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# SPECIFIC AND POTENT INHIBITION OF SPERMIDINE SYNTHASE BY THE TRANSITION-STATE ANALOG, S-ADENOSYL-3-THIO-1,8-DIAMINOOCTANE

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## SUMMARY

The title compound (1c), was designed and synthesized based on mechanistic data concerning enzyme-catalyzed alkyl transfer reactions, applied in this case to aminopropyl transferases. The inhibition by 1c of one such enzyme, spermidine synthase, was both potent ($I_{50}$ = 4 x $10^{-7}$M) and specific. A closely related aminopropyltransferase, spermine synthase was only minimally affected by high concentrations of 1c. Similar, although not as marked, specifity between the two aminopropyltransferases was observed with the corresponding methyl sulfonium salt, 2c. Studies with structurally related compounds support the hypothesis that the strong inhibition of spermidine synthase by 1c derives from the incorporation in this compound of important features of the transition-state structure of this enzyme-catalyzed reaction.

## INTRODUCTION

The biosynthesis of the polyamines, putrescine, spermidine and spermine, is of considerable importance in cellular replication, as evidenced by the myriad of stimuli which elicit changes in the intracellular level of the polyamines and/or their biosynthetic enzymes (1). Several effective inhibitors of enzymes involved in polyamine biosynthesis have been reported, and these compounds have served as useful biochemical and pharmacological tools (2). As part of our research on the mechanisms and inhibition of alkyl transfer reactions, we have developed several metabolically stable inhibitors of methyl transferases (3) and aminopropyltransferases (4). Despite the reasonable potency of these new drugs, it became apparent that more specificity would be necessary in order for in vivo experiments to be interpretable. Therefore, we have initiated a program to synthesize a series of compounds, designed to incorporate the salient features of the transition-state structures for a series

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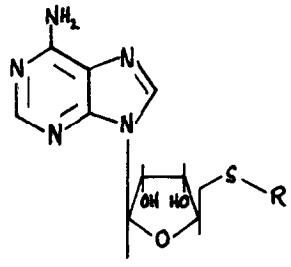
of enzyme-catalyzed group-transfer reactions (5). In the present communication, we report our initial data on the inhibition of purified spermidine synthase from rat ventral prostate by a series of adenosine-5'-thioethers (1), and the corresponding methyl sulfonium salts (2).

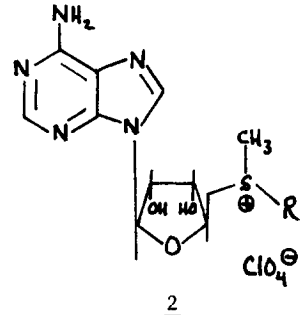


1

a, R = $CH_3$

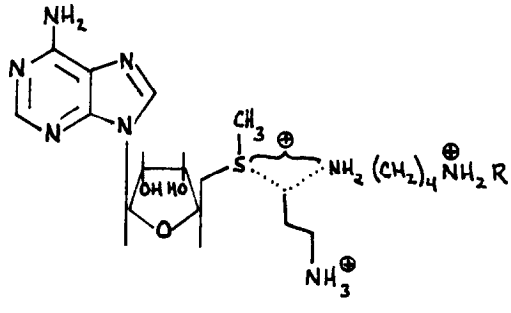
b, R = $CH-(CH_2)_4CH_3$ with $CH_2CH_3$

c, R = $CH-(CH_2)_5-NH_3^{\oplus}$ with $(CH_2)_2NH_3^{\oplus}$



2

These compounds were designed to mimic the hypothetical transition-state involved in direct nucleophilic attack by putrescine on the aminopropyl group of decarboxylated S-adenosylmethionine, as shown in 3a.



3

a, R = H

b, R = $-(CH_2)_3NH_3^{\oplus}$

## MATERIALS AND METHODS

Compounds 1a-c were synthesized by coupling 5'-deoxy-5'-chloroadenosine with the appropriate thiol by a modification of the method previously described for 1a (6). Compounds 2a-c were synthesized by methylation of appropriate thio-

ether precursors in formic acid containing excess methyl iodide. Full details of the synthetic procedures will be published separately (7). Assay of spermidine synthase and spermine synthase were carried out as previously described (4), except that decarboxylated S-adenosylmethionine was maintained at 21 μM. Methylthioadenosine phosphorylase activity was determined as previously described (4), with methylthioadenosine at 100 μM.

## RESULTS AND DISCUSSION

The data given in Table I clearly delineate the structural requirements for inhibition of rat prostate spermidine synthase by compounds of type 1 and 2. The design of these compounds incorporated ideas on transition-state structure in enzyme-catalyzed alkyl transfer reactions, specifically those involving S-adenosyl-L-methionine (SAM) (8,9). Thus, the sulfonium salt, 2c, was synthesized as an analog in which full charge is maintained on sulfur, and dispersed on nitrogen. The thioether analog, 1c, was synthesized as an analog in which charge is completely dispersed on both sulfur and nitrogen. The data of Table I show that the thioether 1c, with no charge at sulfur, is a more potent inhibitor of spermidine synthase than is the corresponding fully-charged methyl sulfonium salt, 2c. This is somewhat surprising when one considers that mechanistic studies of model reactions (8), as well as enzyme-catalyzed methylation (9), indicates that the charge on sulfur is only partially removed in the transition-state. However, it is similar to the observed inhibition of many methylases by the thioether product, S-adenosyl-L-homocysteine, with a $K_i$ considerably lower than the $K_m$ for the corresponding sulfonium substrate, S-adenosyl-L-methionine (10). It could be hypothesized that charge dispersal at sulfur in transmethylation reactions leads to tighter binding by the thioether product than the sulfonium substrate. In addition, recent data (11) show that the methyl group of decarboxylated S-adenosyl-L-methionine can be replaced by an ethyl or propyl group, and still retain most of the substrate activity. Thus it would appear that the methyl group of decarboxylated S-adenosyl-L-methionine is used simply to produce an electrophilic sulfonium salt, susceptible to nucleophilic attack by putrescine (3a) or spermidine (3b). Loss of this charge at sulfur in the transition state, would be facilitated by an active

Table 1. INHIBITION OF RAT PROSTATE SPERMIDINE SYNTHASE AND METHYLTHIOADENOSINE PHOSPHORYLASE BY 1 AND 2[a]

| Compound | Concentration, μM | % Inhibition | | |
|---|---|---|---|---|
| | | Spermidine Synthase | Spermine Synthase | MTA Phosphorylase[d] |
| 1a | 50 | 61 | 80 | Substrate |
| | 100 | 82 | 98 | |
| 1b | 25 | 0 | 11 | — |
| | 100[b] | 0 | 22 | 25 |
| 1c | 10 | 97 | — | 3 |
| | 25 | 97 | 10 | 8 |
| | 50 | 99 | — | — |
| | 100 | 99 | 15 | 10 |
| 2a | 50 | 0 | 68 | — |
| | 250 | 6 | 82 | 30 |
| 2b | 50 | 7 | 35 | — |
| | 100 | 14 | 56[c] | 11 |
| | 250 | 44 | 75 | 21 |
| 2c | 10 | 32 | 7 | — |
| | 50 | 84 | 40 | — |
| | 100 | 92 | 59[c] | 16 |
| | 250 | 96 | 86 | 25 |

[a]Enzyme assays were carried out as described in Materials and Methods, except that decarboxylated S-adenosylmethionine concentration was 42 μM when assaying 1a with spermidine and spermine synthase.

[b]Concentration range of 1b limited by poor solubility.

[c]A second preparation of spermine synthase was inhibited 19% and 28% by 100 μM 2b and 2c, respectively.

[d]MTA = methylthioadenosine

site of low polarity, and this may be the basis for the large difference in inhibition observed between 1c and 2c.

That this inhibition depends on many of the structural features of the transition state, 3a, is shown by the total lack of inhibition of spermidine synthase by the desamino analogs, 1b and 2b. In addition, the non-adenosine fragment of 1c, 1,8-diamino-3-octanol is inactive as an inhibitor of either spermidine or spermine synthase at concentrations as high as 250 μM. More extensive inhibition data are shown in Figure 1, leading to $I_{50}$ values of 0.4 μM and 15 μM for 1c and 2c respectively. Although more complete kinetic studies are required to further characterize this inhibition, a comparison of these $I_{50}$ values with literature $K_m$ values for mammalian spermidine synthase (6,12) shows that 1c is effective at concentrations well below the $K_m$ for either substrate. This is as expected from consideration of the energetics of substrate binding vs. enzyme catalysis (13). Thus, the value of $I_{50}$ = ca $4 \times 10^{-7}$ M for 1c is lower than either $K_m$ (ca $5 \times 10^{-5}$ M, ca $2 \times 10^{-4}$ M

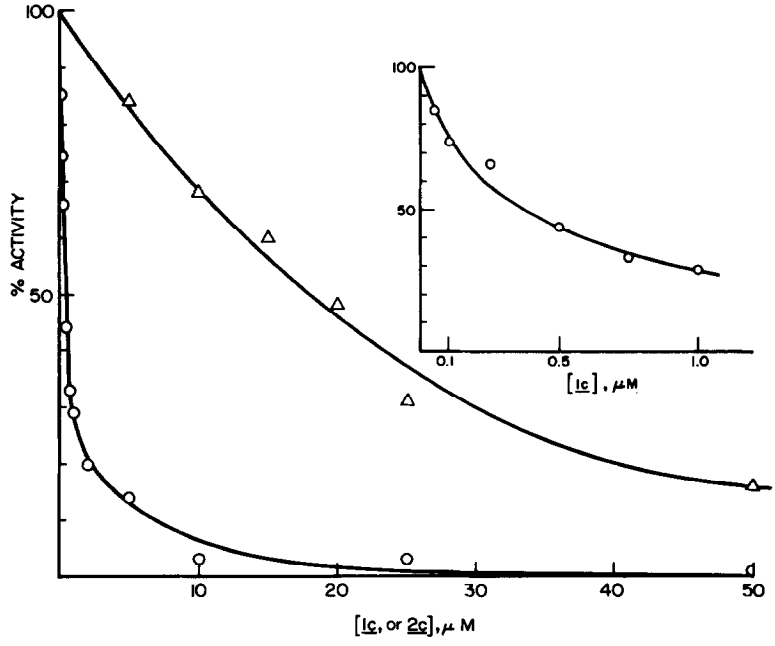



Figure 1. Inhibition of rat prostate spermidine synthase by 1c (O), and 2c (Δ).

(6,11)), but is of somewhat higher magnitude than the product of the $K_m$'s (ca $10^{-8}$ M). Marked substrate inhibition by decarboxylated S-adenosyl-L-methionine (4,6) makes a quantitative comparison of binding energies of questionable validity at this time. A full kinetic study with highly purified enzyme is required.

Of primary importance in the design of these compounds was the goal of obtaining not only potent, but specific inhibition of the target enzyme. To study this point, we evaluated compounds of type 1 and 2 as inhibitors of rat prostate spermine synthase. This enzyme catalyzes the formation of spermine from decarboxylated S-adenosylmethionine and spermidine, presumably by direct nucleophilic attack as shown in 3b. As shown in Table 1, compounds 1b and 1c were nearly devoid of inhibitory activity against spermine synthase, whereas compounds 2b and 2c were only modest inhibitors of this enzyme. The fact that both 2b and 2c inhibited spermine synthase to the same extent suggested that this might be due to a general inhibition of this enzyme by adenosyl sulfonium compounds. This has been noted previously with sulfonium salts such as S-adenosyl-L-methionine (4), and in the present work is observed with the simplest adenosyl sulfonium salt, 2a. Thus, the desired specificity of action obtained with 1c against spermidine synthase suggests that this compound mimics transition state 3a and not 3b.

We have previously reported (4) on the inhibition of spermidine synthase and spermine synthase by a wide variety of adenosine thioethers (1) and sulfonium salts (2). These studies, and the work of Pajula and Raina (14) have shown that 1a is a product inhibitor of both spermidine synthase and spermine synthase. Therefore, it was important to establish the effect of compounds 1 and 2 on the activity of methylthioadenosine phosphorylase, as this enzyme could conceivably hydrolyze thioethers of type 1, or be inhibited by sulfoniums of type 2 (15). This could lead to metabolic degradation of the inhibitors and/or increased levels of methylthioadenosine in future cell culture experiments. As shown in Table 1, neither compound 1c nor 2c, had any significant ef

fect on methylthioadenosine phosphorylase. The inhibitory activity of 2a has been reported previously (15). Thus, compound 1c and 2c may be used in biological studies without the concern of metabolic inactivation by this phosphorylase, or of affecting spermidine biosynthesis by increasing intracellular levels of the product inhibitor methylthioadenosine.

The thioether, 1c, may be of great value in biological studies because of the specifity of inhibition between the two aminopropyltransferases studied in this work. This potent and specific inhibition of rat prostate spermidine synthase by 1c and 2c suggests that corresponding analogs of the spermine synthase transition state, 3b, should have similar activity against the latter enzyme. Experiments to test this hypothesis are currently underway in our laboratories.

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## REFERENCES

1. J. Jänne, H. Pösö and A. Raina, Biochim. Biophys. Acta 473, 241-293 (1978).
2. H. G. Williams-Ashman, A. Corti, and B. Tadolini, Ital. J. Biochem. 25 5-32 (1976).
3. J. K. Coward and P. A. Crooks, in "Transmethylation", E. Usdin, R. T. Borchardt, and C. R. Creveling, eds., Elsevier North-Holland, 1979, p. 215-224.
4. H. Hibasami, R. T. Borchardt, S. Y. Chen, J. K. Coward, and A. E. Pegg, Biochem. J. 187, 419-428 (1980).
5. J. K. Coward, in "Drug Action and Design: Mechanism-Based Enzyme Inhibitors", T. Kalman, ed., Elsevier North-Holland, 1979, p. 13-26.
6. J. K. Coward, N. C. Motola, and J. D. Moyer, J. Med. Chem. 20, 500 (1977).
7. K.-C. Tang, R. Mariuzza and J. K. Coward, J. Med. Chem., submitted.
8. a. J. O. Knipe and J. K. Coward, J. Amer. Chem. Soc. 101, 4339-4348 (1979); b. I. Mihel, J. O. Knipe, J. K. Coward, and R. L. Schowen, ibid., 4349-4351 (1979).
9. M. F. Hegazi, R. T. Borchardt, and R. L. Schowen, ibid., 4359-4365 (1979).
10. G. L. Cantoni, H. H. Richards, and P. K. Chiang, in "Transmethylation", E. Usdin, R. T. Borchardt, and C. R. Creveling, eds., Elsevier North-Holland, 1979, p. 155-164.
11. K. Samejima and Y. Nakazawa, Arch. Biochem. Biophys. 201, 241-246 (1980).
12. A. E. Pegg and H. G. Williams-Ashman, J. Biol. Chem. 244, 682-693 (1969).
13. W. P. Jencks, Adv. Enzymol. 43, 219-410 (1975).
14. R.-L. Pajula and A. Raina, FEBS Lett. 99, 343-345 (1979).
15. V. Zappia, A. Oliva, G. Cacciapuoti, P. Galletti, G. Mignucci, and M. Cartini-Farina, Biochem. J. 175, 1043-1050 (1978).