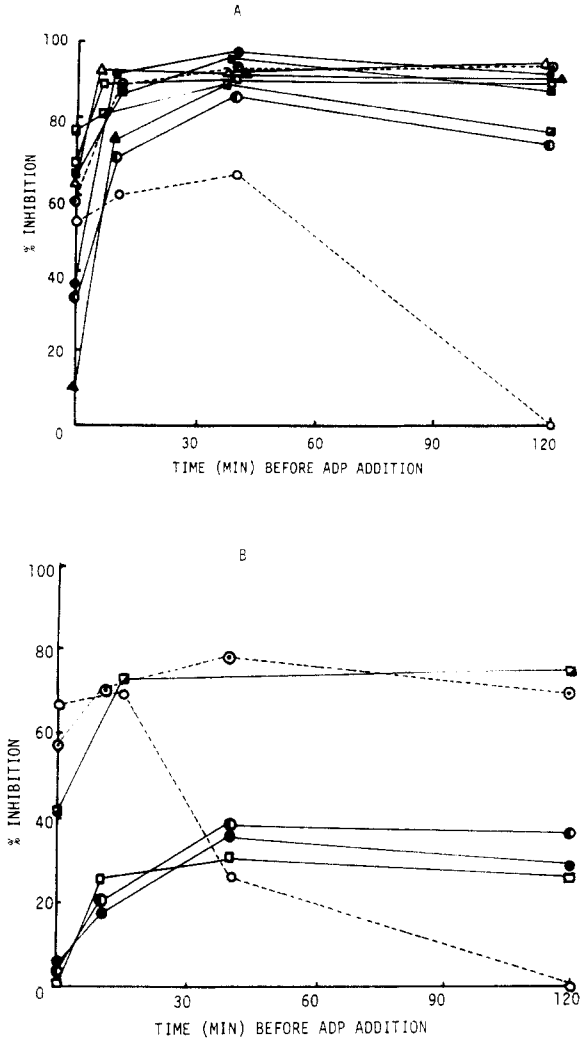


Figure 4. Effect of incubation of S-substituted 2-thioadenosines with rabbit platelet-rich plasma on ADP-induced platelet aggregation. Buffered PRCP (1.0 ml) was incubated at 37° with the test compound in saline without stirring, and after the indicated period it was challenged with ADP. Per cent inhibition of aggregation was plotted *vs.* incubation interval. Extent of aggregation in the control experiment (saline) did not vary during the incubation period of 120 min. 2-*n*-Amyl- (3), □; 2-isoamyl- (4), ■; 2-cyclopentyl- (11), ◪; 2-benzyl- (14), ●; 2-(*p*-chlorobenzyl)- (15), △; 2-(*p*-nitrobenzyl)- (16), ▲; 2-allyladenosine (17), ◐; adenosine, ○; and 2-chloroadenosine (III), ◉. A, test compounds at 10^{-4} *M* (final concentration), and B, test compounds at 10^{-5} *M* (final concentration).

EtOH)]; $R_{f:1}$ 0.37, $R_{f:2}$ 0.67, and $R_{f:3}$ 0.75; nmr (DMSO-d_6) δ 3.6 (C_5·H), 3.9–4.3 (C_4·H and C_3·H or C_2·H), 4.55 (t, C_3·H or C_2·H), 5.82 (d, C_1·H), 7.80 (NH_2), and 8.36 (C_8H). *Anal.* ($C_{10}H_{12}O_4N_5Cl\cdot0.5H_2O$) C, H, N, Cl.

The mixture of syrup II and NH_3–MeOH was treated at 5° overnight and evaporated to dryness. Paper chromatography of the residue revealed two spots ($R_{f:1}$ 0.68 and 0.37). The products were separated and purified through a cellulose column (2.7 × 70 cm) with solvent 1. Fractions containing the product IV were evaporated to dryness and the crystalline residue was recrystallized from EtOH–H_2O to obtain 1.02 g (13.5% based on I) of the pure sample of 2-chloro-6-methoxy-9-β-D-ribofuranosylpurine (IV): mp 129–130.5° (lit. 140°,[22] 124–125° dec[33]); uv λ max (pH 1) 259 nm (ε × 10^{-3} 11.9), (pH 7) 258.5 (12.0), and (pH 13) 259 (12.0) [lit.[22] (pH 1) 259 (10.8), (pH 7) 258 (11.0), and (pH 13) 259 (11.2)]; $[\alpha]^{25}D$ −33.3° (*c* 0.5, H_2O) [lit.[22] $[\alpha]^{26}D$ −30.4 ± 2.3° (*c* 0.6, MeOH)]; $R_{f:1}$ 0.68, $R_{f:2}$ 0.79, and $R_{f:3}$ 0.85; nmr (pyridine-d_5) δ 4.06 (CH_3O), 4.28 (C_5·H), 4.6–5.4 (C_4·H, C_3·H, and C_2·H), 6.74 (d, C_1·H), and 9.13 (C_8H). *Anal.* ($C_{11}H_{13}O_5N_4Cl\cdot0.5H_2O$) C, H, N, Cl. The fractions containing the product III were evaporated to dryness and crystallized from *n*-BuOH–H_2O to afford 1.18 g (16.5% based on I) of III: mp 159–162° dec, 130° shrink.

The product IV (20 mg) was heated at 50° for 2 hr with 0.5 ml of 1 *N* NaOH. Paper chromatography of the mixture showed one spot [$R_{f:1}$ 0.60 and uv λ max (pH 1) 252.5 nm, (pH 7) 255, and (pH 13) 257] corresponding to that of 2-chloroinosine (V).[26,33,34] The product IV (50 mg) was treated with 8 ml of NH_3–MeOH at 100° for 4 hr in a sealed tube and the mixture was evaporated to dryness and recrystallized from *n*-BuOH–H_2O. III was obtained in a quantitative yield.

2-Thioadenosine (VI). Finely divided Na metal (6.0 g, 0.26 mol) was added to anhydrous liquid H_2S (120 ml) pooled in a flask cooled at −60°. Anhydrous dimethylformamide (320 ml) was added to the mixture in 40-ml portions over 3 hr to dissolve Na metal completely. The mixture was set aside at room temperature under anhydrous conditions to remove excess H_2S. To the mixture 8.0 g (25.7 mmol) of 2-chloroadenosine (III) was added and it was heated at 90° for 24 hr under anhydrous conditions. The reaction mixture was cooled in an ice bath, diluted with 320 ml of cold H_2O, and neutralized (pH 6.5–7) with AcOH. The amber-colored suspension was evaporated *in vacuo* at 50° to a small volume. The residue was crystallized by dissolving into 120 ml of *n*-BuOH–H_2O (2:1, v/v) and adding with 60 ml of AcOH. The crude sample of VI (7.07 g) obtained was then suspended in 350 ml of H_2O and adjusted at pH 10–11 with concentrated NH_4OH with stirring. Insoluble material was filtered off and the filtrate was adjusted at pH 2.5 with concentrated HCl. The crystals were collected, washed with H_2O and EtOH, and dried. A pure sample of 2-thioadenosine (VI) was obtained: 5.77 g (yield, 72%); mp 196–199° dec; uv λ max (pH 1) 238.5 nm (ε × 10^{-3} 13.8), 293 (18.4), (pH 12.8) 243 (19.1), and 283 (14.0); ε 293 nm (pH 1)/ε 256.5 nm (pH 1) = 6.42; full spectrum shown in Figure 1; pK_a determined spectrophotometrically was 7.8 and 1.3; $[\alpha]^{25}D$ −43.6° (*c* 0.5, DMSO); $R_{f:1}$ 0.11 and $R_{f:2}$ 0.34; nmr (DMSO-d_6) δ 4.0–4.2 (C_5 H, C_4 H, and C_3 H or C_2 H), 4.50 (t, C_3 H or C_2 H), 5.88 (d, C_1 H), 8.0 (NH_2), and 8.36 (C_8H). *Anal.* ($C_{10}H_{13}O_4N_5S\cdot H_2O$) C, H, N, S.

2-Thioadenosine Disulfide (VII). 2-Thioadenosine (VI) (200 mg, 0.63 mmol) was dissolved in 0.63 ml (0.63 mmol) of 1 *N* NaOH, and the solution was added to 60 ml of 0.1 *M* phosphate buffer (pH 7.5). The mixture was added to 6.3 ml of 0.1 *N* iodine at room temperature with stirring. Crystalline precipitate was collected by filtration after standing the mixture overnight in a refrigerator. Recrystallization from dimethylformamide–H_2O afforded a pure sample of disulfide VII: 170 mg (yield, 85%); mp 255–256° dec; uv λ max (pH 1) 226 nm (ε × 10^{-3} 34.1), 273 (25.4),

Table IV. Conversion of Adenosine and 2-Substituted Adenosines in Rabbit Platelet Suspension

Compound	Optical density (λ max, nm)[a] 0	60	120[b]
Adenosine	0.304 (258)	0.188 (251)	0.212 (250)[c]
2-Chloroadenosine (III)	0.300 (268)	0.265 (267)	0.287 (266)[d]
2-*n*-Amylthioadenosine (**3**)	0.334 (272)	0.321 (273)	0.340 (272)[e]
2-Cyclopentylthioadenosine (**11**)	0.333 (274)	0.321 (273)	0.340 (272)[e]
2-Benzylthioadenosine (**14**)	0.336 (273)	0.299 (273)	0.295 (273)[e]
2-Allylthioadenosine (**17**)	0.371 (273)	0.349 (273)	0.363 (272)[e]

[a]Acidified supernatant from the platelet suspension. [b]Time (min) of incubation of the platelet suspension with the test compounds (10^{-4} *M*). [c]The ratios of the optical density at 250 nm to that at 260 nm were 0.80 (0 min), 1.23 (60 min), and 1.29 (120 min), which indicated adenosine (0.84) was converted into inosine (1.21) or hypoxanthine (1.45). [d]2-Chloroinosine (V) shows λ max 252.5 nm. [e]Ratios of the optical density at 250 and 280 nm to that at 260 nm were constant throughout the incubation, which eliminated the conversion into 2-thioinosine derivatives (λ max ~270 nm): K. Imai, R. Marumoto, K. Kobayashi, Y. Yoshioka, J. Toda, and M. Honjo, *Chem. Pharm. Bull.*, **19**, 576 (1971).

(H_2O) 231.5 (41.3), and 274 (25.6); R_{f1} 0.11 and R_{f2} 0.47; paper electrophoretic mobility, 0.0. *Anal.* ($C_{20}H_{24}O_8N_{10}S_2 \cdot 1.5H_2O$) C, H, N.

Treatment of VII with Alkali. Disulfide VII (32 mg, 0.1 mmol) was suspended in 1 ml (0.3 mmol) of 0.3 *N* NaOH and refluxed for 2 min, and the clear solution was immediately cooled. The uv spectrum (pH 1) of the mixture showed λ max 292 and 239 nm, and the ratio of optical density (pH 1) at 293 nm to that at 256 nm was 2.98. The mixture was evaporated to dryness and the residue was crystallized from 1 ml of *n*-BuOH–AcOH–H_2O (2:1:1, v/v). Crystals of VI were obtained in a yield of 72% (22.9 mg), mp 190–199° dec. Paper electrophoresis of the mother liquor revealed a spot having mobility of +0.60 and uv spectrum of λ max (H^+) 263 nm.

Adenosine 2-Sulfonate (VIII). 2-Thioadenosine (VI) (520 mg, 1.64 mmol) was suspended in 9 ml of H_2O, and the solution was added with NH_4OH to dissolve VI and adjust it to pH 9.5. The mixture cooled in an ice bath was treated with 1.8 ml of 30% H_2O_2. Crystalline precipitate, separated after standing the mixture overnight in a refrigerator, was collected by filtration and was identified as disulfide VII, 65 mg (12.5%), mp 255–259° dec. The filtrate was added with 90 ml of EtOH and 300 mg of 5% Pd/C and after standing at room temperature for 2 days Pd/C was filtered off. The filtrate was evaporated *in vacuo* to dryness, and the residue was crystallized and recrystallized from EtOH–H_2O to afford the adenosine-2-sulfonic acid ammonium salt VIII: 292 mg (yield, 49%); mp >260°; uv λ max (pH 1) 264 nm ($\epsilon \times 10^{-3}$ 13.5), (H_2O) 262 (13.4), and (pH 13) 263 (13.6); R_{f2} 0.19; paper electrophoretic mobility, +0.60; qualitative analysis of sulfur was plus. *Anal.* ($C_{10}H_{13}O_7N_5S \cdot NH_4$) C, H, N.

Conversion of 2-Thioadenosine (VI) in Alkaline Media. (A) At 0.001 *M* Concentration with 2 Equiv of Alkali. 2-Thioadenosine (VI) (320 mg, 1 mmol) was dissolved in 1.0 l. of H_2O containing 2 mmol of NaOH and stored at 50°. The uv absorption maximum of the aliquots (0.1 ml) diluted with 5 ml of 0.1 *N* HCl gradually decreased and reached 20% of the initial optical density at 293 nm, and a new absorption maximum appeared at 263 nm (Figure 1) after 10 days. The mixture was neutralized with Dowex 50 (H^+) at pH 7.5, and the filtrate was evaporated at below 30° to dryness to afford gel. Trituration of the gel with EtOH gave hygroscopic powder, 390.0 mg. Paper electrophoresis revealed a single spot having mobility of +0.60 whereas paper chromatography showed two spots having R_{f2} 0.19 (VIII) and 0.30 (IX); uv λ max (H^-) 264 nm, (H_2O) 262, and (OH^-) 263. Treatment of an aqueous extract of the spot corresponding to IX with H_2O_2 gave VIII, analyzed by paper chromatography. Preparative paper chromatography (solvent 2) to isolate IX was unsuccessful for it was unexpectedly transformed to adenosine in the acidic medium. IX must be adenosine-2-sulfinic acid or -2-sulfenic acid.

(B) At 0.1 *M* Concentration with 1 Equiv of Alkali. VI (250 mg, 0.79 mmol) was dissolved in 7.9 ml of H_2O containing 0.8 mmol of NaOH (pH 9), and the mixture was set aside at 50° for up to 10 days. Crystalline material gradually appeared, and it was confirmed to be VII, 70 mg (28%), mp 262–263° dec. Optical density at 293 nm (pH 1) of the supernatant was reduced to 50% of the initial one.

(C) When VI was submitted to paper electrophoresis at pH 7.5, a single spot having mobility 0.0 [uv λ max (H^-) 227 and 273 nm] corresponding to that of VII was observed.

Conversion of 2-Thioinosine in Alkali Media. At 0.001 *M* concentration 2-thioinosine[27] was also degraded; optical density at 294 nm and pH 1 of the mixture decreased after 10 days at 50° to 39% of the initial one. Paper electrophoresis showed two main spots having mobility +1.15 [uv λ max (H^+) 254 nm] corresponding to that of inosine 2-sulfonate[27] and +0.52 [uv λ max (H^+) 235 and 261 nm] corresponding to that of xanthosine. 2-Thioinosine was intact at 0.1 *M* concentration and on paper electrophoresis.

S-Substituted 2-Thioadenosines (X). 2-Thioadenosine (VI) was added to 1–3 equivalent amounts of 0.25 *N* NaOH and 1–10 equivalent amounts of halide, and the mixture was treated at room temperature or 50° for 1 or 2 days. In the case that the halide could not be readily dissolved, an adequate amount of EtOH was added to dissolve the halide. The synthetic procedures and the physical data of the product (X, **1**–**20**) are given in Table I.

2-*n*-Amylthioadenosine (3). VI (1.0 g, 3.15 mmol) was added to 4.05 ml (10.4 mmol) of 0.25 *N* NaOH and 4.76 g (31.5 mmol) of *n*-amyl bromide and the mixture was stirred at 50° for 2 days. The mixture was extracted with ligroine and the residue was neutralized with HCl and evaporated *in vacuo* to small volume to obtain a crystalline residue. Recrystallization from 400 ml of H_2O gave 909.3 mg (78.1%) of a pure sample of **3**.

2-*n*-Hexylthioadenosine (5). VI (0.20 g, 0.63 mmol) was added to 8.1 ml (2.1 mmol) of 0.25 *N* NaOH, 1.04 g (6.3 mmol) of *n*-hexyl bromide, and 16 ml of EtOH, and the mixture was stirred at room temperature overnight. The mixture neutralized with HCl was evaporated to a small volume and extracted with petroleum ether. The residue was dissolved in EtOH to remove insoluble impurity and salt. The filtrate was added to H_2O to afford an oily precipitate which grew crystals in a refrigerator. Recrystallization from EtOH–H_2O gave 127.3 mg (52.7%) of a pure sample of **5**.

2-Cyclohexylthioadenosine (12). VI (0.20 g) was added to 8.1 ml of 0.25 *N* NaOH, 1.027 g (6.3 mmol) of cyclohexyl bromide, and 20 ml of EtOH, and the homogeneous mixture was heated at 50° for 2 days. The mixture was neutralized with HCl and was evaporated to a gum and washed with petroleum ether. Crystallization from EtOH and recrystallization from EtOH–H_2O gave 99.4 mg (41.4%) of **12**.

2-Benzylthioadenosine (14). VI (1.0 g) was added to 13.0 ml (3.47 mmol) of 0.25 *N* NaOH and 540 mg (3.15 mmol) of distilled benzyl bromide, and the mixture was stirred at room temperature overnight. Crystals (1.21 g) which separated were collected by filtration and recrystallized from aqueous EtOH to obtain 960.9 mg (72.8%) of a pure sample of **14**.

2-Diethylaminoethylthioadenosine Hydrochloride (20). VI (0.10 g, 0.315 mmol) was added to 3.12 ml (0.78 mmol) of 0.25 *N* NaOH and 54.2 mg (0.315 mmol) of diethylaminoethyl chloride hydrochloride, and the mixture was stirred at room temperature overnight. Preparative paper chromatography (solvent 2) was performed, and the uv absorbing band (R_{f2} 0.66) corresponding to that of **20** was extracted with H_2O, and the extract was passed through a column of Dowex 1-X2 (Cl^-) (1 ml). The effluent and washings were evaporated to dryness. The residue was crystallized from *i*-PrOH and recrystallized from EtOH, 36.4 mg (26.5%).

Pharmacological Methods. Methods and materials have been partly described in the previous papers.[14,35] All glassware coming into contact with blood or PRCP was siliconized with Siliconizer N-A (Fuji Ko-bunshi Kogyo Co., Ltd.). Collagen was a lyophilized preparation from bovine achilles tendon (Sigma Chemical Co., Ltd.). Approximately 100 mg of collagen was placed in a glass homogenizer covered with 5 ml of saline and homogenized to a fine suspension with a Teflon covered piston at 2000 rpm for 2.5 hr. The turbid supernatant was removed and stored in a refrigerator. The amount of collagen was estimated by the optical density at 420 nm. ADP (Na_2) was purchased from Sigma Chemical Co., Ltd.

Platelet Aggregation. Platelet aggregation was measured by the optical density method of Born and Cross[28] by use of Evans EEL 169 aggregation meter with stirring at 1000 rpm.

Rabbit Platelet-Rich Plasma. Platelet-rich citrated plasma (PRCP) (pH 7.7–7.9) obtained from a male rabbit[12] was immediately added to an equal volume (in the case of ADP-induced aggregation) or to a half-volume (in the case of collagen-induced aggregation) of isotonic barbital buffer (pH 7.3) in order to prevent increase of pH of the plasma.[36] The pH of the buffered plasma was 7.7 ± 0.1 during platelet aggregation assays. The buffered PRCP was stored at 20° for use within 5 hr. A cuvette containing 1.0 ml of buffered PRCP preincubated at 37° for 5 min was placed in an aggregation meter set at 37° and allowed to stir with a 10- or 100-μl solution of the test sample in saline or DMSO. It was challenged with 10 μl of a solution of ADP (10^{-5} *M* final concentration) or 100 μl of a solution of collagen in saline. As the amount of collagen required for aggregation varied by the preparation of PRCP, the proper concentration of collagen was selected in every case. Inhibition percentage of aggregation by a test compound was calculated by dividing the maximum deflection in the optical density curve by that observed with the control solvent (saline, 10 or 100 μl, or DMSO, 10 μl) and then multiplying by 100. The inhibition percentages thus obtained were not absolute as the sensitivity of platelets to aggregating agents varied from preparation to preparation and by minor changes of the experimental conditions. Accordingly, when a different preparation of PRCP was used to compare the potency a reference standard, adenosine, was tested in every experiment for comparison of potency of inhibition, and the relative potency (Rad) of inhibition of the compound to adenosine at the same concentration was a direct measure of potency of inhibition. Rad was calculated by dividing the per cent inhibition of the test compound with that of adenosine at the same concentration.

Human Platelet-Rich Plasma.[14] Blood samples were obtained from laboratory staffs by clean venipuncture, and the PRCP was

stored at 20° for use within 3 hr after blood sampling. Aggregation and its inhibition were investigated with PRCP in the usual way.

Rabbit Washed Platelet Suspension. The rabbit washed platelet suspension was prepared according to the method of Ardlie, *et al.*[37] Blood collected from a male rabbit was added to a $\frac{1}{6}$ vol of ACD solution (Na citrate, 2.5 g; citric acid, 1.37 g; dextrose, 2.0 g; and H_2O, 100 ml). The mixture (*ca.* 60 ml) showing pH 6.3–6.5 was centrifuged at 1000 rpm for 10 min at room temperature to obtain about 20 ml of acid platelet-rich plasma. The plasma was then centrifuged at 0° and 3000 rpm for 10 min to obtain platelet pellet. The pellet was suspended in 20 ml of Tyrode A, and after keeping at 20° for 15 min it was centrifuged again to obtain the platelet pellet. The pellet was washed with 20 ml of Tyrode B similarly, and it was suspended finally in 20 ml of Tyrode C and stored at 20° for use within 2 hr. The washed platelet suspension contained 3×10^8 cells/ml, and the pH of the suspension showed 7.0–7.5. Aggregation and its inhibition were investigated with the suspension in the usual way. Tyrode A contained 8.0 g of NaCl, 0.2 g of KCl, 0.4 g of $MgCl_2$, 1.0 g of dextrose, 0.1 g of $NaHCO_3$, 0.08 g of EGTA, 1.0 l. of H_2O, and 0.35% of bovine serum albumin powder, fraction V (Armour Pharmaceutical Co.), and showed pH 6.5. Tyrode B was Tyrode A minus EGTA. Tyrode C was Tyrode B plus 0.2 g of $CaCl_2$ and showed pH 7.5.

Conversion of Adenosine and Its Derivatives in Rabbit Washed Platelet Suspension. The washed platelets prepared as above were suspended in 20 ml of 0.05 *M* Tris–HCl–0.15 *M* NaCl (pH 7.4) instead of Tyrode C. The suspension (3.0 ml) was incubated with 0.3 ml of a solution of each of the test compounds in saline (10^{-4} *M* final concentration) at 37° for the indicated periods. The suspension was centrifuged at 3000 rpm for 10 min to remove platelets, and the supernatant fluid was added to 4 vol of 1 *N* HCl as rapidly as possible after the centrifugation and full uv spectrum ranging from 220 to 320 nm was recorded against the control supernatant obtained from the suspension incubated with saline for the same period.

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Supplementary Material Available. Inhibitory patterns of **3**, **5**, **11**, and **12** dissolved in saline and DMSO and the deaggregation pattern of ADP-induced aggregates by **3**, **11**, and **14** will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 20× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JMED-73-1381.

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