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# Platelet Aggregation Inhibitors. 4.[1] $N^6$-Substituted Adenosines

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Various $N^6$-substituted adenosines **1–21** were synthesized in 20–100% yields by reaction of 6-chloropurine ribonucleoside or 2-amino-6-chloropurine ribonucleoside with the requisite substituent amine in EtOH. $N^6$-Benzyl- (or allyl-) adenosine 5′-monophosphate **27** and **28** was prepared by treatment of adenosine 5′-monophosphate with benzyl (or allyl) bromide and alkali. Among the $N^6$-substituted adenosines **1–26** and $N^6$-substituted adenosine 5′-monophosphate **27** and **28**, $N^6$-phenyl- (**1**), $N^6$-*o*-chlorophenyl- (**2**), $N^6$-*p*-chlorophenyl- (**4**), $N^6$-*p*-methoxyphenyl- (**7**), $N^6$-cyclopentyl- (**10**), $N^6$-cyclohexyl- (**11**), $N^6$-(*p*-hydroxyphenylethyl)- (**23**), $N^6$-(indole-3-ethyl)- (**24**), and $N^6$-allyl- (**26**) adenosines showed strong inhibitory activity at $10^{-4}$ *M* against adenosine 5′-diphosphate and collagen induced rabbit platelet aggregation. Incubation of these active compounds with rabbit platelet-rich plasma for a period exceeding 2 hr did not lead to loss of activity. Rabbit platelet adhesiveness to glass beads was inhibited by compounds **1** and **11**. These also inhibited adenosine 5′-diphosphate induced platelet aggregation in plasmas obtained from rabbits given single intravenous doses of 8 mg/kg. Compounds **1** and **11** were also active as inhibitors of human platelet aggregation.

Platelet aggregation or platelet thrombus formation is of primary importance in arterial thrombogenesis.[2] Agents that inhibit platelet aggregation are of interest as potential drugs, and a number of compounds have been tested as inhibitors of adenosine 5′-diphosphate (ADP) and/or collagen induced platelet aggregation.[3–14] Among them, prostaglandin $E_1$,[4] certain adenosine derivatives,[5–7] pyrimidopyrimidines,[8,9] thieno compounds,[10] thiazolo compounds,[11] [(dialkylamino)-alkyl]thio heterocyclic compounds,[12,13] and a fluorene derivative[14] are typical strong inhibitors of platelet aggregation, but few have been evaluated as antithrombotic agents owing to their undesirable side effects or toxicity.

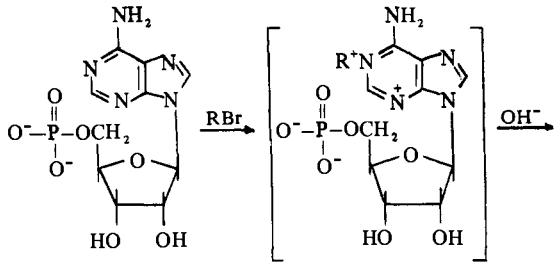
Among the derivatives of adenosine, 2-chloroadenosine[5] has been found to be a powerful inhibitor of platelet aggregation, but it is very toxic.[15] Recently, 2-methylthioadenosine 5′-monophosphate which is less toxic has been evaluated to be an antithrombotic agent, although it showed rather weak inhibitory activity against platelet aggregation.[16] In the course of our studies on platelet aggregation inhibitors, we have found 6-hydroxyaminopurine ribonucleosides to be ten times as potent as adenosine in the inhibition of rabbit plasma platelet aggregation.[7] However, they showed little activity against human platelet aggregation.[7,17]

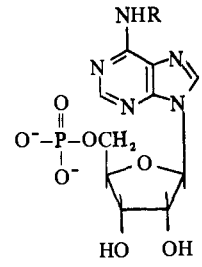
In order to secure further information on the structure–activity relationships in this series of adenosine derivatives, some additional $N^6$-substituted adenosines and $N^6$-substituted adenosine 5′-monophosphates were prepared and examined for the inhibitory activity of platelet aggregation induced by ADP and collagen.

Chemistry. 6-Substituted purine ribonucleosides have been synthesized by treatment of 6-halogenopurine ribonucleosides,[7,18–22] 6-methyl- (or benzyl-) thiopurine ribonucleosides,[23] or 6-trimethylsilyloxypurine ribonucleosides[24] with appropriate amines or *via* $N^1$-substituted adenosines obtained by reaction of adenosine with appropriate halides.[21,25] Fleysher, *et al.*,[20–22] have synthesized several $N^6$-substituted adenosines by treatment of 6-chloropurine ribonucleoside with amines in the presence of $CaCO_3$ or $Et_3N$ and also by the $N^1$ quaternization of adenosine with the appropriate halides. In the present study, 6-chloropurine ribonucleoside[18] or 2-amino-6-chloropurine ribonucleoside[26] was allowed to react with large excesses of various amines and the corresponding $N^6$-substituted adenosines **1–21** were obtained. The reaction was performed in EtOH in the absence of an auxiliary acid acceptor which facilitated the isolation of the products. The structures and the physical data of **1–21** are given in Table I. Compounds **8–10** and **12–21** have not been reported thus far, while compounds **1**,[21] **11**,[22] **22**,[18,27] **25**,[21] and **26**[20] have been reported in the literature and compounds **2–7** have been described in the patent field.[28] $N^6$-Substituted adenosine 5′-monophosphates **27** and **28** were prepared by reaction of adenosine 5′-monophosphate (AMP) with benzyl (or allyl) bromide and subsequent treatment of the intermediate $N^1$-benzyl- (or allyl-) AMP with alkali (Scheme I).

**Pharmacology.** The $N^6$-substituted adenosines **1–26** and $N^6$-substituted AMP **27** and **28** were tested *in vitro* as inhibitors of ADP- and collagen-induced rabbit platelet aggregation according to the method of Born and Cross.[29] The

Scheme I





27, R = $PhCH_2$-
28, R = $CH_2$=$CHCH_2$-

inhibitory activity of every compound was estimated by the extent of the decrease in the optical density of platelet-rich citrated plasma (PRCP) after the addition of ADP or collagen.

Rabbit PRCP was buffered at pH 7.7 ± 0.1 in order to keep pH of the plasma constant, since the extent and the capacity of aggregation of platelets were largely influenced by pH of the plasma.[30] In order to test the inhibitory effect of compounds insoluble in saline such as **9**, aprotic solvents were examined as solvents of inhibitors of aggregation (Table II). Dimethylformamide (DMF), hexamethylphosphoramide (HMPA), and formamide (FA) were not proper solvents, since aggregation was greatly influenced by DMF and HMPA, and certain inhibitors such as hydroxylamine[31] were inactivated by FA. Dimethyl sulfoxide (DMSO) did not show inhibition greater than 50% against ADP- and collagen-induced aggregation and was found to be useful as a solvent for inhibitors. Comparisons of the inhibitory activities of several compounds relative to adenosine (Rad) in DMSO with those in saline (Table III) indicated that DMSO was a proper solvent of inhibitors.

Among the compounds **1-28**, $N^6$-phenyl- (**1**), $N^6$-*o*-chlorophenyl- (**2**), $N^6$-*p*-chlorophenyl- (**4**), $N^6$-*p*-methoxyphenyl- (**7**), $N^6$-cyclopentyl- (**10**), $N^6$-cyclohexyl- (**11**), $N^6$-(*p*-hydroxyphenylethyl)- (**23**), $N^6$-(indole-3-ethyl)- (**24**), and $N^6$-allyl- (**26**) adenosines showed strong inhibitory activity at $10^{-4}$ *M* against ADP- and collagen-induced rabbit platelet aggregation (Table III). The relative potency (Rad) of each of these to adenosine at $10^{-4}$ *M* was greater than 0.6, and these were as potent as the parent compound, adenosine. Other compounds except $N^6$-hydroxyethyladenosine (**22**) had little or no inhibitory activity at $10^{-4}$ *M*. Inhibitory effects of the active compounds were concentration dependent and each at $10^{-5}$ *M* was less active than at $10^{-4}$ *M* and than adenosine at $10^{-5}$ *M*.

The inhibitory profiles of **1** and **11** at $10^{-4}$ *M* against ADP-induced aggregation are presented in Figure 1A, which demonstrated that the inhibitory effect of these compounds was similar to that of adenosine and different from that of previously reported compounds, 6-hydroxyaminopurine ribonucleosides,[7] which induced very strong deaggregation after the maximal aggregation. Deaggregation potencies of **1** and **11** against platelet aggregates mediated by ADP were

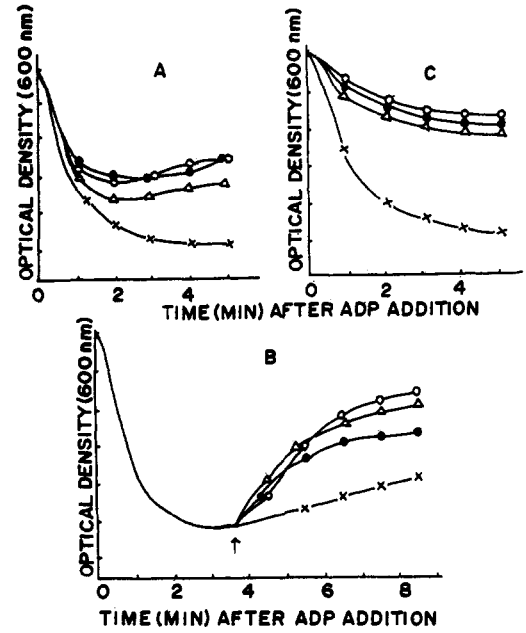



Figure 1. Inhibitory profiles of $N^6$-phenyl- (1) and $N^6$-cyclohexyl- (**11**) adenosines against ADP-induced platelet aggregation. A: buffered PRCP (rabbit) was treated with each of the test samples in saline for 3 min and challenged with ADP. B: buffered PRCP (rabbit) was challenged with ADP. After the maximum aggregation (indicated by an arrow) each of the test samples in saline was added. C: PRCP (human) was treated with each of the test samples in saline for 3 min and challenged with ADP. Adenosine ($10^{-4}$ *M*), ○; $N^6$-phenyladenosine (**1**, $10^{-4}$ *M*), △; $N^6$-cyclohexyladenosine (**11**, $10^{-4}$ *M*), ●; and saline (control), X.

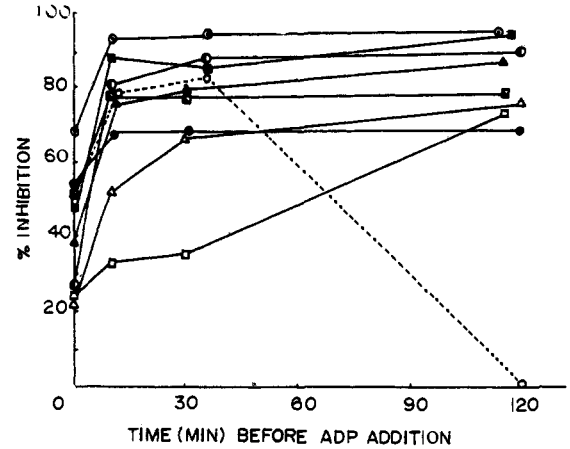


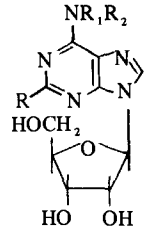

Figure 2. Incubation effect of $N^6$-substituted adenosines with rabbit platelet-rich plasma on ADP-induced platelet aggregation. Buffered PRCP was treated with the test sample ($10^{-4}$ *M*) for the indicated period and challenged with ADP. $N^6$-Phenyladenosine (**1**), △; $N^6$-*o*-chlorophenyladenosine (**2**), □; $N^6$-*p*-chlorophenyladenosine (**4**), ■; $N^6$-*p*-methoxyphenyladenosine (**7**), ◐; $N^6$-cyclopentyladenosine (**10**), ▲; $N^6$-cyclohexyladenosine (**11**), ●; $N^6$-(*p*-hydroxyphenylethyl)adenosine (**23**), ◪; adenosine, ○; and 2-chloroadenosine, ⊙.

investigated (Figure 1B), and they were as strong as that of adenosine at $10^{-4}$ *M* though weaker than those of 6-hydroxyaminopurine ribonucleosides.[7]

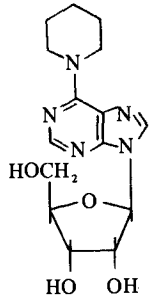
6-Hydroxyaminopurine ribonucleosides which strongly inhibited rabbit platelet aggregation have been ineffective against human platelet aggregation.[7,17] Compounds **1** and **11** were active as adenosine against human platelet aggregation induced by ADP. Profiles are shown in Figure 1C.

The compounds **1**, **2**, **4**, **7**, **10**, **11**, and **23** were incubated at 37° with buffered rabbit PRCP over the longer intervals

Table I. $N^6$-Substituted Adenosines



1-20



21

| No. | Compound | R | $R_1$ | $R_2$ | Method[a] | Yield, % | Purifcn solvent | Mp, °C[b] | Paper chromatography[c] $R_{f_A}$ | Paper chromatography[c] $R_{f_B}$ | Uv, λ max (ε × $10^{-3}$), nm pH 1.0 | Uv, λ max (ε × $10^{-3}$), nm $H_2O$ | Uv, λ max (ε × $10^{-3}$), nm pH 13.0 | Formula and analyses[d] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $N^6$-Phenyladenosine[e] | H | H | $C_6H_5$- | A | 88.3 | EtOH-$H_2O$ | 195-196 | 0.82 | 0.89 | 275 (17.8) | 290 (20.8) | 290 (20.9) | ($C_{16}H_{17}O_4N_5$) C, H, N |
| 2 | $N^6$-*o*-Chlorophenyl-adenosine | H | H | *o*-$ClC_6H_4$- | A | 52.3 | EtOH-$H_2O$ | 190-192[f] | 0.85 | | 274 (21.7) | 279 (20.7) | 281.5 (20.1) | ($C_{16}H_{16}O_4N_5Cl \cdot 0.5H_2O \cdot 0.5C_2H_5OH$) C, H, N |
| 3 | $N^6$-*m*-Chlorophenyl-adenosine | H | H | *m*-$ClC_6H_4$- | A | 74.8 | EtOH | 208-210[f] | 0.86 | | 277 (18.6) | 293.5 (23.5) | 294.5 (23.8) | ($C_{16}H_{16}O_4N_5Cl \cdot 0.5C_2H_5OH$) C, H, N |
| 4 | $N^6$-*p*-Chlorophenyl-adenosine | H | H | *p*-$ClC_6H_4$- | A | 82.0 | EtOH | 205-206[f] | 0.84 | | 277 (20.4) | 294.5 (25.3) | 294.5 (25.5) | ($C_{16}H_{16}O_4N_5Cl \cdot 0.5C_2H_5OH$) C, H, N |
| 5 | $N^6$-*o*-Methoxyphenyl-adenosine | H | H | *o*-$OMeC_6H_4$- | A | 82.9 | EtOH | 229-230[f] | 0.79 | | 272 (18.6) | 282.5 (17.4) | 282.5 (17.9) | ($C_{17}H_{19}O_5N_5 \cdot 0.5C_2H_5OH$) C, H, N |
| 6 | $N^6$-*m*-Methoxyphenyl-adenosine | H | H | *m*-$OMeC_6H_4$- | A | 90.1 | EtOH | 201-203[f] | 0.76 | | 276 (18.5) | 298 (21.1) | 298 (21.6) | ($C_{17}H_{19}O_5N_5 \cdot 0.5C_2H_5OH$) C, H, N |
| 7 | $N^6$-*p*-Methoxyphenyl-adenosine | H | H | *p*-$OMeC_6H_4$- | A | 88.5 | EtOH | 213-217[f] | 0.74 | 0.92 | 275 (21.0) | 288.5 (21.8) | 288.5 (22.3) | ($C_{17}H_{19}O_5N_5 \cdot C_2H_5OH$) C, H, N |
| 8 | $N^6$-(α-Naphthyl-adenosine | H | H | α-Naphthyl | A | 78.9 | EtOH | 170-172 | 0.82 | | 265 (19.1)<br>283 sh (17.7) | 273 sh (16.6)<br>283 (17.3) | 273 sh (16.6)<br>283 (17.2) | ($C_{20}H_{19}O_4N_5 \cdot 0.5C_2H_5OH$) C, H, N |
| 9 | $N^6$-(β-Naphthyl)-adenosine | H | H | β-Naphthyl | A | 96.1 | EtOH | 228-230 | 0.79 | | 277 (29.6) | 272 sh (22.9)<br>279.5 (26.2)<br>309.5 (20.8) | 272 sh (24.0)<br>279.5 (27.5)<br>309.5 (22.4) | ($C_{20}H_{19}O_4N_5 \cdot 0.5C_2H_5OH$) C, H, N |
| 10 | $N^6$-Cyclopentyl-adenosine | H | H | *c*-$C_5H_9$- | B | 74.8 | $H_2O$ | 77-81 | | 0.93 | 266 (22.7) | 270 (19.9) | 269.5 (20.4) | ($C_{15}H_{21}O_4N_5$) C, H, N |
| 11 | $N^6$-Cyclohexyl-adenosine[e] | H | H | *c*-$C_6H_{11}$- | B | 100 | AcOEt-EtOH | 187-188 | 0.83 | | 265.5 (19.0) | 270 (18.3) | 270 (18.0) | ($C_{16}H_{23}O_4N_5$) C, H, N |
| 12 | $N^6$-Cycloheptyl-adenosine | H | H | *c*-$C_7H_{13}$- | C | 22.0 | EtOH | 93-97 | | 0.84 | 265.5 (21.8) | 271 (19.3) | 271 (21.0) | ($C_{17}H_{25}O_4N_5 \cdot C_2H_5OH$) C, H, N |
| 13 | $N^6$-Cyclooctyl-adenosine | H | H | *c*-$C_8H_{15}$- | B | 54.0 | $H_2O$ | 167-168 | 0.85 | | 265 (21.7) | 270.5 (19.2) | 270.5 (21.1) | ($C_{18}H_{27}O_4N_5 \cdot 0.5H_2O$) C, H, N |
| 14 | $N^6$-(4-Cyclohexyl-butyl)adenosine | H | H | *c*-$C_6H_{11}(C_2H_2)_4$- | A | 76.0 | EtOH-$H_2O$ | 108-114 | 0.88 | | 264.5 (21.1) | 270 (17.0) | 269.5 (17.7) | ($C_{20}H_{31}O_4N_5 \cdot 0.5C_2H_5OH$) C, H, N |
| 15 | $N^6$-Di(hydroxyethyl)-adenosine | H | $HOC_2H_4$- | $HOC_2H_4$- | A | 84.5 | EtOH-$H_2O$ | 228-230 | 0.40 | 0.66 | 274 (18.3) | 279 (20.2) | 279 (20.5) | ($C_{14}H_{21}O_6N_5$) C, H, N |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 16 | $N^6$-(3-Dimethylamino-propyl)adenosine | H | H | $(CH_3)_2N(CH_2)_3$– | D | 36.7 | EtOH-$H_2O$ | 160 dec | 0.05 | 0.62 | 264 (18.8) | 267 (19.2) | 269 (20.0) | $(C_{15}H_{24}O_4N_6 \cdot 2HCl \cdot H_2O)$ C, H, N |
| 17 | $N^6$-Diethylaminoethyl-adenosine | H | H | $(C_2H_5)_2N(C_2H_4)$– | D | 24.9 | EtOH-$H_2O$ | 145–155 dec | 0.07 | 0.69 | 269 (16.9) | 267 (19.2) | 269.5 (19.4) | $(C_{16}H_{26}O_4N_6 \cdot 2HCl \cdot 0.5H_2O)$ C, H, N |
| 18 | 2-Amino-$N^6$-phenyl-adenosine | $NH_2$ | H | $C_6H_5$– | E | 80.2 | EtOH-$H_2O$ | 269–270 dec | 0.66 | 0.83 | 256.5 (12.9) 306 (17.6) | | 251.5 (13.8) 305 (23.8) | $(C_{16}H_{18}O_4N_6)$ C, H, N |
| 19 | 2-Amino-$N^6$-dihydroxyethyl)adenosine | $NH_2$ | $HOC_2H_4$– | $HOC_2H_4$– | E | 70.5 | EtOH-$H_2O$ | 123–124 dec | 0.26 | 0.60 | 232 (12.3) 238 (13.0) 259 (13.5) 292.5 (12.8) | 231.5 (19.0) 288 (16.1) | 230 (20.4) 288 (16.6) | $(C_{14}H_{22}O_6N_6 \cdot H_2O)$ C, H, N |
| 20 | 2-Amino-$N^6$-(p-hydroxyphenylethyl)-adenosine | $NH_2$ | H | p-HO-$C_6H_4(CH_2)_2$– | F | 56.4 | $H_2O$ | 137–139 dec | 0.58 | 0.76 | 256 (14.8) 295 (12.0) | 283 (15.4) | 285 (18.1) | $(C_{18}H_{22}O_5N_6 \cdot H_2O)$ C, H, N |
| 21 | 6-Piperidinopurine ribonucleoside | | | | B | 73.0 | EtOH-$H_2O$ | 180–181 | 0.79 | 0.88 | 274 (21.9) | 282 (22.8) | 282 (23.4) | $(C_{15}H_{21}O_4N_5 \cdot H_2O)$ C, H, N |

[a]Methods A–F were described in the Experimental Section. [b]Melting points were determined on a Büchi-Tottoli melting point apparatus and are uncorrected. [c]$R_{f_A}$ (solvent, *n*-BuOH-$H_2O$, 84:16), $R_{f_B}$ (solvent, *n*-BuOH-AcOH-$H_2O$, 2:1:1). [d]Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. [e]Synthesized according to the modified method of Fleysher, *et al.*[21,22] [f]Melting points were described in a German Patent.[28]

before the addition of ADP (Figure 2). Inhibitory activity of these compounds increased for 10–30 min, as in the cases of adenosine and 2-chloroadenosine. Although adenosine completely lost its activity after 2-hr incubation, the inhibitory potencies of these compounds were maintained even after the interval of 2 hr as in the case of 2-chloroadenosine. It has been explained by Born[5] that the loss of the inhibitory activity of adenosine was probably due to the susceptibility of the nucleoside to plasma adenosine deaminase, and the retention of the activity of 2-chloroadenosine was attributed to the resistance of this compound to the enzyme. In order to explore inactivation of adenosine by plasma deaminase, the inhibitory activity of adenosine which was incubated at 37° for 2 hr with rabbit platelet-poor plasma was examined. The results (Table IV) indicated that the activity of adenosine was not lost in the platelet-poor plasma. Ultraviolet absorption and paper chromatography estimation of the incubated mixture showed that adenosine was intact and not even a trace of inosine could be detected. It seems, therefore, that the loss of the inhibitory activity of adenosine in PRCP could not be attributed to plasma adenosine deaminase but to the interaction with some factors concerned with platelets. 2-Chloroadenosine, **1**, and **11** were also intact with incubation with the plasma. In any event, it is evident that the active compounds synthesized had long-lasting inhibitory activity and were evaluated as strong inhibitors.

Compounds **1** and **11** were tested as inhibitors of rabbit platelet adhesiveness according to the method of Hellem.[32] These effectively inhibited platelet adhesiveness to glass beads (Table V). In order to establish the *in vivo* inhibitory effect of platelet aggregation of **1** and **11**, *in vivo-in vitro* test[11,33] was performed. Thus, single doses of 8 mg/kg each of **1**, **11**, adenosine, and 2-chloroadenosine were infused over a period of 30 min, through an ear vein, into anesthetized rabbits. Buffered PRCP obtained 0–150 min after the infusion was submitted to a test of aggregation induced by ADP. The results shown in Figure 3 indicated that **1** and **11** reduced platelet aggregation as effectively as adenosine and 2-chloroadenosine.

In conclusion, several adenosine derivatives substituted at the $N^6$ position were found as potent inhibitors of platelet




Figure 3. Rabbit platelet aggregation after intravenous infusion of adenosine derivatives. $N^6$-Phenyladenosine (**1**, Δ), $N^6$-cyclohexyladenosine (**11**, ●), adenosine (○), 2-chloroadenosine (⊙), and saline (control, X) were infused at a single dose of 8 mg/kg. Buffered PRCP collected at the indicated period after infusion was challenged with ADP. The results of more than two experiments were used to plot the average values of the aggregation percentage.

Table II. Effect of Aprotic Solvents on Rabbit Platelet Aggregation

| Solvent | Amount of solvent: Buffered PRCP, μl/ml | Amount of solvent: Concn, M | % aggregation[a]: ADP induced | % aggregation[a]: Collagen induced |
|---|---|---|---|---|
| Saline | 10 | | 100 | 100 |
| Dimethyl sulfoxide (DMSO)[b] | 10 | 0.14 | 57 | 59 |
| Dimethylformamide (DMF) | 10 | 0.13 | 17 | 12 |
| Hexamethylphosphoramide (HMPA) | 10 | 0.06 | 30 | 12 |
| Formamide (FA) | 10 | 0.25 | 67 | 89 |

[a]Expressed by per cent aggregation compared with saline. [b]DMSO has been shown to be inhibitory against ADP-induced rat platelet aggregation: P. Gorog and I. B. Kovacs, *Proc. Int. Symp., Inflammation Biochem. Drug Interaction*, 197 (1968).

Table III. Inhibition of Rabbit Platelet Aggregation by $N^6$-Substituted Adenosines[a]

| Compound | Solvent[b] | Inhibition of ADP-induced aggregation[c]: $10^{-4}$ M % | $10^{-4}$ M Rad | $10^{-5}$ M % | $10^{-5}$ M Rad | Inhibition of collagen-induced aggregation[c]: $10^{-4}$ M % | $10^{-4}$ M Rad | $10^{-5}$ M % | $10^{-5}$ M Rad |
|---|---|---|---|---|---|---|---|---|---|
| $N^6$-Phenyladenosine (1) | 1 | 82 | 0.9 | 30 | 1.0 | 41 | 0.6 | 18 | 0.3 |
| | 3 | 86 | 0.7 | | | | | | |
| $N^6$-*o*-Chlorophenyladenosine (2) | 1 | 71 | 0.8 | 23 | 0.3 | 45 | 0.8 | 5 | 0.1 |
| | 3 | 53 | 0.7 | | | 48 | 0.9 | | |
| $N^6$-*p*-Chlorophenyladenosine (4) | 2 | 73 | 0.8 | 27 | 0.5 | 72 | 0.8 | 37 | 0.7 |
| | 3 | 85 | 1.0 | | | 78 | 1.2 | | |
| $N^6$-*p*-Methoxyphenyladenosine (7) | 2 | 72 | 0.9 | 47 | 0.6 | 94 | 1.2 | 18 | 0.4 |
| | 3 | 87 | 1.0 | | | 57 | 0.9 | | |
| $N^6$-(β-Naphthyl)adenosine (9)[d] | 3 | 28 | 0.4 | | | 59 | 0.8 | | |
| $N^6$-Cyclopentyladenosine (10) | 1 | 64 | 0.9 | 8 | 0.1 | 75 | 0.9 | 42 | 0.6 |
| | 3 | 56 | 0.7 | | | | | | |
| $N^6$-Cyclohexyladenosine (11) | 1 | 84 | 1.1 | 49 | 0.7 | 75 | 0.9 | 27 | 0.4 |
| | 3 | 71 | 1.0 | | | | | | |
| $N^6$-Hydroxyethyladenosine (22) | 2 | 21 | 0.4 | 0 | 0 | 12 | 0.3 | 7 | 0.3 |
| $N^6$-(*p*-Hydroxyphenylethyl)adenosine (23) | 2 | 62 | 0.9 | 17 | 0.4 | 31 | 1.0 | 4 | 0.2 |
| $N^6$-(Indole-3-ethyl)adenosine (24) | 2 | 41 | 0.6 | 13 | 0.3 | 27 | 0.6 | 0 | 0 |
| $N^6$-Allyladenosine (26) | 1 | 33 | 1.0 | 4 | 0.2 | 37 | 1.0 | 7 | 0.2 |
| Adenosine | 1 | 30–80 | 1.0 | 20–80 | 1.0 | 30–90 | 1.0 | 20–70 | 1.0 |
| | 2 | 50–80 | 1.0 | 20–80 | 1.0 | 30–90 | 1.0 | 20–70 | 1.0 |
| | 3 | 70–100 | 1.0 | | | 50–90 | 1.0 | | |

[a]Among the $N^6$-substituted adenosines 1–26 and $N^6$-substituted 5'-AMP's 27 and 28, compounds unlisted in Table III were inactive (Rad <0.3). [b]Compounds were dissolved in the following solvents at $10^{-4}$ or $10^{-5}$ M when added to buffered PRCP (1 ml): (1) saline (10 μl), (2) saline (100 μl), and (3) DMSO (10 μl). [c]Inhibition percentages were not absolute (see Experimental Section). Relative potency (Rad) of inhibition of every compound to adenosine compared in the same solvent under the same conditions was a direct measure of potency of inhibition. [d]Insoluble in saline.

Table IV. Inhibition of Rabbit Platelet Aggregation by Adenosine Derivatives Incubated with Platelet-Poor Plasma

| Sample incubated with platelet-poor plasma | % inhibition of aggregation[a]: 0 | 60 | 120 |
|---|---|---|---|
| Saline | 2 | −5 | 3 |
| Adenosine | 83 | 91 | 81 |
| 2-Chloroadenosine | 85 | 86 | 85 |
| $N^6$-Phenyladenosine (1) | 54 | 59 | 81 |
| $N^6$-Cyclohexyladenosine (11) | 76 | 78 | 78 |

[a]Incubation time in minutes.

aggregation induced by ADP and collagen. These were active against both rabbit and human platelet aggregation and were characterized by long-lasting activity. Availability of these active compounds synthesized depends on further pharmacological investigations, because there has been found a good correlation between vasodilative and antithrombotic activity with some derivatives of adenosine.[34]

## Experimental Section

**Chemical Methods and Materials.** Paper chromatography was performed using Tōyō Roshi No. 51A paper in an ascending technique with solvent systems: A, *n*-BuOH–$H_2O$ (84:16); B, *n*-BuOH–AcOH–$H_2O$ (2:1:1); C, *i*-PrOH–$NH_4OH$–$H_2O$ (7:1:2); D, EtOH–0.5 M $NH_4OAc$ (5:2); and E, $NH_4OH$–$H_2O$ (pH 10). Uv spectra were measured with a Hitachi recording spectrophotometer, ESP-3T. Aniline, *o*-, *m*-, and *p*-chloroanilines, *o*-, *m*-, and *p*-anisidines, α- and β-naphthylamines, cyclohexylamine, *N,N*-diethanolamine, *N,N*-dimethyl-1,3-propanediamine, tyramine hydrochloride, and piperidine were purchased from Wako Pure Chemical Industries, Ltd. *N,N*-Diethylethylenediamine was from Tokyo Kasei Kogyo Co., Ltd. Cyclopentyl-, cycloheptyl-, cyclooctyl-, and cyclohexanebutylamines were the products of Aldrich Chemical Co., Inc. Calf intestinal phosphomonoesterase was purchased from Tokyo Kasei Kogyo Co., Ltd.

$N^6$-Hydroxyethyladenosine (**22**),[27b] $N^6$-(*p*-hydroxyphenylethyl)-adenosine (**23**), and $N^6$-(indole-3-ethyl)adenosine (**24**) were gifts of Dr. M. Shikita. $N^6$-Benzyladenosine (**25**) and $N^6$-allyladenosine (**26**) were originally prepared according to ref 20 and 21 and were now products of this company. 2-Chloroadenosine was synthesized according to the modified method of Montgomery and Hewson.[19b]

**$N^6$-Substituted Adenosines 1–21. Method A.** $N^6$-(α-Naphthyl)-adenosine (**8**). 6-Chloropurine ribonucleoside (2.0 g, 7 mmol) and α-naphthylamine (6.01 g, 42 mmol) were dissolved in 42 ml of EtOH. After the mixture was refluxed for 5 hr, it was kept overnight in a refrigerator. The crystals of 8 which separated were collected by filtration.

**Method B.** $N^6$-Cyclooctyladenosine (**13**). A mixture of 6-chloropurine ribonucleoside (2.0 g), cyclooctylamine (5.35 g, 42 mmol), and EtOH (42 ml) was refluxed for 5 hr and it was evaporated *in vacuo* to dryness. The residue was triturated with a small amount of EtOH, and the crystals of 13 were obtained.

**Method C.** $N^6$-Cycloheptyladenosine (**12**). A mixture of 6-chloropurine ribonucleoside (2.0 g), cycloheptylamine (4.8 g, 42 mmol), and EtOH (42 ml) was refluxed for 5 hr and it was cooled in an ice bath. A crystalline precipitate of cycloheptylamine hydrochloride was filtered off, and the filtrate was evaporated *in vacuo* to one-half of the original volume. The precipitate which separated con-

Table V. Inhibition of Platelet Adhesiveness by Adenosine Derivatives

| Compound | At 24° | | At 37° | |
|---|---|---|---|---|
| | Adhesiveness, % | Inhibition, % | Adhesiveness, % | Inhibition, % |
| Control (saline) | 44 | 0 | 45 | 0 |
| Adenosine | 21 | 52 | 34 | 24 |
| $N^6$-Phenyladenosine (1) | 23 | 47 | 23 | 49 |
| $N^6$-Cyclohexyladenosine (11) | 18 | 60 | 7 | 85 |

taining 17 was collected, washed with $H_2O$, and crystallized from EtOH.

**Method D.** $N^6$-(3-Dimethylaminopropyl)adenosine (16). 6-chloropurine ribonucleoside (2.0 g) and *N,N*-dimethyl-1,3-propanediamine (14.3 g, 140 mmol) were dissolved in 42 ml of EtOH. The mixture was refluxed for 5 hr and evaporated *in vacuo* to dryness. The residue was applied onto a cellulose column (1.7 × 40 cm) and eluted with solvent B. Fractions containing **16** examined by paper chromatography (solvent B) were evaporated *in vacuo* to dryness. The residue was redissolved in 200 ml of $H_2O$ and passed through a Dowex 1X4 ($Cl^-$) column (40 ml). The effluent was evaporated *in vacuo* to dryness and the residue was crystallized from EtOH to afford a crystalline powder of $N^6$-(3-dimethylaminopropyl)adenosine hydrochloride (16).

$N^6$-Diethylaminoethyladenosine (17). A mixture of 6-chloropurine ribonucleoside (2.0 g), *N,N*-diethylethylenediamine (16.3 g, 140 mmol), and EtOH (42 ml) was refluxed for 5 hr and was evaporated *in vacuo* to dryness. The residue was triturated with EtOH to afford a hygroscopic crystalline powder. The powder was purified using a cellulose column and a Dowex 1 column successively as described above and the crystalline $N^6$-diethylaminoethyladenosine hydrochloride (17) was obtained from EtOH–acetone.

**Method E.** 2-Amino-$N^6$-phenyladenosine (18). 2-Amino-6-chloropurine ribonucleoside (2.0 g, 6.6 mmol) and aniline (3.92 g, 42 mmol) were dissolved in 42 ml of EtOH and refluxed for 6 hr. The mixture was kept in a refrigerator overnight. The crystals of 18 which separated were collected by filtration.

**Method F.** 2-Amino-$N^6$-(*p*-hydroxyphenylethyl)adenosine (20). Tyramine hydrochloride (2.42 g, 14 mmol) was dissolved in 41 ml of hot absolute EtOH and the mixture was added with 784 mg (14 mmol) of KOH dissolved in 3 ml of absolute EtOH. White precipitate of KCl was filtered off and to the filtrate was added 2.0 g (6.6 mmol) of 2-amino-6-chloropurine ribonucleoside. The mixture was refluxed for 12 hr and was evaporated *in vacuo* to a small volume. The product (20) which separated was collected by filtration.

$N^6$-Benzyladenosine 5'-Monophosphate (27). 5'-AMPNa$_2$ (1.9 g, 4.8 mmol) was suspended in 12 ml of anhydrous DMF and the mixture was added to 2 ml (16 mmol) of benzyl bromide. After the mixture was stirred at 40° for 48 hr, it was added to an additional 2 ml of benzyl bromide and the reaction was continued for 24 hr. Paper chromatography (solvent C) of the reaction mixture revealed a major spot having a $R_f$ of 0.35 ($R_f$ of 5'-AMP, 0.20). The uv spectrum of the aqueous extract of the spot showed λ max ($H^+$) 260 and λ max ($H_2O$, $OH^-$) 261 nm, which was identified as that of $N^1$-benzyladenosine 5'-monophosphate. The reaction mixture was evaporated *in vacuo* and the residue was washed with 50 ml of $CHCl_3$ twice to remove excess benzyl bromide. The residue was dissolved in 50 ml of concentrated $NH_4OH$ and heated at 60° for 3.5 hr. Paper chromatography (solvent C) revealed a major spot having a higher $R_f$ value of 0.50. The mixture was evaporated *in vacuo* to dryness and the residue was purified through a cellulose column (1.5 × 50 cm) with solvent C. The fractions containing the product were evaporated *in vacuo* to dryness to afford 711 mg of crude ammonium salt of 27. The salt was redissolved in 100 ml of $H_2O$ and absorbed to diethylaminoethyl cellulose ($HCO_3^-$) column (2 × 20 cm) and eluted with 0.01 *M* $Et_3N \cdot HCO_3$. Fractions (1000–2000 ml) containing the product were evaporated to dryness, and the residue was redissolved in 10 ml of $H_2O$ and absorbed to a column of 10 ml of Dowex 1X2 ($OH^-$). After elution of the column with 200 ml of 2 *N* HCOOH, the eluate was evaporated *in vacuo* (below 35°) to dryness. Coevaporation with EtOH several times furnished a crystalline powder of 27. It was dried at 40° *in vacuo* over NaOH and weighed 598 mg (yield, 28%): uv λ max (pH 1) 266 nm (εp 19,700), (pH 7) 270 (19,700), and (pH 13) 270 (19,900); paper chromatography $R_f$ 0.50 (solvent C); purity 102.5% (phosphorus assay). The compound (27) was treated with calf intestinal phosphomonoesterase at pH 7.5 and 37° for 1 hr, and paper chromatography of the dephosphorylated mixture showed a spot having $R_f$'s of 0.78 (solvent C) and 0.87 (solvent D) which were identical with those of an authentic $N^6$-benzyladenosine (25).[21]

$N^6$-Allyladenosine 5'-Monophosphate (28). 5'-AMPNa$_2$ (946 mg, 2.4 mmol) was treated with allyl bromide in DMF as described above. Paper chromatography (solvent C) of the reaction mixture revealed a major spot having a $R_f$ of 0.45. The mixture was evaporated *in vacuo* to dryness to obtain a gum, which was subsequently treated with 50 ml of concentrated $NH_4OH$ at 60° for 3 hr. Paper chromatography (solvent C) of the mixture showed a major spot having a $R_f$ of 0.30. The mixture was evaporated *in vacuo* to dryness and the residue was purified through a cellulose column as described in the preparation of 27. The yield of 28 was 28% (296 mg); uv λ max (pH 1) 264 nm (εp 20,500), (pH 7) 267 (20,000), and (pH 13) 268 (20,300); paper chromatography $R_f$ 0.30 (solvent C); purity 87% (phosphorus assay). The compound (28) was treated with phosphomonoesterase, and the paper chromatography (solvents A, C, and D) of the dephosphorylated mixture showed that the product was identical with an authentic sample of $N^6$-allyladenosine (26).[20]

**Pharmacological Methods.** Methods and materials have been partly described in the previous papers.[1,7,31] All glassware coming into contact with blood or PRCP was siliconized with Siliconizer N-A (Fuji Ko-bunshi Kogyo Co., Ltd.).

Collagen was a lyophilized preparation from bovine achilles tendon (Sigma Chemical Co., Ltd.). Approximately 100 mg of collagen was placed in a glass homogenizer covered with 20 ml of saline and homogenized to a fine suspension with a Teflon covered piston. The collagen suspension was kept in a refrigerator and mixed well before use.

**Platelet Aggregation (Rabbit).** Platelet aggregation were measured by the optical density method of Born and Cross[29] by use of Evans EEL 169 aggregation meter. Platelet-rich citrated plasma (PRCP) obtained from a male rabbit was immediately buffered with an equal volume (in the case of ADP-induced aggregation) or with a half volume (in the case of collagen-induced aggregation) of isotonic barbital buffer (pH 7.3).[30] The pH of the buffered PRCP was 7.7 ± 0.1. The buffered PRCP was stored at 25° for use within 8 hr.

A cuvette containing 1.0 ml of buffered PRCP preincubated at 37° for 3–5 min was placed in an aggregation meter set at 37° and allowed to incubate with a 10-μl or 100-μl solution of the test sample in saline with stirring for 3 min. At this point, the buffered PRCP was challenged with 10 μl of a solution of ADP or 100 μl of a solution of collagen in saline. The final concentration of ADP and collagen required for aggregation were $10^{-5}$ *M* and 0.5 mg/ml, respectively. Inhibition percentage of aggregation by a test compound was calculated by dividing the maximum deflection in the optical density curve by that observed in the absence of a test compound and then multiplying by 100. When DMSO was used as a solvent of a test sample, buffered PRCP was allowed to incubate with a 10-μl solution of test sample in DMSO with stirring for 3 min and then challenged with ADP or collagen similarly. Inhibition percentage was calculated by dividing the maximum deflection by that observed with 10 μl of the control solvent, DMSO. The inhibition percentages thus obtained were not absolute as the sensitivity of platelets to aggregating agents varied from preparation to preparation and by minor changes in the experimental conditions. Accordingly, a reference standard, adenosine, was tested in every experiment for comparison of the inhibition. Relative potency of inhibition to adenosine (Rad value) was a direct measure of inhibition.

**Platelet Aggregation (Human).** 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. Whole blood (18 ml) was immediately transferred into a 30-ml centrifuge tube containing 2 ml of 3.8% Na citrate in saline. PRCP was prepared by centrifugation at 1000 rpm for 10 min and stored at 25° for use within 3 hr after blood sampling. Two PRCP preparations used in the experiment contained 2.5 and 2.9 × $10^8$ platelets/ml. A cuvette containing 1.0 ml of PRCP preincubated at 37° for 5 min was placed in an aggregation meter and

allowed to incubate with a test sample in saline for 3 min. It was then challenged with ADP in the usual way.

Effect of Incubation of $N^6$-Substituted Adenosines with Platelet-Rich Plasma on Platelet Aggregation. A cuvette containing 1.0 ml of buffered PRCP (rabbit) was preincubated at 37° for 3 min and added with 100 μl of a solution of the test compound in saline. The mixture was allowed to incubate at 37° without stirring during the period of up to 120 min and it was challenged with ADP in an aggregation meter with stirring. Inhibition percentage of ADP-induced platelet aggregation was plotted *vs.* incubation interval (Figure 2). Extent of aggregation in the control experiment (saline, 100 μl) did not vary during the incubation period of 120 min.

Effect of Incubation of Adenosine Derivatives with Platelet-Poor Plasma on Platelet Aggregation. Platelet-poor plasma was obtained from buffered PRCP (rabbit) by centrifugation at 3000 rpm for 20 min. A solution (1 ml) of $10^{-3}$ *M* test compound in saline was incubated with 1 ml of the platelet-poor plasma at 37° for up to 120 min. Buffered PRCP (1.0 ml) was mixed with 0.2 ml of the above mixture in an aggregation meter and was challenged with ADP in the usual way. Inhibitory effect of the test compounds is listed in Table IV.

The optical density unit (260 nm) of the mixture of adenosine and platelet-poor plasma was 15.0 before and after the incubation, whereas that of the control (a mixture of saline and platelet-poor plasma) was 7.2 throughout the incubation period. The incubated mixture of adenosine and platelet-poor plasma was submitted to paper chromatography (solvent E). A single spot of adenosine ($R_f$ 0.40) was observed and not even a trace of inosine ($R_f$ 0.78) could be detected on a chromatogram after the incubation of 120 min. The results indicated that adenosine was not degraded under the conditions.

Platelet Adhesiveness to Glass Beads.[32] A tube containing 0.8 ml of PRCP (rabbit) and 0.1 ml of a solution of the test compound in saline was kept at 24° for 30 min (or 37° for 10 min). After it was added with 0.1 ml of a solution of ADP in saline, an aliquot (0.5 ml) of the mixture was immediately passed through the glass beads column[7] during 20 sec, and the platelets were counted within 5 min with an automatic microcell counter, PA-701 (Toa Electric Co., Ltd.). The final concentration of the test sample was $10^{-4}$ *M* at 24° and $10^{-3}$ *M* at 37° and that of ADP was $10^{-7}$ *M*.

Effect of Infused Adenosine Derivatives on ADP-Induced Platelet Aggregation.[11,33] Male rabbits weighing 2–3 kg were anesthetized with $Et_2O$. Using siliconized polyethylene tubing washed with heparin, a carotid artery was cannulated for blood sampling. A test compound ($10^{-2}$ *M*) in saline was infused through an ear vein during a period of 30 min. Blood samples (3.7 ml of whole blood, 0.3 ml of 3.8% Na citrate) were drawn prior to and at 30-, 60-, 90-, 120-, and 150-min intervals post treatment and were immediately buffered with an equal volume of isotonic barbital buffer (pH 7.3). Buffered PRCP's were obtained by centrifugation of the above blood samples at 1000 rpm for 5 min, stored at 20° for 30 min, and were submitted to ADP-induced aggregation in the usual manner. Aggregation percentage of platelets was calculated on the basis of the aggregation just before the infusion.

Acknowledgments. The authors wish to express their sincere thanks to Messrs. Y. Higuchi and H. Suehiro and Miss R. Sugata for their technical assistances and to Dr. M. Shikita of National Institute of Radiological Sciences for his kindness in supplying compounds 22-24.

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