The Specificity of S-Adenosylmethionine Derivatives in Methyl Transfer Reactions*

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SUMMARY

The preparation, purification, and analysis of a variety of new sulfonium derivatives of methionine is reported. Deamination of S-adenosyl-D-methionine with nitrous acid yielded, depending on experimental conditions, S-adenosyl L-(2-hydroxy-4-methylthio)butyric acid or S-inosyl-L-(2-hydroxy-4-methylthio)butyric acid. S-Inosyl-L-methionine was obtained by enzymatic deamination of S-adenosyl-L-homocysteine to S-inosyl-L-homocysteine and methylation of the latter by methyl iodide. 4-Dimethylsulfonium-L-(2-hydroxy)butyric acid has been prepared from S-methyl-L-methionine sulfonium salt. The sulfonium derivatives were purified by ion exchange chromatography and were characterized by ultraviolet spectrophotometry, paper and thin layer chromatography, electrophoresis, and hydrolysis to known fragments.

The new methionine sulfonium derivatives and S-adenosyl-(5')-3-methylthiopropylamine were investigated as methyl donors and as inhibitors with three purified methyltransferases: histamine methyltransferase, acetylserotonin methyltransferase, and homocysteine methyltransferase. The replacement of the 2-amino group of the methionine moiety by the hydroxyl group resulted in complete loss of activity in all systems investigated; these deaminated derivatives were also inactive as inhibitors of S-adenosyl-L-methionine in the transmethylations. The methyl donor capacity of inosine derivatives and of decarboxylated S-adenosylmethionine ranged between inactivity and an effect equal to that of the biological methyl donor. Homocysteine methyltransferase was the least specific of the three enzymes. Experiments with S-inosylmethionine, racemic with respect to the sulfonium pole, established that both sulfonium diastereomers were utilized in the methylation of homocysteine. S-Adenosyl-L-homocysteine was found to be a very effective inhibitor of the systems investigated; the enzymatic removal of its amino group in the adenine part led to S-inosylhomocysteine, which was without effect as an inhibitor. 5'-Methylthioadenosine, a catabolite of S-adenosyl-

Analogues and derivatives of metabolic intermediates have contributed much to the understanding of biochemical reaction mechanisms. The possibilities of modifying the biological methyl donor, S-adenosylmethionine, have been explored, so far, only with regard to the methyl sulfonium group. The sulfur atom has been replaced by selenium (1) and some properties of the selenonium derivative have been described (2). S-Adenosyl-selenium has become available by biosynthesis from ethionine with yeast (3); this derivative has been investigated extensively by Stekol (2). Chemical synthesis of (+)S-adenosyl-L-methionine has permitted a study of the sulfonium stereoisomerism in relation to enzymatic activity in transmethylations (4).

The present experiments deal with the modification of other regions of the S-adenosylmethionine molecule, specifically, the two amino groups and the carboxyl group. Several derivatives (Fig. 1) of S-adenosylmethionine (I) have been obtained. Replacement of the 2-amino group of the methionine part by a hydroxyl group gave S-adenosyl-L-(2-hydroxy-4-methylthio)butyric acid (II); more rigorous deaminating conditions led to the totally deaminated derivative, S-inosyl-L-(2-hydroxy-4-

Fig. 1. S-Adenosylmethionine (I) and some of its derivatives (II to V).
methylthio)butyric acid (III). In a circulatory way, S-mesyI-L
methionine was prepared. For this, S-adenosylhomocysteine was converted by enzymatic deamination to S-mesyI-
homocysteine; the latter was methylated to S-adenosylmethionine (IV). By enzymatic removal of the carboxyl group, using the procedure of Tabor (5), S-adenosyl-(5')-S-methylthiopropyl-amine (V) was obtained. S-Methyl-L-methionine sulfonium salt, which contains an enzymatic methyl donor toward L-homocysteine (6), has been included in this study. Its deamination led to 4-
dimethylsulfonium-L-(2-hydroxy)butyric acid, (CH₂)₂SCTLCH₂-
CH(OH)₂COOH.

Three enzymes, catalyzing nitrogen, oxygen, and sulfur methylation, were selected for testing these compounds as methyl donors and as inhibitors; the systems were, histamine, thiol transfer, acetylsalicylic methyltransferase, and homocysteine methyltransferase, respectively.

S-Adenosylhomocysteine is a product of all transmethylation with S-adenosylmethionine as methyl donor (7, 8); similarly, S-mesyIhomocysteine would result from transmethylation with S-adenosylmethionine. S-Methylthioadenosine represents another enzymatic derived from S-adenosylmethionine through separate pathways (9-11). The inhibitory effect of these reaction products on transmethylation has been examined.

**EXPERIMENTAL PROCEDURE**

**Compounds**—The quality of S-adenosyl-L-methionine used as starting material for the preparation and isolation of derivatives is of great importance. The commercial radioactive preparations in acid solution were satisfactory, but numerous impurities were found in some nonlabeled samples by paper and thin layer chromatography. By dehydroxylase or deamination of such mixtures, the number of products is increased beyond the possibility of resolution by single step ion exchange chromatography. For the present investigation, S-adenosylmethionine was prepared by biosynthesis with yeast (12) and isolated by the procedures developed in this laboratory (13, 14). The material was concentrated under reduced pressure to a level of 15 to 20 μmoles per ml, the pH was adjusted to a value between 2.5 and 3.0, and the solution was kept in the frozen state. More recently, a satisfactory quality of S-adenosylhomocysteine sulfate has been made available to the authors by the Research Laboratories of Boehringer Mannheim.

S-Adenosyl-L-homocysteine was produced by enzymatic synthesis from adenosine and L-homocysteine (15) and isolated by ion exchange chromatography (16).

S-Methyl-L-methionine sulfonium salt was obtained as iodide by the method of Toonnie and Kolb (17). In the nitrous acid treatment used for the deamination of the compound, iodide interferes because it is oxidized to iodine. The material was converted, therefore, into the sulfide by adsorption on a weakly acidic cation exchange resin, followed by elution with sulfurous acid. The procedure also removes methionine which occasionally presents as an impurity in the sulfonium preparation. For the present experiments, ¹⁴C-labeled material was used because 4-dimethylsulfonium-L-(2-hydroxy)butyric acid (deaminated S-methylmethionine) cannot be detected by ninhydrin tests or spectrophotometry. The purification steps are exemplified by the following procedure: 160 μmoles of (¹⁴C₂H₂)₂S-methyl-L-
methionine sulfonium iodide were applied to a column of Amberlite IRC-50 (resin bed, 2 × 10 cm) in H⁺ form, previously washed with water until the effluent showed a pH value above 5. The column was washed with 200 ml of water, and fractions of 25 ml were collected and assayed for radioactivity. The contaminating ¹⁴CH₃-methionine was contained in the first 75 ml and the following fractions declined to an insignificant counting rate. The sulfonium compound was then eluted with 0.1 × LSO₄₃ nearly all of it being contained in the first 75 ml of eluate as judged by the radioactivity. These fractions were pooled and adjusted to pH 3 to 4 with 0.3 × NaOH, the precipitate was removed by centrifugation, and the solution was concentrated to a small volume. The compound was pure as judged by paper and thin layer chromatography and by electrophoresis.

S-Methylthioadenosine was prepared by hydrolysis of S-adenosylmethionine (18). Commercial L-homocysteine thiolactone hydrochloride was converted to L-homocysteine by alkali (19). Histamine dihydrochloride and N-acetylsalicylic were obtained from Mann.

**Enzymes and Assay**—Non-specific adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4) from *Aspergillus oryzae* (20) was prepared from Sozyme (Sankyo, Japan) which was obtained from Calbiochem. The material was purified according to the directions of Sharpless and Wolfenden (21) through the alcohol fractionation. This product was satisfactory for the deamination of S-adenosylhomocysteine (22).

S-Adenosylhomocysteine decarboxylase was purified from *Escherichia coli* by the method of Tabor (5) including the first ammonium sulfate precipitation.

S-Adenosylmethionine:histamine N-methyltransferase (EC 2.1.1.8) was purified by the procedure of Brown, Tomchick, and Axelrod (23). Frozen guinea pig brains were obtained from Pel-Freez Biologicals, Inc., Rogers, Arkansas. The labeled methylhistamine formed in the reaction was measured by the procedure of Snyder, Baldessarini, and Axelrod (24), and the amount of ¹³C-methylhistamine produced was calculated on the basis of the specific radioactivity of the methyl donor. The sulfonium compounds tested as donors were retained quantitatively in the aqueous phase, as shown by the absence of radioactivity in the control experiments without acceptor. A slight correction was necessary in experiments with S-adenosyl (5')-S-methylthiopropylamine because some decomposition occurred in the control experiments and traces of radioactivity were extracted. ¹³C-Methylhistamine was measured also by radioactivity scanning after separation by paper chromatography.

Acetylserotonin methyltransferase (S-adenosylmethionine:N-acetylsalicylic O-methyltransferase, EC 2.1.1.4) was purified by the procedure of Axelrod and Weisshen (25) from pineal glands, obtained from Pel-Freez Biologicals. ¹³C-Melatonin, separated from the methyl donor by extraction into CHCl₃ at neutrality (25), was measured by scintillation counting. The calculation of the yield of melatonin was based on the specific activity of the methyl donor.

S-Adenosylmethionine:homocysteine S-methyltransferase (EC 2.1.1.10), purified from *Saccharomyces cerevisiae* (19), was generously furnished by Dr. S. K. Shapiro. Methionine formation was measured by the tracer assay of Shapiro and Yphantis (26). All sulfonium derivatives were retained by the Dowex 50, Li⁺ form columns, except those without the 2-amino group of methionine. For S-adenosyl- and S-mesyI-L-(2-hydroxy-4-methylthio)butyric acid, the procedure has been modified as follows. The chromatographic separation was performed with Dowex 50, H⁺-form columns, instead of Li⁺ form, as in the orig-
inal method. The samples were acidified to pH 3 before chromatography, and methionine was eluted with 8 ml of 1 x H2SO4, whereas the sulfonium compounds were retained. The samples with 4-dimethyl-sulfonium-L-(2-hydroxy)butyric acid as methyl donor were analyzed for labeled methionine and 2-hydroxy-4-methylthiobutyric acid after paper and thin layer chromatography of the incubation mixture.

In some experiments, transmethylation was studied by coupling the methyl transfer reaction with deamination of the resulting adenosine thioether. The deamination was followed spectrophotometrically (22). For S-adenosyl-L-(2-hydroxy-4-mercapto)butyric acid the reactivity with deaminase was implied in analogy with other S-adenosylthioethers (22).

Protein concentration was estimated by the method of Lowry et al. (27), with bovine serum albumin as standard.

Analytical Procedures—For spectrophotometric assay of purine compounds, the following molar absorbances were used: for all adenosyl compounds, 15,400 at 260 mμ as in neutral solution, and 14,700 at 257 mμ in strongly acid solution (12, 14); for all isosyl compounds, 19,900 at 249 mμ in neutral solution, and 10,900 at 250 mμ in acid solution (21, 22).

Radioactivity was measured in a Beckman Scintillation Spectrometer, model LS II. A 0.4% solution of 2,5-diphenyloxazole in a mixture of equal volumes of toluene and ethanol was used. The quenching was corrected by external standardization. A Packard radiochromatogram scanner, model 7200, was employed for scanning of the paper chromatograms. In some instances, thin layer chromatograms were examined for radioactivity by radioautography with Gevaert X-ray films; an exposure time of 5 days was sufficient for the levels of radioactivity used in the experiments.

For ion exchange chromatography, Dowex 50 resin, 8°C, cross-linked, 100 to 200 mesh size, was employed in most experiments. The resin bed was 12 X 1 cm. For the elution of sulfonium derivatives, hydrochloric acid was used and then removed from the desired fractions by distillation under reduced pressure. An alternative method is the elution with H2SO4 and isolation of the compounds by precipitation with phosphotungstic acid (12) or Reineke acid (13). However, this method gave lower yields because the precipitates of deaminated derivatives with the complexing agents are more soluble than those of S-adenosylmethionine. After removal of HCl, the oily residue were taken up twice in small quantities of water, re-evaporated, and kept in the frozen state. These solutions still contained some HCl which contributed to the stability of the sulfonium compounds. All derivatives, like S-adenosylmethionine (28), have been found unstable when kept for prolonged periods in neutral solution. If neutralization is required for enzymatic experiments, the pH can be adjusted to the desired value with K2HPO4 or KH2PO4 shortly before use.

For paper chromatography, Whatman No. 1 paper was used with ascending technique. Silica gel plates (Eastman Chromagram Sheets 6060, with fluorescent indicator) were employed for thin layer chromatography. Before use, the Chromagram Sheets were activated by heating for 20 min at 80°C. The solvent systems had the following composition, by volume: Solvent A, 1-butanol-acetic acid-water (12:3:5); Solvent B, 1 liter of 4.55 M (NH4)2SO4-0.1 M sodium phosphate, pH 6.8, + 20 ml of 2-propanol; Solvent C, 1-butanol-ethanol-propionic acid-water (10:5:2:5); Solvent D, 2-propanol-water (16:9); Solvent E, 2-propanol-HCl-water (14:0:41:39); Solvent F, 2,4-lutidine-9,4,6-soludine-water (6:5:5). All compounds were neutralized to pH 7 before the chromatographic separation. For detection, ultraviolet quenching (254 mμ), ninhydrin spray, platinum iodide spray (29), and radioactivity scanning were used. Table I gives the RF values of the principal compounds and of some of their hydrolysis products.

For paper ionophoresis, a Savant high voltage electrophoresis apparatus, model LT-48A, was used with a distance of 90 cm between the electrodes. The separations (Table II) were performed for 1 or 2 hours at 3000 volts on strips of Whatman No. 3MM paper. After migration, the spots were identified as described above.

**Sulfonium Compounds—Deamination of S-adenosylmethionine**

by enzymes would be the method of choice, but experiments with various deaminases including the nonspecific adenosine deaminase from A. oryzae, which deaminates S-adenosylhomocysteine, were unsuccessful (22). Likewise, L-amino acid oxidase from snake venom, while active toward L-methionine, is without effect on the amino group of methionine when the latter is bound in the sulfonium structure (20). It was necessary, therefore, to employ nitrous acid as the deaminating agent.

Cantoni (30) has shown that the amino group of the adenine part of S-adenosylmethionine can be removed by nitrous acid, but the product was not isolated. The pK values of the two amino groups in S-adenosylmethionine are similar to those in adenine and methionine, respectively. Klee and Mudd (31) have reported pK 3.5 for the amino group of the adenine, and pK 7.8 for the 2-amino group of the methionine part of the sulfonium compound. We have taken advantage of this difference, and by reducing the acidity, nitrite concentration,
The separations were carried out at 25° on Whatman No. 3MM paper in a Savant high voltage electrophoresis apparatus. Negative numbers indicate migration toward the anode. Each spot contained 20 μmole.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Buffer and pH values</th>
<th>( \text{cm volt}^{-1}\text{hr}^{-1} \times 10^3 )</th>
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</thead>
<tbody>
<tr>
<td>S-Adenosyl-L-methionine (I)</td>
<td>0.1 M formate, 3.0</td>
<td>-3.4</td>
</tr>
<tr>
<td>S-Adenosyl-L-(2-hydroxy-4-methylthio)butyric acid (II)</td>
<td>0.1 M acetate, 5.0</td>
<td>-3.4</td>
</tr>
<tr>
<td>S-Inosyl-L-(2-hydroxy-4-methylthio)butyric acid (III)</td>
<td>0.2 M phosphate, 7.0</td>
<td>-3.4</td>
</tr>
<tr>
<td>S-Inosyl-L-methionine (IV)</td>
<td>0.1 M borate, 8.5</td>
<td>-3.4</td>
</tr>
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</table>

Fig. 2. Hydrolyses of S-adenosylmethionine and its derivatives. Details are given in the text.

and reaction time, as compared with the conditions leading to complete deamination, we have succeeded in making the removal of the amino group of the methionine the predominant reaction.

For identification of the new sulfonium compounds, the procedures elaborated for S-adenosylmethionine were used (28, 31). Like the parent substance, the derivatives were not obtained in the crystalline state; and the analytical tests were restricted by the small quantities available. Spectrophotometry permitted us to distinguish between adenosine and inosine by the specific numbers indicated migration toward the anode. Each spot contained 20 μmolecules.

S-Adenosyl-L-(2-hydroxy-4-methylthio)butyric acid.—The partial deamination of S-adenosylmethionine was carried out in citrate-phosphate buffer solutions at pH 2.5 to 3.0 with a 10-fold excess of NaNO₂. The buffer solution was similar to that specified by McIlvaine (32): it contained 4.4 ml of 1.0 M citric acid and 1.1 ml of 1.0 M Na₂HPO₄, diluted with water to 10 ml; the pH was 2.5. A quantity of 48 mg of NaNO₂ was dissolved with minimal agitation in 3.0 ml of this buffer in an ice bath. To this, 4.0 ml of cold S-adenosylmethionine solution (17.5 μmolecules per ml; pH 2.3) were added and the mixture was kept without agitation at 25° for 45 min. The final pH was 2.9. Residual nitrous acid was removed from the reaction mixture by evaporation under reduced pressure to one-half of its original volume. The material was transferred to a cation exchange column (H⁺ form), and the elution was finished by a gradient obtained with 100 ml of 1.8 N HCl and 100 ml of 3.5 N HCl. The elution was carried out with 0.5 ml of Na₂HPO₄ in the mixing flask and 400 ml of 0.5 N HCl in the feeding reservoir. Fractions, 1 ml each, were collected and assayed by spectrophotometry at 260 and 257 nm. The fractions of elution Peak A were concentrated under reduced pressure to one-half of its original volume. The material was transferred to a cation exchange column (H⁺ form), and the elution was carried out with Na₂HPO₄ shown in Fig. 3. The elution peaks was identified by high pressure liquid chromatography as: A, S-inosyl-L-(2-hydroxy-4-methylthio)butyric acid \( (A_{max} = 250 \text{ mn}) \); B, S-adenosyl-L-(2-hydroxy-4-methylthio) butyric acid; and C, residual, nondeaminated S-adenosylmethionine.

The compound was identified by the following tests: spectrophotometry gave \( e_{250 \text{ nm}} = 16,400 \text{ in } 0.02 \text{ M phosphate at pH } 7.4 \), and \( e_{257 \text{ nm}} = 14,700 \text{ in } 1 \text{ N HSO₄} \). These values are identical with those of S-adenosylmethionine (14, 33) which can be considered as evidence that the adenosine moiety was not altered. Paper and thin layer chromatography (Table I), as well as paper electrophoresis (Table II), gave only one spot which distinguished the material from the parent substance, and its failure to respond to the ninhydrin reagent indicated the absence of the 2-amino group. The presence of the sulfonium structure was indicated by the hydrolytic behavior, which

![Fig. 3. Chromatography of deaminated derivatives of S-adenosylmethionine.](http://www.jbc.org/)

**TABLE II**

<table>
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resembled closely that of other adenosine sulfonium compounds in the extreme lability of the glycosidic bond in the alkaline range and unusual stability in the acid range (28, 39).

Hydrolysis according to Fig. 2, under the conditions specified above, yielded adenine (Split a), 2-hydroxy-4-methylthiobutyric acid (Split b), and 5'-methylthioadenosine (Split c). The release of adenine by alkaline hydrolysis was shown by spectrophotometry ($\epsilon_{260} = 12,300$) and paper chromatography. $S$-Pentoxy-($2$-hydroxy-$4$-methylthio)butyric acid was an additional product of alkaline hydrolysis at low temperature; it was located on the paper chromatograms by anisidine spray ($R_F 0.12$ acid (Split b), and 5'-methylthioadenosine (Split c). The release of adenine by alkaline hydrolysis was shown by spectrophotometry ($\epsilon_{260} = 12,300$) and paper chromatography. $S$-Pentoxy-($2$-hydroxy-$4$-methylthio)butyric acid was an additional product of alkaline hydrolysis at low temperature; it was located on the paper chromatograms by anisidine spray ($R_F 0.12$ Solvent System A, and $R_F 0.95$ in Solvent System B). 2-Hydroxy-4-methylthiobutyric acid was formed in $0.1 \ \text{N NaOH}$ at $100^\circ$ after 10 min; it was identified by paper chromatography and comparison with a commercial sample, giving $R_F 0.84$ and $0.57$ in Solvent Systems A and B. No other spots were found. These data characterized the new compound as $S$-adenosyl-$L$-(2-hydroxy-$4$-methylthio)butyric acid (Formula II in Fig. 1).

$S$-Inosyl-$L$-(2-hydroxy-$4$-methylthio)butyric Acid—For the preparation of doubly deaminated $S$-adenosylmethionine (34), a quantity of 50 $\mu$moles of $S$-adenosylmethionine in 3.4 ml of $H_2O$ was mixed with 0.6 ml of glacial acetic acid and cooled in ice. One milliliter of 3 M $\text{HNO}_3$ was added, drop by drop, over a period of 10 min. The solution was kept in ice for 24 hours. Paper chromatography of 5-$\mu$l samples with Solvent System B showed that all $S$-adenosylmethionine had disappeared after 30 min, while $S$-adenosyl-(2-hydroxy-$4$-methylthio)butyric acid had become the principal product. The latter gradually was converted to $S$-inosyl-$L$-(2-hydroxy-$4$-methylthio)butyric acid. After 24 hours, the excess of nitrous acid was removed by concentrating the solution under reduced pressure to one-half of its original volume. It was transferred to a Dowex 50-H$^+$ column as specified under "Analytical Procedures." The development was carried out with $\text{HICl}$ in a gradient provided by 100 ml of $H_2O$ to 300 ml of 2 N $\text{HCl}$ in the feeding reservoir. Initially, a small peak of 5'-methylthioinosine was observed by monitoring the eluates at 250 nm; $S$-inosyl-$L$-(2-hydroxy-$4$-methylthio)butyric acid was eluted with 1.0 to 1.3 N $\text{HCl}$. The requisite fractions were pooled, concentrated, and stored in the frozen state.

The yields ranged between 70 and 80%, and the same procedure was used to obtain the labeled derivative from $^{14}$CH$_3$-$S$-adenosylmethionine. The fully deaminated derivative of $S$-adenosylmethionine was identified by paper and thin layer chromatography (Table I), paper electrophoresis (Table II), and by its absorbance spectrum ($\epsilon_{260} = 10,900$ in 1.0 N $\text{H}_{2}\text{SO}_4; \epsilon_{230} = 12,200$ in 0.02 M phosphate buffer). Alkaline hydrolysis at $100^\circ$ for 10 min (Split b, Fig. 2) gave hypoxanthine and 2-hydroxy-4-methylthiobutyric acid. Hydrolysis at pH 4 yielded 5'-methylthioinosine (35, 36). No other spots were detected with the reagents employed.

$S$-Inosyl-$L$-methionine—$S$-Adenosyl-$L$-homocysteine was converted to $S$-inosyl-$L$-homocysteine; the methyl sulfoxonium derivative of the latter was obtained by treatment with methyl iodide. Deamination of $S$-adenosylhomocysteine to $S$-inosylhomocysteine cannot be accomplished in a satisfactory way with nitrous acid, because experimental conditions that lead to replacement of the 6-amino group of adenine remove the amino group of the homocysteine part as well. The thioether group is also affected by nitrous acid, leading to the sulfoxide. It was possible, however, to convert $S$-adenosylhomocysteine to $S$-inosylhomocysteine by enzymatic deamination with the non-specific adenosine deaminase from $A$. $\text{oryzae}$. This enzyme did not remove the amino group of the homocysteine part, and $S$-inosylhomocysteine was obtained (22). This compound was characterized by the absorption spectrum, which was typical of an inosine compound, and by a positive response to ninhydrin which showed the presence of the 2-amino group of the homocysteine part. For further identification, the product was hydrolyzed with 0.1 N $\text{HCl}$ for 30 min at $100^\circ$. Paper chromatography (see Table I) showed the formation of hypoxanthine and $S$-ribosylhomocysteine; a reference sample of the latter was obtained from $S$-adenosylhomocysteine (16, 37).

The synthesis of $S$-inosoylmethionine was accomplished as follows. $S$-Adenosyl-$L$-homocysteine (45 mg) was dissolved in 45 ml of 0.01 N potassium phosphate buffer, pH 6.8, and 0.3 ml of adenosine deaminase purified from $A$. $\text{oryzae}$ (see "Enzymes and Assays"), 2.0 mg of protein per ml, was added. The progress of deamination was followed by spectrophotometry at 265 nm (22). After 20 min the readings were constant and the absorbance at 265 nm was 40% of the initial value. The solution was passed through an Amberlite IRC-50 column, resin bed 8 X 1 cm, previously treated with 100 ml of 1 N $\text{HCl}$, followed by water until the effluent was at or above pH 5. $S$-Inosylhomocysteine was not adsorbed to the resin; its elution was completed by water, and spectrophotometry at 250 nm showed the material to be present in the first 100 ml of eluate; the enzyme was retained by the column. After the addition of 0.15 ml of 88% formic acid to the eluate, $S$-inosylhomocysteine was isolated by evaporation to the dry state. Its methylation was patterned after the procedure outlined by Toennies and Kolb (17) for the synthesis of $S$-methylmethionine. The material was taken up in 0.65 ml of 88% formic acid and 0.2 ml of glacial acetic acid. After cooling in ice, 0.1 ml of $\text{CH}_3\text{I}$ was added, the container was sealed tightly and kept at room temperature for 4 days with exclusion of light. After dilution with a few milliliters of $H_2O$, the residual $\text{CH}_3\text{I}$ was removed by distillation under reduced pressure. The solution was cooled and neutralized to pH 6.2 by slow addition of about 24 ml of 1.0 N $\text{K}_2\text{HPO}_4$. The separation of $S$-inosyl-$L$-methionine from $S$-inosyl-$L$-homocysteine is illustrated in Fig. 4. The yield of $S$-inosylmethionine was 48.4 $\mu$moles (41% of the theoretical value).

The product had the typical absorbance spectrum of an inosine compound ($\epsilon_{260} = 10,900$ in 1.0 N $\text{H}_2\text{SO}_4; \epsilon_{230} = 12,200$ in 0.02 M phosphate buffer, pH 7.4). By the hydrolytic procedures outlined in Fig. 2, hypoxanthine, methionine, 5'-methylthioinosine, and homocysteine was obtained. The presence of the sulfonium group in the molecule rendered the glycosidic bond labile. After 10 min in 0.1 N $\text{NaOH}$ at $25^\circ$, the absorbance shifted to that of hypoxanthine ($\epsilon_{260} = 11,500$).

Baddiley and Jamieson (38) have shown that the methylation of $S$-adenosylhomocysteine with methyl iodide leads to $S$-adenosylmethionine which is racemic at the sulfonium center. In analogy, the compound obtained in the present experiments should be designated as ($\pm$)$S$-inosyl-$L$-methionine.

$S$-Adenosyl-(5')-$S$-methylthiopropylamine—$S$-Adenosylmethionine was decarboxylated with the specific decarboxylase obtained from $E$. $\text{coli}$ (5) which converted 60 to 85% of the starting material. For detection of the residual $S$-adenosylmethionine, paper electrophoresis was used; especially at low pH values,
decarboxylated S-adenosylmethione migrates toward the cathode faster than its parent material (Table II).

A solution of 5.0 μmoles of S-adenosyl-L-methionine-3H, 0.3 μCi per μmole, 1 mmole of Tris-HCl buffer, pH 7.4, 40 ng of decarboxylating enzyme, and 0.1 mmole of MgSO₄ in a final volume of 7.0 ml, was incubated for 2 hours at 37°C. The mixture was deproteinized with 2.0 ml of 1.5 x perchloric acid. After centrifugation, the supernatant fluid was applied to a Dowex 50-H⁺ column as described under "Analytical Procedures." After initial treatment with 300 ml of 2 x HCl, an acid gradient ranging from 3 x to 3.5 x HCl was applied which separated S-adenosylmethionine and its decarboxylated product (Fig. 5). The fractions of elution Peak B were pooled and evaporated under reduced pressure; the material was pure as judged by isomophoretic analysis. The specific radioactivity of the parent material, S-adenosyl-L-methionine-3H, was recovered in the decarboxylated compound and the yield was 65% of the starting material.

The glycosidic bond of S-adenosyl-(5')-3-methylthiopropylamine showed the characteristic stability of S-adenosylsulfonium compounds toward acid, and unusual lability toward alkali. Chromatography with strong acid was tolerated for prolonged periods. Hydrolysis in 0.1 x NaOH for 10 min at 25°C severed adenine, which was observed by the shift of the maximum of absorbance from 260 to 268 mp, and by the drop of the molar absorbance value. The spectrophotometric data of the decarboxylated compound resembled those shown by S-adenosylmethionine (14) and by S-adenosyl-L-(2-hydroxy-4-methylthio)butyric acid. Hydrolysis for 25 min at 100°C and pH 4 yielded 5'-methylthioadenosine and 3-aminopropanol. The absence of homoserine in the hydrolysate further proved the removal of S-adenosylmethionine from the decarboxylated product. Solvent System A (Table I) permitted the separation of homoserine (R₂ 0.31) and 3-aminopropanol (R₂ 0.21).

Deamination of S-Methyl-L-methionine Sulfonium Salt—The conditions of deamination of S-adenosylmethionine by nitrous acid have been found adequate also for the removal of the amino group of S-methylmethionine. To a solution of 52 μmoles of the sulfate of purified S-methyl-L-methionine sulfonium (see "Compounds"), 9 mmoles of acetic acid were added to a final volume of 6.5 ml. After cooling in an ice bath, 1.0 ml of 3 x NaNO₂ was added, drop by drop, without agitation. The mixture was removed from the ice bath and left at room temperature for 30 min. Residual nitrous acid was removed by concentration of the solution under reduced pressure to about one half of the original volume. The material was transferred to a column of Dowex 50-H⁺ resin. Gradient elution with HCl, ranging from 0 to 1.0 x, was started, and fractions of 14 ml were collected and assayed for radioactivity. Most of the latter appeared in the Fractions 8 to 10, with an acidity near 0.5 x; they were pooled and evaporated under reduced pressure. The material was taken up twice in a few milliliters of water and re-evaporated. It was dissolved in 2.0 ml of water and stored in the frozen state. The yield of the deaminated product, as judged by radioactivity, was 40.3 μmoles. Its structure as 4-dimethylsulfonium-L-(2-hydroxy)-butyric acid was indicated by the absence of ninhydrin response and by the recovery in a single fraction by ion exchange chromatography. The material proved to be uniform, as judged by radioactivity scanning of paper chromatograms and electrophoretograms (Tables I and II).

RESULTS

Various sulfonium compounds were tested for their ability to act as methyl donors in the formation of methylhistamine (Table III). In addition to the natural methyl donor, S-adenosyl-L-methionine, only S-inosyl-L-methionine showed significant activity. The other compounds were found to be inactive within the limits of accuracy of the analytical tests.

The same compounds were checked for their inhibitory action in the presence of S-adenosylmethionine (Table IV). Inhibition
TABLE III
Sulfonium compounds as methyl donors for histamine N-methyltransferase

Purified histamine methyltransferase, 0.4 mg of protein, was incubated in the presence of 0.2 μmole of histamine, 50 μmoles of sodium phosphate buffer, pH 7.5, and the labeled methyl donor as indicated in the table; the final volume was 0.2 ml. Reaction mixtures without histamine were prepared as controls. The mixtures were incubated for 30 min at 37° in glass-stoppered tubes. The samples were assayed for methylhistamine as outlined under "Experimental Procedure."

<table>
<thead>
<tr>
<th>Methyl donor, 1CH3-labeled</th>
<th>Specific radioactivity (cpm/μmole × 103)</th>
<th>Concentration used (μM)</th>
<th>Methyl-histamine formed (μmole/sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-Adenosyl-L-methionine</td>
<td>1.4</td>
<td>0.25</td>
<td>4.5</td>
</tr>
<tr>
<td>S-Adenosyl-(L-2-hydroxy-4-methylthio)butyric acid</td>
<td>1.5</td>
<td>1.00*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>S-Adenosyl-L-methionine</td>
<td>1.8</td>
<td>0.25</td>
<td>0.5</td>
</tr>
<tr>
<td>S-Inosyl-L-methionine</td>
<td>1.8</td>
<td>0.50</td>
<td>0.8</td>
</tr>
<tr>
<td>S-Inosyl-(L-2-hydroxy-4-methylthio)butyric acid</td>
<td>1.1</td>
<td>1.00*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>S-Adenosyl-(5'S)-3-methylthiopropylamine</td>
<td>1.4</td>
<td>1.00*</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>S-Methyl-L-methionine</td>
<td>1.6</td>
<td>1.00*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>4-Dimethylsulfonium-L-(2-hydroxy)butyric acid</td>
<td>1.6</td>
<td>0.50</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

* The same result was obtained with concentrations of 0.25 and 0.5 μM.

TABLE IV
Inhibition of histamine methylation by S-adenosyl-L-methionine analogues and derivatives

The conditions of the reaction are reported in Table III. The concentration of S-adenosyl-L-methionine was 0.25 μM in each experiment.

<table>
<thead>
<tr>
<th>Additions</th>
<th>Concentration tested (μM)</th>
<th>Methyl-histamine formed (μmole/sample)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-Adenosyl-L-(2-hydroxy-4-methylthio)butyric acid</td>
<td>0.25</td>
<td>4.58</td>
<td>0</td>
</tr>
<tr>
<td>S-Inosyl-L-methionine</td>
<td>0.45</td>
<td>4.12</td>
<td>100</td>
</tr>
<tr>
<td>S-Inosyl-(L-2-hydroxy-4-methylthio)butyric acid</td>
<td>1.00</td>
<td>3.66</td>
<td>20</td>
</tr>
<tr>
<td>S-Adenosyl-(5'S)-3-methylthiopropylamine</td>
<td>0.25</td>
<td>4.37</td>
<td>0.3</td>
</tr>
<tr>
<td>S-Methyl-L-methionine</td>
<td>1.00</td>
<td>3.75</td>
<td>18</td>
</tr>
<tr>
<td>S-Adenosyl-L-homocysteine</td>
<td>0.25</td>
<td>3.41</td>
<td>27</td>
</tr>
<tr>
<td>S-Inosyl-L-homocysteine</td>
<td>0.50</td>
<td>3.66</td>
<td>90</td>
</tr>
<tr>
<td>5'-Methylthiodenosine</td>
<td>0.45</td>
<td>3.65</td>
<td>80</td>
</tr>
</tbody>
</table>

* For these values the inhibition is statistically significant.

Fig. 6. Effect of S-adenosyl-L-homocysteine (○) and S-inosyl-L-homocysteine (△) on histamine methylation. The reaction conditions and methylhistamine assay are reported in Tables III and IV and under "Experimental Procedure." was observed with S-adenosyl-(5'S)-3-methylthiopropylamine (decarboxylated S-adenosylmethionine) and with S-inosyl-L-methionine. In the latter case, a correction has been made for the low methyl donor activity of this compound (Table III). Several thioethers, derived from the sulfonium compounds, were included in the survey. S-Adenosyl-L-homocysteine was found to be an effective inhibitor. The corresponding deaminated compound, S-inosyl-L-homocysteine, gave only negligible inhibition which illustrates the importance of the 5-amino group of the purine part of the molecule. 5' Methylthiodenosine also inhibited the methylation of histamine significantly (Table IV).

The difference between S-inosyl-L-homocysteine and S-adenosyl-L-homocysteine as inhibitors was examined in more detail (Fig. 6). At a concentration of 10-5 M the reaction was strongly inhibited by S-adenosyl-L-homocysteine whereas the inosyl derivative exerted only 10% inhibition. At a level of 5 × 10-3 M, S-adenosyl-L-homocysteine still caused a significant inhibition.

Experiments on the methylation of acetylserotonin by the requisite methyltransferase are summarized in Table V. Of the enzymes tested in this investigation, this was the most specific. All derivatives of S-adenosylmethionine were found to be inactive as methyl donors. When S-adenosylmethionine was used as methyl donor, high concentrations of S-inosylmethionine, S-adenosyl-(5'S)-3-methylthiopropylamine, or 5'-methylthiodenosine were required for inhibition of the enzyme. S-Inosylhomocysteine did not cause significant inhibition whereas S-adenosylhomocysteine was found to be a very effective inhibitor (Fig. 7). This again emphasizes the significance of the 5-amino group of the purine moiety for this class of reactions.

The methylsulfonium donor specificity in the enzymatic methylation of L-homocysteine is much wider (Table VI). In fact, all sulfonium compounds examined acted as methyl donors with the exception of those that lacked the 5-amino group of the
TABLE V

S-Adenosylmethionine derivatives as substrates and as inhibitors for acetylserotonin methyltransferase

Purified acetylserotonin methyltransferase (50 μg of protein) was incubated with 30 μmoles of sodium phosphate buffer, pH 7.9, 0.2 μmole of N-acetylserotonin, and the labeled methyl donors (specific activity 2.8 × 10^3 cpm per pmole) in the concentrations listed in the table; the final volume was 0.2 ml. The concentration of the additions is indicated in the table. Reaction mixtures without N-acetylserotonin were prepared as controls. After 1 hour of incubation at 37°C, the reaction mixtures were assayed for labeled melatonin as described under "Experimental Procedure."

<table>
<thead>
<tr>
<th>Substrate, 1CH3-labeled</th>
<th>Concentration</th>
<th>Additions</th>
<th>Concentration</th>
<th>Melatonin formed</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-Adenosyl-L-methionine</td>
<td>0.05</td>
<td>0.10</td>
<td>0.10</td>
<td>2.10</td>
<td>43.2%</td>
</tr>
<tr>
<td>S-Adenosyl-(2-hydroxy-4-methylthio)butyric acid</td>
<td>0.10</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-Adenosyl-L-methionine</td>
<td>0.05</td>
<td>S-Adenosyl-L-(2-hydroxy-4-methylthio)butyric acid</td>
<td>0.20</td>
<td>2.11</td>
<td>0</td>
</tr>
<tr>
<td>S-Inosyl-L-methionine</td>
<td>0.10</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-Adenosyl-(5')-3-methylthiopropylamine</td>
<td>0.10</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-Adenosyl-(5')-3-methylthiopropylamine</td>
<td>0.05</td>
<td>0.10</td>
<td>2.06</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>S-Adenosyl-(5')-3-methylthiopropylamine</td>
<td>0.05</td>
<td>S-Adenosyl-(5')-3-methylthiopropylamine</td>
<td>0.20</td>
<td>1.85</td>
<td>12%</td>
</tr>
<tr>
<td>S-Adenosyl-(5')-3-methylthiopropylamine</td>
<td>0.05</td>
<td>0.50</td>
<td>1.83</td>
<td>13%</td>
<td></td>
</tr>
<tr>
<td>S-Methyl-L-methionine</td>
<td>0.10</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-Adenosyl-L-methionine</td>
<td>0.05</td>
<td>0.05</td>
<td>0.53</td>
<td>75%</td>
<td></td>
</tr>
<tr>
<td>S-Adenosyl-L-homocysteine</td>
<td>0.05</td>
<td>0.05</td>
<td>1.95</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>S-Methylthioadenosine</td>
<td>0.05</td>
<td>0.05</td>
<td>1.60</td>
<td>24%</td>
<td></td>
</tr>
<tr>
<td>S-Methylthioadenosine</td>
<td>0.05</td>
<td>0.50</td>
<td>1.49</td>
<td>29%</td>
<td></td>
</tr>
</tbody>
</table>

a The same result was obtained with 0.05 mM concentration.

b For these values the inhibition is significant on a statistical basis.

c The same result was obtained with 0.05 mM concentration.

The value represents the total formation of methionine according to the equation, S-methyl-L-methionine + L-homocysteine → 2 L-methionine + H+. Thus, one-half of the methionine is formed by transmethylation, the other by demethylation of the donor.

FIG. 7. Effect of S-adenosyl-L-homocysteine (○) and S-inosyl-L-homocysteine (□) on the methylation of N-acetylserotonin. The experimental conditions are reported in Table V and under "Experimental Procedure." The concentration of S-adenosyl-L-methionine was 0.05 mM in all experiments.

TABLE VI

Sulfonium compounds as methyl donors for S-adenosylmethionine-homocysteine methyltransferase

Purified S-adenosylmethionine-homocysteine methyltransferase (1.4 mg of protein) was incubated with the 1CH3-labeled sulfonium compounds in the concentrations indicated above, 10 pmole of L-homocysteine, 0.06 pmole of Zn++, 60 pmole of sodium phosphate buffer, pH 7.2, in a final volume of 0.6 ml. The incubation time was 1 hour at 37°C. The specific radioactivity of the sulfonium compounds ranged between 1.4 and 1.8 × 10^6 cpm per pmole.

<table>
<thead>
<tr>
<th>Methyl donor, 1CH3-labeled</th>
<th>Concentration</th>
<th>Methionine formed</th>
<th>Transmethylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-Adenosyl-L-methionine</td>
<td>1.5</td>
<td>0.25</td>
<td>31</td>
</tr>
<tr>
<td>S-Adenosyl-L-(2-hydroxy-4-methylthio)butyric acid</td>
<td>3.0</td>
<td>&lt;0.01</td>
<td>0</td>
</tr>
<tr>
<td>S-Inosyl-L-homocysteine</td>
<td>1.5</td>
<td>0.23</td>
<td>26</td>
</tr>
<tr>
<td>S-Adenosyl-(5')-3-methylthiopropylamine</td>
<td>3.0</td>
<td>&lt;0.01</td>
<td>0</td>
</tr>
<tr>
<td>S-Methyl-L-methionine</td>
<td>1.5</td>
<td>0.19</td>
<td>21</td>
</tr>
<tr>
<td>S-Adenosyl-(5')-3-methylthiopropylamine</td>
<td>1.5</td>
<td>0.30</td>
<td>33</td>
</tr>
<tr>
<td>S-Adenosyl-(5')-3-methylthiopropylamine</td>
<td>3.4</td>
<td>&lt;0.01</td>
<td>0</td>
</tr>
</tbody>
</table>

a The same result was obtained with 0.05 mM concentration.
The methionine sulfoxonium compound as a methyl donor in this system was studied in some detail with respect to their interaction with the enzyme. The results of the kinetic experiments are illustrated in Fig. 9. The $K_m$ values of decarboxylated S-adenosylmethionine as compared with those of S-adenosylmethionine is relatively high. A $K_m$ value of 21.3 x $10^{-8}$ M was calculated for S-inosyl-L-methionine in the same manner.

The methyl donor activity of S-inosyl-L-methionine in the biosynthesis of methionine was studied in more detail to clarify the relationship between the steric configuration of the sulfoxonium pole and the enzymatic activity (Fig. 10). The synthesis of this compound by methylation of S-inosyl-L-homocysteine, in analogy to the earlier synthesis of S-adenosylmethionine from S-adenosylhomocysteine (4, 38), results in a compound racemic with respect to the sulfoxonium pole. With an excess of enzyme, (+)-S-inosyl-L-methionine gave virtually complete transmethylation (Fig. 10), which indicates that both diastereoisomers are utilized by this methyltransferase. This is in agreement with the results obtained with (+)-S-adenosyl-L-methionine in this reaction (39); only the (-)-sulfonium component was found active in several other transmethylations (4).

The methionine sulfoxonium compound as a methyl donor toward L-homocysteine whereas 70% transmethylation was attained under the same conditions with S-adenosyl-L-methionine (Fig. 8).

In Dry Ice. The quantity of $^{14}CH_3$-methionine formed was assayed with 0.1 ml of the incubation mixture by the method specified in Table VI and under "Experimental Procedure." The reaction velocity has been expressed as millimicromoles of methionine formed per mg of protein per hour. The lines were fitted by the methods of the least squares.
DISCUSSION

The preparation of three deaminated derivatives of S-adenosylmethionine and the availability of its decarboxylation product has made possible an examination of the function of these groups in the activity of S-adenosylmethionine as methyl donor. The structure of the deaminated compounds was established by hydrolysis to known fragments. However, the possibility of racemization or Walden inversion at carbon atom 2 of the amino acid moiety of S-adenosylmethionine in the process of deamination has to be considered. Conclusive proof for the retention of the L-configuration has not yet been provided, and the formulation of the L-configuration as belonging to the L-series is tentative. However, for the deamination of amino acids Brewer et al. (40) have furnished proof for the retention of the configuration; the presence of the adjoining carboxyl group was found to be important. The problem has been reviewed extensively by Greenstein and Winitz (41). The different behavior of β-protonated amino acids and amino acid esters has been reported recently by Yamada, Taniguchi, and Koga (42).

Deaminated derivatives, particularly of coenzymes, have played an important role in investigations on the interaction of the amino group with enzymes. Extensive studies with DPN, TPN, ATP, and FAD (43-46) have shown that the 6-amino group of the adenine moiety is essential for biological activity in many instances.

The occurrence of decarboxylated S-adenosylmethionine in nature and its function in enzymatic transfer of the propylamino group has been established by Tabor, Rosenthal, and Tabor (47), but there are, as yet, no indications that the deaminated derivatives of S-adenosylmethionine are natural compounds.

The enzymes used for testing the methionine sulfonium derivatives as methyl donors and inhibitors provide examples of methyl transfer to oxygen, nitrogen, and sulfur atoms of the acceptors (6, 25, 18, 40).

In the present experiments, the 2-amino group of the methionine moiety has been found indispensable in all three methyl transfer reactions, and the 2-deamino derivatives are inactive as methyl donors as well as inhibitors. Similarly, deaminated S-methylmethionine is inactive in the methylation of homocysteine. There are exceptions, however, to the requirement of an amino group for methyl donor activity in transmethylation, since dimethylacetothetin, (CH$_3$)$_2$SCH$_2$COOH, and dimethylpropiothetin, (CH$_3$)$_2$SCH$_2$CH$_2$COOH, methylate homoacetine in the presence of the specific enzyme (50). It appears, therefore, that the amino group of methionine is required for binding to the protein rather than for an interaction with the sulfonium group to facilitate methyl transfer.

The 6-amino group of the adenine part of S-adenosylmethionine was found indispensable in acetylserotonin methyltransferase. In histamine methyltransferase, S-methylmethionine showed about one-tenth of the methyl donor activity of S-adenosylmethionine; the doubly deaminated derivative, however, was inactive. In the S-adenosylhomocysteine-methyltransferase system, the presence of the adenine moiety is not a necessity; S-methylmethionine was found active although the $K_m$ value is higher than that of S-adenosylmethionine. The lack of specificity is not surprising because S-methylmethionine is also an efficient methyl donor in this system. The sulfonium pole and the 2-amino group appear to be the essential requirements in this case.

Decarboxylation of S-adenosylmethionine occurs in microorganisms (11) as well as in mammals (51). The ability of this compound to act as a methyl donor apparently has not been previously examined. Here again, the inactivity in the histamine methyltransferase and the acetylserotonin methyltransferase systems is contrasted with its activity as methyl donor in the enzymatic formation of methionine from homocysteine; however, in the latter system the $K_m$ value is relatively high compared with that of S-adenosylmethionine. On the other hand, in the O-methyl- and N-methyltransferases, the decarboxylated derivative exhibits a significant inhibition on the reaction with S-adenosylmethionine as methyl donor. This suggests that in cellular metabolism adenosylmethionine decarboxylase not only competes with the methyl transfer reactions for the utilization of the same substrate, but also indirectly inhibits this class of reactions.

The inhibitory effect of S-adenosylhomocysteine in the S-adenosylmethionine-homocysteine methyltransferase system had been observed earlier (52). In the present study on N- and O-methyltransferase, the effect is particularly evident. This product inhibition seems to be a general feature of methyltransferase systems, and a regulation of this class of reactions by S-adenosylhomocysteine is indicated. The recent observation by Salvatore, Zappia, and Shapiro (53) that the concentrations of this thioether in liver are of the same order of magnitude as those of S-adenosylmethionine further suggests the biological significance of this inhibition.

The inhibitory effect of 5'-methylthioadenosine on the methylation of histamine and N-acetylsperotonin is of particular interest considering the origin of this compound from S-adenosylmethionine (9-11).

The role of the amino groups and of the carboxyl group of S-adenosylmethionine in relation to the enzyme systems studied is represented in a schematized form in Fig. 11. Binding site 2 appears to be particularly important in all instances. In the
homocysteine S-methyltransferase, binding sites 1 and 4 appear to be of minor importance. Attachment of the sulfonium group at site 2 is not readily amenable to tests. However, if the sulfonium group by itself would be a major factor in the attachment of the methyl donor to the enzyme proteins, one would expect strong inhibition of S-adenosylmethionine-dependent transmethylations by the deaminated derivatives. The scheme does not take into consideration additional binding sites which determine the acceptor specificity.

Acknowledgments—The advice of Dr. John F. Thomson in the kinetic experiments is greatly appreciated. The help of Mr. M. H. Dipert in writing the computer program for the statistical elaboration of the results is gratefully acknowledged.

REFERENCES

The Specificity of S-Adenosylmethionine Derivatives in Methyl Transfer Reactions
Vincenzo Zappia, Cynthia R. Zydek-Cwick and F. Schlenk

J. Biol. Chem. 1969, 244:4499-4509.

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