15%[12] and the spectra were taken at room temperature (23°) within a few hours.[13] As shown in Fig. 1—4, in each case the resonances of the interfering hydrogens were displaced to much lower field than those of anomeric protons which remained almost in the original field. Olefinic proton(s) and/or hydrogen(s) next to ether oxygen, if any, in the aglycone gave the signal(s) also in the region 4.2—5.5 ppm, but they could be distinguished by comparison with the spectrum of the aglycone. Therefore all the anomeric proton signals of the component monosaccharides were easily and clearly located on the spectrum.

The method has so far been applied to thirty-five known oligoglycosides[14] (steroid saponins 8, cardiac glycosides 7, pregnane glycosides 4, hydroxyfatty acid glycosides 4) and it seems to be generally applicable.

Experimental

A sample (10—15 mg) was dissolved in 0.4 ml of a mixture of $CDCl_3$–CD_3OD or $CDCl_3$–CD_3OD–D_2O, and CF_3COOH was added up to 5—15%.[13] NMR spectra were taken at room temperature (23°) within a few hours on a JEOL PS-100 (100 MHz) spectrometer using tetramethylsilane as internal reference.

14) Mostly isolated and characterized in this laboratory. Five cardiac glycosides including oleandrin gentiobioside (ref. 6) and three pregnane glycosides were kindly supplied by Prof. T. Yamauchi of Fukuoka University, to whom the authors' thanks are due.

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Hypotensive and Radioprotective Properties of N^6-Substituted Adenosine Derivatives

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Previous studies of Asakura[2,3] and of ours[4,5] have been concerned with N^6-substituted adenosines which are effective in protecting experimental animals from deleterious actions of ionizing radiations. It seems likely that the radioprotective action of these compounds is attributable partly to hypoxia caused secondarily by their cardiovascular actions,[3] and partly to their direct radioprotective action on cellular components.[4,5] The present paper reports similar radioprotective action of several other adenosine derivatives. N^6-(*p*-Hydroxyphenylethyl)adenosine (**1**) and N^6-(indole-3-ethyl)adenosine (**2**) are the compounds of choice as they possess the structure of tyramine and tryptamine in the molecule. Special attention has been directed to the hypotensive action of these compounds, because neither of the compounds has been reported thus far in this respect. Adenosine has been known as a potent vasodilator.[6]

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5) Y. Takagi, M. Okazaki, and M. Shikita, *J. Radiat. Res.*, **12**, 100 (1971).
6) R.B. Philp and V. Lemieux, *Nature*, **218**, 1072 (1968).

Experimental

Chemicals——6-(2-*p*-Hydroxyphenylethyl)amino-9-*β*-D-ribofuranosylpurine (N^6-(*p*-hydroxyphenylethyl) adenosine) (**1**) was synthesized by heating at reflux (6 hr) an ethanol solution (15 ml) containing tyramine (60 mg) and 6-chloro-9-*β*-D-ribofuranosylpurine (115 mg). Crude product was recrystallized from ethanol to give 78 mg (yield 50%), mp, 199° (uncorrected). $\lambda_{max}^{0.1N\ NaOH}$ 267 mμ (ε, 18500); $\lambda_{max}^{20\%\ EtOH}$ 270 mμ (ε, 20600); $\lambda_{max}^{0.1N\ NaOH}$ 269 mμ (ε, 20100). *Anal.* Calcd. for $C_{18}H_{21}O_5N_5 \cdot H_2O$: C, 53.32; H, 5.72; N, 17.28. Found: C, 53.52; H, 5.34; N, 17.75.

6-(2-(3-Indoyl)ethyl)amino-9-*β*-D-ribofuranosylpurine (N^6-(indole-3-ethyl)adenosine) (**2**) was prepared by the procedure similar to above. A solution of tryptamine HCl (215 mg) and 6-chloro-9-*β*-D-ribofuranosylpurine (287 mg) in a mixture of isopropanol (15 ml) and triethylamine (0.5 ml) was refluxed for 6 hr. The product was recrystallized from ethanol–water to afford 283 mg (69%), mp 170° (uncorrected). $\lambda_{max}^{0.1N\ HCl}$ 267 mμ (ε, 17750), 215 mμ (ε, 35300); $\lambda_{max}^{20\%\ EtOH}$ 272 mμ (ε, 21300), 218 mμ (ε, 40500); $\lambda_{max}^{0.1N\ NaOH}$ 272 mμ (ε, 20200). *Anal.* Calcd. for $C_{20}H_{22}O_4N_6 \cdot 1/2H_2O$: C, 57.27; H, 5.53; N, 20.04. Found: C, 57.64; H, 5.68; N, 19.78.

Compounds (**3** and **4**) were gifts of Dr. R. Nishiwaki of Morishita Pharmaceut. Co., Ltd. (Osaka). Compounds (**5** to **10**) were donated by Dr. M. Saneyoshi of the National Cancer Center Research Institute (Tokyo). Compounds (**11** and **12**) were gifts from Dr. K. Kikugawa of Tokyo Research Laboratory of Kohjin Co., Ltd. (Tokyo). 9-*β*-D-Ribofuranosylpurine (nebularine, **13**) was prepared from thioinosine by the method of Fox, *et al.*[7]

Blood Pressure Determination——Male rats of the Wistar strain averaging 400 g were used under anesthesia by sodium pentobarbital (Nembutal, Abbott, 30—40 mg/kg body weight *i.p.*). Right carotid artery was cannulated with a fine polyethylene tubing and connected to a pressure transducer (MPU-0.5). Blood pressure change was recorded on a polygraph (Nihon Kohden Kogyo Co., Model RM-150). Respiration rate was recorded with use of a force-displacement transducer (SB-1T) to which breast of the animal was strung by thread. The adenosine derivatives were injected intraperitoneally in the animals in solutions in 20% ethanol.

Radiation Protection——Male mice of the JCL-ICR strain (Japan Clea Co., Ltd., Tokyo) were given X-radiation (700 R) at the age of 5 weeks and observed for up to 30 days following irradiation. The radioprotective substances were dissolved in 0.2M borate buffer of pH 7.4 by warming or in 20% ethanol and injected intraperitoneally in the animals 15 min prior to irradiation. The radioprotective effect of the compound was expressed by the average of survival time of the animals. The survival time of the animal which survived longer than 30 days after irradiation was regarded as 30 days. Further details of the experimental conditions were described in a previous paper.[8] Acute toxicity of the compounds was determined in a manner similar to that described above, but the mice were not X-irradiated. LD_{50} values were estimated from the results obtained by injection of 2 or 3 doses of each compound. Although scantness of supply of the chemicals hampered statistical analysis for establishing LD_{50} values, the compounds were screened for the radioprotective action at doses of 1/5 to 1/10 of the presumable LD_{50} values.

Result and Discussion

Fig. 1 shows changes of systolic blood pressure after the injection of compounds (**1** and **2**) in rats. A typical example of actual records of the blood pressure change is shown in Fig. 2. Injection of the compound caused transient lowering of blood pressure within 1 min. This response was observed after the injection of 20% ethanol alone and recovered to normal quickly. On the other hand, in the animals which received the adenosine derivative, the transient response was followed by great vasodepression which lasted as long as 2 hr. There was a significant decrease of pulse pressure a few minutes after the injection, but it was normalized within half an hour, or sometimes increased above normal at which time blood pressure was still lower than normal. Respiration rate was decreased with concomitant over-breathing (Fig. 1). Analogous response was observed after the injection of compound (**2**). Fig. 3 shows the minimum value of systolic blood pressure as a function of the dose of the compound. It may be concluded that both compounds are more than 100 times as active as adenosine which has a vasodepressor effect at both coronary and systemic levels.[9]

Table 1 shows the radioprotective effect of the adenosine derivatives in mice. Five N^6-substituted adenosines (compounds **1** to **4**, and **11**) were effective, while four others (compounds

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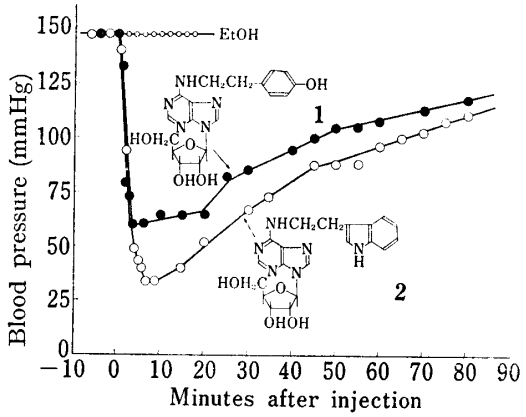

Fig. 1. Lowering of Systolic Blood Pressure by the Injection of N^6-(*p*-Hydroxyphenylethyl) adenosine (**1**) and N^6-(Indole-3-ethyl) adenosine (**2**) in Rats

The compounds (1 mg/animal) were injected *i.p.* in solutions (0.2 ml/animal) in 20% ethanol. The control animal received 0.2 ml of 20% ethanol alone.

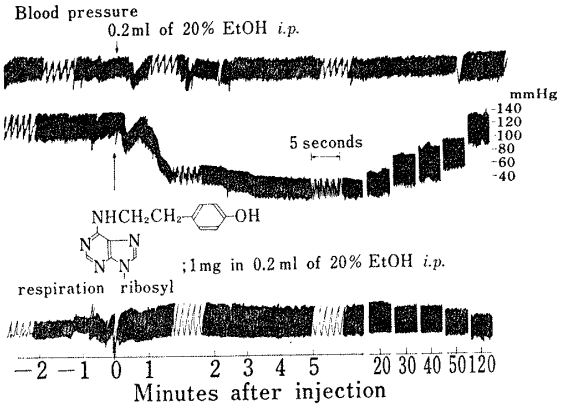

Fig. 2. Records of Changes of Blood Pressure and Respiration Rate after the Injection of N^6-(*p*-Hydroxyphenylethyl) adenosine in Rats

Half an hour after the injection of 0.2 ml of 20% ethanol, the animal received the injection of the adenosine derivative. There was no significant change of respiration rate after the injection of the ethanol solution alone (the datum is not shown in the figure). The pressure change was calibrated with use of a Hg manometer.

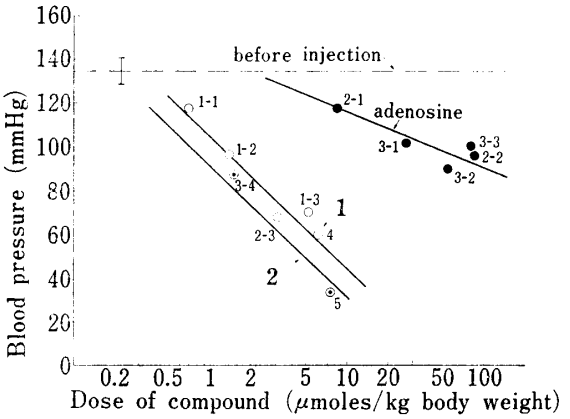

Fig. 3. Minimum Value of Systolic Blood Pressure attained after the Injection of Adenosine and Its Derivatives

Effect of the dose of the compound. Five rats were used in total. Some of them received injections of the compound repeatedly with intervals long enough for recovery each time; *e.g.*, 3—2 represents the blood pressure change observed when animal 3 received the injection of 56 μmoles/kg body weight of adenosine for the second time after the effect of the first injection (28 μmoles/kg body weight) was recovered. Compound (**1**)=N^6-(*p*-hydroxyphenylethyl) adenosine, compound (**2**)=N^6-(indole-3-ethyl)adenosine

6 to **9**) were not. Adenosine, the mother compound, was ineffective so far as examined in the present experiment. Both nebularin (**13**) and inosine (**14**) were also without significant effect. On the other hand, it was noted that 2-chloroadenosine (**12**) was strongly radioprotective. It has been known that 2-chloroadenosine is a potent hypotensive compound which is 2.4 times (in dogs[10]) and 9 to 200 times (in cats[11]) stronger than adenosine. As it was demonstrated above, compounds (**1** and **2**) are also powerfully hypotensive. Six N^6-substituted adenosines including compounds (**3** and **5**) of this paper were reported to cause lowering of blood pressure and produce radioprotection.[2] However, it seems that the hypotensive action *per se* is not directly related to the mechanism of radiation protection. Compound (**2**) was slightly more effective than compound (**1**) in lowering blood pressure (Fig. 1 and 2), but apparently less effective in radiation protection (Table I). It is likely that blood flow decreases because of relaxation of systemic blood vessels,[12] which consequently produces hypoxia in tissues and facilitates the intrinsic radioprotective action of the compound.

Adenosine and its derivatives such as 2-chloroadenosine are known as powerful inhibitors of platelet aggregation.[13] Various N^6-substituted adenosines including compounds (**1** and **2**)

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Table I. Radioprotective Effect of Adenosine Derivatives in Mice

Experiment	Compound[a]	Dose of compound (mg/animal)	Survival time of animals (days)
1	20% ethanol alone	—	10.6±0.6
	N^6-(*p*-hydroxyphenylethyl)adenosine (**1**)	0.05	17.5±2.2*[b]
		0.30	20.8±1.8**
	N^6-(indole-3-ethyl)adenosine (**2**)	0.05	11.6±0.7
		0.10	16.0±2.5*
		0.30	15.9±2.8
2	noninjection	—	7.0±0.6
	borate buffer alone	—	7.5±0.6
	N^6-(3-hydroxypropyl)adenosine (**3**)	0.50	13.4±3.1*
		1.0	14.4±3.1*
	N^6-(3-aminopropyl)adenosine (**4**)	0.50	17.0±3.6*
3	noninjection	—	10.7±1.4
	6-methylthioinosine (**5**)	3.0	7.1±0.9
	adenosine N-oxide (**6**)	3.0	11.6±0.7
	N^6-aminoadenosine (**7**)	3.0	7.7±0.7
	N^6-dimethyladenosine (**8**)	3.0	12.0±1.1
	N^6-methyladenosine (**9**)	3.0	10.6±1.1
	N^1-methyladenosine (**10**)	3.0	11.2±1.0
4	noninjection	—	11.1±1.5
	N^6-hydroxyadenosine (**11**)	1.0	10.3±0.8
		3.0	18.7±3.1*
	2-chloroadenosine (**12**)	0.20	24.2±3.0**
		0.50	23.7±3.3**
5	saline	—	9.9±0.8
	nebularin (**13**)	1.0	7.8±0.8
		2.0	7.6±0.6
	inosine (**14**)	12.0	11.2±0.9
	adenosine (**15**)	10.0	11.8±1.0

a) Injected intraperitoneally 15 min prior to X-irradiation (700R).
b) The difference from the control value is statistically significant at 5% (*) and 1% (**) level. Each group consists of 10 to 20 animals.

of this paper also possess such an action.[14] In summary, pharmacological actions of the adenosine derivatives are (1) lowering of blood pressure, (2) relaxation of muscles, (3) inhibition of platelet aggregation, and (4) radioprotection. Correlation between these four actions is obscure at present.

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