Discussion

As anticipated the 2-methyl- and 2,6-dimethylbenzoates of 4-[N,N-bis(2-chloroethyl)amino]phenol (**21** and **22**, Table V) and 4-[N,N-bis(2-bromoethyl)amino]phenol (**2** and **3**, Table V) were less toxic than the unsubstituted compounds (**1** and **20**, Table V), particularly dramatic decreases being observed with the 2,6-dimethyl analogs. That these decreases in toxicity may be due to increased resistance to hydrolysis is suggested by the data of Table IV which reveal that the 2,6-dimethylbenzoate of 4-(N,N-diethylamino)phenol[8] is hydrolyzed only very slowly by the crude liver esterase preparation. These results are paralleled by the antitumor activities (Table V) which show that the 2,6-dimethylbenzoates (**3** and **22**) are active only at markedly higher dose levels. These findings are thus consistent with the previously expressed hypothesis[2] that hydrolysis of esters of 4-[N,N-bis(2-haloethyl)amino]phenol is a prerequisite for significant antitumor activity.

Examination of the enzyme and animal data for the series of *meta*- and *para*-substituted benzoates of 4-[N,N-bis(2-halogenoethyl)amino]phenol shows that, as anticipated,[9] hydrolysis is facilitated by electron-withdrawing substituents and decreased by electron-releasing substituents. However, these substituents did not exert a large effect upon the sensitivity of the compounds to enzymatic hydrolysis (Table IV). In view of this finding, large differences in the biological activities of these compounds would not be expected. In fact, the toxicities and antitumor activities of the esters of 4-[N,N-bis(2-bromoethyl)amino]phenol (**1**, **6**–**16**, Table V) are not significantly different from those of the parent phenol (B, Table V). Similarly, there is little difference in the biological activities of the members of the chloro series (**19**, **22**–**29**, Table V; **23** appears to be an exception), although because of their lower reactivity these compounds are significantly less toxic than their bromo analogs.

Finally, it will be observed that the more favorable therapeutic indices (as measured by LD_{50}/ED_{90}) are found with the esters of 4-[N,N-bis(2-chloroethyl)-amino]phenol. This is, in part, probably due to the fact that the derived phenol, A (Table V), has a more favorable activity than the bromo analog (B).

(8) Esters of 4-(N,N-diethylamino)phenol were used as appropriate model compounds in the enzyme studies to avoid the complication of using the alkylating agents in this system.[2]

(9) J. Hine, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1962, Chapter 12.

Acknowledgments.—We wish to thank Miss Carole A. Hayden and Mr. D. R. Garrison for their assistance. This work was supported by a grant (CA 06645) from the National Cancer Institute, National Institutes of Health.

2-Chloroadenosine 5′-Phosphate and 2-Chloroadenosine 5′-Diphosphate, Pharmacologically Active Nucleotide Analogs

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Received January 15, 1969

2-Chloroadenosine 5′-phosphate and diammonium 2-chloroadenosine 5′-diphosphate have been synthesized, and isolated analytically pure. Their effects on the *in vitro* aggregation of sheep platelets in platelet-rich plasma and on rat arterial blood pressure were compared with those of adenosine, 2-chloroadenosine, adenosine 5′-phosphate (AMP), and adenosine 5′-diphosphate (ADP). 2-Chloroadenosine 5′-phosphate (2-chloro-AMP) inhibited the ADP-mediated aggregation of sheep platelets and was initially equipotent with AMP and was longer acting; 2-chloro-AMP was a more potent and longer acting vasodepressor than AMP. 2-Chloroadenosine 5′-diphosphate (2-chloro-ADP) was of similar potency to ADP as a vasodepressor and was longer acting; 2-chloro-ADP reversibly aggregated sheep platelets and was nine times as potent as ADP.

Adenosine, adenosine 5′-phosphate (AMP), and adenosine 5′-diphosphate (ADP) are physiologically active in a number of *in vivo* and *in vitro* preparations. For example, adenosine on intravenous administration in the cat[1] caused a transitory drop in blood pressure, and in the anesthetized open-chested dog adenosine, AMP, and ADP have been found to have brief coronary vasodilator effects.[2] ADP in concentrations as low as 10^{-6} *M* causes mammalian platelets in plasma to aggregate reversibly,[3] a phenomenon which is believed to play a key role in hemostasis.[4] Adenosine and AMP have been shown to inhibit the ADP-mediated aggregation of platelets,[3] although their inhibitory effects are of short duration. The analog 2-chloroadenosine has more potent effects than adenosine, both on the vascular[1,5] and platelet-aggregation systems,[6] and its action in both systems is of greater duration than that of adenosine. The ready deamination of adenosine to inosine by adenosine deaminase is thought to explain the transient nature of the adenosine effect, since inosine is without activity either as a vasodilator[1] or as an inhibitor of platelet aggregation.[3] 2-Chloroadenosine is not deaminated by adenosine deaminase,[1,7] and this resistance to deamination may explain the greater duration of action of 2-chloroadenosine; it does not, however, ex-

(1) D. A. Clarke, J. Davoll, F. S. Philips, and G. B. Brown, *J. Pharmacol. Exp. Ther.*, **106**, 291 (1952).

(2) M. M. Winbury, D. H. Papierski, M. L. Hemmer, and W. E. Hambourger, *ibid.*, **109**, 255 (1953).

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

(4) S. A. Johnson in "Blood Clotting Enzymology," W. H. Seegers, Ed., Academic Press, New York, N. Y., 1967, p 393.

(5) R. H. Thorp and L. B. Cobbin, *Arch. Int. Pharmacodyn. Ther.*, **118**, 95 (1959).

(6) G. V. R. Born, *Nature*, **202**, 95 (1964); G. V. R. Born, A. J. Honour, and J. R. A. Mitchell, *ibid.*, **202**, 761 (1964).

(7) M. Rockwell and M. H. Maguire, *Mol. Pharmacol.*, **2**, 574 (1966).

plain the greater potency of the analog compared to adenosine.

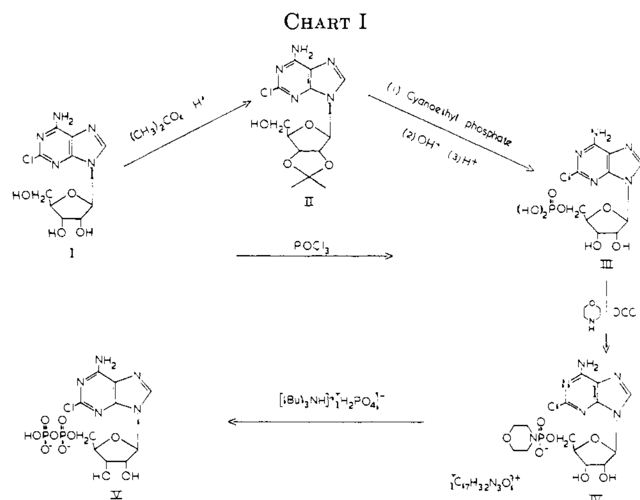
In order to investigate the influence of similar 2-chloro substitution on the pharmacological activity of AMP and ADP we have synthesized 2-chloroadenosine 5'-phosphate (2-chloro-AMP) and 2-chloroadenosine 5'-diphosphate (2-chloro-ADP) and compared the effects of these analogs with those of AMP and ADP on the arterial blood pressure of rats and on the aggregation of sheep platelets. In addition, a preliminary study has been made of the substrate specificity of these analog nucleotides for the enzyme adenylate kinase (E.C. 2.7.4.3) which catalyzes the reaction

$$2ADP^{3-} \rightleftharpoons AMP^{2-} + ATP^{4-}$$

Adenylate kinase is present in many tissues[8] and in plasma[9] and could conceivably be responsible for the interconversion of 2-chloro-AMP and 2-chloro-ADP *in vivo*.

Chemistry.—2-Chloro-AMP was prepared from 2-chloroadenosine by the two routes outlined in Chart I. In the first, 2-chloroadenosine (I) was aceton-

CHART I



ated by the method of Hampton,[10] and the resulting cyclic ketal (II) was phosphorylated by the procedure of Tener[11] to give pure crystalline 2-chloroadenosine-5'-monophosphoric acid (III) in 61% yield from I.

2-Chloro-AMP was also obtained by the direct phosphorylation of 2-chloroadenosine with $POCl_3$ in trimethyl phosphate, a procedure based on that reported recently by Yoshikawa, *et al.*[12] 2-Chloroadenosine was only partially phosphorylated under the reaction conditions described by Yoshikawa, *et al.*, for the synthesis of AMP from adenosine, and it was found necessary to use a large excess of $POCl_3$ and a prolonged reaction time in order to obtain complete phosphorylation of the analog. In this way 2-chloro-AMP was isolated in 42% yield.

2-Chloro-ADP (V) was synthesized from 2-chloro-AMP by the morpholidate procedure of Moffatt and Khorana.[13] Purification of the crude reaction product by ion-exchange chromatography followed by preparative paper chromatography resulted in a 32% yield of pure diammonium 2-chloro-ADP.

Pharmacology.—Arterial blood pressure was measured in rats anesthetized with sodium pentobarbital. A cannula was inserted into the left common carotid artery and the blood pressure was recorded *via* a Statham pressure transducer on a Grass polygraph. Compounds dissolved in normal saline were injected into the jugular vein. 2-Chloro-AMP caused a fall in arterial blood pressure which was greater than that caused by AMP, and which lasted considerably longer. 2-Chloro-ADP was found to be slightly more potent than ADP but its effect on rat arterial blood pressure was of much greater duration. These results and those obtained with 2-chloroadenosine are compared in Table I with the effects of adenosine.

TABLE I
EFFECTS OF NUCLEOSIDES AND NUCLEOTIDES ON RAT BLOOD PRESSURE

Compd	Molar potency ratio[a]	Av duration of blood press. fall, min
Adenosine	1.0	0.7
2-Chloroadenosine	8.2 ± 0.7	15
AMP	1.1 ± 0.13	0.5
2-Chloro-AMP	6.6 ± 0.4	6
ADP	23.5 ± 4.9	1
2-Chloro-ADP	30.7 ± 4.8	10

[a] Mean results (±SE) obtained by comparing the effectiveness of the compound with that of adenosine on four or more animals. Doses used were such as to cause a 30% fall in arterial blood pressure.

The aggregation of platelets in citrated sheep platelet-rich plasma was studied by a turbidimetric technique[14,15] in which the changes in the optical density of the platelet suspension caused by added ADP were recorded on a potentiometric recorder. Inhibitors of the ADP-mediated aggregation were incubated in the platelet-rich plasma at 37° for varying periods of time prior to the addition of ADP. Initial rates of aggregation were measured and compared to the rate of the control response to 1.5 μM ADP.

In Table II the potencies of the inhibitors AMP,

TABLE II
EFFECTS OF NUCLEOSIDES AND NUCLEOTIDES ON THE AGGREGATION OF SHEEP PLATELETS

	Molar potency ratio
ADP aggregates	1.0
2-Chloro-ADP aggregates	9.0
Adenosine inhibits aggregation	1.0[a]
2-Chloroadenosine inhibits aggregation	4.0[a]
AMP inhibits aggregation	0.1[a]
2-Chloro-AMP inhibits aggregation	0.1[a]

[a] Inhibitors preincubated for 2 min prior to the addition of ADP.

2-chloro-AMP, and 2-chloroadenosine are compared to that of adenosine. The preincubation period was 2 min. AMP and 2-chloro-AMP were of similar potency and were only one-tenth as active as adenosine

(8) L. Noda, *Enzymes*, **6**, 139 (1962).
(9) R. J. Haslam and D. C. B. Mills, *Biochem. J.*, **103**, 773 (1967).
(10) A. Hampton, *J. Amer. Chem. Soc.*, **83**, 3640 (1961).
(11) G. M. Tener, *ibid.*, **83**, 159 (1961).
(12) M. Yoshikawa, T. Kato, and T. Takenishi, *Tetrahedron Lett.*, 5065 (1967).
(13) J. G. Moffatt and H. G. Khorana, *J. Amer. Chem. Soc.*, **83**, 649 (1961).
(14) G. V. R. Born, *Nature*, **194**, 927 (1962).
(15) M. H. Maguire and F. Michal, *ibid.*, **217**, 571 (1968).

and one-fortieth as active as 2-chloroadenosine. With increasing periods of incubation the inhibitory effect of adenosine in sheep platelet-rich plasma has been shown to reach an optimum value after 2 min of incubation and then to diminish sharply.[16] In contrast, the potency of 2-chloro-AMP was found to increase with incubation, so that after 30 min the inhibitory activity of 2-chloro-AMP was four times that of adenosine at its optimum. Figure 1 shows the effect of increasing periods of preincubation on the inhibitory potencies of adenosine, 2-chloroadenosine, AMP, and 2-chloro-AMP.

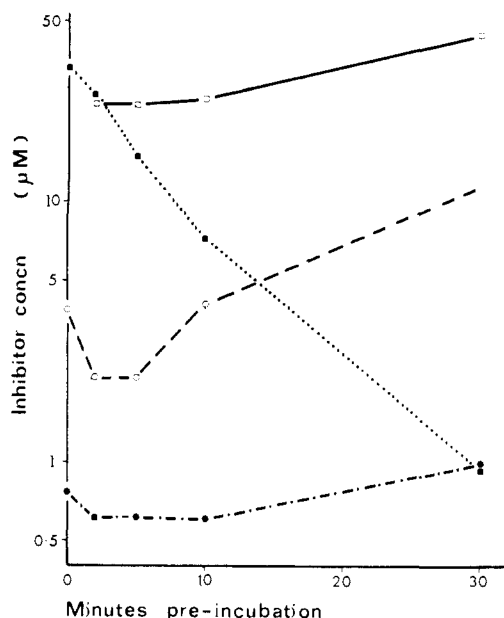


Figure 1.—Effect of preincubation on the potency of inhibitors of ADP-mediated platelet aggregation. Initial rates of aggregation induced by 1.5 μM ADP were measured; adenosine (○ – – – – ○), 2-chloroadenosine (● - · - · - ●), AMP (□——□), and 2-chloro-AMP (■ · · · · · · ■) concentrations are those which caused 50% inhibition.

2-Chloro-ADP has been found to be a very powerful aggregator of sheep and human blood platelets with nine times the aggregating potency of ADP. The aggregation caused by 2-chloro-ADP is reversible. These findings have been reported in detail elsewhere.[15]

Adenylate Kinase.—2-Chloro-AMP and 2-chloro-ADP were substrates for adenylate kinase. When the enzyme from rabbit muscle was incubated with 2-chloro-AMP and ATP and the resulting mixture examined by paper chromatography, three adenine nucleotide components, the monophosphate, diphosphate, and triphosphate, were found to be present. Similarly 2-chloro-ADP when incubated with adenylate kinase gave an equilibrium mixture of 2-chloro-AMP, 2-chloro-ADP, and 2-chloro-ATP.

Discussion

Compared to adenosine at its optimum potency (2 min preincubation) the molar potency ratio of AMP as an inhibitor of ADP-mediated platelet aggregation was 0.1 after 2 min preincubation; this value decreased to 0.04 after 30 min preincubation (*cf.* Figure 1). 2-Chloro-AMP also had a molar potency ratio of 0.1 after 2 min, but on further incubation this value increased until after 30 min it was 3.8, almost equal to that of 2-chloroadenosine. These findings suggest that AMP and 2-chloro-AMP are gradually hydrolyzed by a plasma phosphatase with the release of adenosine and 2-chloroadenosine. 2-Chloroadenosine is a potent and long-lasting inhibitor of ADP-mediated platelet aggregation, and if it were gradually produced from 2-chloro-AMP in platelet-rich plasma a concomitant increase in the inhibition caused by 2-chloro-AMP would be expected. The effect of adenosine produced by similar hydrolysis of AMP would be masked by its simultaneous deamination to inosine, with the result that the inhibitory effect of AMP would be further decreased.

On arterial blood pressure 2-chloro-AMP was six times as potent as AMP which was equipotent with adenosine. It is possible that the effect that is measured in this way is not the direct effect of the nucleotide on the vascular system, but the effect of the free nucleoside produced by hydrolysis of the nucleotide by phosphatases or 5′-nucleotidase in the blood or associated with the walls of the blood vessels. This possibility is supported by the work of Baer and Drummond[17] who found that AMP was converted to inosine during a single passage through isolated perfused rat hearts. 2-Chloro-AMP has been shown to be a substrate for 5′-nucleotidase purified from rat heart.[18]

To summarize, 2-chloro substitution in adenosine leads to an increase in potency and in duration of action both on arterial blood pressure and on the platelet system. 2-Chloro substitution of AMP does not produce an increase in potency compared to AMP in the platelet system, unless it is preincubated for a considerable time in platelet-rich plasma, but 2-chloro substitution does produce an increase in the vasodepressive potency of AMP. In both physiological systems the duration of action of 2-chloro AMP is prolonged compared to that of AMP. 2-Chloro substitution of ADP gives an analog of equivalent potency to ADP in producing an effect on arterial blood pressure, but having almost ten times the potency of ADP as an aggregator of platelets.

Table III

Paper Chromatography and R_f Values

	R_f		
	Solvent systems		
Compd	A	B	C
2-Chloroadenosine (I)	0.47	0.75	0.84
2-Chloro-2′,3′-*O*-isopropylideneadenosine (II)	0.82	0.93	
2-Chloroadenosine 5′-monophosphate (III)		0.22	0.58
2-Chloroadenosine 5′-phosphoromorpholidate (IV)	0.10	0.65	0.82
2-Chloroadenosine 5′-diphosphate (V)			0.46

Experimental Section

Paper chromatography unless otherwise stated was carried out by the ascending technique on Whatman No. 1 paper in the following solvent systems: (A) *n*-BuOH–H_2O (86:14), (B) *i*-PrOH–0.25 *M* NH_4HCO_3 (2:1), and (C) isobutyric acid–1 *M* NH_4OH (100:60). Purine derivatives were located on paper by

(16) F. Michal and R. H. Thorp, *Nature*, **209**, 208 (1966).

(17) H. P. Baer and G. I. Drummond, *Proc. Soc. Exp. Biol. Med.*, **127**, 33 (1968).

(18) M. Edwards and M. H. Maguire, unpublished.

observation under uv light, and phosphates were detected by spraying with molybdate–$HClO_4$.[19] Relevant R_f values are shown in Table III.

Melting points were determined on a Reichert apparatus and are uncorrected. Uv spectra were measured on a Perkin-Elmer 350 spectrophotometer. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. Evaporations were carried out under reduced pressure at temperatures less than 35°, and solids were dried *in vacuo* over P_2O_5 at room temperature, unless otherwise noted.

Reagent purity pyridine was rendered anhydrous by storing it over CaH_2. Commercial grade trimethyl phosphate was distilled and the fraction of bp 192–193° was stored over Linde Molecular Sieve (Type 4A). 2-Chloroadenosine was synthesized in this institute by a modification of the procedure of Montgomery and Hewson,[20] in which 2,6-dichloropurine was fused with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose[21] without a catalyst and the product was treated with methanolic NH_3.

Adenylate kinase (2 mg/ml of solution, Boehringer Corp.) was used undiluted.

2-Chloro-2′,3′-*O*-isopropylideneadenosine (II) was prepared in 85% yield from 2-chloroadenosine (I) by the method of of Hampton.[10] The product (mp 238–241°) was chromatographically homogeneous in solvent systems A and B and was used for the synthesis of III without further purification. A small amount was recrystallized once from Me_2CO and three times from H_2O to give the analytical sample, mp 238–241°. *Anal.* ($C_{13}H_{16}ClN_5O_4$) C, H, N.

2-Chloroadenosine 5′-Phosphate (III). (i) A solution of II (2.74 g, 8 mmoles) in pyridine (50 ml) was treated with pyridinium β-cyanoethyl phosphate (32 ml of a 1 mmole/ml of pyridine stock solution)[11] and the resulting mixture was evaporated to an oil. Traces of moisture were removed by three additions and evaporations of anhydrous pyridine (each 30 ml). The residue was then dissolved in anhydrous pyridine (50 ml), treated with dicyclohexylcarbodiimide (16 g), and allowed to stand at room temperature for 18 hr. H_2O (10 ml) was then added and after 1 hr the reaction mixture was concentrated to dryness. Following a further addition and evaporation of H_2O (50 ml), the white solid residue was suspended in 0.4 *M* LiOH (320 ml) and refluxed for 1 hr. The mixture was cooled and filtered. The filtrate was applied to a column (3.2 × 25 cm) of Bio-Rad AG50W-X4 (H^+) ion-exchange resin and washed through with H_2O until the effluent was neutral. The eluate was adjusted to pH 2.8 with aqueous $Ba(OH)_2$, heated under reflux for 1.5 hr, then concentrated to *ca.* 400 ml. Aqueous $Ba(OH)_2$ was added to pH 7.5 and the precipitated barium phosphate was removed by centrifugation. The supernatant was treated with twice its volume of EtOH and, after 1 hr at room temperature, the white precipitated barium salt of III was collected by centrifugation and washed with EtOH, Me_2CO, and Et_2O to give 4.69 g of the dry salt. Paper chromatography in solvent system B revealed the presence of a minor unidentified contaminant (R_f 0.07).

The Ba salt (400 mg) was dissolved in H_2O (*ca.* 10 ml) with the aid of a little Bio-Rad AG50W-X4 (H^+) and passed through a column (1 × 20 cm) of the same resin. The combined effluent and washings were neutralized with NH_4OH, evaporated to a glass, and dissolved in 3 ml of solvent B. This solution was applied to a column (2.5 × 35 cm) of Whatman cellulose powder packed in solvent B and the column was eluted with the same solvent. Fractions of 20 ml were collected; III emerged in fractions 12–16. These were pooled and concentrated to dryness. Repeated evaporations of H_2O removed residual NH_4HCO_3 and the glasslike NH_4 salt was dissolved in H_2O (5 ml) and converted to the free acid by passage through the 1 × 20 cm cation-exchange column (H^+ form). Evaporation of the eluate yielded a white crystalline residue of III (199 mg, 72% from II) which was chromatographically pure and free from inorganic phosphate in solvent systems B and C. It was recrystallized twice from H_2O to give the analytical sample as white needles, mp 159–160° dec, λ_{max} (0.1 *N* HCl) 265 mμ (ε 14,200). *Anal.* ($C_{10}H_{13}ClN_5O_7P \cdot 1.5H_2O$) C, H, N.

(ii) I (755 mg, 2.5 mmoles) was dissolved with stirring in an ice-cold solution of $POCl_3$ (2.28 ml, 25 mmoles), H_2O (0.045 ml, 2.5 mmoles), and 30 ml of dry trimethyl phosphate. After 24 hr at 3°, the clear solution was poured over ice (100 g) with stirring and the pH was brought to 9 with concentrated aqueous LiOH. The mixture was kept at room temperature for 2 hr and the pH was maintained at 9 by further additions of LiOH. Lithium phosphate was removed by centrifugation and washed (H_2O) until no further uv-absorbing material could be extracted. The combined supernatants (*ca.* 180 ml) were extracted twice with 100 ml of $CHCl_3$ to remove $(MeO)_3PO$, diluted to 300 ml, treated with $Ba(OAc)_2$ (1.28 g, 5 mmoles), then allowed to stand overnight at room temperature. The resulting precipitate of $Ba_3(PO_4)_2$ was removed by centrifugation and washed (H_2O), and the supernatant and washings were concentrated to *ca.* 50 ml. Two volumes of EtOH were added and the precipitated nucleotide Ba salt was collected by centrifugation and washed once with EtOH–H_2O (2:1, 30 ml), then with EtOH, Me_2CO, and Et_2O to give 1.57 g of product. Paper chromatography in solvent B showed two uv-absorbing spots, one corresponding in R_f to III and the other at R_f 0.05. The two spots were eluted with H_2O and shown to have identical λ_{max} values, 264 mμ.

The Ba salt (300 mg) was subjected to column chromatography in a manner identical with that described in (i) above. Fractions 12–17 from the cellulose column were pooled and worked up to give 82 mg (42% yield) of a white crystalline solid, which was chromatographically homogeneous and had the same R_f value as III in solvent systems B and C.

2-Chloroadenosine 5′-phosphoromorpholidate (IV) was prepared on a 0.25-mmole scale from III by the general method for synthesis of nucleoside 5′-phosphoromorpholidates described by Moffatt and Khorana.[13] It was obtained in 83% yield as the 4-morpholine-*N,N′*-dicyclohexylcarboxamidinium salt and appeared as a single blue fluorescent spot on chromatography in solvent systems A and B. *Anal.* ($C_{31}H_{51}ClN_9O_8P \cdot 6.5H_2O$) C, H, N.

2-Chloroadenosine 5′-Diphosphate (V).—The phosphoromorpholidate (IV) (120 mg, 0.14 mmole) was dried by three successive additions and evaporations of 10 ml of anhydrous pyridine. A mixture of 89% orthophosphoric acid (0.03 ml, 0.48 mmole) and $(n\text{-}Bu)_3N$ (0.11 ml, 0.48 mmole) in pyridine (10 ml) was prepared and dried in the same way to give a residue which was dissolved in pyridine (10 ml) and added to the phosphoromorpholidate. Two further evaporations were carried out to free the reaction mixture from residual traces of H_2O. The residue was dissolved in 5 ml of pyridine and allowed to stand at room temperature for 3 days with exclusion of moisture. Pyridine was removed *in vacuo* and H_2O (10 ml) was added. The resultant cloudy yellow solution was applied to a column of DEAE cellulose (2 × 20 cm, HCO_3^- form) and followed by 100 ml of H_2O. Stepwise elution was then begun with 0.025 *M* NH_4HCO_3 (100 ml) and continued with ten successive 100-ml aliquots, each increasing in concentration by 0.025 *M*; 100-ml fractions were collected and examined by paper chromatography in solvent C. Uv-absorbing material with an R_f identical with that of III emerged in fractions 5 and 6.

Fractions 7 and 8 which contained V together with an unidentified fluorescent contaminant (R_f 0.62) were pooled and evaporated to dryness. NH_4HCO_3 was removed by three evaporations with H_2O (each 30 ml). The oily residue was dissolved in H_2O (0.25 ml) and applied to the origin line of two 15-cm wide sheets of washed Whatman No. 3 chromatography paper. After ascending development in solvent C the uv-absorbing band was cut out and eluted with *i*-PrOH to remove ammonium isobutyrate. Subsequent elution with H_2O extracted the nucleotide material. The aqueous extract was filtered, evaporated to *ca.* 20 ml, and lyophilized to yield (after drying) 26 mg (32%) of the diammonium salt of V as a white fluffy powder, λ_{max} (0.1 *N* HCl) 266 mμ (ε 14,000). *Anal.* Calcd for $C_{10}H_{20}ClN_7O_{10}P_2 \cdot 2.5H_2O$: C, 22.21; H, 4.62; N, 18.12; P, 11.45. Found: C, 22.45; H, 4.98; N, 17.81; P, 11.25.

Adenylate Kinase.—Adenylate kinase (10 μl) was added to a solution of V (0.5 mg) in 0.15 ml of 0.1 *M* Tris–HCl buffer pH 8 containing 0.01 *M* $MgCl_2$. The solution was incubated for 30 min at 37°, and an aliquot was chromatographed by the descending technique in solvent C together with markers of III and ATP. The incubation mixture gave three uv-absorbing spots, corresponding in R_f to III, V, and 2-chloro-ATP.[22] A similar experiment was carried out using III (1 mg) and sodium ATP (1.5 mg)

(19) C. S. Hanes and F. A. Isherwood, *Nature*, **164**, 1107 (1949).

(20) J. A. Montgomery and K. Hewson, *J. Heterocycl. Chem.*, **1**, 213 (1964).

(21) E. F. Recondo and H. Rinderknecht, *Helv. Chim. Acta*, **42**, 1171 (1959).

(22) In solvent C the R_f values of AMP and III are identical, as are the R_f values of ADP and V. 2-Chloro-ATP was not available for use as a marker but has since been synthesized and shown to have the same R_f as ATP in solvent C.

in 0.4 ml of buffer. Chromatography of the incubation mixture showed the presence of a third uv-absorbing spot corresponding in R_f to ADP and 2-chloro-ADP.

Acknowledgments.—The authors are grateful to Dr. J. Fildes (Australian National University) and Dr. E. Challen (University of New South Wales) who carried out the microanalyses. Mr. D. Nobbs synthesized 2-chloroadenosine.

Nucleosides of 2-Fluoroadenine[1]

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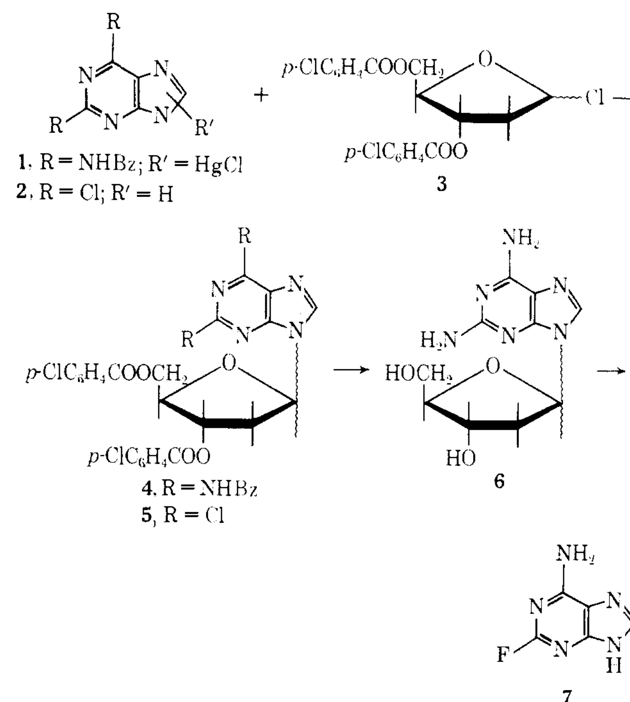
Received December 23, 1968

The preparation of the anomeric 9-(2-deoxy-D-*erythro*-pentofuranosyl)-2-fluoroadenines and 9-D-arabinofuranosyl-2-fluoroadenines from 2,6-dichloropurine is described. The cytotoxicity of these compounds, and also of 3′-deoxy-2-fluoroadenosine and 9-β-D-xylofuranosyl-2-fluoroadenine, to a number of HEp-2 cell lines in culture has been determined. The data permit certain conclusions concerning the probable metabolism and mechanism of action of these nucleosides.

2-Fluoroadenosine[2] is readily anabolized[3-5] but not catabolized[6-9] in cells in culture or *in vivo*. It is highly cytotoxic,[2] highly toxic to rodents,[10] and has broad-spectrum antibacterial activity.[11,12] It has a synergistic effect on the antimicrobial action of actinobolin[13] and is an inhibitor of blood-platelet aggregation.[14] The broad and high-level biologic activity of 2-fluoroadenosine has made the study of other nucleosides of 2-fluoroadenine desirable.

The preparation of 2-amino-2′-deoxyadenosine (β-**6**) in 1.7% over-all yield and its α anomer in 1.5% over-all yield from 2-amino-6-chloropurine by the conventional chloromercuri procedure has been reported.[15] In an effort to improve the yields of both anomers of **6** and to obtain analytical samples of these compounds, their preparation from the chloromercuri derivative of 2-benzamido-N-benzoyladenine[16] and 3,5-di-O-(*p*-chlorobenzoyl)-2-deoxy-D-*erythro*-pentofuranosyl chloride[17] was investigated and found to give **4** as an approximately 1:1 mixture of α and β anomers in a total yield of 36% (Scheme I). Treatment of **4** with NaOMe in the usual manner resulted in decomposition of the nucleoside, whereas treatment with methanolic NH_3 at 5° removed only the *p*-chlorobenzoyl groups. The anomeric mixture of 9-(2-deoxy-D-ribofuranosyl)-2-aminoadenine (**6**) was finally obtained in 31% yield by heating **4** with methanolic NH_3 at 100° for 6 hr in a bomb. The α and β anomers of **6**[15] were separated by fractional crystallization; about 8 parts α to 1 part β were isolated. Treatment of α-**6** with $NaNO_2$ in 48% fluoroboric acid resulted in replacement of the 2-amino group by fluorine but also in cleavage of the glycosyl linkage giving only 2-fluoroadenine (**7**),[18] a result not too unexpected in view of the known acid lability of purine 2′-deoxyribonucleosides.[19]

SCHEME I



Because of the acid lability of **6** and because of the difficulties in obtaining pure β-**6**[15] from the reaction of **1** and **3**, another route[20] to 2-fluoro-2′-deoxyadenosine

(1) This work was supported by funds from the Southern Research Institute, the C. F. Kettering Foundation, and the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Contract No. PH43-64-51.

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