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Platelet Aggregation Inhibitors. X.[1] S-Substituted 2-Thioadenosines and Their Derivatives

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A series of S-substituted 2-thioadenosines (IV) were prepared by treatment of 2-thioadenosine (III) with a requisite halide in NaOH–H_2O, NaOH–H_2O–EtOH, Na/dimethylformamide, NaOMe/dimethylformamide or NaH/dimethylformamide. N-Substituted 2-aminoadenosines (V) were obtained by treatment of 2-chloroadenosine (II) with a requisite amine. Compounds (IV and V) and N-oxide derivatives of them were tested as inhibitors of adenosine 5′-diphosphate- and collagen-induced rabbit platelet aggregation. 2-Cycloalkyl- or 2-polycycloalkylthioadenosines (IV_{7-9}), water-soluble 2-piperazinoethylthioadenosines (IV_{16-19}) and 2-thioadenosine N-oxide (X_2) were found effective. Most of them showed long lasting activity during the incubation with rabbit plasma. Compounds (IV_{7-9}) were also inhibitory against human platelet aggregation.

Keywords——2-thioadenosine; S-substituted 2-thioadenosines; 2-thioadenosine N-oxide; N-substituted 2-aminoadenosines; mass spectrum; NMR spectrum; platelet aggregation; inhibition of platelet aggregation; platelet aggregometer

Adenosine, a structural analog of a blood platelet aggregation inducer, adenosine 5′-diphosphate (ADP), is known to be a potent inhibitor of blood platelet aggregation.[3] But its effects are of relatively short duration because it is rapidly deaminated by blood cells.[4,5] Among the derivatives of adenosine, 2-chloroadenosine (II) is a potent inhibitor with long-durating activity[6] but it is toxic.[7] In the course of our investigations of the adenosine derivatives as effective inhibitors of platelet aggregation,[8–12] S-substituted 2-thioadenosine[10] and their 5′-monophosphate derivatives[11] have been found to show potent and long-lasting inhibitory activity against ADP- and collagen-induced platelet aggregation. These compounds have been readily synthesized from 2-thioadenosine (III). Although the preparation of the key intermediate (III) had been very troublesome at that time,[10] a facile synthetic procedure has been demonstrated[1,13] that it is prepared from adenosine *via* 2-steps in an overall yield of 60%. The availability of III prompted us to synthesize a wider variety of S-substituted 2-thioadenosines and to investigate inhibitory activity of them towards platelet aggregation.

The present paper deals with the synthesis of S-substituted 2-thioadenosines and the inhibitory activity of them against platelet aggregation.

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2) Location: *Higashi-hama, Saiki, Oita*; *a*) To whom correspondence should be addressed: *2–301, Takahata-dai, Hino-city, Tokyo.*
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Synthesis

2-Thioadenosine (III) was prepared from adenosine (I)[1] or from 2-chloroadenosine (II).[10] Reaction of II with NaSH/dimethylformamide[10] gave 2-dimethylaminoadenosine (V_1)[14] as a by-product.

Treatment of 2-thioadenosine (III) with a requisite alkyl halide (R_1X) gave S-substituted 2-thioadenosines (IV). 2-*n*-Propylthio- (IV_2),[15] 2-(β-hydroxyethyl)thio- (IV_3),[16a] 2-(γ-hydroxypropyl)thio- (IV_4), 2-carboxymethylthio- (IV_5) and 2-cinnamylthio- (IV_6) adenosines were obtained by reaction with a halide in NaOH–H_2O or NaOH–H_2O–EtOH according to the previous methods.[10]

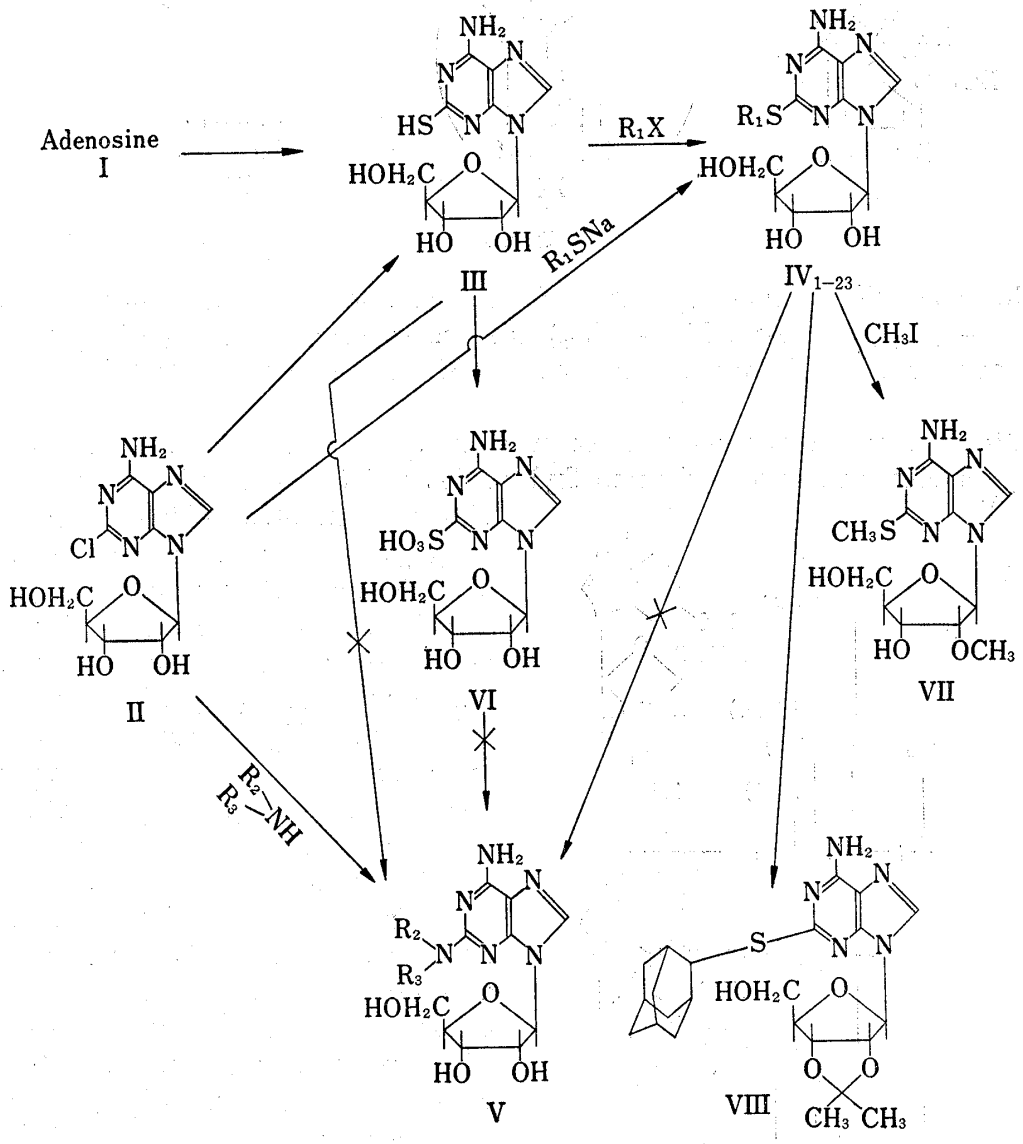

Chart 1

An introduction of a polycycloalkyl substituent was difficult when III was treated in NaOH–H_2O–EtOH at 50° or Et_3N–dioxane–dimethylformamide at reflux. 2-(Adamantan-2-yl)thio- (IV_7) and 2-(*exo*-norbornan-2-yl)thio- (IV_8) adenosines were prepared by reaction of

14) H.J. Schaeffer and J. Thomas, *J. Am. Chem. Soc.*, **80**, 3738 (1958).
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III with the corresponding halide in Na–dimethylformamide or NaOMe–dimethylformamide at reflux. 2-Cyclopentylthio-, 2-cyclohexylthio- and 2-cycloheptylthio- (IV_9) adenosines were prepared in a good yield in NaOMe-dimethylformamide. Reaction of III with 1-bromoadamantane or cyclopropyl bromide was unsuccessful. Treatment of III with an aromatic halide such as iodobenzene and *p*-bromonitrobenzene in NaOMe–dimethylformamide gave 2-phenylthio- (IV_{10})[16a] and 2-(*p*-nitrophenyl)thio- (IV_{11}) adenosines, respectively, yielding 2-methylthioadenosine (IV_1) as a by-product. The compound (IV_1) was yielded by treatment of III

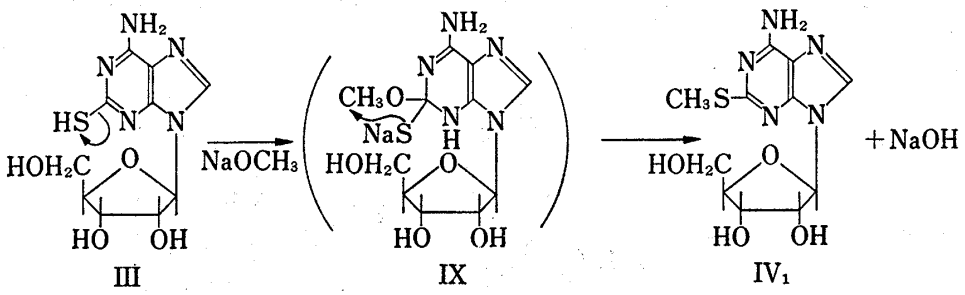

Chart 2

in NaOMe–dimethylformamide, probably *via* the intermediate (IX) shown in the chart. 2-Phenylthio- (IV_{10}) and 2-tolylthio- (IV_{12}) adenosines were also prepared by reaction of 2-chloroadenosine (II) with an aryl mercaptan (R_1SNa), benzenethiol and thiocresol, respectively.

An introduction of a water-soluble substituent was conducted by treatment of III with aminoalkyl halides such as dialkylaminoalkyl halides and 4-substituted piperazinoethyl halides[12] in NaOH–H_2O–EtOH, and compounds (IV_{13-19}) were obtained. Secondary alkyl substituents containing nitrogen atom were introduced by treatment of III with a requisite halide in NaH–dimethylformamide, and compounds (IV_{20-22}) were obtained. Compound (IV_{23}) having a water-soluble aromatic substituent was obtained by treatment of III with 2-bromopyridine in NaOMe–dimethylformamide.

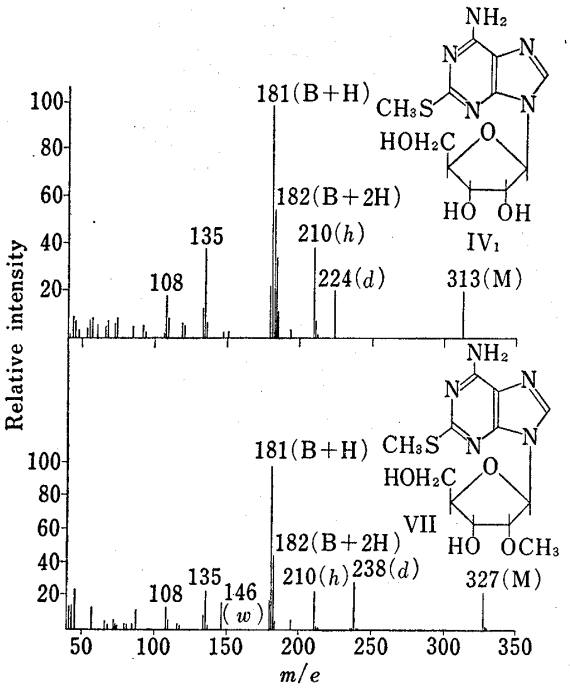

Fig. 1. Mass Spectrum of 2-Methylthioadenosine (IV_1) and 2-Methylthio-2′-O-methyladenosine (VII)

Attempts to convert III, IV or adenosine-2-sulfonate (VI)[10] into 2-aminoadenosine derivatives (V) failed. 2-Aminoadenosine derivatives such as 2-cyclohexylamino- (V_2)[16b] and 2-cyclooctylamino-(V_3) adenosines were prepared by reaction of II with a requisite amine (R_2R_3NH).[14]

Treatment of 2-methylthioadenosine (IV_1) with CH_3I–NaOH gave VII, whose structure was confirmed to be 2-methylthio-2′-O-methyladenosine by UV spectrum, NMR spectrum and mass spectrum. It consumed no metaperiodate. The fragmentation pattern of the mass spectrum of VII compared to that of IV_1 is presented in Fig. 1. Molecular ion peak of VII appeared at *m/e* 327 and the characteristic ion peaks[17]

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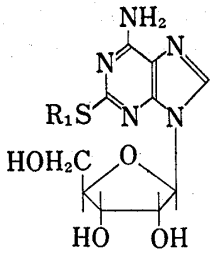
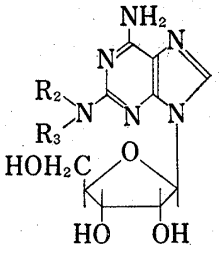
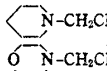
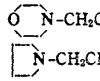
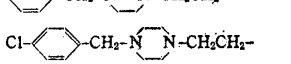
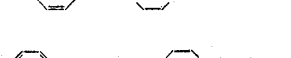
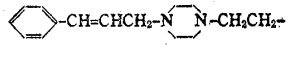
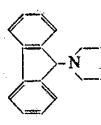
TABLE I. 2-Substituted Adenosines



IV



V

No.	R_1 or $\begin{matrix}R_2\\R_3\end{matrix}$	mp (°C)	Formula	Analysis (%) Calcd. (Found) C	H	N	UV: λ_{max} ($\varepsilon \times 10^{-3}$) nm[a] pH 1	H_2O	pH 13	EtOH	*Rf* in solvent 1	2
V2[b]	$CH_3(CH_2)_2$-	165—167.5	$C_{13}H_{19}N_5O_4S \cdot 1/2H_2O$	44.56 (44.56)	5.75 (5.40)	19.99 (20.05)	272(16.2)	236(20.2) 278(14.4)	236(20.0) 278(14.0)		0.70	0.84
V3[c]	$HOCH_2CH_2$-	211—212.5 dec.	$C_{12}H_{17}N_5O_5S$	41.97 (41.71)	4.99 (4.93)	20.40 (20.24)	271(16.0)	235(21.5) 277(14.8)	234(21.3) 277(14.6)		0.35	0.61
V4	$HOCH_2CH_2CH_2$-	117—119	$C_{13}H_{19}N_5O_5S \cdot 1/4H_2O$	43.14 (43.08)	5.43 (5.41)	19.35 (19.09)	271(15.9)	235.5(20.7) 277.5(14.4)	235.5(21.0) 277.5(14.4)		0.46	0.67
V5	$HOOCCH_2$-	236—238 dec.	$C_{12}H_{15}N_5O_6S \cdot 1/4H_2O$	39.83 (39.89)	4.32 (4.21)	19.36 (19.45)	269(14.7)	235(21.5) 277(14.4)	235(21.5) 277(14.5)		—	0.53
V6		87— 90	$C_{19}H_{21}N_5O_4S \cdot 1/2H_2O$	53.76 (53.69)	5.22 (5.08)	16.50 (16.51)	258(25.8)[d] 285s(17.0) 295s(13.6)	238s(23.6)[d] 258(26.9) 285s(17.0)	240s(24.3)[d] 259(27.2) 285s(18.0)		0.77	0.91
V7		274.5—277 dec.	$C_{20}H_{27}N_5O_4S$	55.41 (55.30)	6.28 (6.24)	16.16 (16.05)				240(23.2) 280.5(14.7)	0.87	0.90
V8		272—274.5 dec.	$C_{17}H_{23}N_5O_4S$	51.89 (51.77)	5.89 (5.92)	17.80 (17.94)				240(23.6) 280.5(15.6)	0.85	0.90
V9		234—237	$C_{17}H_{25}N_5O_4S$	51.63 (51.66)	6.37 (6.29)	17.71 (17.79)	273(15.9)	237.5(20.0) 279(14.3)	237(20.2) 279(14.3)		0.86	0.89
V10[e]		210	$C_{16}H_{17}N_5O_4S \cdot 2/3H_2O$	49.60 (49.48)	4.77 (4.52)	18.08 (18.41)				236(24.3) 281(16.1)	0.72	0.83
V11	NO_2- 	238—246 dec.	$C_{16}H_{16}N_6O_6S$	45.71 (45.45)	3.84 (3.91)	19.99 (20.03)				232(22.6) 275(19.5) 343(7.95)	0.67	0.82
V12		250—270 dec.	$C_{17}H_{19}N_5O_4S \cdot 1/3H_2O$	51.63 (51.74)	5.01 (4.91)	17.71 (17.50)				236(26.7) 280(16.8)	0.77	0.87
V13		210.5—213.5 dec.	$C_{17}H_{26}N_6O_4S \cdot HCl$	45.68 (45.58)	6.09 (6.00)	18.80 (19.20)	270(15.6)	231.5(23.3) 275(15.0)	235(21.4) 277(15.1)		0.12	0.63
V14		191.5—193 eff.	$C_{16}H_{24}N_6O_5S$	46.59 (46.61)	5.86 (5.88)	20.38 (20.29)	270(14.6)	233(21.5) 276(14.4)	234.5(21.4) 277(14.6)		—	0.57
V15		215—218 dec.	$C_{16}H_{24}N_6O_4S \cdot HCl$	44.39 (44.14)	5.82 (5.74)	19.41 (19.04)	270(15.1)	231.5(23.0) 275(15.1)	235(21.3) 276(15.2)		0.09	0.55
V16		159—163 eff.	$C_{23}H_{31}N_7O_4S \cdot 5/3HCl \cdot 2/3EtOH$	49.28 (49.28)	6.23 (6.19)	16.56 (16.89)	269.5(13.5)	234(18.1) 276(12.9)	234.5(19.5) 278(13.5)		0.18	0.56
V17		110—120 eff.	$C_{23}H_{30}ClN_7O_4S \cdot 3HCl \cdot 3/2H_2O$	41.08 (40.63)	5.40 (5.38)	14.58 (15.00)	221.5(26.2) 241(14.4) 280(11.6)	223(29.1) 235s(21.1) 276(13.2)	235s(21.1) 280(11.9)		—	0.65
V18		95—105 eff.	$C_{23}H_{33}N_7O_4S \cdot 3HCl \cdot H_2O$	45.84 (46.27)	5.85 (6.16)	14.97 (15.06)	215(30.9) 255(30.3) 281s(14.7) 291s(10.5)	237(30.4) 255(28.6) 280s(14.9)	239(30.3) 253s(25.7) 262s(21.7) 276s(14.4) 281s(13.6)		—	0.69
V19		191 eff.	$C_{19}H_{33}N_7O_4S \cdot 2HCl \cdot 3/2H_2O$	51.55 (51.77)	5.67 (5.36)	14.51 (14.60)	220s(38.1) 227(38.8) 234(34.6) 271(27.0) 282s(23.7)	225(33.7) 232(31.7) 270(26.5) 306(2.4)	231(28.2) 271(25.1) 305(2.8)		0.34	0.76
V20		138—142 dec.	$C_{17}H_{24}N_6O_4S \cdot HCl \cdot H_2O \cdot 1/2EtOH$	44.48 (44.68)	6.22 (5.87)	17.29 (17.16)	219s(18.6) 271(15.2) 287s(12.6)	231.5(21.8) 276(14.8)	237.5(18.7) 279(14.8)		0.08	—
V21	CH_3- 	151—165 dec.	$C_{16}H_{24}N_6O_4S \cdot HCl \cdot 3/2H_2O$	41.78 (41.85)	6.14 (5.94)	18.27 (17.81)	271(14.7) 291s(11.0)	233.5(19.6) 276(14.0)	236(20.0) 278(14.0)		0.07	0.52
V22	CH_3CH_2- 	97—111 dec.	$C_{17}H_{26}N_6O_4S \cdot HCl \cdot 3/2H_2O$	43.08 (43.08)	6.38 (6.12)	17.73 (17.74)	270(12.9) 287s(10.9)	232(20.1) 275(12.8)	235.5(19.8) 279(12.8)		—	—
V23		208—212.5 dec.	$C_{15}H_{16}N_6O_4S \cdot 1/2H_2O$	46.75 (46.42)	4.45 (4.31)	21.81 (22.19)	269(17.3) 318(6.4)	232(20.3) 273(16.1)	231(20.7) 274(15.9)		0.44	0.70
V1[f]	CH_3, CH_3	217—218 dec.	$C_{12}H_{18}N_6O_4 \cdot 1/2H_2O$	45.14 (44.75)	6.00 (5.89)	26.32 (26.23)	216 261 305	226.5 263 295	226 263 295		—	0.61
V2[g]		147—150.5	$C_{16}H_{24}N_6O_4 \cdot 1/2H_2O$	51.46 (51.58)	6.75 (6.51)	22.51 (22.41)	257(15.1) 302(8.9)	222(22.8) 259(12.0) 290.5(7.7)	260(12.2) 290(8.6)		0.79	0.83
V3		219—222	$C_{18}H_{28}N_6O_4 \cdot 1/2H_2O$	53.85 (54.19)	7.28 (7.14)	20.93 (20.76)	257(15.3) 302.5(8.9)	223.5(23.6) 260(12.2) 290(8.1)	260(11.9) 290(8.3)		0.84	0.86

a) Symbol, s, indicates Shoulder; *b*) Ref. 15: mp 168°; *c*) Ref. 16a: mp 207—208°; *d*) Taken in solvent containing 3% EtOH; *e*) Ref. 16a: mp 125—126°; *f*) Ref. 14: mp 213°(dec.); *g*) Ref. 16b: mp 148—150°.

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to 2′-O-methyladenosine derivatives, *d* and *w*, appeared at *m/e* 238 and 146, respectively. An ion peak characteristic to 3′-O-methyl derivatives corresponding to M-61 (*c*-OMe)[17] was not observed.

S-Substituted 2-thioadenosine such as IV_7 could be readily converted into 2′,3′-O-isopropylidene derivative (VIII) by the known procedure.[18]

Synthesis of adenosine N-oxide derivatives (X, XI) shown in the chart have been described in the previous paper.[1]

Physicochemical properties of the compounds (IV and V) synthesized, and NMR spectrum of the representative IV, V, VII and VIII are presented in Table I and II, respectively.

Table II. NMR Spectrum of 2-Substituted Adenosines

Compound	Solvent[a] (standard)	δ, ppm Substituent	C8H[b]	Cl′H[c]	C2′ H[d]
IV_1	DMSO (TMS)	3.50 (s, CH_3)	8.23	5.86(6)	4.66(6)
	Py-D_2O (DSS)	2.66 (s, CH_3)	8.40	6.30(6)	4.83(6)
2ESAR[e]	DMSO (TMS)	3.10 (q, SCH_2–, J_{CH_2,CH_3}=8Hz) 1.33 (t, CH_3, J_{CH_2,CH_3}=8Hz)	8.23	5.82(6)	4.57(6)
IV_2	DMSO (TMS)	3.10 (t, SCH_2–, J_{CH_2,CH_2}=7Hz) 1.70 (m, $-CH_2$–) 1.00 (t, CH_3, J_{CH_2,CH_3}=7Hz)	8.23	5.88(6)	4.62(6)
2nASAR[f]	DMSO (TMS)	3.10 (t, SCH_2–, J_{CH_2,CH_2}=6Hz) 1.4 (bm, $-(CH_2)_2$–) 0.90 (t, CH_3, J_{CH_2,CH_3}=6Hz)	8.23	5.85(6)	4.62(6)
2iASAR[g]	DMSO (TMS)	3.10 (t, SCH_2–, J_{CH_2,CH_2}=7Hz) 1.6 (bm, $-CHCH_2$–) 0.95 (d, CH_3, J_{CH,CH_3} =6Hz)	8.23	5.83(6)	4.63(6)
IV_3	DMSO (TMS)	3.6 (OCH_2), 3.20 (t, SCH_2–, J_{CH_2,CH_2}=6Hz)	8.20	5.83(6)	4.63(6)
IV_4	DMSO (TMS)	3.6 (OCH_2), 3.17 (t, SCH_2–, J_{CH_2,CH_2}=6Hz), 1.83 (m, $-CH_2$–)	8.23	5.85(6)	4.63(6)
2AlSAR[h]	DMSO (TMS)	5.97 (m, =CH–), 5.32 (d, CH_2=*trans*, $J_{CH_2,CH}$ =16Hz), 5.08 (d, CH_2=*cis*, $J_{CH_2,CH}$=9Hz) 3.80 (d, SCH_2–, J_{CH,CH_2}=7Hz)	8.23	5.85(6)	4.60(6)
IV_6	DMSO (TMS)	7.40 (bm, PhCH=), 6.60 (m, CH=), 3.97 (d, SCH_2–, J_{CH,CH_2}=6Hz)	8.27	5.92(5)	4.63(5)
2DSAR[i]	DMSO (TMS)	3.10 (bs, SCH_2–), 1.26 (b, $-(CH_2)_8$–), 0.87 (b, CH_3)	8.23	5.83(5)	4.63(5)
IV_7	DMSO (TMS)	1.9 (bm, Adam)	8.23	5.83(5)	4.73(5)
IV_8	DMSO (TMS)	1.5 (bm, Norbor)	8.23	5.83(5)	4.63(5)
IV_9	DMSO (TMS)	1.6 (b, $-(CH_2)_6$–)	8.20	5.80(5)	4.63(5)
2CPSAR[j]	DMSO (TMS)	1.7 (b, $-(CH_2)_4$–)	8.22	5.82(5)	4.63(5)
IV_{11}	DMSO (TMS)	8.27, 7.86 (d and d, phenyl, $J_{CH,CH}$=8Hz)	8.30	5.75(5)	4.58(5)
IV_{12}	Py (TMS)	7.2 (b, phenyl), 2.50, 2.23 (s, CH_3)	8.55	6.50	
IV_{13}	DMSO (TMS)	1.80 (bm, Piper)	8.30	5.92(6)	4.50(6)
IV_{23}	DMSO (TMS)	8.5, 7.98, 7.4 (bm, Pyri)	8.30	5.78(6)	4.57(6)
V_1	DMSO (TMS)	3.10 (s, CH_3)	7.96	5.80(6)	
V_2	DMSO (TMS)	1.0—2.0 (bm, $-(CH_2)_5$–)	7.87	5.73(6)	4.63(6)
V_3	DMSO (TMS)	1.57 (bm, $-(CH_2)_7$–)	7.90	5.73(5)	4.63(6)
VII	Py-D_2O (DSS)	3.66 (s, OCH_3), 2.66 (s, SCH_3)	8.43	6.23(5)	
VIII	DMSO (TMS)	1.9 (bm, Adam), 1.53 (s, CH_3), 1.33 (s, CH_3)	8.20	6.10(3)	5.47(q,3)

a) DMSO: d_6-dimethylsulfoxide and Py: d_5-pyridine.
b) All appeared as a singlet.
c) All appeared as a doublet having ($J_{C_1'H,C_2'H}$ Hz).
d) All except VIII appeared as a triplet having ($J_{C_1'H,C_2'H}$ Hz).
e) 2-Ethylthio-. *f*) 2-*n*-Amylthio-. *g*) 2-iso-Amylthio-. *h*) 2-Allylthio-. *i*) 2-*n*-Decylthio-.
j) 2-Cyclopentylthioadenosines in Ref. 10.

18) J.A. Zderic, J.G. Moffatt, D-K.K. Gerzon, and W.E. Fitzgibbon, *J. Med. Chem.*, **8**, 275 (1965).

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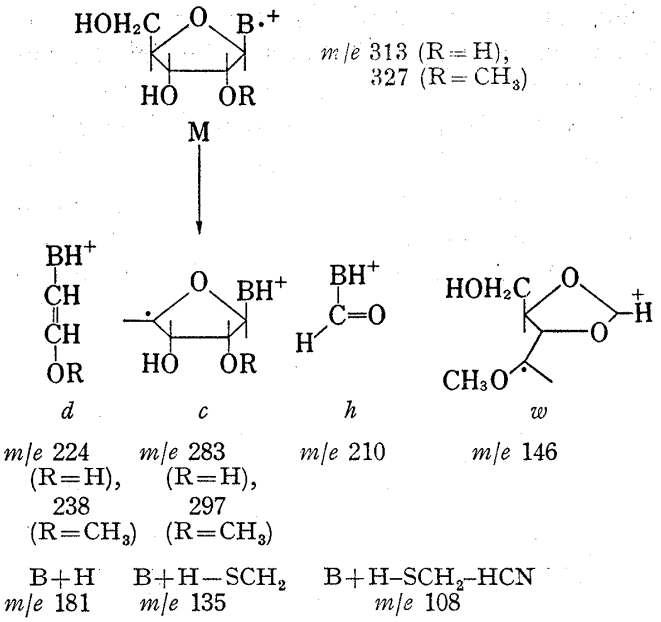

Chart 3

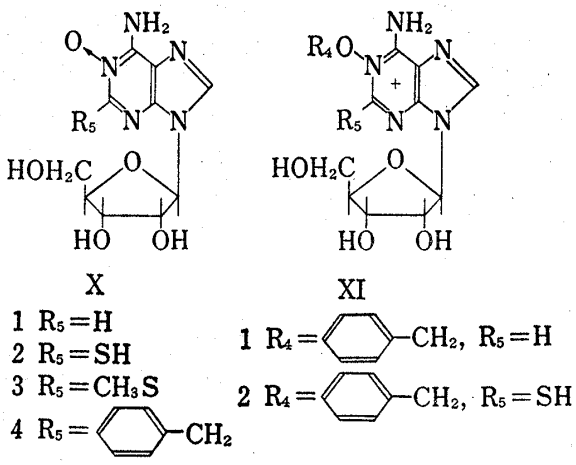

Chart 4

Pharmacological Results and Discussions

S-Substituted 2-thioadenosines (IV), N-substituted 2-aminoadenosines (V), VII and adenosine N-oxide derivatives (X,XI) were tested as inhibitors of ADP- and collagen-induced rabbit platelet aggregation according to the method of Born and Cross.[10,19] Prior to test of the compounds, aggregation and its inhibition profiles of rabbit platelet-rich plasma were compared with two types of the aggregometer, Bryston and Evans EEL 169. Platelet-rich plasma was pretreated with or without an inhibitor, and subsequently challenged with ADP or collagen. Inhibition percentages of adenosine in ADP-induced aggregation were 36% on a Bryston aggregometer and 82% on an Evans aggregometer, and those in collagen-induced aggregation were 15% and 43%, respectively. Differences of the degree of inhibition might be due to the differences of the stirring method, stirring speed and the light source of the meters. As a matter of convenience, the experiments on ADP-induced aggregation were performed on a Bryston aggregometer and those on collagen-induced aggregation on an Evans aggregometer.

Platelet-rich plasma was pretreated with a test compound (10^{-4} M and 10^{-5} M) at 37° for 3 min. The inhibitory activity of every compound was estimated by the extent of the decrease in the optical density of platelet-rich plasma after the addition of 10^{-5} M ADP or collagen. Compounds insoluble in saline were tested with solutions in dimethylsulfoxide (DMSO).[9] Inhibition percentages of every compound were calculated based on the extent of aggregation with the control solvent. As inhibition percentages differed from preparation to preparation of platelet-rich plasma and with the type of aggregometers, relative potency (RAD) to a reference standard, adenosine, in the same concentration was a direct measure of potency of inhibition. The results are summarized in Table III.

2-*n*-Propylthioadenosine (IV_2), described to be inactive against sheep platelet aggregation,[15] showed relatively potent activity as did 2-ethylthio- and 2-*n*-amylthioadenosine.[10] 2-Hydroxyalkylthio- or 2-carboxymethylthioadenosine (IV_{3-5}) were as or less effective than the corresponding 2-alkylthioadenosines.[10] 2-(Adamantan-2-yl) thio- (IV_7), 2-(*exo*-norbornan-2-yl)thio- (IV_8) and 2-cycloheptylthio- (IV_9) adenosines were as potent as 2-cyclopentylthio- or 2-cyclohexylthioadenosines,[10] all bearing branched α-carbon atoms in the substituents (Fig. 2A and Fig. 3A). 2-Arylthioadenosines (IV_{10-12}) were less potent than IV_{7-9}.

19) G.V.R. Born and M.J. Cross, *J. Physiol.*, **168**, 178 (1963).

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Among the water-soluble compounds having nitrogen-containing substituents (IV_{13-23}), 2-piperazinoethylthioadenosines (IV_{16-19}) showed much stronger activity at 10^{-4} M than adenosine against both ADP- and collagen-induced aggregation. Compound (IV_{16}) showed high potency: RAD 1.9 (ADP) and 2.2 (collagen) at 10^{-4} M, but it showed much lower potency: RAD 0.6 (ADP) and 0.4 (collagen) at 10^{-5} M (Fig. 2B and Fig. 3B). Most adenosine derivatives having high RAD value at 10^{-4} M showed lower RAD value at 10^{-5} M,[9,10] and this concentration dependency was much more obvious in these compounds (IV_{16-19}). Nitrogen-containing compounds (IV_{13-23}) had rather stronger activity against collagen-induced than against ADP-induced aggregation.

TABLE III. Inhibition of Rabbit Platelet Aggregation by 2-Substituted Adenosines

Compound	Solvent[a]	ADP-induced aggregation[b]					Collagen-induced aggregation[c]			
		% aggregation of solvent	10^{-4} M		10^{-5} M		10^{-4} M		10^{-5} M	
			% inhibition	RAD[d]	% inhibition	RAD[d]	% inhibition	RAD[d]	% inhibition	RAD[d]
IV_2	A	75	44	1.0	17	0.5	43	1.0		
IV_3	A	69	36	0.6			50	0.6		
IV_4	A	69	19	0.3			69	0.8		
IV_5	A	69	12	0.2			0	0		
IV_6	C	58	52	0.8						
IV_7	B	54	54	1.3	13	0.3	81	1.2	53	1.1
IV_8	B	54	71	1.7	19	0.5	83	1.3	47	1.0
IV_9	B	54	65	1.5	22	0.6	74	1.1	38	0.8
2CPSAR[e]	B	54	50	1.2	22	0.6	81	1.2	45	1.0
2CHSAR[f]	B	54	46	1.1	24	0.6	76	1.2	51	1.1
IV_{10}	B	57	58	0.9			89	1.0		
IV_{11}	B	57	82	1.2			87	1.0		
IV_{12}	B	57	62	0.9			87	1.0		
IV_{13}	A	69	52	0.9			74	0.9		
IV_{14}	A	69	17	0.3			77	0.9		
IV_{15}	A	75	27	0.6						
IV_{16}	A	73	100	1.9	21	0.6	96	2.2	15	0.4
IV_{17}	A	73	55	1.1	12	0.4	96	2.2	4	0.1
IV_{18}	A	73	49	0.9	10	0.3	89	2.0	4	0.1
IV_{19}	A	73	74	1.4	8	0.2	96	2.2	2	0
IV_{20}	A	73	37	0.7	14	0.4	39	0.9		
IV_{21}	A	75	12	0.3						
IV_{22}	A	73	42	0.8	7	0.2	37	0.9		
IV_{23}	A	69	38	0.7			74	0.9		
V_1	B	67	16	0.5			0	0		
V_2	B	67	25	0.8	15	0.6	83	1.0	67	1.0
V_3	B	67	25	0.8	10	0.4	85	1.0	44	0.6
VII	A	69	0	0						
X_1	A	75	39	0.9	17	0.5	43	1.0		
XI_1	A	51	10	0.1						
X_2	A	73	52	1.0	45	1.4	61	1.4	50	1.4
X_3	A	51	0	0						
X_4	C	58	0	0						
XI_2	C	58	33	0.5						

a) A: 10 μl of saline, B: 1 μl of DMSO and C: 10 μl of DMSO.
b) ADP-induced aggregation was measured on a Bryston aggregometer and the final concentration of ADP was 10^{-5} M (See Experimental).
c) Collagen-induced aggregation was measured on an Evans EEL 169 aggregometer and total optical density of collagen added was 0.15—0.20 (See Experimental).
d) RAD represents the relative potency to adenosine at the same concentration.
e) 2-Cyclopentylthio- and *f*) 2-Cyclohexylthioadenosines in Ref. 10.

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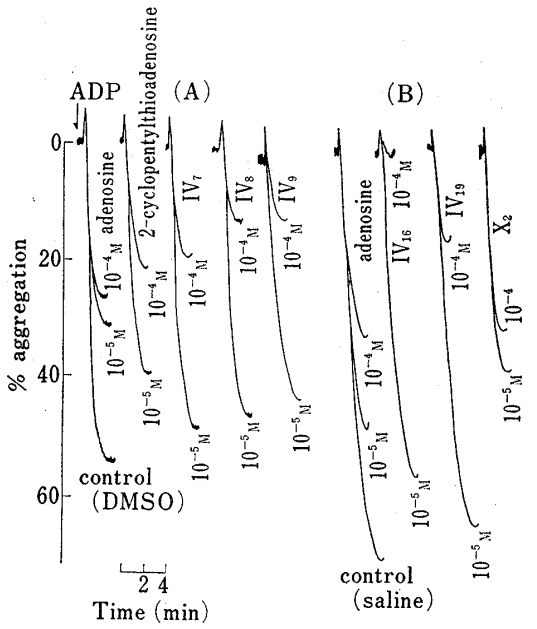

Fig. 2. Inhibitory Profiles of ADP-induced Rabbit Platelet Aggregation by S-Substituted 2-Thioadenosines (IV) and Adenosine N-Oxide Derivative (X) on a Bryston Aggregometer

(A) Platelet-rich plasma (1.0 ml) was treated with a compound in 1 μl of DMSO at 37° for 3 min, and then challenged with 10^{-5} M ADP.
(B) Platelet-rich plasma (1.0 ml) was treated with a compound in 10 μl of saline at 37° for 3 min, and then challenged with 10^{-5} M ADP.

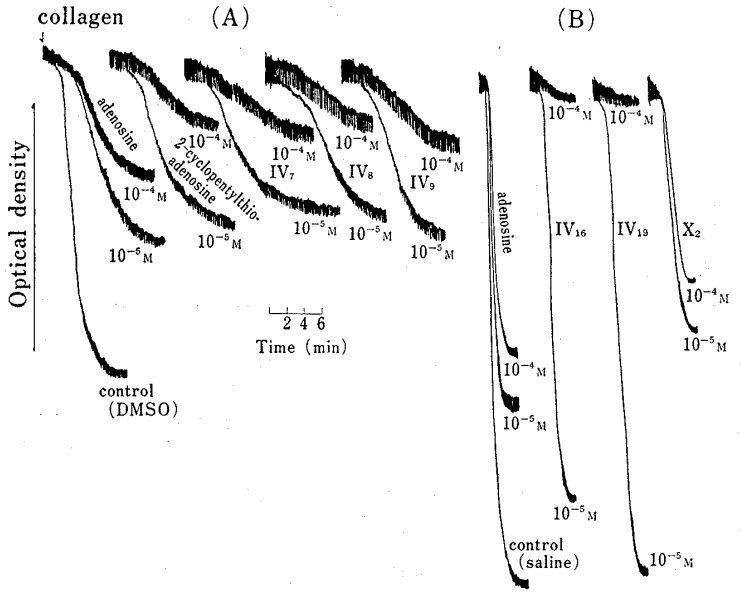

Fig. 3. Inhibitory Profiles of Collagen-induced Rabbit Platelet Aggregation by S-Substituted 2-Thioadenosines (IV) and Adenosine N-Oxide Derivative (X) on an Evans Aggregometer

(A) Platelet-rich plasma was treated with a compound in 1 μl of DMSO at 37° for 3 min, and then challenged with collagen.
(B) Platelet-rich plasma was treated with a compound in 10 μl of saline at 37° for 3 min, and then challenged with collagen.

2-Cycloalkylaminoadenosines ($V_{2,3}$), derivatives of 2-cycloalkylthioadenosines, were potent but less effective.

Adenosine N-oxide (X_1), inhibitory against human[20] and turtle platelet aggregation[21], was effective to rabbit platelet aggregation, but its benzyl derivative (XI_1) was much less. Although III has been found inactive,[10] its N-oxide (X_2) was found effective as adenosine at 10^{-4} and 10^{-5} M in both ADP- and collagen-induced rabbit platelet aggregation. Compound (X_2) had little concentration dependency compared with other derivatives (Fig. 2B and Fig. 3B). 2-Alkylthioadenosine N-oxides ($X_{3,4}$) were less effective than the corresponding 2-methylthio- and 2-benzylthioadenosines.[10] N_1-Benzyloxy-2-thioadenosine (XI_2) was less effective.

Among the compounds tested, 2-cycloalkylthio- or 2-polycycloalkylthioadenosines (IV_{7-9}), 2-piperazinoethylthioadenosines (IV_{16-19}) and 2-thioadenosine N-oxide (X_3) were found to be potent inhibitors. Effect of incubation of these compounds with rabbit plasma on platelet aggregation was investigated. Compounds (IV_{7-9}) were as effective as 2-cyclopentylthio- and 2-cyclohexylthioadenosine (Fig. 4), and 2-[2-(4-benzylpiperazino)ethyl]thioadenosine (IV_6) was also effective (Fig. 5) during the incubation period of 120 min, while adenosine lost its activity owing to its susceptibility to adenosine deaminase.[10] 2-Thioadenosine N-oxide (X_2) showed rather different profiles and it lost its activity during the incubation probably due to its chemical unstability (Fig. 5).

2-Cycloalkyl- and 2-polycycloalkylthioadenosines including IV_{7-9} were tested as inhibitors of ADP-induced human platelet aggregation, and found effective at 10^{-4} M (Fig. 6).

20) G.V.R. Born, R.J. Haslam, and M. Goldman, *Nature* (London), **205**, 607 (1965).
21) M. Kien, F.A. Belamarich, and D. Shepro, *Am. J. Physiol.*, **220**, 604 (1971).

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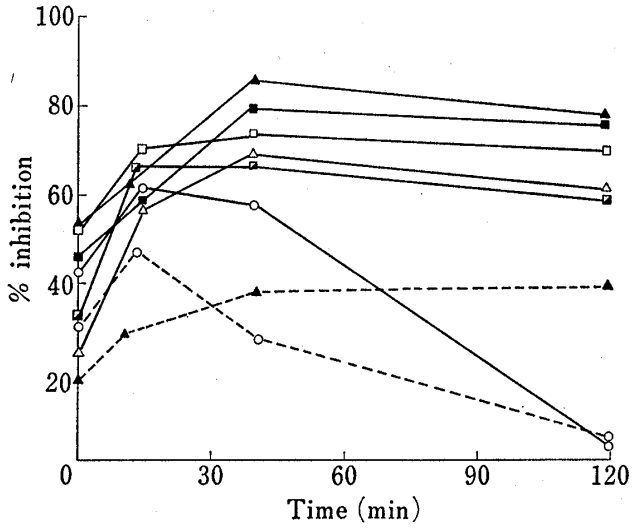

Fig. 4. Effect of Incubation of S-Substituted 2-thioadenosines (IV) with Rabbit Platelet-rich Plasma on ADP-induced Platelet Aggregation

Platelet-rich plasma (1.0 ml) was incubated at 37° with the test sample in 1 μl of DMSO without stirring, and after the indicated period it was challenged with 10^{-5} M ADP. % Inhibition was plotted *vs.* incubation interval. Extent of aggregation in the control experiment (DMSO, 1 μl) did not vary during 120 min, and was quite identical with that obtained with saline (1 μl).

Adenosine, ○; 2-cyclopentylthioadenosine, ◪; 2-cyclohexylthioadenosine, △; 2-(adamantan-2-yl)thioadenosine (IV_7), ▲; 2-(*exo*-norbornan-2-yl)thioadenosine (IV_8), ■; and 2-cycloheptylthioadenosine (IV_9), □.

10^{-4} M, ——; and 10^{-5} M, ------.

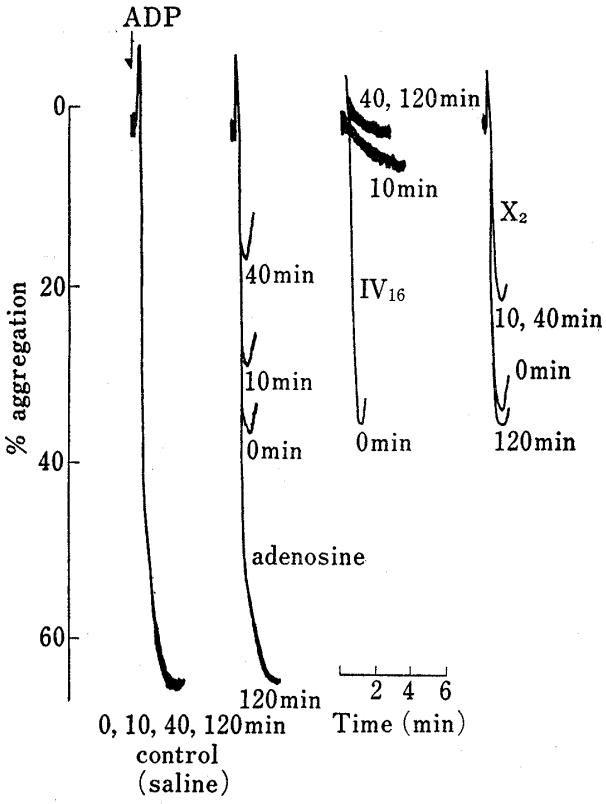

Fig. 5. Effect of Incubation of IV_{16} and X_2 with Rabbit Platelet-rich Plasma on ADP-induced Aggregation

Platelet-rich plasma (1.0 ml) was incubated at 37° with the test sample in 10 μl [of saline (10^{-4}M final concentration) without stirring, and after the indicated period it was challenged with 10^{-5} M ADP.

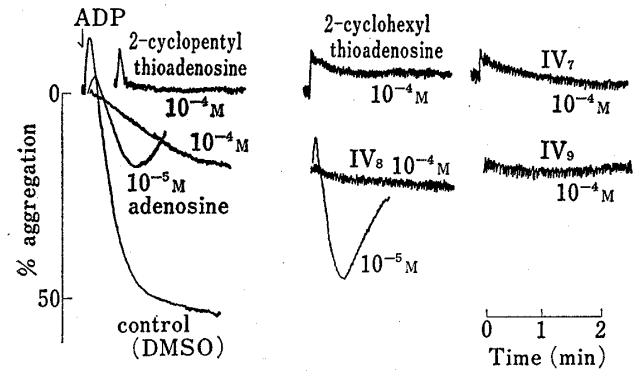

Fig. 6. Inhibitory Profiles of Human Platelet Aggregation by S-Substituted 2-Thioadenosines

Platelet-rich plasma (1.0 ml) was treated with each of the test compounds (adenosine, 2-cyclopentylthioadenosine, 2-cyclohexylthioadenosine, IV_7, IV_8 and IV_9) in 1 μl of DMSO (10^{-4} or 10^{-5} M final concentration) at 37° for 3 min and was challenged with 10^{-5} M ADP.

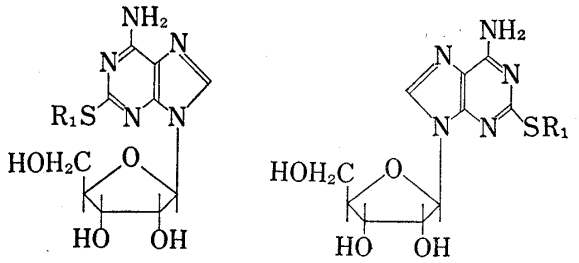

Chart 5

Among the S-substituted 2-thioadenosines (IV) including those described in the previous papers,[10,11] the compounds with bulkier substituents at the 2-position had a potent inhibitory activity. The circular dichroism spectra of S-substituted 2-thioadenosines have been report-

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ed,[22] and the inversion of the sign of the circular dichroism band for S-substituted 2-thioadenosines with bulkier substituents indicated the predominance of *anti*- rather than *syn*-conformation in aqueous solution. The results indicate that the pharmacological activity of 2-thioadenosine derivatives synthesized must be due to the *anti*-conformation of the molecules.

Experimental[23]

2-*n*-Propylthioadenosine (IV_2)——To a solution of 1.0 g (3.16 mmol) of 2-thioadenosine (III) in a mixture of 2.4 ml (9.6 mmol) of 4 N NaOH and 12.6 ml of H_2O was added 583 mg (4.74 mmol) of *n*-propyl bromide. The mixture was stirred at room temperature for 10 hr. The neutral reaction mixture was evaporated to dryness, and the residue was purified through a cellulose column (2.5 $\phi \times 55$ cm) with elution by *n*-BuOH–H_2O (84: 16) and successively through a silica gel column (10 g) with elution by $CHCl_3$–MeOH (49: 1) and subsequently $CHCl_3$–MeOH (24: 1). Fractions containing the product were combined and evaporated to dryness. The residue was crystallized from MeOH giving 800.4 mg of the product. Recrystallization from EtOH–H_2O gave 532 mg (48.0%) of IV_2.

2-(β-Hydroxyethyl)thioadenosine (IV_3)——To a solution of 200 mg of III in 8.1 ml of 0.25 N NaOH was added 790 mg (6.3 mmol) of ethylene bromohydrin. The mixture was stirred at room temperature for 3 hr, and it was neutrallized with 1 N HCl. The aqueous reaction mixture was extracted with 20 ml of petroleum ether twice and it was evaporated to dryness. The residue was crystallized from 30 ml of H_2O to yield 188 mg of IV_3. Recrystallization from H_2O gave 174.1 mg (80.5%) of white columns of IV_3.

2-(γ-Hydroxypropyl)thiadenosine (IV_4)——To a solution of III (200 mg) in 0.25 N NaOH (8.1 ml) were added 880 mg (6.3 mmol) of 3-bromo-1-propanol and 15 ml of EtOH. The mixture was stirred at room temperature for 3 hr. It was then evaporated to small volume, neutrallized with 1 N HCl, extracted with petroleum ether and evaporated to dryness. The residue was crystallized from EtOH containing small amount of H_2O to give 144 mg of the product. Recrystallization from *n*-BuOH–H_2O afforded 81.8 mg (36%) of IV_4.

2-Carboxymethylthioadenosine (IV_5)——To a solution of 200 mg of III in 8.1 ml of 0.25 N NaOH was added 880 mg (6.3 mmol) of bromoacetic acid. The mixture was stirred at room temperature for 3 hr. The precipitate separated was collected by filtration and recrystallized from H_2O to afford 173.5 mg (yield 76.1%) of IV_5.

2-Cinnamylthioadenosine (IV_6)——To a solution of 600 mg of III in 7.8 ml of 0.25 N NaOH were added 290 mg (1.89 mmol) of cinnamyl chloride and 20 ml of EtOH. The mixture was allowed to stand at room temperature for 2 days and then cooled. The precipitate separated was collected by filtration which was then recrystallized from EtOH containing small amount of H_2O gave 392 mg (yield 49%) of fine needles of IV_6.

2-(Adamantan-2-yl)thioadenosine (IV_7)——To a suspension of 11.0 g of III in 150 ml of anhydrous dimethylformamide were added 14.9 g (69.3 mmol) of 2-bromoadamantane (Aldrich Chemical Company, Inc.) and 1.21 g (52.6 mmol) of Na metal. The mixture was refluxed overnight. The slightly alkaline reaction mixture was filtered through cotton fibers and the filtrate was evaporated *in vacuo* to a syrup which was extracted with 200 ml of ligroin twice. The residue was suspended in 200 ml of H_2O and the suspension was extracted with 200 ml of ethyl acetate several times. The extracts were combined and evaporated. The residue was dissolved in 300 ml of hot EtOH and to the hot solution was added 700 ml of H_2O. Crystals of IV_7 separated were collected by filtration giving 19.785 g. Recrystallization from 1.0 liter of EtOH–H_2O (3: 7) gave 9.67 g (yield 64.3%) of a pure sample of IV_7.

2-(*exo*-Norbornan-2-yl)thioadenosine (IV_8)——To a suspension of 11.0 g of III in 150 ml of anhydrous dimethylformamide were added 70 ml of 1 M NaOMe/MeOH and *exo*-2-bromonorbornane (Aldrich Chemical Company, Inc.) (12.1 g, 69 mmol). The mixture was refluxed overnight. The slightly acidic reaction mixture

22) S. Higuchi and K. Kikugawa, 3rd Symposium on Nucleic Acid Chemistry, Sapporo, September, 1975.

23) Melting points were determined on a Buchi–Tottoli apparatus and are uncorrected. UV spectra and NMR spectra (TMS: tetramethylsilane or DSS: 4,4-dimethyl-4-silapentanesulfonic acid sodium salt as an internal standard) were measured with a Hitachi recording spectrophotometer EPS-3T and a Varian-T 60 spectrometer, respectively. High and low resolution mass spectrum was obtained on a CEC-110B double focus mass spectrometer at the National Chemical Laboratory for Industry. Paper chromatography was performed with solvent systems: solvent 1, *n*-BuOH–H_2O (84: 16) and solvent 2, *n*-BuOH–acetic acid–H_2O (2: 1: 1). Preparative paper chromatography was done with Toyo Roshi No. 51A paper. Cellulose column chromatography was performed with cellulose powder A (Toyo Roshi Kaisha, Ltd.). Thin-layer and preparative thin-layer chromatography were performed using Merck Kieselgel HF_{254} nach Stahl (Type 60). Silica gel and silicic acid column chromatographies were carried out with Merck Kieselgel 60 (70—230 mesh) and silicic acid from Mallinckrodt Chemical Works, respectively. A qualitative metaperiodate consumption test on the chromatogram was done with coloration by metaperiodate-benzidine reagent.

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was neutrallized with aqueous NaOH and filtered through cotton fibers. The filtrate was evaporated to a syrup, which was extracted with 200 ml of ligroin. The residue was evaporated to dryness and dissolved in 300 ml of hot EtOH. To the hot solution was added 1.7 liter of H_2O. Crystals separated were collected by filtration, 6.034 g. Recrystallization from EtOH–H_2O (3: 7) gave 5.522 g (yield 40.4%) of a pure sample of IV_8.

2-Cycloheptylthioadenosine (IV_9)——To a suspension of 11.0 g of III in 150 ml of anhydrous dimethylformamide were added 70 ml of 1 M NaOMe/MeOH and 12.3 g (69 mmol) of freshly distilled cycloheptyl bromide (Aldrich Chemical Company, Inc.). The mixture was refluxed overnight. The slightly acidic reaction mixture was neutrallized with 4 N NaOH and filtered through cotton fibers. The filtrate was evaporated to dryness and the residue was dissolved in 70 ml of hot EtOH. To the solution was added 400 ml of H_2O, yielding 12.147 g of crystals of IV_9. Recrystallization from EtOH–H_2O (3: 7) afforded 7.89 g (yield (57.5%) of a pure sample of IV_9.

2-Cyclopentylthioadenosine——A mixture of 15.0 g of III, 71 ml of 1 M NaOMe/MeOH and 9.90 g (94.6 mmol) of cyclopentyl chloride in 200 ml of dimethylformamide was refluxed overnight. The mixture was neutrallized with 4 N NaOH and filtered. The filtrate was evaporated to dryness and the residue was crystallized from EtOH–H_2O to yield 15.135 g of the product. Recrystallization from *n*-BuOH gave 11.15 g (yield 64.2%) of a pure sample of the product, mp 217—220° (lit.[10] 223—224.5°).

2-Cyclohexylthioadenosine——A mixture of 11.0 g of III, 52 ml of 1 M NaOMe/MeOH and 11.3 g (69 mmol) of cyclohexyl bromide in 150 ml of dimethylformamide was refluxed overnight. The mixture was filtered after neutrallization with 4 N NaOH. The filtrate was evaporated to dryness and the residue was crystallized from EtOH–H_2O to yield 8.708 g of the product. Recrystallization from EtOH–H_2O (4: 6) gave 7.425 g (yield 55.4%) of a pure sample of the product, mp 226—227.5° (lit.[10] 225—227.5°).

2-Phenylthioadenosine (IV_{10}): Method A——A mixture of 200 mg of III, 0.63 ml of 1 M NaOMe/MeOH and 257 mg (1.26 mmol) of iodobenzene in 3 ml of anhydrous dimethylformamide was refluxed for 18 hr. Paper chromatography (solvent 2) of the reaction mixture revealed two spots having *Rf* value of 0.68 and 0.83. The products were separated by preparative paper chromatography (solvent 2). 2-Methylthioadenosine (IV_1), 36.6 mg, mp 228.5—230° (lit.[10] 228—229.5°), was obtained from the aqueous EtOH-extract of the lower spots of the chromatogram. NMR (D_2O-d_5-pyridine, DSS) ppm; 8.40 (1H, s, C_8H), 6.30 (1H, d, $C_{1'}H$), 2.66 (3H, s, CH_3). *Anal.* Calcd. for $C_{11}H_{15}N_5O_4S \cdot 1/2H_2O$; C, 40.99; H, 4.96; N, 21.73. Found: C, 40.98; H, 4.84; N, 21.67. The product, IV_{10}, 11.3 mg, was obtained from the aqueous EtOH-extract of the higher spots of the chromatogram with successive purification by preparative thin-layer chromatography (solvent, $CHCl_3$–MeOH, 17: 3).

Method B——To a mixture of 881 mg (8 mmol) of benzene thiol and 150 mg (6.5 mmol) of Na metal in 8 ml of anhydrous dimethylformamide was added 200 mg (0.65 mmol) of 2-chloroadenosine (II).[10] The mixture was heated at reflux for 4 hr. To the solution was added an ice-water mixture and the aqueous solution was evaporated to a small volume, which was neutrallized with 6 N HCl. The solution was extracted with petroleum ether and evaporated to dryness. The residue was purified by preparative thin-layer chromatography (solvent, $CHCl_3$–MeOH, 9: 1). The ethyl acetate extract of the band having *Rf* of 0.5 was evaporated to dryness and the residue was crystallized and recrystallized from EtOH–H_2O (1: 9) to afford 23.5 mg (yield 9.3%) of a pure sample of IV_{10}.

2-(*p*-Nitrophenyl)thioadenosine (IV_{11})——A mixture of 200 mg of III, 0.95 ml of 1 M NaOMe/MeOH and 255 mg (1.26 mmol) of *p*-bromonitrobenzene in 3.0 ml of anhydrous dimethylformamide was refluxed for 18 hr. The mixture was evaporated to dryness and the residue was purified by preparative thin-layer chromatography (solvent, $CHCl_3$–MeOH, 9: 1). From the ethyl acetate-extract of the band having *Rf* of 0.4, 111.2 mg of IV_{11} was obtained. Recrystallization from EtOH gave 87.2 mg (yield 32.9%) of a pure sample of IV_{11}.

2-Tolylthioadenosine (IV_{12})——Commercial *o*-thiocresol was used after steam distillation, $bp_{17\ mm\ Hg}$ 73°. A mixture of 2.5 g of *o*-thiocresol, 375 mg of Na metal and 500 mg of II in 20 ml of anhydrous dimethylformamide was refluxed for 5 hr. To this was added ice–water, and the aqueous mixture was evaporated to dryness. The residue was purified by preparative thin-layer chromatography (solvent, $CHCl_3$–MeOH, 9: 1). Ethyl acetate-extract of the band having *Rf* of 0.3 was evaporated to dryness and the residue was crystallized from EtOH–H_2O, 85.6 mg. Recrystallization from EtOH–H_2O gave 60.7 mg (yield 9.2%) of a pure sample of IV_{12}. NMR spectrum of IV_{12} (Table II) showed 2 proton signals corresponding to 2 methyl groups, indicating that IV_{12} was a mixture of 2 methyl isomers of *ortho*, *para* and *metha*.

Reaction of 2-Thioadenosine (III) with NaOMe——A mixture of 200 mg of III, 1.0 ml of 1 M NaOMe/MeOH in 3.0 ml of anhydrous dimethylformamide was heated at reflux overnight. Paper chromatography (solvent 1) revealed a spot (*Rf* 0.46) corresponding to 2-methylthioadenosine (IV_1). The aqueous extract of the spot showed UV-absorption spectrum identical with that of IV_1.

2-(2-Piperidinoethyl)thioadenosine·HCl (IV_{13})——A mixture of 5.0 g of III, 9.83 ml (39.4 mmol) of 4 N NaOH, 65 ml of H_2O, 2.90 g (15.75 mmol) of N-(2-chloroethyl)piperidine·HCl (Aldrich Chemical Company, Inc.) and 50 ml of EtOH was allowed to stand at room temperature for 3 days. The mixture was then evaporated *in vacuo* to dryness, and the residue was extracted with 100 ml of hot EtOH three times. The

NII-Electronic Library Service

ethanolic extract was neutrallized with 1 N HCl and evaporated to dryness. The residue was crystallized and recrystallized from MeOH to yield 4.75 g (yield 58%) of pure IV_{13}.

2-(2-Morpholinoethyl)thioadenosine (IV_{14})——A mixture of 200 mg of III, 0.393 ml of 4 N NaOH, 2.61 ml of H_2O, 117.2 mg (0.63 mmol) of N-(2-chloroethyl)morpholine·HCl (Aldrich Chemical Company, Inc.) and 2 ml of EtOH was allowed to stand at room temperature for 3 days. The mixture was then evaporated to a small volume and neutrallized with 1 N HCl. It was evaporated to dryness and extracted with 10 ml of EtOH twice. The alcoholic extract was evaporated to dryness and the residue was dissolved in 20 ml of H_2O. The solution was passed through a column of 5 ml of Dowex 1×2 (HCO_3^-) and the effluent was evaporated to dryness. The residue was crystallized and recrystallized from EtOH to afford 38.3 mg (yield 14.7%) of a pure sample of IV_{14}.

2-(2-Pyrrolidinoethyl)thioadenosine·HCl (IV_{15})——A mixture of 200 mg of III, 0.39 ml of 4 N NaOH, 2.6 ml of H_2O, 107 mg (0.63 mmol) of N-(2-chloroethyl)pyrrolidine·HCl (Aldrich Chemical Company, Inc.) and 1.0 ml of EtOH was allowed to stand at room temperature for 5 days. The mixture was then evaporated to dryness and the residue was extracted with 10 ml of hot EtOH three times. The extract was neutrallized with 0.1 N HCl and evaporated. The residue was crystallized and recrystallized from MeOH–H_2O to yield 100 mg (yield 37%) of a pure sample of IV_{15}.

2-[2-(4-Benzylpiperazino)ethyl]thioadenosine·5/3HCl (IV_{16})——A mixture of 3.60 g (11.34 mmol) of III, 11.4 ml of 4 N NaOH, 43.2 ml of H_2O, 4.26 g (13.68 mmol) of 1-benzyl-4-(β-chloroethyl)piperazine·2HCl[12] and 60 ml of EtOH was kept at 50° for 2 days. The reaction mixture was evaporated to dryness and the residue was extracted with 50 ml of hot EtOH three times. To the alcoholic extract was added 200 ml of H_2O and the mixture was adjusted to pH 4 with 1 N HCl. It was decolorized with 1.5 g of active carbon and evaporated to dryness. The residue was dissolved in 80 ml of EtOH and the insoluble NaCl was filtered off. On cooling the filtrate gave 4.15 g (yield 61.7%) of IV_{16}. Recrystallization from EtOH gave a hygroscopic sample of IV_{16}. It had three pK_a values; 2.0, 3.5 and 7.0 estimated by titration with acid and alkali.

2-[2-(4-*p*-Chlorobenzylpiperazino)ethyl]thioadenosine·3HCl (IV_{17})——A mixture of 200 mg of III, 0.63 ml of 4 N NaOH, 2.4 ml of H_2O, 263 mg (0.76 mmol) of 1-(*p*-chlorobenzyl)-4-(β-chloroethyl)piperazine·2HCl[12] and 5.0 ml of EtOH was allowed to stand at room temperature for 5 days. The mixture was then evaporated to dryness and the residue was extracted with 10 ml of hot EtOH three times. The alcoholic extract was evaporated to dryness and the residue was purified through a column of silica gel (10 g) by elution with 1.0 liter of $CHCl_3$–MeOH (49: 1) and successively with 1.0 liter of $CHCl_3$–MeOH (19: 1). The fractions containing the product were pooled and evaporated to dryness. The residue was dissolved in 10 ml of EtOH and to this was added a few drops of 40% HCl/EtOH. The precipitate separated was collected by filtration and recrystallized from EtOH–H_2O giving 150 mg (35%) of IV_{17}.

2-[2-(4-Cinnamylpiperazino)ethyl]thioadenosine·3HCl (IV_{18})——A mixture of 200 mg of III, 0.63 ml of 4 N NaOH, 2.4 ml of H_2O, 257 mg (0.76 mmol) of 1-cinnamyl-4-(β-chloroethyl)piperazine·2HCl[12] and 3 ml of EtOH was allowed to stand at room temperature for 5 days. The reaction mixture was treated as the preparation of IV_{17}. A pure sample of IV_{18} was obtained in a yield of 45% (185 mg).

2-[2-(4-Fluoren-9-yl-piperazino)ethyl]thioadenosine·2HCl (IV_{19})——A mixture of 200 mg of III, 0.63 ml of 4 N NaOH, 2.4 ml of H_2O, 293 mg (0.76 mmol) of 1-(fluoren-9-yl)-4-(β-chloroethyl)piperazine·2HCl[12] and 5 ml of EtOH was allowed to stand at room temperature for 5 days. The reaction mixture was purified through a silica gel column as described. The fractions containing the product were evaporated to dryness and the residue dissolved in EtOH–H_2O was neutrallized with 0.1 N HCl to yield IV_{19}. Recrystallization from EtOH–H_2O gave 98 mg (23%) of a pure sample of IV_{19}.

2-(Quinuclidin-3-yl)thioadenosine·HCl (IV_{20})——To a solution of 4.54 mmol of NaH, which was obtained from 50% NaH in oil by washing with anhydrous petroleum ether, in 20 ml of anhydrous dimethylformamide was added 600 mg of III. The mixture was stirred at room temperature for 3 hr. Dry 3-chloroquinuclidine·HCl (Aldrich Chemical Company, Inc.), 413 mg (2.27 mmol), was added to the mixture and it was refluxed overnight. The reaction mixture was evaporated to dryness and the residue was purified through a column (2.7 ϕ×55 cm) of cellulose by elution with *n*-BuOH–H_2O (84: 16). The fractions containing the product were combined and evaporated to dryness. The residue was dissolved in EtOH–H_2O (1: 1) and the solution was neutrallized with 0.1 N HCl. It was then evaporated to dryness and the residue was crystallized from EtOH giving 200.4 mg (21.8%) of IV_{20}. Recrystallization from EtOH–MeOH gave a pure sample of 70.4 mg (7.7%) of IV_{20}.

2-(N-Methylpiperidin-4-yl)thioadenosine·HCl (IV_{21})——To a solution of 4.54 mmol of NaH in 20 ml of anhydrous dimethylformamide was added 600 mg of III. After the mixture was sirred at room temperature for 3 hr, to this was added 386 mg (2.27 mmol) of 4-chloro-N-methylpiperidine·HCl (Aldrich Chemical Company, Inc.). The mixture was refluxed overnight. The reaction mixture was evaporated to dryness and the residue was purified through a cellulose column by elution with *n*-BuOH–H_2O (84: 16). Fractions containing the product were pooled and neutrallized with 1 N HCl and evaporated to dryness. Crystallization of the residue from EtOH gave 177.4 mg (20.4%) of the product. Recrystallization from EtOH gave a pure sample of IV_{21}.

2-(N-Ethylpiperidin-3-yl)thioadenosine·HCl (IV_{22})——To a solution of 4.45 mmol of NaH and 600 mg of III in anhydrous dimethylformamide stirred for 3 hr was added 418 mg (2.27 mmol) of 3-chloro-N-ethyl-

NII-Electronic Library Service

piperidine·HCl (Pfaltz and Bauer, Inc.). The mixture was refluxed overnight. The reaction mixture was evaporated to dryness and the residue was purified through a cellulose column by elution with *n*-BuOH–H_2O (84: 16). The fractions containing the product were combined and evaporated to dryness. After the residue dissolved in EtOH–H_2O (1: 1) was adjusted to pH 4 with HCOOH, the solution was evaporated to dryness. The residue was dissolved in 20 ml of H_2O and passed through a column of Dowex 1×2 (Cl^-) (5 ml). The effluent and washings were combined (40 ml) and decolorized with 0.5 g of active carbon. The carbon was filtered off and the filtrate was evaporated to dryness. The residue was crystallized from *iso*-PrOH–MeOH (8: 2) yielding 238.2 mg (26.6%) of IV_{22}. Recrystallization from EtOH–ethyl acetate gave a pure sample of IV_{22}.

2-(Pyridin-2-yl)thioadenosine (IV_{23})——To a suspension of 200 mg of III and 0.63 ml of 1 M NaOMe/MeOH in 3.0 ml of dimethylformamide was added 200 mg (1.26 mmol) of 2-bromopyridine, and the mixture was refluxed for 8 hr. The reaction mixture was evaporated to dryness and the residue was purified by preparative paper chromatography (solvent 2). The spots corresponding to the product were extracted with H_2O. The aqueous extract was adsorbed to a column of Dowex 50×4 (H^+) (5 ml) which was subsequently washed with H_2O. The column was eluted with 20 ml of 10% NH_4OH–H_2O and the effluent was evaporated to dryness. The residue was crystallized from H_2O yielding 101.2 mg (26.3%) of a pure specimen of IV_{23}.

Reaction of 2-Chloroadenosine (II) with NaSH/Dimethylformamide——2-Chloroadenosine (II), 8.0 g, was treated with NaSH in dimethylformamide as described in the previous paper.[10] Paper chromatography (solvent 2) of the reaction mixture showed 2 spots having *Rf* of 0.39 and 0.61. A mother liquor from 2-thioadenosine (III), 4.44 g (recrystallization gave 3.86 g, yield 47%), was evaporated to dryness. One twenty fifth of the residue was applied onto a cellulose column (1.7 ϕ×50 cm) with elution by *n*-BuOH–H_2O (84: 16). The fractions containing the product having *Rf* of 0.61 (solvent 2) were pooled and evaporated to dryness. The residue was crystallized and recrystallized from H_2O giving 83 mg (yield 25.3%) of a pure sample of 2-dimethylaminoadenosine (V_1).[14]

2-Cyclohexylaminoadenosine (V_2)——A solution of 500 mg (1.6 mmol) of II in 5.0 ml of a freshly distilled cyclohexylamine was refluxed for 5 hr. The mixture was evaporated to a gum, which was dissolved in 10 ml of EtOH and subsequently added with 5 ml of ethyl acetate. Needles of cyclohexylamine hydrochloride separated were filtered off and the filtrate was evaporated to dryness. The residue was purified through a silicic acid column (20 g) by elution with ethyl acetate and successive ethyl acetate–EtOH (9: 1). Fractions containing the product were combined and evaporated to dryness. The residue was crystallized and recrystallized from EtOH–H_2O giving 161 mg (27%) of a pure sample of V_2.

2-Cyclooctylaminoadenosine (V_3)——A solution of 500 mg of II in 5 ml of cyclooctylamine (Aldrich Chemical Company, Inc.) was heated at 160° for 4 hr. The reaction mixture was evaporated to dryness, and the residue was purified through a silicic acid column. Crystallization and recrystallization from EtOH–H_2O gave 115 mg (yield 16.4%) of a pure specimen of V_3.

2-Methylthio-2′-O-methyladenosine (VII)——A mixture of 200 mg (0.62 mmol) of 2-methylthioadenosine (IV_1), 0.465 ml of 4 N NaOH, 2.525 ml of H_2O, 5 ml of EtOH and 1.76 g (12.4 mmol) of CH_3I was allowed to stand at room temperature for several days. The reaction mixture was evaporated to dryness. The residue was dissolved in a mixture of H_2O and ethyl ether. The aqueous layer was purified by preparatuve paper chromatography (solvent 1). The band having *Rf* of 0.70 was extracted with H_2O and the aqueous extract was evaporated to dryness. Crystallization and recrystallization of the residue from H_2O gave 9.5 mg (yield 3.0%) of a pure sample of VII, mp 100° shrink, 189.5—192°. UV: $\lambda_{max}^{pH\,1}$ (ε) nm, 270 (16300), $\lambda_{max}^{H_2O}$ 235 (20900), 277 (14500), $\lambda_{max}^{pH\,13}$ 235 (21400), 277 (14800). Paper chromatographic *Rf*: 0.70 (solvent 1) and 0.82 (solvent 2). It consumed no metaperiodate. Mass spectrum and NMR spectrum are shown in Fig. 1 and Table II, respectively. *Anal.* Calcd. for $C_{12}H_{17}N_5O_4S\cdot H_2O$; C, 41.73; H, 5.55; N, 20.28. Found: C, 41.94; H, 5.41; N, 20.29.

2′,3′-O-Isopropylidene-2-(adamantan-2-yl)thioadenosine (VIII)——A mixture of 7 ml of anhydrous acetone, 0.7 ml of 2,2-dimethoxypropane and 0.08 ml of 70% $HClO_4$ was kept at room temperature for 5 min. To this was added 200 mg of IV_7. The solution was stirred at room temperature for 2.5 hr. To the mixture was added 300 mg of K_2CO_3 and the suspension was stirred for 1 hr. The solid was removed by filtration and the filtrate was evaporated to dryness. The residue was triturated with H_2O and then recrystallized from MeOH–H_2O yielding 150 mg (yield 69%) of VIII, mp 216—221°. UV: λ_{max}^{EtOH} (ε) nm, 239.5 (25100), 279 (15300). NMR spectrum is shown in Table II. *Anal.* Calcd. for $C_{23}H_{31}N_5O_4S$; C, 58.33; H, 6.60; N, 14.79. Found: C, 58.16; H, 6.58; N, 14.65.

Pharmacological Methods and Materials——All glasswares and tubes coming into contact with blood or platelet-rich plasma were siliconized with Siliconizer N-A (Fuji Kobunshi Kogyo Company, Ltd.). Collagen was a lyophilized preparation of bovine Achilles tendon (Sigma Chemical Company, Ltd.), and 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 about 2000 rpm. The turbid supernatant was removed, estimated by optical density at 420 nm, stored in a refrigerator, and mixed well before use. Adenosine 5′-diphosphate disodium salt (ADP) was a product of Sigma Chemical Company, Ltd.

Rabbit Platelet-rich Plasma——A rabbit (1.8—3.0 kg) was anesthetized with ethyl ether. The carotid artery was carefully cut and cannulated with a 20 cm-piece of polyethylene tubing. The first 5—10 ml

NII-Electronic Library Service

portion of blood was discarded, and about 60—90 ml of blood was transferred into 50 ml-polyethylene centrifuge tubes covered with 0.1 volume of 3.8% sodium citrate (pH 7.3) stored at 37°. It was centrifuged at 1000 rpm for 10 min. The supernatant platelet-rich plasma (20—40 ml, pH 7.7—7.9) was immediately diluted with an equal volume of isotonic barbital buffer (pH 7.3) in order to prevent the increase of the pH of the plasma.[24] The pH of the buffered platelet-rich plasma was 7.7 ± 0.1 during aggregation assays. The buffered platelet-rich plasma containing $3—4 \times 10^8$ cells/ml was stored at 20° and used during the period of 1—5 hr. Platelet-poor plasma was obtained by centrifugation of the buffered platelet-rich plasma at 3000 rpm for 30 min.

ADP-induced Rabbit Platelet Aggregation——ADP-induced platelet aggregation test was carried out by use of a Bryston aggregometer attached to a Hitachi Recorder, Type 056, or a Rikadenki Auto-pen Recorder N-14 (10 mV). The absorbancy of buffered platelet-rich plasma was set at 0% while that of platelet-poor plasma was set at 100% aggregation rate. A siliconized glass cuvette (ϕ 7 mm) containing 1.0 ml of buffered platelet-rich plasma preincubated at 37° for 3—5 min was placed in an aggregometer set at 37°. It was allowed to stir at 1100 rpm for 3 min by a siliconized stirring bar with 1 or 10 μl of a solution of a test compound in saline or dimethylsulfoxide (DMSO). It was then challenged with 10 μl of a solution of ADP (10^{-5} M, final concentration) in saline. Aggregation percentage relative to platelet-poor plasma was calculated in every case from the maximum deflection of the optical density curve. Inhibition percentage by a test compound was calculated from the maxumum deflection relative to that observed with the control solvent. The inhibition percentages thus obtained were not absolute as the sensitivity of platelets to the aggregating agent varied from preparation to preparation, and a reference standard, adenosine, was tested in every experiment for comparison of potency of inhibition. The relative potency (RAD) of inhibition of a test compound to adenosine at the same concentration was a direct measure of potency of inhibition (Table III, Fig. 2).

Effect of Incubation of the Adenosine Derivatives with Rabbit Platelet-rich Plasma on Platelet Aggregation——A cuvette containing 1.0 ml of buffered platelet-rich plasma was preincubated at 37° for 3—5 min and mixed with 1 or 10 μl of a solution of a test compound in saline or DMSO. The mixture was allowed to incubate at 37° without stirring during the period of up to 120 min and it was challenged with 10^{-5} M ADP in a Bryston aggregometer set at 37° with stirring at 1100 rpm. (Fig. 4, Fig. 5).

Collagen-induced Rabbit Platelet Aggregation——Collagen-induced platelet aggregation tests were carried out by use of an Evans EEL 169 aggregometer attached to a Rikadenki Auto-pen Recorder N-14 (10 mV). A plastic cuvette containing 1.0 ml of buffered platelet-rich plasma preincubated at 37° for 3—5 min was mixed with 1 or 10 μl of a solution of a test compound in saline or DMSO and allowed to incubate at 37° for 3 min without stirring. It was then placed in an aggregation meter set at 37° and challenged with 100 μl of a solution of collagen in saline (total optical density at 420 nm: 0.15—0.20) with stirring. RAD was a direct measure of potency of inhibition (Table III, Fig. 3).

Human Platelet Aggregation——Blood samples were obtained from healthy laboratory staffs by clean venipuncture with a 22-gauge Terumo sterile disposable needle connected to a 20 ml disposable plastic syringe containing 2.0 ml of 3.8 w/v% sodium citrate. The whole blood (20 ml) was immediately transferred to a 30 ml-polyethylene centrifuge tube. Platelet-rich plasma was obtained by centrifugation at 1000 rpm for 10 min, and stored at 25° for use during the period of 1—2 hr. It contained $2—3 \times 10^8$ cells/ml. A cuvette containing 1.0 ml of platelet-rich plasma preincubated at 37° for 5 min was placed in a Bryston aggregometer set at 37° and allowed to stir at 1100 rpm with a test sample in 1 μl of DMSO for 3 min. It was then challenged with ADP in the usual way (Fig. 6).

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NII-Electronic Library Service