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# Steric Constraints in Intramolecular Reactions at $sp^3$ Carbon Implications for Methylase Mechanisms[1]

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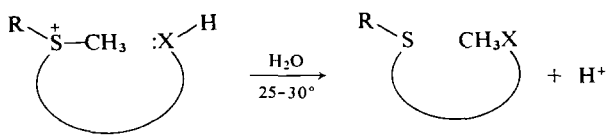
A series of methyl sulfonium compounds, containing appropriately positioned nucleophilic moieties, has been synthesized and studied as models for methylase enzymes in which the methyl sulfonium compound, *S*-adenosyl-L-methionine, serves as the methyl donor. The results of these studies show that there is a strict requirement for a linear transition state in intramolecular transmethylation reactions, and even slight deviations from this linear transition state are not permitted. These conclusions are pertinent in understanding the steric controls which appear to be operative in enzyme-catalyzed transmethylation reactions.

## INTRODUCTION

Despite the apparent simplicity of the overall reaction, there are few data available in the literature on the mechanism of enzyme-catalyzed transmethylation reactions in which the methylsulfonium compound, *S*-adenosyl-L-methionine (SAM) serves as the methyl donor (*1*). We have investigated the kinetics of nonenzymic (*2*) and enzyme-catalyzed (*3*) transmethylations involving sulfonium compounds in order to provide a basis for a detailed chemical mechanism of this simple, yet critical, biochemical transformation. The conclusion which is pertinent to the present study may be stated as follows: Nucleophilic attack at the methyl group of a methyl sulfonium compound occurs early along the reaction coordinate, is associated with a large (approx. 20 kcal/mole) energy of activation, and is facilitated by media of low dielectric constant (*2*). It would be of interest if one could study a facile intramolecular transmethylation




(1)

(Eq. (1)) under mild conditions so that the questions of proximity effects, nature of catalysis, etc. could be investigated thoroughly. Therefore, we have synthesized a series of methylsulfonium compounds (Fig. 1) designed to undergo facile intramolecular transmethylation and have investigated their behavior under a variety of reaction conditions.

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The results of these studies demonstrate a strict steric requirement for nucleophilic attack at methyl carbon in contrast to the latitude permitted in the orientation of nucleophilic attack at $sp^2$ carbon (*4*).

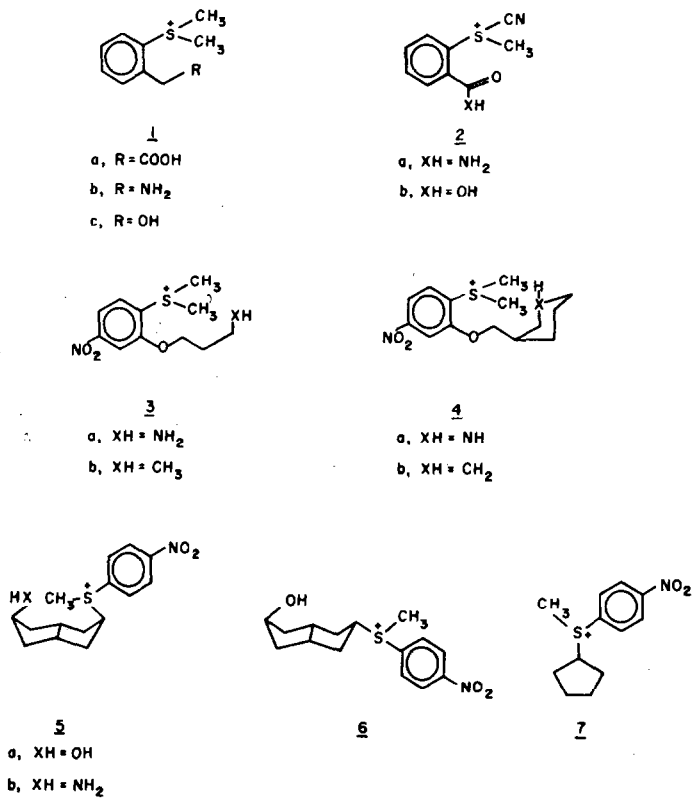
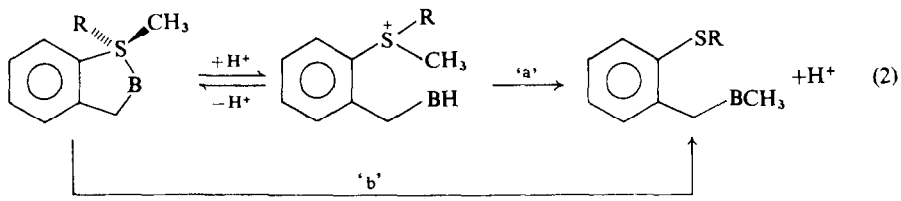



FIG. 1. Structures of compounds synthesized for this work.

## RESULTS AND DISCUSSION

As previously noted (*2*), we were unable to effect any intramolecular transmethylation with **1** under a variety of conditions. Similarly, no conditions could be found to effect nucleophilic attack by the carbonyl oxygen of **2** on the more electron-deficient adjacent methyl sulfonium moiety (*2*). Inspection of space-filling molecular models revealed that the nucleophiles in both **1** and **2** are unable to approach the methyl group so as to attain the required linear transition state for a classic $S_N2$ attack. These findings are in agreement with those independently reported by Tenud et al (*5*) in reactions which they termed "endocyclic" intramolecular alkylations. However, there are data in the literature which support the existence of so-called "pentacoordinate" intermediates in nucleophilic attack on sulfonium compounds. These sulfurane intermediates result from attack of the nucleophile on sulfur rather than on carbon. In the decomposition of triarylsulfonium compounds by organo-lithium reagents (*6*, *7*), breakdown of the

sulfurane intermediate is assisted by coordination with the lithium cation. More closely related to the present work is the proposal of similar sulfurane intermediates in the reaction of sulfonium compounds with much weaker nucleophiles such as chloride (*8*) or hypochlorite (*9*) ion. The proposed sulfurane intermediates were suggested by data accumulated from nuclear magnetic resonance spectra. These spectral data showed, on mixing of the reagents at low temperature, the buildup of a new signal which disappeared on raising the temperature to form product. This type of data does not, however, rule out the possibility that the observed intermediate is a "dead-end complex" which is in equilibrium with starting material, but does not go on directly to form product. This is depicted in Eq. (2). Inspection of molecular models reveals that the nucleophilic atom,B, can readily bond to sulfur to form the proposed sulfurane



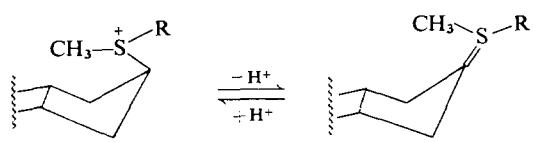

(2)

intermediate. The fact that both **1** and **2** do not undergo intramolecular transmethylation, suggests that orbital overlap between B and the methyl group of the proposed sulfurane intermediate is insufficient to permit formation of the B–$CH_3$ bond via path 'b.' This is analogous to steric arguments advanced in phosphorane chemistry (*10*), and invoked to explain the product distribution in reactions thought to involve sulfurane intermediates (*7*). However, it should be noted that all attempts to isolate the sulfurane postulated from nucleophilic attack on triaryl sulfonium compounds failed (*7*). Since there are no compelling data to support the existence of sulfurane intermediates in the nucleophilic attack at sulfonium compounds bearing aryl and/or alkyl substituents such as those studied in this work, the discussion which follows will be concerned primarily with steric constraints in nucleophilic attack at carbon, i.e., a classic $S_N2$ displacement. Similar steric restrictions may be expected to be operative if the reactions involve sulfurane intermediates, i.e., nucleophilic attack on sulfur. This latter pathway, of course, cannot be ruled out in the enzyme-catalyzed methylations involving SAM. However, kinetic studies on nonenzymic reactions of sulfonium compounds are consistent with a mechanism involving nucleophilic attack on carbon (*2*).

Following the realization that the stability of **1** and **2** was steric in origin, we synthesized **3** and **4**, in which the nucleophile was attached to a longer "arm," thus allowing for correct alignment of the nucleophile and electron-deficient methyl group in a linear transition state. Although we could not observe the desired intramolecular transmethylation with a flexible arm, as in **3a**, the constraint introduced by including the nitrogen nucleophile in a piperidine ring, as in **4a**, resulted in a facile intramolecular reaction in dioxane ($t_{\frac{1}{2}} = 566$ sec) at 25°C. A more detailed kinetic analysis of this reaction was not possible due to competing intermolecular processes which led to nonlinear first-order or second-order plots. The reaction is assumed to proceed as depicted in Eq. (1), in which the piperidino moiety acts as a nucleophile rather than a general base. The latter pathway is rare in $S_N2$ reactions (*11*). The corresponding

intermolecular reaction of **4b** and piperidine in dioxane gave a second-order rate constant, $k_2 = 4.19 \times 10^{-1}\ M^{-1}\ \text{sec}^{-1}$. From these data, one can calculate that at a concentration of $10^{-4}\ M$, the intramolecular reaction of **4a** proceeds approx. 40 times faster than the intermolecular reaction of **4b** and equimolar piperidine ($10^{-4}\ M$). The effective molarity ($k_{\text{intra}}/k_{\text{inter}}$) is $2.9 \times 10^{-3} M$. All attempts to observe the reaction of **4** in aqueous media failed, presumably a consequence of the large solvent effects previously noted in nucleophilic reactions with this type of charged onium compound (*2*). The fact that **4a** undergoes a facile intramolecular demethylation reaction, as predicted from studies of molecular models, lends support to the idea that a strictly linear approach of the nucleophile to the methyl carbon is required in this type of reaction. This fact also argues against the involvement of a sulfurane intermediate in the reaction of **4a**, since there appears to be no mode of decomposition of the pentacoordinate intermediate in which molecular models show a substantial improvement in the orbital overlap between the nucleophilic base and the methyl group in the sulfurane derived from **4a** vs those derived from **1** and **2**. In addition, recent data suggest that sulfuranes formed from aryl or alkyl sulfonium ions would not be stable due to the low apicophilicity of the aryl and/or alkyl substituents (*12*).

Presumably the major reason for the slow rate of reaction of **4a** is the flexibility in the side chain which contains the piperidine nucleophile. In order to obtain a molecule in which there is less conformational flexibility between the nucleophile and the methylsulfonium moiety, we chose to synthesize **5**. Inspection of molecular models indicated that although a strictly 180° approach angle between the nucleophile and the methyl group was not possible, the deviation from 180° was slight (approx. 20°). When preliminary kinetic studies with **5** and **6** revealed them to be stable to reaction conditions identical to those used in the studies with **4a**, and in a variety of other aprotic and protic solvents, it became apparent that even this deviation of less than 20° from collinearity was not tolerated in the transition state. A possible criticism of the present work is that in the basic media sometimes employed, we might be forming appreciable amounts of




**8**

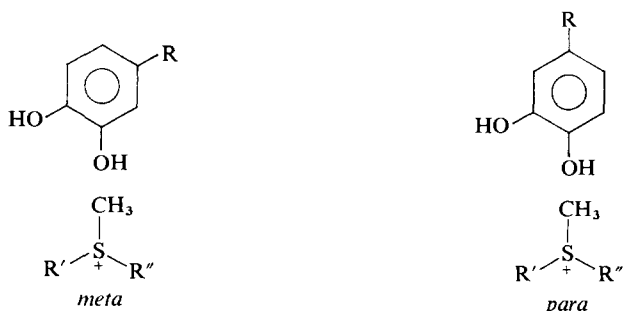
unreactive ylide, **8**. In an attempt to answer this question, we used **7** as a model compound for the less readily available **5** and **6**. Experiments using nmr spectroscopy failed to provide any evidence for ylide formation, as measured by exchange of the methine proton in deuterated methanol containing $10^{-2}\ M$ NaOD. The reaction conditions for the nmr experiments were similar to conditions employed in the kinetic studies with **5** and **6** in aqueous methanol.

Thus, it would appear that intramolecular nucleophilic attack at $sp^3$ methyl carbon requires a 180° linear transition state, and even slight deviations from this collinear array are not permitted. In contrast, Martin and Basalay (*13*) reported a deviation of 17° was permitted in the reaction shown in Eq. (3), involving intra-

molecular nucleophilic attack on $sp^3$ methylene carbon rather than methyl carbon. We have also shown recently that intramolecular alkylation of methylene carbon by a hydroxyl moiety can be observed at 25°C in aqueous media over a wide range of pH (*11*).

(3)

Consideration of these results leads to some interesting conclusions regarding enzyme-catalyzed methylation reactions. Most methylases which have been purified catalyze the methylation of one particular nucleophilic substrate. However, in the case of substrates which contain more than one nucleophilic center, it remains to be shown how the enzyme distinguishes one nucleophilic center from another. In the case of catechol-*O*-methyltransferase (COMT, EC 2.1.1.6), several lines of evidence suggest that the catechol substrate binds in two different modes, as depicted in **9**, depending



*meta* *para*

**9**

on whether *meta*- or *para*-methylation occurs. This concept for COMT is supported by product analysis studies with numerous substrates (*14*), steady-state kinetic studies (*3*), and differential labeling of the enzyme (*15*). Thus, the present work would suggest that a similar mechanism should apply to all methylases. For example, the base methylations of t-RNA must be effected by either separate enzymes, or by substrates binding in particular modes as in the COMT reaction discussed above. Purified base-specific t-RNA methylases have been described (*16*), but it remains to be seen whether any purified t-RNA methylase will catalyze the methylation of different nucleophilic centers on the same base, as observed in the COMT reaction.

## EXPERIMENTAL SECTION

The synthesis of sulfonium compounds from appropriate thioether precursors was effected by one of several methods, depending on the properties of the desired product. Compounds **1** and **2** were prepared as previously described (*2*), as were com-

pounds **3b** and **4a** (*17*). Compounds **4b** and **7** were prepared by a general method previously described (*2*), except toluene was used as the solvent. At the end of the reaction, toluene was removed by decantation, and the insoluble sulfonium salt dissolved in $CH_2Cl_2$. After filtration, the solution was concentrated *in vacuo* and the residue recrystallized; this procedure is listed as method "A" in Table 1. Compounds **5a** and **6** were prepared as follows (Method "B," Table 1). To a solution of the hydroxy sulfide

TABLE 1

PHYSICAL PROPERTIES OF SULFONIUM SALTS AND THIOETHER PRECURSORS

| Compound | $Y^-$ | Method of Preparation | mp | Analysis | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Calculated | | | Found | | |
| | | | | (%C) | (%H) | (%N) | (%C) | (%H) | (%N) |
| *4b* | $ClO_4^-$ | A[a] | 155°C | 45.54 | 5.56 | 3.54 | 45.39 | 5.74 | 3.42 |
| *5a* | $BF_4^-$ | B | oil | | | n.d.[c] | | | |
| *5b*·$HClO_4$ | $ClO_4^-$ | C | hygr. | 36.52 | 4.50 | 5.68 | 36.69 | 4.77 | 5.46 |
| *6* | $BF_4^-$ | B | oil | | | n.d.[c] | | | |
| *7* | $ClO_4^-$ | A[b] | 101–103°C | 42.67 | 4.77 | 4.15 | 42.82 | 4.87 | 4.26 |

[a] Thioether precursor prepared by coupling cyclohexylmethanol and 2-methylthio-5-nitrophenol in the presence of dicyclohexylcarbodiimide (*17*); mp 85–87; *Anal.* Calcd for $C_{14}H_{19}NO_3S$: C, 59.75; H, 6.81; N, 4.98; Found: C, 59.52; H, 6.68; N, 5.06.

[b] Thioether precursor prepared by alkylation of cyclopentyl bromide with *p*-nitrobenzene thiol; oil; *Anal.* Calcd for $C_{11}H_{13}NO_2S$: C, 59.14; H, 5.88; N, 6.28; Found: C, 59.12, H, 5.73; N, 6.51.

[c] Unstable.

(1 equiv) in toluene–methylene chloride was added a large excess of methyl iodide followed by $AgBF_4$ (1 equiv) in toluene. The mixture was stirred for 1 to 2 hr, and the methylene chloride evaporated *in vacuo*. The remaining toluene was removed by decantation and the residue washed several times with toluene. Finally, the flask contents were dried *in vacuo*, triturated with water, and filtered. Water from the filtrate was removed by lyophilization to give a residue with the following characteristics: uv $\lambda_{max}$ 249 nm; nmr ($D_2O$, acetone as ref.) $\delta$ 8.4, 8.07 (4 H, two sets of doublets, $J =$ 9 Hz, ArH), 3.26 (3 H, s, SMe). Ammonio-sulfonium compound **5b**·$HClO_4$ was prepared as previously described for the preparation of **4a**·$HClO_4$ (*17*); this procedure is listed as "C" in Table 1. Thioether precursors were prepared as previously described (*17*, *18*) or as indicated in the footnotes to Table 1. The physical properties of sulfonium salts **4–7** are listed in Table 1. All sulfonium compounds were homogenous by tlc on silica gel (BuOH:HOAc:$H_2O$, 12:3:5), and had the expected spectral characteristics.

All uv spectra were obtained using a Cary Model 15 spectrophotometer equipped with a repetitive scan accessory. All nmr spectra were obtained using a Varian T-60A spectrophotomer, and chemical shift data are given in parts per million (ppm) downfield from tetramethylsilane. Reactions of the sulfonium salts **1–7** with nucleophiles (intermolecular or intramolecular) were monitored spectrophotometrically by scanning

the 400–220 nm region repetitively during the course of the reaction. Ammonio–sulfonium compounds, **4a**·$HClO_4$ and **5b**·$HClO_4$, were converted *in situ* to the corresponding free amines, **4a** and **5b**, by addition of 1 equiv of 1 *M* NaOH to the cuvette. In all reactions studied, regardless of solvent, sufficient base was added to insure neutralization of the proton released (Eq. (1)). In the case of **4a**, more detailed kinetic studies were carried out by monitoring the appearance of the *p*-nitrophenylthioether at 360 nm using a Gilford Model 2400 spectrophotometer ($T = 25°C$). All organic solvents used in kinetic studies were freshly distilled or chromatographed, and doubly distilled water was used in all aqueous solutions. The ionic strength of aqueous buffers was maintained at 1.0 *M* with KCl, and buffers were prepared from reagent grade chemicals, which were freshly distilled or recrystallized where possible.

*Note added in proof.* The Structure of **1a** (Fig. 1) is in error. Compound **1a** is a substituted benzoic acid, not a substituted phenylacetic acid.

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