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Ethyl Adenosine-5′-carboxylate, A Potent Vasoactive Agent in the Dog

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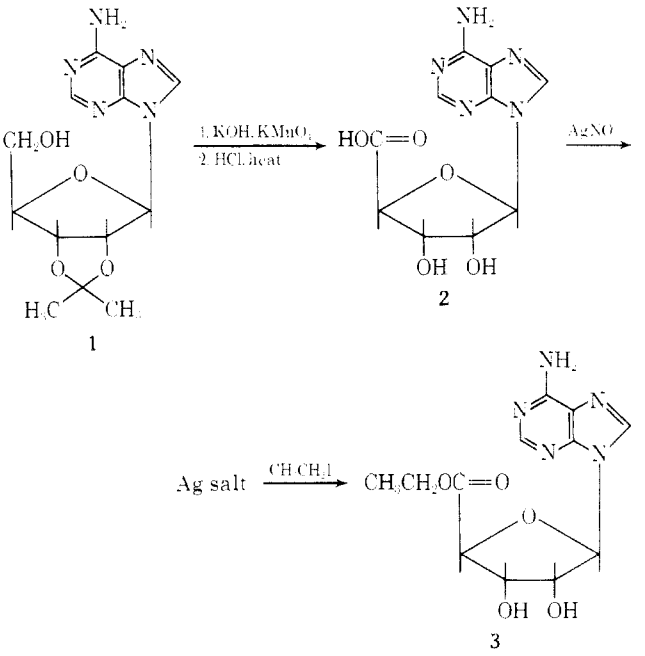
The activity of exogenous adenosine and adenosine nucleotides as coronary vasodilators is well documented.[1-3] The effect is of a short duration, however, probably because the compounds are rapidly metabolized and very quickly reach low, steady-state concentrations; adenosine itself is taken up rapidly by red blood cells and tissues[4] and converted to inosine[5] while phosphorylated derivatives do not cross biological membranes readily. Indeed, the hemodynamic effects are usually studied by administration *via* the coronary artery or by direct injection into the atrium. Berne, *et al.*,[6-9] argue that adenosine is an important physiological mediator of vasodilation. Although the exact role of adenosine has not been completely elucidated, there is little doubt that it is a potent, vasoactive substance.

As part of a program to prepare related compounds with similar activity but with a longer duration of action, ethyl adenosine-5′-carboxylate (3) was synthesized by the reaction sequence shown in Scheme I. The cardiovascular effects were tested in the dog preparation described by Schoepke, *et al.*,[10] in which blood pressure, heart rate, and coronary sinus Po_2 are continuously monitored. Changes in the last variable can be related to the coronary blood flow when oxygen extraction by the myocardium does not alter markedly. The *in vitro* effects on adenosine and adenylate deaminase were also assessed.

Scheme I. Synthesis of Ethyl Adenosine-5′-carboxylate



Results and Discussion

Typical cardiovascular data obtained are shown in Table I. As expected adenosine elicited marked responses of a short duration when given intravenously and essentially no effects when administered intraduodenally at doses as high as 30 mg/kg. On the other hand, 3 exhibited a marked, long-acting effect in the Po_2 test which, like intravenous adenosine, was initiated essentially instantaneously after intravenous administration of 0.10 mg/kg doses and lower; the intraduodenal activity, which became evident approximately 5 min after administration, was of particular significance since it indicated that 3 was well absorbed when given by this route and that it was not hydrolyzed rapidly to adenosine-5′-carboxylic acid. The latter was orders of magnitude less active in the preparation.

Table I. Effect on Cardiovascular Parameters in Anesthetized Dogs[a]

Compd	Injection route[c]	Dose, mg/kg	Po_2,[b] mm Change, %	Po_2,[b] mm Duration, min	Mean aortic pressure, mm Change, %	Mean aortic pressure, mm Duration, min	Heart rate, beats/min Change, %	Heart rate, beats/min Duration, min
Adenosine	iv	2	150	4	−50	2	−40	3
	id	15	3	15	0	0	−2	15
	id	30	8	20	0	0	0	0
3	iv	0.01	19	18	−1	9	5	5
	iv	0.05	104	32	−1	8	17	32
	iv	0.10	145	50	−5	26	17	50
	id	0.025	24	83	−3	60	13	65
	id	0.10	57	89	−2	60	2	36
	id	0.50	118	102	−25	88	34	75

[a] Peak changes are shown; the duration is the total time elapsed from the initiation of the change until return to pretest value. The range of the initial basal values of all the preparations was: Po_2, 15–25 mm; blood pressure, 90–120 mm; heart rate, 100–140 beats/min. The values cited are the averages of three measurements with a deviation of the order of 40%; iv dose responses were obtained in a given animal in three different preparations, while a total of six dogs were utilized to obtain the id data. [b] Po_2 = partial pressure of oxygen. [c] iv = intravenous; id = intraduodenal.

Table II. Studies with Deaminases

Addition, $M \times 10^4$	Incubation time, min	NH_3 formed, μmol
Adenylate Deaminase		
2.67 5′-AMP	8.0	0.32
2.67 5′-AMP +		
6.67 **3**	8.0	0.32
9.70 **3**	25.0	0.01
Adenosine Deaminase		
5.37 adenosine	3.0	0.72
5.37 adenosine +		
6.67 **3**	3.0	0.75
6.67 **3**	15.0	0.00

The blood pressure and heart rate effects of **3** were variable in that low doses given slowly usually caused a small change in both parameters; rapid administration, however, resulted in a fall in pressure and a compensatory rise in heart rate. Subsequent pharmacological studies have verified that it is a coronary vasodilator and that prolonged increases in coronary sinus Po_2 are observed when it is administered to unanesthetized dogs, orally, in doses as low as 0.15 mg/kg.[11]

A possible mechanism for the action of **3** might stem from an ability to inhibit the deamination of adenosine and/or adenylic acid, thereby increasing the respective steady-state levels and in some way increasing the adenosine concentration at a receptor. To test this hypothesis, the effect on adenosine deaminase from calf gut and adenylate (5′-AMP) deaminase from rabbit muscle was investigated. These data are shown in Table II. In neither system was it an inhibitor, nor was it a substrate for the enzymatic reaction as seen by the longer incubation without the respective substrates. It can be concluded then that inhibition of deaminase does not play a significant role in the mechanism of action. Additionally, it has been shown that **3** does not potentiate the coronary dilator or systemic hemodynamic effects of adenosine,[11] so a mechanism involving blockade of uptake of adenosine by the lung or red blood cells is not likely.

The two major pathways for the metabolism of adenosine involve deamination to inosine and phosphorylation at the 5′-hydroxyl group to form 5′-AMP. Since **3** has been shown to be a very poor substrate for both adenosine and 5′-AMP deaminase, and the 5′ terminus has been blocked so that phosphorylation would not be expected to occur, it is reasonable to assume that neither of these reactions plays an important role in its metabolism. Because the cardiovascular effects are observed almost instantaneously after intravenous administration, it is tempting to speculate that it exerts a direct action, perhaps on an "adenosine receptor." The similarity of **3** to adenosine and the fact that it would not be expected to be metabolized by the normal, primary purine reactions fit the concept that it could be a "long-acting adenosine." Further studies involving blockade of the cardiovascular effects and physiological half-life are in progress in an attempt to clarify the mechanism of action.

The inability of **3** to be phosphorylated at the 5′ terminus precludes the possibility of its entering the normal metabolic pool and being transformed into an aberrant nucleoside triphosphate with a potential for incorporation into RNA and/or DNA. This is an important consideration from the viewpoint of long-term toxicity. It is interesting that the toxicity of **3** is quite low; the acute LD_{50} in mice is greater than 1000 mg/kg by the oral route and approximately 700 mg/kg, intravenously. Doses as high as 500 mg/kg, orally, and 100 mg/kg, intravenously, are well tolerated by both dogs and monkeys; decreased activity is the major symptomatology observed.

Experimental Section

Adenosine-5′-carboxylic Acid (2). The acid was prepared from 2′,3′-*O*-isopropylideneadenosine (**1**, P-L Biochemicals)[12] with a yield of 64%. The melting point was greater than 300° (lit.[13] mp >320°).

Ethyl Adenosine-5′-carboxylate (3). Typically, 2.2 g of **2** was suspended in 5 ml of H_2O and 2.7 ml of 3.0 *M* NH_3 was added with stirring. After essentially all of the solid had dissolved, the solution was filtered and 1.47 g of $AgNO_3$ in 10 ml of H_2O was added dropwise with stirring to the filtrate. The resulting solid was filtered, washed, and dried *in vacuo*, in the dark, at 60° for 16 hr to yield 3.2 g of the silver salt. This solid was suspended in 70 ml of dry EtOH, 1.0 ml of EtI was added, and the mixture was refluxed in the dark for 6 hr. The suspension was filtered and upon cooling a white solid separated which was collected and dried to yield 0.53 g of **3**: mp 196–199° dec (uncorrected, Thomas-Hoover capillary melting point apparatus); nmr δ (DMSO-d_6, TMS) 8.41 (s, 1, H-8), 8.16 (s, 1, H-2), 7.29 (s, 2, NH_2), 6.08 (d, 1, $J_{1',2'}$ = 6.2 Hz, H-1′), 5.2–6.2 (b, 2, OH), 4.64 (m, 1, $J_{2',3'}$ = 4.5 Hz, H-2′), 4.49 (d, 1, $J_{3',4'}$ = 2.5 Hz, H-4′), 4.39 (m, 1, H-3′), 4.20 (q, 2, *J* = 7.0 Hz, OCH_2), 1.23 ppm (t, 3, CH_3); λ_{max} (pH 7.0) 259 nm (ε 16,000); λ_{min} 227 nm; mass spectrum, found *m/e* 309. *Anal.* ($C_{12}H_{15}N_5O_5$) C, H, N, O.

Cardiovascular Measurements. Dogs of either sex were anesthetized with 250 mg/kg of barbital sodium, intravenously, after sedation with 3 mg/kg, subcutaneously, of morphine sulfate. Under artificial respiration, an incision was made in the fourth right intercostal space and the pericardium was incised. A Beckman polarographic Po_2 microelectrode (in a Riley needle) was placed in the coronary sinus *via* the superior vena cava. Continuous recordings of the blood pressure, heart rate, and coronary sinus Po_2 were made on a Grass Model 7 polygraph. Compound, dissolved in isotonic saline, was administered either intravenously (jugular) or intraduodenally through an indwelling catheter.

Studies with Deaminases. Adenylate deaminase in glycerol was obtained from Sigma Chemical Co.; the incubation mixture contained 0.44 μg of protein in 3.0 ml of 0.14 *M* NaCl, 0.01 *M* citrate, and 2.67×10^{-4} *M* 5′-AMP, adjusted to pH 6.5. After 5 min preincubation at 37° with **3**, the reaction was started by the addition of 5′-AMP and 8 min later was terminated by the addition of 0.2 ml of Nessler's reagent. The amount of ammonia formed was determined by measuring the absorbance at 425 nm. Adenosine deaminase in glycerol was obtained from Boehringer-Mannheim; the incubation mixture contained 0.40 μg of protein in 3.0 ml of 0.11 *M* NaCl, 0.05 *M* Na_2HPO_4, and 5.37×10^{-4} *M* adenosine, adjusted to pH 7.4. The same procedure was used as with adenylate deaminase except that the reaction was terminated after 3 min. In both systems the conditions were chosen such that the reaction rate was linear with respect to enzyme concentration and incubation time. All tests were run in duplicate and the differences were of the order of 3%.

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Nitrofuryl Heterocyclics. 3[1]

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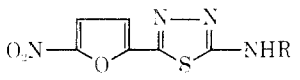
The synthesis and antibacterial properties of some 1-[4-(5-nitro-2-furyl)-2-thiazolyl]hydantoins and -hydrouracils have been described earlier.[2] As part of a search for new chemotherapeutic nitrofurans, the preparation of hydantoin and hydrouracil derivatives of 2-amino-5-(5-nitro-2-furyl)-1,3,4-thiadiazole (I, R = H) was examined, since it has been established that this latter nitrofuran and related compounds (*e.g.*, I, R = CHO) have potent antibacterial activities.[3]



I

Chemistry. The preparation of ureas II from I (R = H) and various acyl isocyanates, the cyclization of 2- or 3-haloacylureas to the corresponding hydantoins or hydrouracils III with NaH in DMF, and the alkylation of these last compounds to the 3-substituted derivatives IV are described in the Experimental Section.

Screening Results. Nitrofurans **1**-**27** were tested *in vitro* against a variety of bacteria according to procedures described previously.† It can be seen from Table I, which records the more active compounds, that most of the nitrofurans possessed efficacy against *Staphylococcus aureus* and *Streptococcus pyogenes*. Selected compounds were evaluated further against *S. aureus* and *S. pyogenes* infections in mice by oral and subcutaneous administration. Although some of these derivatives were active *in vivo* against *S. pyogenes* (Table II), none was effective against *S. aureus*.

Nitrofurans **1**-**27** were also evaluated against *Trichomonas vaginalis in vitro* and in mice.‡ Most of the compounds had some activity in the *in vitro* screen, and when tested against *T. vaginalis* infections in mice, hydantoins **16**, **17**, and **21** were found to be *ca.* four times more active than metronidazole.§ Further experiments showed, however, that none of the compounds appeared in measurable quantities in the urine.

In conclusion, when the nitrofurylthiadiazoles above are compared with the analogous 1-[4-(5-nitro-2-furyl)-2-thiazolyl]hydantoins and -hydrouracils discussed earlier,[2] two points of interest emerge. Firstly, as antibacterial agents the two series have comparable potencies against most organisms in the *in vitro* screen. *In vivo*, activity against *S. aureus* and *S. pyogenes* is relatively widespread among nitrofurylthiazole congeners, and in these compounds hydantoins and hydrouracils (*i.e.*, derivatives equivalent to III and IV) appear to be more active than the acylureas corresponding to II. In thiadiazoles II-IV, however, no compound appears to be effective against *S. aureus in vivo*, and activity against *S. pyogenes in vivo* is of a lower order than that shown by the analogous nitrofurylthiazoles. Finally, as trichomonicidal agents, the nitrofurylthiadiazoles above are very much more potent *in vivo* than the nitrofurylthiazoles discussed earlier.[2] In this respect, maximum activity appears to reside in the nitrothi-

Table I. *In Vitro* Antibacterial and Trichomonicidal Activity of Nitrofurans **1**-**25**[a]

	Minimum inhibitory concentration, μg/ml					
Compd	*Staph. aureus*, UC-76	*Diplococcus pneumoniae*	*Strep. pyogenes*, C203	*Salmonella typhimurium*, V-31	*Shigella sonnei*, C-10	*T. vaginalis*
1	1.25	0.31	1.25	2.5	2.5	>25 (6.25)[b]
2	5	0.16	0.16	20	20	>25
3	5	0.16	0.63	10	20	>25
4	5	20	0.31	20	20	>25
5	0.63	0.31	1.25	2.5	5	6.25
6	0.63	>20	0.16	20	5	>25 (1.56)
9	1.25	*c*	0.63	5	10	>25
11	2.5	5	0.08	>20	>20	>25 (6.25)
13	1.25	*c*	0.63	>20	>20	25
15	0.63	>20	0.08	>20	>20	25
16	1.25	10	0.63	>20	10	25
17	2.5	*c*	1.25	>20	20	25
21	5	*c*	5	>20	>20	>25 (6.25)
23	1.25	1.25	>20	>20	>20	>25 (6.25)
24	1.25	10	5	20	20	25
25	0.63	1.25	2.5	>20	20	*c*

[a] See ref 4 and 5. [b] Figures in parentheses indicate 90-99.9% inhibition at stated levels. [c] Indicates test not done.

Table II. *In Vivo* Activity[a]

	S. pyogenes, ED_{50} (mice), mg/kg		*T. vaginalis*, metronidazole equiv, *M*[b]
Compd	po	sc	
2	200	50	*c*
3	35	50	<0.25
11	42.5	10.2	*c*
16	*c*	*c*	4
17	*c*	*c*	4
21	*c*	*c*	2-4

[a] See ref 4 and 5. [b] *M* = ED_{50} of metronidazole/ED_{50} of test compound. [c] Indicates test not done.

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† For a description of the general *in vitro* and *in vivo* test procedures, see ref 4.

‡ For a description of test methods, see ref 5.

§ Flagyl.