N,N-dimethyltryptamine has been found to be a potent psychotomimetic agent when administered parenterally or by inhalation to man.[8,9] This compound has recently been reported to occur in blood and urine of acute schizophrenics.[10,11] The present report provides evidence for the existence of an enzyme in human lung tissue which catalyzes the formation *in vitro* of this hallucinogen from *N*-methyltryptamine and S-adenosylmethionine. A similar enzyme activity had previously been detected in small amounts in one out of four human lungs.[1] The role of indoleamine-*N*-methyl transferase and of its reaction products *N,N*-dimethyltryptamine and bufotenin in the etiology of mental aberrations remains to be established. Studies on the relationship of *N,N*-dimethylated indoleamines to psychoses are awaited with interest.

Merck Sharp & Dohme Research Laboratories,
Rahway, N.J. 07065, *U.S.A.*

LEWIS R. MANDEL
HO SAM AHN
W. J. A. VANDENHEUVEL
ROBERT W. WALKER

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Inhibition of catechol-*O*-methyltransferase by S-adenosylhomocysteine and S-adenosylhomocysteine sulfoxide, a potential transition-state analog*

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WE HAVE been investigating several aspects of nonenzymic[1] and enzymic transmethylation reactions. One of our main goals in these studies has been the elucidation of a molecular mechanism for the transmethylation reaction in order to design more effective regulators of this important biological process. The recent report of Deguchi and Barchas[2] concerning the inhibition of phenethanolamine *N*-methyltransferase by S-adenosylhomocysteine (SAH) prompts us to report similar findings with catechol-*O*-methyltransferase (COMT), EC 2.1.1.6. Based on our conclusions regarding the nature of the transition state in nonenzymic methyl transfer reactions,[1] we have also investigated the inhibition of COMT by S-adenosylhomocysteine sulfoxide (SAHO), a possible transition-state analog for the enzyme-catalyzed process.

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S-adenosylmethionine (SAM) and SAH were purchased from Boehringer Mannheim; $^{14}CH_3$-SAM (specific activity 44·5–52·3 mc/m-mole) was purchased from New England Nuclear Corp.; and 3,4-dihydroxybenzoic acid (DHBA) was purchased from Sigma Chemical Company. COMT was isolated from rat liver, purified and assayed by the method of Nikodejevic *et al.*,[3] with minor modifications (see figure legends). SAHO was prepared by the method of Duerre *et al.*,[4] and its homogeneity established by spectral and chromatographic properties. Thin-layer chromatography on cellulose in 71·5 per cent aqueous ethanol; $R_f = 0{\cdot}18$.

The effect of added SAH on the initial velocity, v', of the COMT-catalyzed transmethylation reaction is shown in Fig. 1. It is obvious from these data that any accumulation of SAH formed during

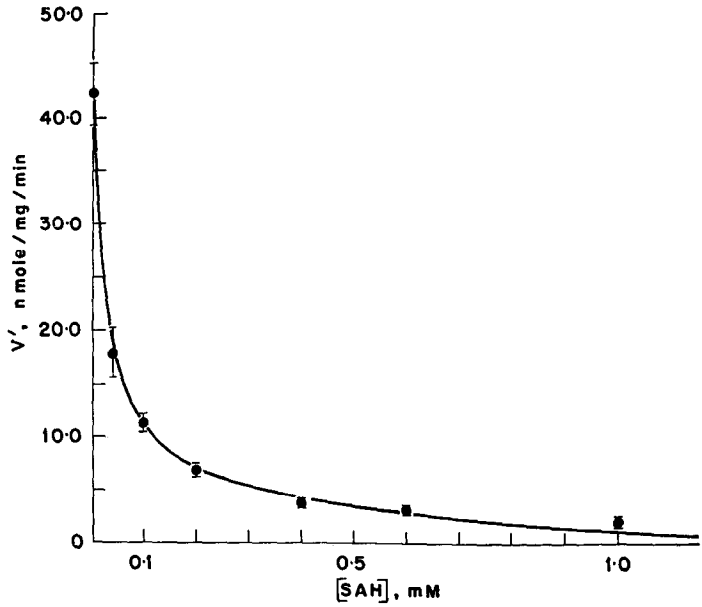


FIG. 1. Assays were run as described by Nikodejevic *et al.*,[3] except that [SAM] = 6×10^{-4} M (containing 3×10^{-3} μc $^{14}CH_3$-SAM), and pH was maintained at 7·9 with 0·1 M Tris buffer. Error bars indicate one standard deviation.

the transmethylation reaction will lead to nonlinear zero-order plots, and a sharply decreased value of v' derived from the initial slope of such plots. Detailed kinetic studies of the interaction of substrates and inhibitors with various transmethylases should provide valuable information which is necessary to determine the molecular mechanism of these reactions. However, due to the potent inhibition by SAH, it is imperative to establish conditions which keep the conversion to product below 5 per cent during the assay period. Inspection of data in the literature[3,5] indicates that routine kinetic analyses of the COMT-catalyzed reaction involve greater than 5 per cent conversion, and therefore considerable inhibition by SAH occurs. All data presented in Figs. 1 and 2 were obtained under saturating conditions such that conversion to product was always kept below 5 per cent, and in most cases below 3 per cent. Unfortunately, these criteria could not be met at low concentrations of SAM and DHBA, and therefore, kinetic constants such as $K_i = ca.\ 3 \times 10^{-5}$ M obtained for SAH acting as an inhibitor of SAM ($K_m = ca.\ 6 \times 10^{-5}$ M) and of DHBA ($K_m = ca.\ 2 \times 10^{-4}$ M) must be considered as only approximate values. For the same reason, it is not yet possible to state with certainty the exact nature of this product inhibition. Inhibition by the second product of the reaction, 3-methoxy-4-hydroxybenzoic acid, is much less potent ($K_i = ca.\ 3 \times 10^{-3}$ M) than that observed with SAH. An alternative method of evaluating the effectiveness of SAH as an inhibitor of the COMT-catalyzed transmethylation is shown in Fig. 2 (closed circles). From this type of plot, the concentration of SAH required to effect a 50 per cent inhibition of the reaction ($I_{/50}$) is seen to be $3{\cdot}0 \times 10^{-5}$ M. It can also be seen from Fig. 2 that at equimolar concentrations of SAH and SAM more than 90 per cent inhibition of the reaction is observed.

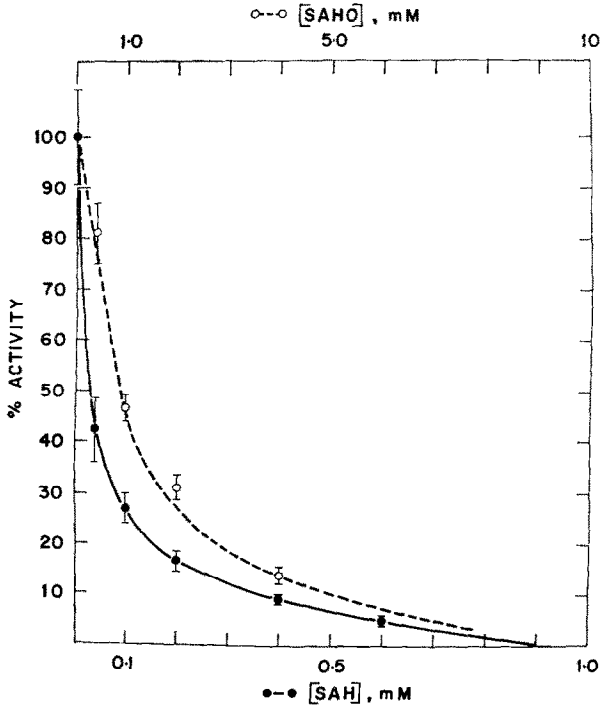
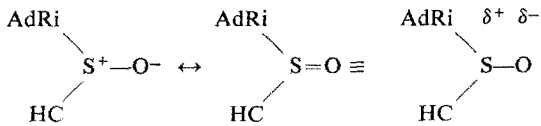


FIG. 2. Assay conditions as described for Fig. 1. Inhibitors added were SAH (closed circles, solid line) and SAHO (open circles, dashed line) at the concentrations indicated. Note that the concentration scale for SAHO is 10 times that for SAH.

Based on previous kinetic studies involving several nonenzymic transmethylation reactions, we have concluded that the transition state for such a process is structurally very similar to that of the starting materials, as shown in structure 1.[1] Considering the potent inhibition by SAH discussed above, we

(1)

decided to synthesize a transition-state analog[6,7] incorporating the SAH backbone, but containing a nonreactive trivalent sulfur atom, as a possible potent inhibitor of COMT. We chose to evaluate the sulfoxide of SAH,[4] which is shown in structure 2, where AdRi symbolizes adenosine, and HC indicates a homocysteine residue. The data of Fig. 2 (open circles) show that SAHO is a good



(2)

inhibitor of COMT ($I_{50} = 8{\cdot}6 \times 10^{-4}$ M), but it not as effective as SAH. Two explanations for this result can be offered. The first is that the doubly bonded > S=O form of structure 2 contributes most heavily to the resonance hybrid, and therefore SAHO does not resemble the transition state, either sterically or electronically. However, this is contrary to most reports in the literature,[8,9] which indicate that the semipolar > $S^+ - O^-$ form contributes most heavily to the sulfoxide resonance hybrid. Thus, the second, and more plausible, explanation is that the formal negative charge on the oxygen of SAHO leads to an unfavorable electrostatic interaction with groups in the enzyme active site.

The importance of S-adenosylhomocysteine as an inhibitor of biological transmethylations has been suggested previously,[10,11] and the recent findings involving biogenic amine[2] and transfer RNA[12] methylation, together with the present work, tend to support this hypothesis. The hydrolysis of SAM to adenosine and homocysteine serves to regulate the activity of methylases in a brain homogenate.[2] More stable analogs of SAH would be useful as inhibitors of methylation reactions, and the first series of such analogs has recently been synthesized in this laboratory.[13]

Department of Pharmacology,
Yale University School of Medicine,
New Haven, Conn. 06510, *U.S.A.*

JAMES K. COWARD
MARIA D'URSO-SCOTT
WILLIAM D. SWEET

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Inhibition of purine biosynthesis *de novo* and of Ehrlich ascites tumor cell growth by 6-methylmercaptopurine ribonucleoside*

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6-METHYLMERCAPTOPURINE ribonucleoside (6 MeMPR) is metabolized to its 5'-phosphate,[1–4] and inhibits purine biosynthesis *de novo* in Ehrlich ascites tumor cells ,[5,6] H. Ep. No. 2 cells,[1] leukemia L1210 cells,[1] adenocarcinoma 755 cells,[7] and in normal human fibroblasts[8–10] and several mouse tissues.[11] The first enzyme in this pathway, PP-ribose-P amidotransferase (EC 2.4.1.14), is strongly inhibited by 6 MeMPR-phosphate.[12,13] The idea that such inhibition is the basis of the growth-inhibitory activity of 6 MeMPR is very attractive, but as Mandel[14] has pointed out, such a conclusion cannot be fully accepted without being subjected to a number of tests. This is particularly relevant because recent studies have shown that 6 MeMPR does have other biochemical effects, including inhibition of the conversion of purine bases to nucleotides in cultured adenocarcinoma 755 cells.[15]

The most conclusive evidence presented to date to support inhibition of purine biosynthesis *de novo* as the mechanism of action of 6 MeMPR was the demonstration by Bennett and Adamson[7] that hypoxanthine and aminoimidazole carboxamide could completely reverse the growth-inhibitory effects of 3·5 μM 6 MeMPR, using cultured adenocarcinoma 755 cells. Similar observations have been made using cultured lymphoma L5178Y cells.†

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