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Platelet Aggregation Inhibitors. VIII.[1] 2-Thioadenine Derivatives

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A series of S-substituted 2-thioadenines (VII) and S,9-di-substituted 2-thioadenines (VIII) were prepared by direct alkylation of 2-thioadenine (IV). Among the compounds synthesized, 2-cyclopentylthio-9-methyladenine ($VIII_7$), 2-cyclopentylthio-9-cyclopentyladenine ($VIII_{20}$) and 2-[2-(4-benzylpiperazino)ethyl]thio-9-[2-(4-benzylpiperazino)ethyl]-adenine ($VIII_{23}$) were inhibitory against adenosine 5′-diphosphate induced platelet aggregation but these were less potent than adenosine.

Keywords——2-thioadenine; S-substituted 2-thioadenine; S,9-disubstituted 2-thioadenine; S,7-disubstituted 2-thioadenine; 1-substituted 4-(2-hydroxyethyl)piperazine; 1-substituted 4-(2-chloroethyl)piperazine; platelet aggregation; inhibition of platelet aggregation

2-Thioadenosine derivatives have been known to exhibit several pharmacological activities such as platelet aggregation inhibition and coronary vasodilation.[3-8] Synthesis of these 2-thioadenosine derivatives were, however, very tedious and expensive for they must be prepared from the ribose linked nucleosides[3,7-10] or the condensation of a base and a ribose derivative.[3]

This paper deals with the studies directed to the synthesis of water-soluble and less expensive 2-thioadenine derivatives having ionic substituents at the 9-position, and with the comparisons of their inhibitory activity against platelet aggregation with those of 2-thioadenosine series.

Synthesis

S-Substituted 2-thioadenines such as 2-methylthio-,[3,11-14] 2-ethylthio,[3] 2-benzylthio-,[15] 2-carboxymethylthio-[16] and 2-aminoethylthio-[17] adenines and S,9-disubstituted 2-thioadenines such as 2-methylthio-9-methyl-[11,14] and 2-methylthio-9-ethyl-[14] adenines have been prepared *via* one of the following three routes: route 1, ring closure of 2-alkylthio-4-amino-

1) Part VII: K. Kikugawa, H. Suehiro, and M. Ichino, *J. Med. Chem.*, **16**, 1389 (1973).
2) Location: *Higashihama, Saiki-city, Oita*; *a*) To whom correspondence should be addressed: *2-301, Takahata-dai, Hino-city, Tokyo.*
3) M.H. Maguire, D.M. Nobbs, R. Einstein, and J.C. Middleton, *J. Med. Chem.*, **14**, 415 (1971).
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17) V.D. Lyashenko, M.B. Kolesova, K.L. Aleksandr, and V.A. Sheremet'eva, *Zh. Obshch. Khim.*, **34**, 2752 (1964) [*Chem. Abstr.*, **61**, 14674*c* (1964)].

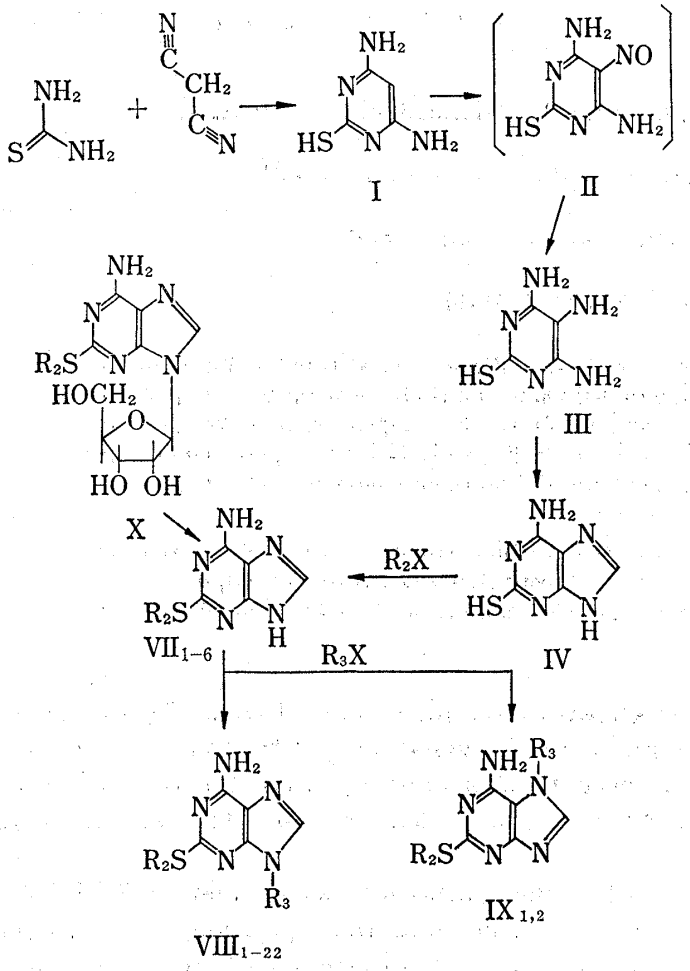

Chart 1

6-alkylaminopyrimidine;[11–13,15] route 2, alkylation of 2-thioadenine;[11,16,17] and route 3, synthesis from 7-aminofurazano[3,4-*d*]pyrimidine.[14]

This time a series of S-substituted 2-thioadenines and S,9-disubstituted 2-thioadenines were prepared *via* route 2; direct alkylation of 2-thioadenine. The synthesis was directed mainly to the derivatives having S-cyclopentyl substituent at 2-position, for 2-cyclopentylthioadenosine was the most potent compound in the 2-thioadenosine series against inhibition of platelet aggregation.[7]

2-Thioadenine (IV)[16,18] was prepared in an overall yield of 16.3% from thiourea and malononitrile *via* 4,6-diamino-2-thiopyrimidine (I), 5-nitroso derivative (II) and 4,5,6-triamino-2-thiopyrimidine (III). Alkylating agents (R_2X and R_3X) of IV included piperazine derivatives which were synthesized as follows.[19] Treatment of N-(2-hydroxyethyl)-piperazine with alkyl halides (R_1X) afforded 1-substituted 4-(2-hydroxyethyl)piperazines(V_{1-5}), which were subsequently converted into 1-substituted 4-(2-chloroethyl)piperazines (VI_{1-5}) by treatment with thionyl chloride. Compounds (V_{2-4}) and compounds (VI_{2-5}) were hitherto unreported. Physicochemical properties of these piperazine derivatives are listed in Table I.

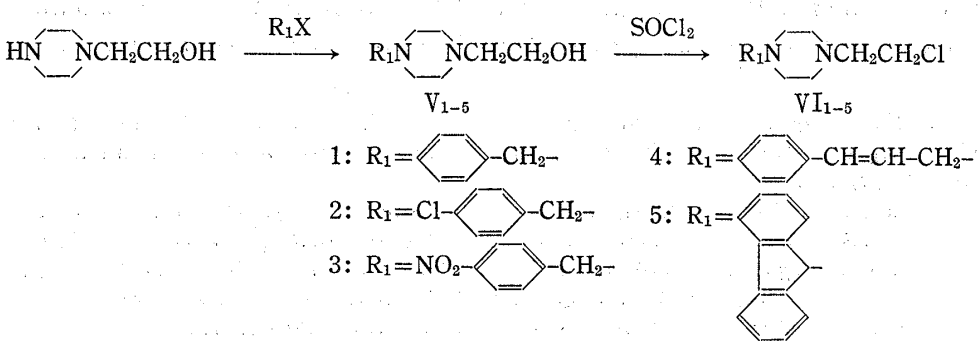

Chart 2

Alkylation of IV did not occur in the present studies when IV was treated with a halide (R_2X) in aqueous alkali, while carboxymethylation[16] and aminoethylation[17] of IV had been performed under rather mild conditions. S-Alkylation of IV was performed in the presence of NaOMe/dimethylformamide under reflux conditions. Treatment of IV with an equal amount of an alkyl halide (R_2X) such as *n*-propyl bromide in the presence of an equal amount

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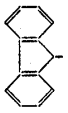
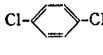
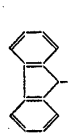
19) Y. Ikeda, Y. Nitta, and Y. Yamada, *Yakugaku Zasshi*, **89**, 669 (1969).

TABLE I. 1-Substituted 4-(2-Hydroxyethyl)piperazine (V) and 1-Substituted 4-(2-Chloroethyl)piperazine (VI)

V VI

	R_1	Starting halide	Method[a]	Yield (%)	mp(°C) (appearance)	Formula	Analysis (%) Calcd. (Found) C	H	N	NMR (D_2O)[b] ppm	UV ($\varepsilon \times 10^{-3}$) nm $\lambda_{max}^{pH\,1}$	$\lambda_{max}^{H_2O}$	Reference[c]
V-1		Cl	A	77.3	228—232 eff. (plates)	$C_{13}H_{20}N_2O\cdot 2HCl$	53.25 (53.36)	7.56 (7.54)	9.55 (9.62)	3.98(m, O-CH_2-) 3.50(m, N-CH_2-) 3.77(s, N N-) 4.53(s, >CH_2-) 7.60(s, phenyl)			1—5
V-2		Cl	A	60.7	248—260 dec. (plates)	$C_{13}H_{19}ClN_2O\cdot 2HCl$	47.65 (47.58)	6.46 (6.43)	8.55 (8.50)	3.97(m, O-CH_2-) 3.48(m, N-CH_2-) 3.73(s, N N) 4.52(s, >CH_2-) 7.57(s, phenyl)			—
V-3		Br	B	81.5	174—176 (plates)	$C_{13}H_{19}N_3O_3\cdot HBr$	45.10 (45.32)	5.82 (5.75)	12.14 (12.24)	4.07(t, O-CH_2-) 3.50, 3.03 (m, m, -CH_2-N N-) 3.93(s, >CH_2-) 8.26, 7.68(d, d, phenyl)			—
V-4		Cl	A	54.7	224—240 dec. (plates)	$C_{15}H_{22}N_2O\cdot 2HCl$	56.42 (56.31)	7.58 (7.47)	8.77 (8.82)	4.02(m, O-CH_2-) 3.50(m, N-CH_2-) 3.80(s, N N) 4.12(d, N-CH_2-) 6.25(m, -CH_2=) 7.03(d, =CH-<) 7.50(m, phenyl)			—
V-5		Br	C	101.0	238—240 (needles) Base: 140—141 (needles)	$C_{15}H_{23}N_2O\cdot HCl\cdot HBr$ $C_{15}H_{22}N_2O$	55.42 (55.53) 77.52 (77.59)	5.87 (5.81) 7.53 (7.53)	6.80 (6.85) 9.52 (9.51)	3.43—4.15 (m, -CH_2CH_2N N-) 5.45(s, >CH-N-) 7.33—7.93(m, phenyl)			6
VI-1			a	90.0	Above 270 (needles)	$C_{13}H_{19}ClN_2\cdot 2HCl$	50.09 (50.10)	6.79 (6.82)	8.99 (9.22)	4.00(m, N-CH_2-) 3.73(m, $ClCH_2$-) 3.75(s, N N) 4.53(s, >CH_2-) 7.58(s, phenyl)	257(0.68) 262(0.64) 269(0.46)		3, 5
VI-2			a	59.2	Above 270 (plates)	$C_{13}H_{18}Cl_2N_2\cdot 2HCl$	45.11 (45.24)	5.82 (5.79)	8.09 (8.19)	3.98(m, N-CH_2-) 3.70(m, $ClCH_2$-) 3.72(s, N N) 4.50(s, >CH_2-) 7.55(s, phenyl)	222(13.2)	221(12.4)	—
VI-3			b	53.0	Above 270 (needles)	$C_{13}H_{18}ClN_3O_2\cdot 2HCl$	43.77 (43.54)	5.65 (5.71)	11.78 (11.85)	3.98(m, N-CH_2-) 3.60(m, $ClCH_2$-) 3.70(s, N N) 4.58(s, >CH_2-) 8.38, 7.78(d, d, phenyl)	262(10.1)	270(9.2)	—
VI-4			a	47.5	Above 270 (needles)	$C_{15}H_{21}ClN_2\cdot 2HCl$	53.35 (53.29)	6.86 (6.82)	8.30 (8.52)	4.00(m, N-CH_2-) 3.75(m, $ClCH_2$-) 3.80(s, N N) 4.12(d, N-CH_2-) 6.27(m, -CH=) 7.03(d, =CH-<) 7.53(m, phenyl)	215(14.1) 254(19.6) 282(2.0) 293(1.1)	215(13.6) 252(19.4) 283(1.7) 293(1.0)	—
VI-5			c	53.4	Above 270 (needles)	$C_{19}H_{21}ClN_2\cdot 2HCl$	59.16 (59.18)	6.01 (6.07)	7.26 (7.16)	3.43—4.30 (m, -CH_2CH_2N N-) 5.48(s, >CHN-) 7.43—7.93(m, phenyl)	220(21.0) 227(26.0) 234(23.4) 272(13.1) 283(11.3)	224s(16.8) 233(11.5) 269(13.8) 294(3.2) 306(1.9)	—

a) See Experimental section.
b) Internal standard: 2,2-dimethylsilapentane-5-sulfonic acid Na salt.
c) 1: R. Baltzly, J.S. Buck, E. Lorz, and W. Schon, *J. Am. Chem. Soc.*, **66**, 263 (1944).
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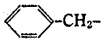
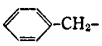
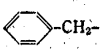
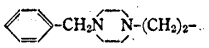
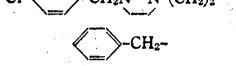
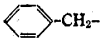
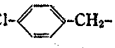
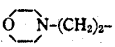
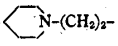
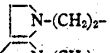
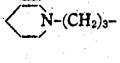
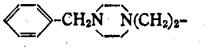
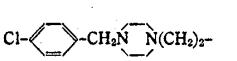
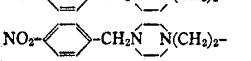
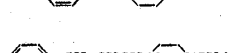
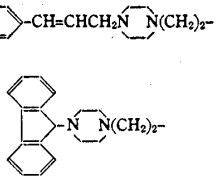
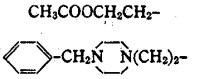
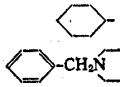
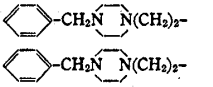
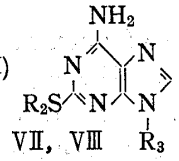
of NaOMe gave 2-*n*-propylthioadenine(VII_1) in a good yield. Treatment of IV with 2 equivalent amount of a halide (R_2X) such as *n*-propyl bromide, *n*-hexyl bromide and benzyl bromide in the presence of 1.2 equivalent amount of NaOMe gave S-substitutrd 2-thioadenines (VII_{1-3}) together with S,9-disubstituted 2-thioadenines ($VIII_{1-3}$). Treatment of IV with 2 moles of a cycloalkyl halide (R_2X) in the presence of 1.2 mole of NaOMe gave 2-cycloalkylthioadenines ($VII_{4,5}$). A cycloalkylthioadenine such as 2-cycloheptylthioadenine (VII_6) was also obtained by an acid hydrolysis of an adenosine derivative (X_1).

TABLE II. S-Substituted 2-Thioadenines (VII) and

	R_2	R_3	mp (°C)	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N
VII-1	$CH_3(CH_2)_2$-	H	231—233	$C_6H_{11}N_5S \cdot 1/2H_2O$	44.02	5.54	32.09	43.89	5.46	32.26
VII-2	$CH_3(CH_2)_6$-	H	239—240	$C_{11}H_{17}N_5S$	51.64	6.89	27.37	51.67	6.69	27.65
VII-3		H	259—263[c]	$C_{12}H_{11}N_5S$	56.01	4.31	27.22	55.95	4.46	27.02
VII-4		H	277—280	$C_{10}H_{13}N_5S$	51.04	5.57	29.76	51.36	5.58	29.73
VII-5		H	271—273	$C_{11}H_{15}N_5S \cdot 1/2H_2O$	51.14	6.24	27.11	51.37	5.96	27.51
VII-6		H	260—263 dec.	$C_{12}H_{17}N_5S$	54.72	6.51	26.59	54.71	6.49	26.23
VIII-1	$CH_3(CH_2)_2$-	$CH_3(CH_2)_2$-	148—150	$C_{11}H_{17}N_5S$	52.56	6.82	27.86	52.50	6.76	28.13
VIII-2	$CH_3(CH_2)_5$-	$CH_3(CH_2)_5$-	124—126	$C_{17}H_{29}N_5S$	60.86	8.71	20.87	60.64	8.59	21.40
VIII-3			193—197	$C_{19}H_{17}N_5S$	65.68	4.93	20.16	65.19	4.97	20.46
VIII-4	$CH_3(CH_2)_2$-		120—122	$C_{21}H_{29}N_5S \cdot 1/4H_2O$	60.62	7.14	23.57	60.68	6.93	23.61
VIII-5	$CH_3(CH_2)_2$-		128—129	$C_{21}H_{28}ClN_7S$	56.55	6.33	21.98	56.56	6.29	22.33
VIII-6[g]	CH_3-									
VIII-7		CH_3-	187—192	$C_{11}H_{15}N_5S$	52.99	6.06	28.09	53.18	6.06	28.01
VIII-8			261—263	$C_{17}H_{19}N_5S \cdot HBr$	50.24	4.96	17.24	50.42	4.94	17.35
VIII-9			196—198	$C_{17}H_{18}ClN_5S$	56.74	5.04	19.46	56.56	5.06	19.25
VIII-10			134—137	$C_{16}H_{24}N_6OS$	55.15	6.94	24.12	55.23	6.88	24.17
VIII-11			127—129	$C_{17}H_{26}N_6S \cdot 1/2H_2O$	57.43	7.66	23.64	57.70	7.48	24.02
VIII-12			118—120	$C_{16}H_{24}N_6S \cdot 1/4H_2O$	57.02	7.33	24.94	57.11	7.21	25.12
VIII-13			134—137	$C_{18}H_{28}N_6S$	59.97	7.83	23.31	60.01	7.07	23.39
VIII-14			258—261 dec.	$C_{23}H_{31}N_7S \cdot 2HCl \cdot H_2O$	52.26	6.67	18.55	52.61	6.35	18.65
VIII-15			126—130 resolidify 167—170	$C_{23}H_{30}ClN_7S$	58.52	6.40	20.77	58.52	6.40	20.79
VIII-16			108—110 dec.	$C_{23}H_{30}N_8O_2S \cdot 1/2H_2O$	56.19	6.36	22.79	55.83	6.28	22.67
VIII-17			150—154	$C_{25}H_{33}N_7S \cdot 1/4H_2O$	64.14	7.21	20.94	64.28	7.08	20.95
VIII-18			109—112 dec.	$C_{29}H_{33}N_7S \cdot 1/2H_2O$	66.90	6.58	18.83	66.90	6.50	18.66
VIII-19		HOOC-CH_2-	267—269 dec.	$C_{12}H_{15}N_5O_2S \cdot 1/2H_2O$	47.51	5.32	23.09	47.57	4.96	23.22
VIII-20			161—165	$C_{16}H_{21}N_5S$	59.38	6.98	23.08	59.97	6.94	23.32
VIII-21		$CH_3COOCH_2CH_2$-	129—131	$C_{14}H_{19}N_5O_2S$	52.32	5.96	21.79	52.35	5.89	21.88
VIII-22			179—182	$C_{24}H_{33}N_7S$	63.82	7.37	21.71	63.93	7.40	21.78
VIII-23			263—268 eff.	$C_{31}H_{41}N_9S \cdot 5HCl \cdot 1.5H_2O$	47.67	6.32	16.14	47.77	6.00	16.59

a) Internal standard: tetramethylsilane.
b) See Experimental section. R_{AD}: relative potency of inhibition to adenosine.
c) lit. 15: mp 238—240°.
d) pH 1 H_2O. *e*) H_2O. *f*) pH 13 H_2O.
g) The compound was kindly supplied by Dr. Y. Nitta of this company.

S,9-Disubstituted 2-Thioadenines (VIII)



VII, VIII

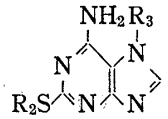
	UV ($\varepsilon \times 10^{-3}$) nm			NMR(d_6-dimethyl sulfoxide), ppm[a]			Inhibition of platelet aggregation[b]	
	$\lambda_{max}^{0.1N\ HCl(5)\ EtOH(1)}$	$\lambda_{max}^{H_2O(5)\ EtOH(1)}$	λ_{max}^{EtOH}	H_8	2-Substituent	9-Substituent	Solvent	Rad
VII-1	220 (10.8) 248.5(13.5) 287.5(11.5)	236 (19.6) 277 (11.6)		7.93(s)	3.05(t, $-CH_2S-$) 1.67(m, $-CH_2-$) 0.98(t, CH_3-)		D	0
VII-2	221 (11.8) 249 (14.5) 288 (12.5)	236 (21.0) 277.5(12.3)					D	0
VII-3	248.5(14.9) 287 (12.7)	233.5(22.5) 277.5(13.0)					D	0
VII-4	220.5(13.0) 250 (14.2) 288 (12.9)	237 (20.9) 278 (12.6)		7.97(s)	3.95(m, >CHS-) 1.65(bs, $-(CH_2)_4-$)		D	0.2
VII-5	221 (12.6) 250 (14.3) 288 (13.0)	237 (20.8) 278 (12.7)					D	0.3
VII-6	250 (12.8)[d] 287 (12.4)	237 (18.9)[e] 278 (12.1)	283 (11.4)[f]				D	0.2
VIII-1	272 (14.4)	236.5(21.4) 279 (13.7)		7.80(s)	3.07(t, $-CH_2S-$) 1.78(m, $-CH_2-$) 0.98(t, CH_3-)	4.05(t, $-CH_2N$) 1.78(m, $-CH_2-$) 0.83(t, CH_3-)	D	0
VIII-2			237.5(26.4) 279 (13.8)				D	0
VIII-3	274 (14.9)	235 (20.5) 278.5(12.1)					D	0
VIII-4	272 (14.6)	237 (20.1) 279 (12 9)		7.96(s)	3.05(t, $-CH_2S-$) 1.67(m, $-CH_2-$) 0.97(t, CH_3-)	4.18(t, $-CH_2N$) 2.70(t, $-CH_2HNpip$) 2.40(m, piperazine) 3.43(s, $-CH_2-\langle$) 7.28(m, phenyl)	H	0
VIII-5	222 (27.9) 273 (15.5)	222 (25.4) 236 (22.0) 278 (14.1)					H	0
VIII-6							D	0.2
VIII-7	273 (15.0)	237.5(20.7) 280 (13.9)		7.93(s)	3.98(m, >CHS-) 1.67(bs, $-(CH_2)_4-$)	3.67(s, CH_3-)	D	0.4
VIII-8	274 (15.2)	238 (21.1) 279.5(14.3)					D	0
VIII-9			222 (23.2) 226 (23.4) 239 (23.0) 279.5(15.2)	8.12(s)	3.87(m, >CHS-) 1.63(bs, $-(CH_2)_4-$)	5.30(s, $-CH_2-\langle$) 7.40(s, phenyl)	D	0
VIII-10	274 (16.5)	238 (21.9) 279 (14.2)					H	0
VIII-11	274 (16.7)	238.5(21.7) 279 (14.6)		7.93(s)	3.85(m, >CHS-) 1.65(bs, $-(CH_2)_4-$)	4.17(t, $-CH_2N$) 2.65(t, $-CH_2-Npip$) 1.40(m, piperidine)	H	0
VIII-12	273.5(15.3)	238.5(19.6) 279 (13.3)					H	0
VIII-13	273 (15.7)	238 (24.4) 279 (14.1)					H	0
VIII-14	273 (15.2)	237.5(20.5) 279.5(13.4)		8.00(s)	3.93(m, >CHS-) 1.65(bs, $-(CH_2)_4-$)	4.18(t, $-CH_2N$) 2.70(t, $-CH_2Npip$) 2.42(m, piperazine) 3.43(s, $-CH_2-\langle$) 7.28(s, phenyl)	H	0
VIII-15	222 (29.0) 274 (16.2)	222 (25.6) 237.5(21.5) 279 (14.0)					H	0
VIII-16	230 (16.2) 271 (24.0)	239 (24.4) 277 (22.0)					H	0
VIII-17	215s (30.7) 233 (19.0) 258 (28.2) 283s (16.3) 292s (13.7)	215s (26.0) 240 (32.8) 255 (26.3) 283s (14.8)		7.95(s)	3.92(m, >CHS-) 1.63(bs, $-(CH_2)_4-$)	4.18(t, $-CH_2N-$) 2.70(t, $-CH_2Npip$) 2.43(m, piperazine) 3.05(d, $NCH_2CH=$) 6.42(m, $-CH=CH-\langle$) 7.35(m, phenyl-CH=)	H	0
VIII-18			222 (36.0) 230.5(32.4) 239 (29.4) 270 (26.6) 308 (3.8)	7.88(s)	3.90(m, >CHS-) 1.60(bs, $-(CH_2)_4-$)	4.12(t, $-CH_2N$) 2.68(t, $-CH_2Npip$) 2.45(m, piperazine) 4.87(s, fluoren H_9) 7.33(m, fluoren)	H	0
VIII-19	273 (14.6)	237 (18.8) 278 (13.2)					N	0
VIII-20	274 (15.5)	237 (22.8) 279 (14.5)		8.05(s)	3.97(m, >CHS-) 1.67(bs, $-(CH_2)_4-$)	4.80(m, >CHN-) 1.80(bs, $-(CH_2)_4-$)	D	0.4
VIII-21	273 (15.5)	237.5(19.5) 278.5(13.5)					D	0
VII-22	274 (15.4)	215s (18.8) 237 (22.0) 279 (14.0)					H	0
VIII-23	230s (14.9)[d] 278 (13.4)	233 (21.5)[e] 278 (14.2)					H	0.5

Specific alkylation at the 9-position of adenine in the presence of a base catalyst has been described,[20] and the methylation at the 9-position of 2-methylthioadenine has been conducted with MeI in alcoholic NaOH.[11] Treatment of 2-*n*-propylthioadenine (VII_1) with an alkyl halide (R_3X) in alcoholic NaOH gave 2-*n*-propylthio-9-substituted adenines ($VIII_{4,5}$). Treatment of 2-cyclopentylthioadenine(VII_4) with a halide (R_3X) in alcoholic NaOH gave a wide variety of 2-cyclopentylthio-9-substituted adenines ($VIII_{7-19}$), and in case of methylation 7-methyl derivative (IX_1) was obtained together with 9-methyl derivative ($VIII_7$). VII_5 gave 9-substituted derivative ($VIII_{22}$) under the conditions. Reaction of VII_4 with a halide such as cyclopentyl chloride, cinnamyl chloride, diphenylmethyl chloride, fluorenyl chloride and hydroxyethyl bromide in alocholic NaOH failed. Treatment of VII_4 with 5 molar excess of cyclopenyl chloride in the presence of NaOMe/dimethylformamide gave 2-cyclopentylthio-9-cyclopentyladenine ($VIII_{20}$). Treatment of VII_4 with 2-chloroethyl acetate in K_2CO_3/dimethylacetamide gave $VIII_{21}$.

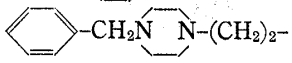
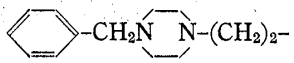
A direct treatment of IV with VI_1 in NaOMe/dimethylformamide gave S,9-disubstituted ($VIII_{23}$) and S,7-disubstituted(IX_2) derivatives.

Physicochemical properties of the compounds synthesized (VII, VIII and IX) are listed in Table II and III. The structures of VII and VIII were confirmed by the comparisons of their ultraviolet (UV) spectra with those of the reported 2-methylthioadenine,[12,13] 2-methylthio-9-methyl(or ethyl)adenine[14] and 2-alkylthioadenosines.[3,7,14] UV spectra of $VIII_{5,9,15-18}$ were different from those of other S,9-disubstituted 2-thioadenines owing to their UV-absorbing 9-substituents.

Table III. S,7-Disubstituted 2-Thioadenines (IX)



IX

	R_2	R_3	mp(°C)	Formula
IX_1		CH_3–	Above 270	$C_{11}H_{15}N_5S \cdot 1/2H_2O$
IX_2			200—210 eff.	$C_{31}H_{14}N_9S \cdot 5HCl \cdot 1.5H_2O$

	Analysis (%) Calcd. C	H	N	Found C	H	N	UV ($\varepsilon \times 10^{-3}$) nm λ_{max} {0.1N HCl(5) / EtOH(1)}	λ_{max} {H_2O(5) / EtOH(1)}	Inhibition of platelet aggregation[a] Solvent	RAD
IX_1	51.14	6.24	27.11	51.03	6.23	27.28	248.5(23.3) 290 (17.1)	245(23.7) 293(16.8)	H	0
IX_2	47.67	6.32	16.14	47.76	5.96	16.24	244.5(22.1)[b] 288 (17.1)	247(20.7)[c] 290(16.1)	H	0

a) See Experimental section. RAD: relative potency of inhibition to adenosine.
b) pH 1 H_2O. *c*) H_2O.

Pharmacology and Discussion

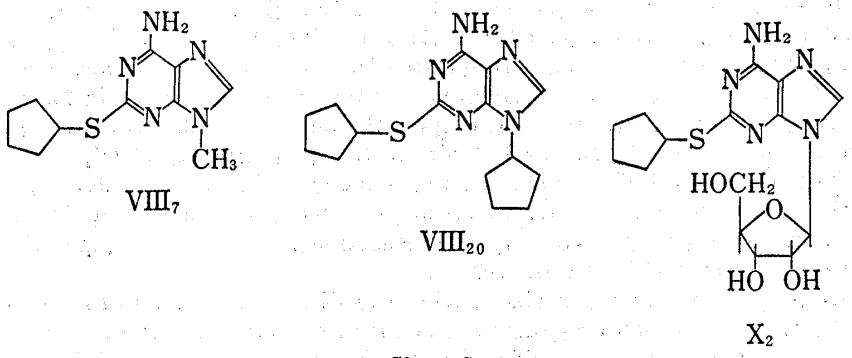
S–Substituted 2-thioadenines (VII_{1-6}), S,9-disubstituted 2-thioadenines ($VIII_{1-23}$) and S,7-disubstituted 2-thioadenines ($IX_{1,2}$) were tested *in vitro* as inhibitors of adenosine 5′-

20) "The Chemistry of Heterocyclic Compounds, Fused Pyrimidines, Part II, Purines," ed. by D.J. Brown, Wiley-Interscience, 1971, p. 342.

diphosphate (ADP) induced rabbit platelet aggregation according to the method of Born and Cross.[7,21] Platelet-rich citrated plasma was pretreated with a test compound (10^{-4} 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 the plasma after the addition of ADP (10^{-5} M). Relative potency of inhibition (RAD) of every compound to adenosine is listed in Table II and Table III.

Among the tested compounds, 2-cyclopentylthio-9-methyladenine ($VIII_7$) (RAD=0.4), 2-cyclopentylthio-9-cyclopentyladenine ($VIII_{20}$) (RAD=0.4) and 2-[2-(4-benzylpiperazino)ethyl]-thio-9-[2-(4-benzylpiperazino)ethyl]adenine ($VIII_{23}$) (RAD=0.5) were slightly inhibitory but less potent than adenosine. All other compounds were much less potent than adenosine or quite impotent.

Comparisons of an inhibitory activity of a series of 2-cyclopentylthio derivatives were made. Thus, a compound with no substitution at the 9-position (VII_4) and compounds with



$VIII_7$ $VIII_{20}$ X_2

Chart 3

substitution of aralkyl groups ($VIII_{8,9}$), nitrogen containing groups ($VIII_{10-18}$) or carboxyl containing group ($VIII_{19}$) were quite inactive. Compounds with substitution of alkyl groups at 9-position ($VIII_{7,20}$) was slightly effective. The compound with substitution of ribose at the 9-position, 2-cyclopentylthioadenosine (X_2), was far more effective.[7] These results from structure-activity relationship indicated that the ribosyl moiety of 2-thioadenosine derivatives was essential to the effective inhibition of platelet aggregation and could not be replaced by other substituents.

Experimental[22]

2-Thioadenine (IV)——2-Thioadenine (IV) was prepared according to the modified method of Traube synthesis.[16,18]

Sodium metal, 36 g (1.57 mol) was placed in 800 ml of distilled ethanol and to the solution were added 120 g (1.58 mol) of thiourea and 100 g (1.52 mol) of malononitrile. The mixture was refluxed for 3 hr. The precipitates separated on cooling were collected by filtration and were dissolved in 500 ml of water, which was brought to pH 5—6 with glacial acetic acid. The precipitated 4,6-diamino-2-thiopyrimidine (I) was collected by filtration and dried, 131 g (yield 60.7%). UV: $\lambda_{max}^{pH\,1}$ nm, 239, 288, $\lambda_{max}^{H_2O}$ 246.5, 267 shoulder, 294, $\lambda_{max}^{pH\,13}$ 273.

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

22) Melting points were determined with Buchi melting point apparatus and uncorrected. UV spectra were taken with a Hitachi Recording Spectrophotometer EPS-3T. Nuclear magnetic resonance (NMR) spectra were taken with a Varian T-60 spectrometer, and the measurements were greatly acknowledged to Mr. T. Kawashima of the laboratories. Thin-layer chromatography was performed on Merck Kieselgel HF_{254} (Type 60) with $CHCl_3$–MeOH (17: 3). Silica gel column chromatography was carried out with Merck Kiesel gel 60 (0.063—0.200 mm). A cellulose column was prepared from Cellulose Powder A (Toyo Roshi Kaisha, Ltd.). N-(2-chloroethyl)piperidine·HCl, N-(2-chloroethyl)morpholine·HCl, N-(2-chloroethyl)pyrrolidine·HCl, N-(3-chloropropyl)piperidine·HCl, 9-bromofluorene, N-(2-hydroxyethyl)-piperazine and cycloheptyl bromide were the products of Aldrich Chemical Company, Ltd. 2-Methylthio-9-benzyladenine ($VIII_6$) was a gift of Dr. Y. Nitta of this company.

The compound (I), 63.23 g (0.445 mol), was dissolved in a mixture of 1.16 l of glacial acetic acid and 230 ml of water, which was cooled to 5° and stirred. To this was gradually added a solution of 54 g of $NaNO_2$ in 120 ml of water with the temperature of the solution maintained at 5°. It was stirred for further 3 hr under cooling. The green-colored precipitates (II) were collected by filtration and washed with cold water and resuspended in 3.0 l of water. The suspension was brought to boil on an oil bath. $Na_2S_2O_4$, 198 g, was added and it was boiled for 3—5 min. To the hot solution was added 400 ml of 50% H_2SO_4 with stirring and the solution was filtered immediately. Crystalline 4,5,6-triamino-2-thiopyrimidine (III) was obtained from the filtrate, 34.6 g (yield 30.6%). UV: $\lambda_{max}^{pH\ 1}$ nm, 244, 298, $\lambda_{max}^{H_2O}$ 246, 300, $\lambda_{max}^{pH\ 13}$ 273 (lit.[16] $\lambda_{max}^{H_2O}$ nm, 245, 295).

The compound (III), 25.5 g (99.9 mmol), was dissolved in a mixture of 550 ml of formamide and 9.7 g of 99% formic acid. It was heated at 160° for 3 hr. Crystalline 2-thioadenine separated on cooling was collected by filtration. Recrystallization from 1.3 l of 50% H_2SO_4 gave 19.8 g (yield 88.0%) of 2-thioadenine·$1/2H_2SO_4$·$1/2H_2O$ (IV). UV: $\lambda_{max}^{pH\ 1}$ nm, 240, 287, λ_{max}^{H2O} 229, 285, $\lambda_{max}^{pH\ 13}$ 241, 277 (lit.[16] $\lambda_{max}^{pH\ 6.38}$ nm, 230, 285, 255—262 shoulder, lit.[12] $\lambda_{max}^{pH\ 6.8}$ nm, 229, 282). NMR (d_6-dimethylsulfoxide), ppm: 8.13 (s, C_8H).

An overall yield of 2-thioadenine (IV) was 16.3%.

1-Substituted 4-(2-Hydroxyethyl)piperazine (V)——Method A: N-(2-Hydroxyethyl)piperazine (NMR (D_2O) ppm: 3.72 (t, $-CH_2O-$), 2.4—2.9 (m, $-CH_2N<$)), 2.55 g (19.2 mmol), was dissolved in 20 ml of anhydrous benzene, and to this were added 2.43 g (19.2 mmol) of benzyl chloride and 2.04 g (19.2 mmol) of Na_2CO_3. It was refluxed for 10 hr. The precipitated NaCl was removed by filtration and washed with ethanol. The filtrate was evaporated to dryness and the residue was dissolved in 20 ml of ethanol. The mixture (4.0 g) of 4.0 g of hydrogen chloride and 10 g of ethanol was added. The precipitated material was recrystallized from ethanol to give 1-benzyl-4-(2-hydroxyethyl)piperazine–2HCl (V_1).

Method B: N-(2-Hydroxyethyl)piperazine, 5.10 g (38.40 mmol), was dissolved in 30 ml of ethanol and cooled. To this was added 8.30 g (38.4 mmol) of *p*-nitrobenzyl bromide and it was stirred at room temperature for 3 hr. The precipitated 1-(*p*-nitrobenzyl)-4-(2-hydroxyethyl)piperazine·HBr (V_3) was collected by filtration and recrystallized from ethanol.

Method C: N-(2-Hydroxyethyl)piperazine, 2.55 g, was dissolved in 15 ml of ethanol and cooled. To this was added 4.70 g (19.2 mmol) of 9-bromofluorene and it was refluxed for 10 hr. To the cooled reaction mixture was added 4.0 g of the hydrogen chloride–ethanol mixture to afford the precipitates of 1-(fluoren-9-yl)-4-(2-hydroxyethyl)piperazine·HBr·HCl (V_5) which were subsequently recrystallized from ethanol. The salt was converted into free base by treatment with aqueous NaOH and the base was recrystallized from ethanol–water.

1-Substituted 4-(2-Chloroethyl)piperazine (VI)——Method a: The compound (V_1), 1.5 g (5.12 mmol), was treated with 30.02 g (251 mmol) of thionyl chloride for 8 hr under reflux conditions. The reaction mixture was evaporated to dryness and the residue was crystallized from ethanol to give 1-benzyl-4-(2-chloroethyl)piperazine·2HCl (VI_1).

Method b: The compound (V_3), 1.5 g (4.4 mmol), was dissolved in 10 ml of water and to this was added 8.8 ml (8.72 mmol) of 1 N NaOH. The precipitated free base was collected and dried, 1.10 g. The base was refluxed in 26 g (220 mmol) of thionyl chloride for 10 hr. The mixture was evaporated to dryness and the residue was crystallized from 200 ml of ethanol to give 1-(*p*-nitrobenzyl)-4-(2-chloroethyl)piperazine·2HCl (VI_3).

Method c: The compound (V_5), 4.36 mmol, was converted into the free base, which was dissolved into 8.8 ml (8.8 mmol) of 1 N HCl and evaporated *in vacuo* to dryness. The residue was treated with 15 g (126 mmol) of thionyl chloride for 5 hr under reflux conditions. The mixture was evaporated to dryness and the residue was crystallized from ethanol to give 1-(fluoren-9-yl)-4-(2-chloroethyl)piperazine·2HCl (VI_5).

2-*n*-Propylthioadenine (VII_1)——2-Thioadenine (IV), 3.0 g (13.2 mmol), was suspended in 60 ml of dimethylformamide and to this were added 26.4 ml (26.4 mmol) of 1 M NaOMe/MeOH and 1.62 g (13.2 mmol) of *n*-propyl bromide. The solution was refluxed overnight under anhydrous conditions. It was then evaporated to dryness and the residue was triturated with water and the insoluble material was collected, which was crystallized from ethanol–water, 2.25 g (yield 78.1%). Recrystallization from ethanol gave pure sample.

2-*n*-Propylthioadenine (VII_1) and 2-*n*-Propylthio-9-*n*-propyladenine ($VIII_1$)——A mixture of 1.0 g (4.4 mmol) of IV, 10.8 mmol of 1 M NaOMe/MeOH, 1.08 g (8.8 mmol) of *n*-propyl bromide in 20 ml of dimethylformamide was refluxed overnight. The precipitated salt was filtered off and the filtrate was evaporated to dryness and the residue was triturated with water containing 1 ml of glacial acetic acid. The residue was extracted with 30 ml of hot 2.5 N NH_4OH six times. The extracts were evaporated to dryness and the residue was crystallized from ethanol-water to give VII_1, 200 mg (yield 20.8%). The insoluble material was crystallized from ethanol-water to give $VIII_1$, 250 mg (yield 22.6%). VII_1 and $VIII_1$ were distinguishable on thin-layer chromatography.

2-*n*-Hexylthioadenine (VII_2) and 2-*n*-Hexylthio-9-*n*-hexyladenine ($VIII_2$)——IV was treated with *n*-hexyl bromide similarly to the preparation of VII_1 and $VIII_1$. VII_2 was obtained in a yield of 8.3% from ethanol crystallization and $VIII_2$ was obtained in a yield of 3.0% from ethanol–water crystallization.

2-Benzylthioadenine (VII_3) and 2-Benzylthio-9-benzyladenine ($VIII_3$)——IV was treated with benzyl bromide similarly to the preparation of VII_1 and $VIII_1$. VII_3 was obtained in a yield of 16.8% from ethanol and $VIII_3$ was obtained in a yield of 27.5% from ethanol–water.

2-Cyclopentylthioadenine (VII_4)——A mixture of 8.85 g (39.2 mmol) of IV, 94.5 mmol of 1 M NaOMe/MeOH, 8.05 g (77 mmol) of cyclopentyl chloride in 175 ml of dimethylformamide was refluxed overnight. The reaction mixture was evaporated to dryness, and the residue was triturated with water containing glacial acetic acid. The precipitates were collected by filtration, dissolved in 200 ml of 1 N NaOH and filtered. The filtrate was neutralized with glacial acetic acid and the precipitates thus formed were collected by filtration, 8.70 g (yield 94.3%). Recrystallization from dimethylformamide–water gave pure material.

2-Cyclohexylthioadenine (VII_5)——It was prepared in a yield of 67.1% (recrystallization solvent, dimethylformamide-water) by reaction of IV with cyclohexyl bromide in the same procedure of preparation of VII_4.

2-Cycloheptylthioadenine (VII_6)——2-Thioadenosine,[9] 200 mg (0.63 mmol), was dissolved in a mixture of 0.48 ml of 4 N NaOH and 2.52 ml of water. To this were added 1120 mg (6.3 mmol) of cycloheptyl bromide and 15 ml of ethanol. The mixture was allowed to stand at 50° for 2 days. The acidic reaction mixture, which contained a small amount of 2-cycloheptylthioadenosine (X_1)[10] and a large amount of the acid hydrolyzed compound (VII_6), was evaporated *in vacuo* to dryness and submitted to a cellulose column (1.7×40 cm). The column was eluted with *n*-BuOH–H_2O (84: 16). The fractions containing VII_6 were evaporated to dryness and the residue was crystallized and recrystallized from 50% ethanol, 51.4 mg (yield 31.0%).

2-*n*-Propylthio-9-[2-(4-benzylpiperazino)ethyl]adenine ($VIII_4$)——A mixture of 460 mg (2.1 mmol) of VII_1, 520 mg (13 mmol) of NaOH, 1.3 g (4.2 mmol) of VI_1 in 35 ml of ethanol was refluxed overnight. The salt separated was filtered off and the filtrate was evaporated to dryness. The residue was triturated with 50 ml of hot water and then was extracted by a mixture of 0.1 N NaOH (50 ml) and benzene (50 ml). The organic layer was washed with 0.1 N NaOH (50 ml) and water (50 ml, twice) sucessively, and evaporated to dryness. The residue was crystallized and recrystallized from ethanol–water to afford $VIII_4$, 246 mg (yield 28.2%).

2-*n*-Propylthio-9-[2-(4-*p*-chlorobenzylpiperazino)ethyl]adenine ($VIII_5$)——It was prepared in a yield of 49.6% by reaction of VII_1 and VI_2 in a similar manner of preparation of $VIII_4$.

2-Cyclopentylthio-9-methyladenine ($VIII_7$) and 2-Cyclopentylthio-7-methyladenine (IX_1)——A mixture of 500 mg (2.1 mmol) of VII_4, 178 mg (4.96 mmol) of NaOH, 1.08 g (7.59 mmol) of MeI in 34 ml of ethanol was refluxed for 3 hr. Water was added to the reaction mixture to give precipitates, which were collected by filtration, washed with 50 ml of hot 0.1 N NaOH and water dried, 500 mg. It was then applied onto a silica gel column (30 g). The column was eluted with $CHCl_3$ to give $VIII_7$, 234 mg (yield 44.5%), as colorless columns which were subsequently recrystallized from ethanol–water. The column was then eluted with $CHCl_3$–MeOH (25: 1) to give IX_1, 33 mg (yield 6.1%), as colorless columns which were subsequently recrystallized from ethanol–water.

2-Cyclopentylthio-9-benzyladenine ($VIII_8$)——A mixture of 500 mg of VII_4, 89 mg of NaOH and 1.3 g of benzyl bromide in 34 ml of ethanol was refluxed for 3 hr. It was evaporated to dryness and the residue was triturated with water. The precipitates were crystallized from ethanol to give 198 mg (yield 23.2%) of hydrobromide of $VIII_8$.

2-Cyclopentylthio-9-*p*-chlorobenzyladenine ($VIII_9$)——A mixture of 500 mg of VII_4, 89 mg of NaOH and *p*-chlorobenzyl chloride (1.22 g) in 35 ml of ethanol was refluxed for 5 hr. The solution was evaporated *in vacuo* to dryness and the residue was triturated with 10 ml of hot 2.5 N NH_4OH and water sucessively. It was then crystallized and recrystallized from ethanol–water to give 100 mg (yield 13.2%) of $VIII_9$.

2-Cyclopentylthio-9-(2-morpholinoethyl)adenine ($VIII_{10}$)——A mixture of 500 mg of VII_4, 400 mg of NaOH and 1.41 g of N-(2-chloroethyl)morpholine·HCl in 34 ml of ethanol was refluxed for 3 hr. The salt separated was filtered off and the filtrate was evaporated to dryness. The residue was triturated with pH 11 NH_4OH–water and the precipitates was collected by filtration. It was crystallized and recrystallized from ethanol–water to give 183 mg (yield 25.0%) of $VIII_{10}$.

2-Cyclopentylthio-9-(2-piperidinoethyl)adenine ($VIII_{11}$)——It was prepared from VII_4 and N-(2-chloroethyl)piperidine·HCl in a yield of 35.6% in a similar manner of preparation of $VIII_{10}$. Recrystallization solvent was ethanol–water.

2-Cyclopentylthio-9-(2-pyrrolidinoethyl)adenine ($VIII_{12}$)——It was prepared from VII_4 and N-(2-chloroethyl)pyrrolidine·HCl in a yield of 17.1% in a similar manner of preparation of $VIII_{10}$. Recrystallization solvent was ethanol-water.

2-Cyclopentylthio-9-(3-piperidinopropyl)adenine ($VIII_{13}$)——A mixture of 500 mg of VII_4, 400 mg of NaOH and N-(3-chloropropyl) piperidine·HCl (1.50 g) in 30 ml of ethanol was refluxed for 3 hr. The solution was evaporated to dryness and the residue was triturated with 50 ml of 0.1 N NaOH subsequently with water. The precipitates were applied onto a silica gel column (10 g). The column was eluted with 400 ml of $CHCl_3$ and subsequently with 1 l of $CHCl_3$–MeOH (49: 1). The latter eluate was evaporated to dryness and the residue was crystallized and recrystallized to afford 50 mg (yield 6.6%) of $VIII_{13}$.

2-Cyclopentylthio-9-[2-(4-benzylpiperazino)ethyl]adenine ($VIII_{14}$)——A mixture of 500 mg of VII_4, 520 mg of NaOH and 1.30 g of VI_1 in 35 ml of ethanol was refluxed for 8 hr. The salt separated was filtrated

off and the filtrate was evaporated to dryness. After trituration with 2.5 N NH_4OH (30 ml) the residue was extracted with 30 ml of benzene twice. The combined organic extracts were washed with water and evaporated to dryness. The residue was crystallized and recrystallized from ethanol–water to afford 160 mg (yield 17.4%) of $VIII_{14}$, mp 147—150°. *Anal.* Calcd. for $C_{23}H_{31}N_7S$; C, 63.13; H, 7.14; N, 22.41. Found: C, 63.06; H, 7.04; N, 22.56. It was dissolved in ethanol and a mixture of hydrogen chloride and ethanol was added. Crystals separated were collected by filtration, and a small amount of dihydrochloride form of $VIII_{14}$ was obtained.

2-Cyclopentylthio-9-[2-(4-*p*-chlorobenzylpiperazino)ethyl]adenine ($VIII_{15}$)——A mixture of VII_4 (340 mg), NaOH (360 mg) and VI_2 (1.0 g) in 25 ml of ethanol was refluxed for 10 hr. The salt was removed by filtration and the filtrate was evaporated to dryness. The residue was extracted with a mixture of benzene (50 ml) and 1 N NaOH (50 ml). The organic layer was washed with 25 ml of water twice and evaporated to dryness. The residue was applied onto a silica gel column (10 g). The column was eluted with 600 ml of $CHCl_3$ and subsequently with 400 ml of $CHCl_3$–MeOH (49: 1). The latter eluate was evaporated to dryness and the residue was crystallized and recrystallized from ethanol–water to give 230 mg (yield 33.6%) of $VIII_{15}$.

2-Cyclopentylthio-9-[2-(4-*p*-nitrobenzylpiperazino)ethyl]adenine ($VIII_{16}$)——It was prepared from VII_4 and VI_3 in a yield of 40.7% in a similar chromatographic procedures of preparation of $VIII_{15}$.

2-Cyclopentylthio-9-[2-(4-cinnamylpiperazino)ethyl]adenine ($VIII_{17}$)——It was prepared from VII_4 and VI_4 in a yield of 20.3% in a similar manner of preparation of $VIII_{15}$.

2-Cyclopentylthio-9-[2-(4-fluoren-9-ylpiperazino)ethyl]adenine ($VIII_{18}$)——It was prepared from VII_4 and VI_5 in a yield of 43.9% in a similar manner of preparation of $VIII_{15}$.

2-Cyclopentylthio-9-carboxymethyladenine ($VIII_{19}$)——A mixture of VII_4 (500 mg), NaOH (400 mg) and bromoacetic acid (1.06 g) in 34 ml of ethanol was refluxed for 3 hr. The mixture was evaporated to dryness and the residue was dissolved in water. The solution was acidified with glacial acetic acid to give precipitates. The precipitates were crystallized from ethanol to afford 107 mg (yield 16.8%) of $VIII_{19}$. It was recrystallized from ethanol–water.

2-Cyclopentylthio-9-cyclopentyladenine ($VIII_{20}$)——A mixture of VII_4 (3.0 g, 12.7 mmol), 31 mmol of 1 M NaOMe/MeOH, cyclopentyl chloride (6.6 g, 63 mmol) in 60 ml of dimethylformamide was refluxed for 20 hr under anhydrous conditions. A small amount of the salt separated was removed by filtration and the filtrate was evaporated to dryness. The residue was extracted with a mixture of ethylacetate (50 ml) and 1 N NaOH (50 ml). The aqueous layer was extracted with 50 ml of ethylacetate. The combined organic extracts were washed with 50 ml of water twice and evaporated to dryness. The residue was crystallized and recrystallized from ethanol–water to afford 960 mg (yield 24.9%) of $VIII_{20}$.

2-Cyclopentylthio-9-(2-acetoxyethyl)adenine ($VIII_{21}$)——A mixture of VII_4 (1.18 g), anhydrous K_2CO_3 (0.70 g) and 2-chloroethylacetate (1.23 g) in anhydrous dimethylacetamide (50 ml) was stirred at 110° for 13 hr. The salt separated was removed by filtration and the filtrate was evaporated to dryness. After trituration with water the residue was applied onto a silica gel column (15 g). The column was eluted with $CHCl_3$ and the eluate was evaporated to dryness. The residue was crystallized and recrystallized from methanol–water and methanol, respectively, to afford 47.3 mg (yield 3.0%) of $VIII_{21}$.

2-Cyclohexylthio-9-[2-(4-benzylpiperazino)ethyl]adenine ($VIII_{22}$)——It was prepared from VII_5 and VI_1 in a yield of 4.5% in a similar manner of preparation of $VIII_{15}$.

2-[2-(4-Benzylpiperazino)ethyl]thio-9-[2-(4-benzylpiperazino)ethyl]adenine ($VIII_{23}$) and 2-[2-(4-Benzylpiperazino)ethyl]thio-7-[2-(4-benzylpiperazino)ethyl]adenine (IX_2)——To the suspension of IV (1.0 g) in 20 ml of dimethylformamide were added 30 ml of 1 M NaOMe/MeOH and VI_1 (2.75 g). It was refluxed overnight. The solution was then evaporated to dryness and the residue was triturated with water. The gummy residue was applied onto a silica gel column (40 g) after drying over P_2O_5. The column was eluted with $CHCl_3$–MeOH (49: 1) and subsequently with $CHCl_3$–MeOH (19: 1). The former eluate was evaporated to dryness and the residue was triturated with a mixture of hydrogen chloride and ethanol to afford 321.7 mg (yield 9.4%) of 5HCl salt of $VIII_{23}$. The latter eluate was evaporated to dryness and the residue was triturated similarly to afford 125 mg (yield 3.6%) of 5HCl salt of IX_2.

Platelet Aggregation Test——Platelet aggregation studies were performed according to the optical density method of Born and Cross[7,21] by use of Bryston aggregometer attached to a Hitachi Recorder Type 056 or Rikadenki Auto-pen Recorder N-14. ADP(Na_2) was purchased from Sigma Chemical Company, Ltd. Platelet-rich citrated plasma obtained from a rabbit was immediately added to an equal volume of isotonic barbital buffer (pH 7.3). The buffered platelet-rich citrated plasma was stored at near 20° for use within 5 hr. A siliconized glass cuvette (ϕ, 7 mm) containing 1.0 ml of the buffered platelet-rich citrated plasma preincubated at 37° for 3 min was placed in an aggregometer set at 37° and allowed to stir at 1100 rpm for 3 min with a 10 μl solution of a test compound in 0.02 N HCl (solvent H), 0.02 N NaOH (solvent N) or dimethylsulfoxide (solvent D).[23] The concentration of a test compound in the solvent was 10^{-2} M. It was then challenged with 10 μl of a solution of ADP (10^{-5} M, final concentration) in saline. Inhibition percentage of aggregation by a test compound was calculated by dividing the maximum deflection of the optical density

23) K. Kikugawa, K. Iizuka, and M. Ichino, *J. Med. Chem.*, **16**, 308 (1973).

curve by that observed with the control solvent (10 μl of solvent H, N and D) and then multiplying by 100. While the control solvent H and N was non-effective against platelet aggregation, solvent D inhibited aggregation slightly.[23] The inhibition percentages thus obtained were not absolute, and therefore the relative potency of inhibition of a compound to a reference standard, adenosine, at the same concentration (RAD) was a direct measure of potency of inhibition. RAD was calculated by dividing the percent inhibition of a test compound with that of adenosine.

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Studies on Pyrimidine Derivatives. II.[1] Reaction of Some Dimethyl- and Trimethyl-heteroaromatics with Ethyl Nitrite

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Methyl groups at the 4-position of 2,4-dimethyl-, 2,4,6-trimethyl-pyridine, 2,4-dimethyl-, 2,4,6-trimethyl-pyrimidine, 2,4-dimethylquinoline, and 2,4-dimethylquinazoline were nitrosated selectively with ethyl nitrite in the presence of alkali amide in liquid ammonia to give the corresponding 4-aldoximes. On heating with phosphoryl chloride, the aldoximes were converted to 4-carbonitriles except 2-methylquinazoline-4-aldoxime which was sublimated with phosphorous pentoxide to afford 4-cyano-2-methylquinazoline.

Related to these synthetic experiments, the hydrogen-deuterium exchange reaction of the methyl groups was examined by means of the NMR technique. The observed results did not contradict the direction of nitrosation of the testing compounds.

Keywords——hydrogen-deuterium exchange of active methyl groups; nitrosation of active methyl groups; pyridine-4-aldoximes; 2-methylquinoline-4-aldoxime; pyrimidine-4-aldoximes; 2-methylquinazoline-4-aldoxime; 4-cyanopyridines; 4-cyano-2-methylquinoline; 4-cyanopyrimidines; 4-cyano-2-methylquinazoline

Many papers[3-7] related to the reactions of the active methyl groups attached to an electron-deficient heteroaromatic ring were published. For instance, Kato, *et al.*[8] described that the active methyl groups on a pyridine ring were nitrosated with pentyl nitrite under strongly basic conditions to give 2- or 4-pyridinealdoximes. They extended this reaction to some methylpyrimidine derivatives[9] to find that the activity of pyrimidine derivatives for this nitrosation exceeded than that of pyridine derivatives. However, in the case of compounds containing two or three active methyl groups in the same molecule, this reaction has not been well examined.

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