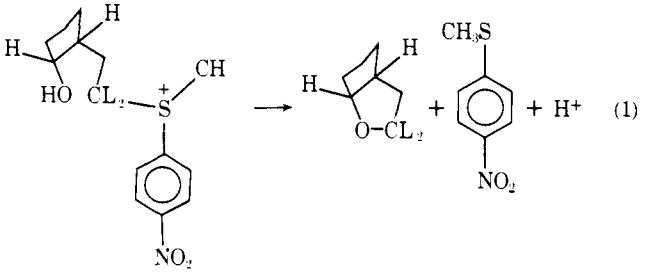
α-Deuterium Isotope Effects and Transition-State Structure in an Intramolecular Model System for Methyl-Transfer Enzymes[1]

Ivan Mihel,[2] Jay O. Knipe, James K. Coward, and Richard L. Schowen*

Contribution from the Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510, and the Department of Chemistry, University of Kansas, Lawrence, Kansas 66045. *Received February 21, 1978*

Abstract: The α-deuterium isotope effects for the general base catalyzed ring-closure reaction of 2-(2-*cis*-hydroxycyclopentyl)ethylmethyl-4-nitrophenylsulfonium tetrafluoroborate (**1**) are $k_{2H}/K_{2D} = 1.17 \pm 0.02$ (water catalysis), 1.104 ± 0.014 (carbonate buffer catalysis), and 0.998 ± 0.001 (hydroxide ion catalysis) in aqueous solution ($\mu = 1.0$) at 40.00 ± 0.05 °C. These are all considerably more normal (i.e., larger in the direction $k_H > k_D$) than the effect for the catechol *O*-methyltransferase reaction ($k_{3H}/k_{3D} = 0.83 \pm 0.05$) for which the reactions of **1** are a model. This indicates the model-reaction transition states to be looser (i.e., to possess a longer oxygen-to-sulfur distance) than the enzymic transition state and suggests that the enzyme may achieve all or part of its catalysis of methyl transfer from processes that are associated with compression of the transition state.

The system of eq 1 exhibits features that make it a useful model for the action of enzymes which catalyze the transfer of methyl groups, such as catechol *O*-methyltransferase (COMT) and tRNA methyltransferases.[3,4] The reaction is very much faster than its intermolecular counterpart, the effective molarity for the model reaction exceeding 10^6 M. In addition, general-base catalysis is observed.



(1)

1

An accompanying paper[5] reports α-deuterium and carbon-13 isotope effects for the action of COMT, which suggest its rate-determining transition state to be that for an S_N2 transfer of the methyl group with a tight, symmetrical structure (i.e., with the carbon about equally bound to entering and leaving groups, thus symmetrical, and with both of these bonds somewhat shorter than for the average S_N2 transition state, and thus tight). Because the enzyme may derive all or part of its catalytic power (which can be estimated[6] to correspond to an acceleration factor of around 10^{16}) from processes concomitant with alteration of the structure of the transition state, a comparison of the transition-state structures for the enzymic and model reactions should be instructive. We have therefore determined the α-deuterium kinetic isotope effects (L = H vs. L = D in eq 1) for the model reaction.

Results

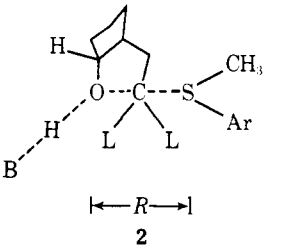
The rates of release of methyl *p*-nitrophenyl sulfide upon ring closure of both isotopic forms (L = H and L = D) of **1** as its tetrafluoroborate salt (eq 1) were measured spectrophotometrically at 380 nm in bicarbonate–carbonate buffers and in sodium hydroxide solutions, the ionic strength being maintained constant at unity by addition of potassium chloride and the temperature held at 40.00 ± 0.05 °C. As Coward, Lok, and Takagi[3] and Knipe and Coward[4] have found, the observed rate constants k_o (Table I) are described by eq 2, which has a "water term", k_w, and terms for catalysis by hydroxide ion, k_{HO}, and carbonate ion, k_B:

$$k_o = k_w + k_B [CO_3^{2-}] + k_{HO} [HO^-] \quad (2)$$

The values of the catalytic constants, shown in Table II, were obtained from linear least-squares fits of the mean values of k_o from Table I to carbonate concentrations (the slope is then k_B) or to hydroxide concentrations (slope k_{HO}, intercept k_w). The kinetic isotope effects for the individual catalytic terms are shown in Table III. The k_B term for carbonate buffers displays some kinetic complexity, currently under study, possibly including a small contribution to k_B from hydroxide ion or a specific effect of potassium chloride. This contributes no more than 15% to the value of k_B for 1:1 buffers, so that the isotope effect for k_B is 85% or more that for simple carbonate catalysis. It does not, however, correspond entirely to this process. As is noted in Table III, the term for hydroxide ion catalysis refers to reaction through a preformed alkoxide ion.[3]

Discussion

The isotope effects obtained in this work are *α-deuterium secondary isotope effects*, which arise in major part from changes in bending force constants at the isotopic centers upon formation of the transition state.[7-10] The effects will be inverse ($k_D > k_H$) if the force constants are greater in the transition state than in the reactant and normal ($k_H > k_D$) if the reverse is true. They should thus reflect the "tight–loose" character of the transition state,[11] relating to the length *R* in **2**. If *R* is



2

sufficiently long, the CL bending force constant will decrease on formation of the transition state, producing a normal α-D effect. If *R* is sufficiently short, the CL bending force constant will increase from reactant to transition state and an inverse α-D will be observed. In fact, the collection of k_{3H}/k_{3D} for S_N2 methyl-transfer reactions, made by Seltzer and Zavitsas,[12,13] contained effects from about 1.25 to about 0.85, suggesting that S_N2 transition states are capable of possessing a range of structures, varying from loose to tight. Inclusion of electrostatic considerations,[5] which may be appropriate with sulfonium

0002-7863/79/1501-4349$01.00/0

Table I. First-Order Rate Constants for Ring Closure of **1** in Aqueous Bicarbonate–Carbonate Buffers and Solutions of Sodium Hydroxide (40.00 ± 0.05 °C, μ = 1.0)

conditions	10^6k_o, s^{-1} protiated substrate (**1**, L = H)	10^6k_o, s^{-1} deuterated substrate (**1**, L = D)
$[Na_2CO_3] = [NaHCO_3] = 0.0150$ M	47.2, 46.2, 46.8, 46.5, 47.0 (mean 46.7, SD 0.4)	43.8, 43.7, 42.5, 42.5, 43.7 (mean 43.2, SD 0.6)
$[Na_2CO_3] = [NaHCO_3] = 0.0750$ M	53.3, 53.0, 53.2, 52.6, 53.2 (mean 53.1, SD 0.3)	49.5, 48.4, 48.6, 49.3, 49.3 (mean 49.0, SD 0.5)
$[Na_2CO_3] = [NaHCO_3] = 0.1500$ M	61.2, 60.6, 60.3, 60.4, 59.8 (mean 60.5, SD 0.5)	56.3, 56.4, 55.4, 55.5, 55.2 (mean 55.7, SD 0.5)
[NaOH] = 0.0100 M	766, 762, 771, 770, 752 (mean 764, SD 8)	761, 759, 773, 759, 761, 762 (mean 762, SD 5)
[NaOH] = 0.0500 M	3703, 3744, 3710, 3669 (mean 3707, SD 30)	3746, 3729, 3671, 3689 (mean 3709, SD 35)
[NaOH] = 0.1000 M	7302, 7450, 7215, 7201, 7457, 7383 (mean 7383, SD 59)	7328, 7349, 7211, 7618, 7314 7638, 7319, 7348, 7414, 7411 (mean 7393, SD 143)

Table II. Catalytic Rate Constants for Ring Closure of **1** in Aqueous Solution (μ = 1.0) at 40.00 ± 0.05 °C

rate constant	protiated substrate (**1**, L = H)	deuterated substrate (**1**, L = D)
10^5k_w, s^{-1}	2.89 ± 0.04	2.47 ± 0.03
10^5k_B, M^{-1} s^{-1}	10.18 ± 0.14	9.24 ± 0.14
10^3k_{HO}, M^{-1} s^{-1}	73.54 ± 0.03	73.68 ± 0.03

Table III. Kinetic Isotope Effects for the Ring Closure of **1** in Aqueous Solution (μ = 1.0) at 40.00 ± 0.05 °C

kinetic term	isotope effect, k_{2H}/k_{2D}	estimated[a] k_{3H}/k_{3D}
"water catalysis," k_w	1.17 ± 0.02	1.27 ± 0.03
general base catalysis, k_B, by carbonate ion	1.104 ± 0.014	1.16 ± 0.02
specific-base catalysis, k_{HO}, by hydroxide ion[b]	0.998 ± 0.001	0.997 ± 0.002

[a] Obtained from $(k_{2H}/k_{2D})^{3/2}$ for comparison with methyl-transfer isotope effects. [b] Previous studies[3] have indicated the intermediacy of the conjugate base of **1** for this term.

substrates, can extend the upper limit of this range to 1.45. Our measurement[5] of $k_{3H}/k_{3D} = 0.83 \pm 0.05$ for COMT-catalyzed transmethylation showed the enzymic transition state to have a structure at the extremely tight end of the S_N2 spectrum so far observed. An accompanying paper[14] presents a much more detailed analysis of α-deuterium isotope effects and transition-state structures for S_N2 reactions, which lends further support to the idea that the enzymic transition state is unusually tight when compared to simple chemical expectations for sulfur-to-oxygen methyl transfer.

The values of k_{2H}/k_{2D} measured in this work can be converted to approximate values of k_{3H}/k_{3D}, for comparison to the methyl-transfer effects, on the assumption that each isotopic center contributes an equal and additive increment to the isotopic difference in free energies of activation. This calculation yields the values shown at the right of Table III. Since the isotopic reactant states are identical for all three of these cases, the isotope-effect variations give a true estimate of force-constant variations among the three transition states. Clearly, the transition state for water catalysis is at the loose-structure end of the scale for these reactions, with the transition state for carbonate catalysis being rather tighter and that for closure of the alkoxide ion the tightest of the three. The effect for the enzymic reaction (0.83 ± 0.05) shows its transition state to be tighter than any of the model-reaction transition states.

An important feature of COMT catalysis thus appears to be the compression of the activated complex in its enzyme-bound form. This compression is almost certainly[15,16] effected by the utilization of the "intrinsic"[15] binding energy of the substrate-derived core of the activated complex to the enzyme in the transition state to cancel the unfavorable contributions to the distortion energy involved in shortening *R*. All or part of the catalytic power of COMT may be correlated with the processes giving rise to this transition-state compression.

Studies intended to make more exact and complete the transition-state structural considerations addressed here are in progress. They involve the use of model vibrational analysis calculations to relate transition-state structure to kinetic isotope effects, the use of quantum-chemical calculations to examine transition-state structures and their distortions, and the use of alternate substrates and inhibitors to elucidate transition-state binding to COMT.

Experimental Section

Materials. Compound **1** (L = H and L = D) as the tetrafluoroborate was prepared at Kansas by methylation of the isotopic sulfides sent from Yale. The latter were synthesized as described before,[3,4] the label being introduced by employment of lithium aluminum deuteride (Merck, 98+% D) to reduce the ethylene ketal of ethyl-(2-oxocyclopentyl) acetate. The deuterated product of this reduction, a clear oil, was collected by distillation (bp 95–98 °C, 0.6–0.7 mm) and had the following NMR characteristics: δ 3.88 (4 H, singlet, ketal protons), 2.62 (1 H, singlet, OH), 2.1–1.4 (9 H, multiplet, ring protons and methylene of side chain). The triplet (2 H) at δ 3.60, observed for the protio compound and assigned[4] to the protons adjacent to OH, was absent. GC/MS analysis showed the compound to be pure and, according to the procedure of Wendt and McCloskey,[17] doubly deuterated to the extent of 97.12%.

The ketal alcohol was converted to the tosylate [NMR $CDCl_3$) δ 7.78, 7.30 (4 H, 2 sets of doublets, aromatic protons), 3.74 (4 H, singlet, ketal protons), 2.40 (3 H, singlet, *p*-CH_3), 2.0–1.2 (9 H, multiplet, ring protons and methylene of side chain)] as described by Lok and Coward[18] and thence by reaction with 4-nitrothiophenol in sodium ethoxide[4] to the ketal sulfide [a yellow oil after preparative TLC; NMR ($CDCl_3$) δ 8.06, 7.25 (4 H, 2 sets of doublets, aromatic protons), 3.84 (4 H, singlet, ketal protons), 2.2–1.4 (9 H, multiplet, ring protons and methylene of side chain)]. The ketal sulfide was converted[4] to the keto sulfide [a yellow oil which solidified on standing; NMR ($CDCl_3$) δ 8.04, 7.23 (4 H, 2 sets of doublets, aromatic protons), 2.3–1.3 (9 H, multiplet, ring protons and methylene of side chain)] and reduced with aluminum isopropoxide[4] to yield, after preparative TLC, a 4:1 endo:exo isomer ratio. The endo isomer had the following NMR ($CDCl_3$) characteristics: δ 8.08, 7.27 (4 H, 2 sets of doublets, aromatic protons), 4.23 (1 H, broad singlet, OH), 2.0–1.3 (10 H, multiplet, ring protons and methylene of side chain). Anal. Calcd for $C_{13}H_{15}D_2NO_3S$: C, 57.97; H, 7.11; N, 5.20. Found: C, 58.51; H, 6.64; N, 4.97.

Methylation of the sulfide was carried out as described before[3,4,20] with methyl iodide and silver tetrafluoroborate in toluene. All materials must be strictly dried to avoid adventitious hydrolysis of **1** in the

course of its formation.[4] If this occurs, microcrystalline hydrolysis product is formed which interferes with the spectrophotometric observations.

Reagent-grade chemicals and double-distilled deionized water were employed throughout the investigation.

Kinetics. The apparatus previously described,[5] in which the photomultiplier signal of a Cary 16 spectrophotometer is digitized and stored in a Hewlett-Packard 2100A computer so as to generate 1000 kinetic points in each run, was used. The points were subjected to weighted, nonlinear least-squares fitting to the first-order kinetic law to produce the observed first-order rate constants. The reactions were carried out in 1-mL cuvettes thermostated by a Lauda circulating constant-temperature bath. Absorbance data were collected at 380 nm. In most cases, runs with protiated and deuterated substrates were conducted in alternation, using the same stock reaction solutions.

References and Notes

(1) This research was supported by the National Institutes of Health through Grants CA 10748, MH 18038 (Yale: J.O.K. and J.K.C.), and GM 20199 (Kansas: I.M. and R.L.S.).
(2) Fulbright Scholar. Permanent address: Pliva Pharmaceutical and Chemical Works, Zagreb, Yugoslavia.
(3) J. K. Coward, R. Lok, and O. Takagi, *J. Am. Chem. Soc.*, **98**, 1057 (1976).
(4) J. O. Knipe and J. K. Coward, *J. Am. Chem. Soc.*, preceding paper in this issue.
(5) M. F. Hegazi, R. T. Borchardt, and R. L. Schowen, *J. Am. Chem. Soc.*, **101**, 4359 (1979).
(6) One of several ways in which this estimate can be made is the following. From the rate constant of 1.7×10^{-6} M^{-1} s^{-1} (80 °C) and ΔH^* of 28.5 kcal/mol for reaction of $C_6H_5O^-$ with $(CH_3)_3S^+$ (C. G. Swain and L. J. Taylor, *J. Am. Chem. Soc.*, **84**, 2456 (1962)), a rate constant for attack of $C_6H_5O^-$ at one methyl center (statistical factor of 3) at 37 °C is estimated as 1.7×10^{-9} M^{-1} s^{-1}. The Brønsted slope for nucleophilic attack, β_{nuc}, is around 0.3 (J. K. Coward and W. D. Sweet, *J. Org. Chem.*, **36**, 2337 (1971)). Taking the pK_a of the conjugate acid of $C_6H_5O^-$ as 9.95 (G. D. Fasman, Ed., "Handbook of Biochemistry and Molecular Biology", 3rd ed., Vol. 1, CRC Press, Cleveland, Ohio, 1976, p 314) and that of C_6H_5OH as −6.7 (E. M. Arnett, *Prog. Phys. Org. Chem.*, **1**, 223 (1963)), the rate constant for reaction of phenol with one methyl center of trimethylsulfonium ion in aqueous solution at 37 °C is estimated as 1.7×10^{-14} M^{-1} s^{-1}. This should approximate the rate constant for uncatalyzed methylation of a catechol by AdoMet. A recent value for the rate constant of the reaction of a complex of COMT, Mg^{2+}, and norepinephrine with AdoMet in aqueous solution at 37 °C (R. T. Borchardt and C. F. Cheng, *Biochim. Biophys. Acta*, **522**, 49 (1978)) is 5×10^2 M^{-1} s^{-1}. The approximate enzymic acceleration factor is thus $(5 \times 10^2)/(1.7 \times 10^{-14}) = 10^{16.5}$.
(7) V. J. Shiner, Jr., in "Isotope Effects in Chemical Reactions", C. J. Collins and N. S. Bowman, Eds., Van Nostrand Reinhold Co., New York, 1971.
(8) J. F. Kirsch, *Isot. Eff. Enzyme-Catalyzed React., Proc. Annu. Harry Steenbock Symp., 6th, 1976* (1977).
(9) J. L. Hogg in "Transition States of Biochemical Processes", R. D. Gandour and R. L. Schowen, Eds., Plenum Press, New York, 1978.
(10) W. E. Buddenbaum and V. J. Shiner, Jr., *Isot. Eff. Enzyme-Catalyzed React., Proc. Annu. Harry Steenbock Symp., 6th, 1976* (1977).
(11) S. R. Hartshorn, "Aliphatic Nucleophilic Substitution", Cambridge University Press, Cambridge, 1973, pp 76–77.
(12) S. Seltzer and A. A. Zavitsas, *Can. J. Chem.*, **45**, 2023 (1967).
(13) The effects for comparison have been determined at various temperatures, but the temperature dependence of small isotope effects is usually quite weak. If the entire effect arose from zero-point energy sources, a value of 1.20 at 0 °C should change only to 1.14 at 100 °C, while an effect of 1.05 at 0 °C would be 1.04 at 100 °C.
(14) C. H. Gray, J. K. Coward, K. B. Schowen, and R. L. Schowen, *J. Am. Chem. Soc.*, following paper in this issue.
(15) W. P. Jencks, *Adv. Enzymol.*, **43**, 219 (1975).
(16) R. L. Schowen, in "Transition States of Biochemical Processes", R. D. Gandour and R. L. Schowen, Eds., Plenum Press, New York, 1978.
(17) G. Wendt and J. A. McCloskey, *Biochemistry*, **9**, 4854 (1970).
(18) R. Lok and J. K. Coward, *J. Org. Chem.*, **39**, 2377 (1974).

α-Deuterium and Carbon-13 Isotope Effects for a Simple, Intermolecular Sulfur-to-Oxygen Methyl-Transfer Reaction. Transition-State Structures and Isotope Effects in Transmethylation and Transalkylation[1]

Carmen H. Gray, James K. Coward, K. Barbara Schowen, and Richard L. Schowen*

Contribution from the Department of Chemistry, University of Kansas, Lawrence, Kansas 66045, and the Department of Pharmacology, Yale University School of Medicine, New Haven, Connecticut 06510.

Received February 21, 1978

Abstract: The transmethylation reaction of isotopically labeled *S*-methyldibenzothiophenium ($^{12}CH_3$-$SC_{12}H_8^+$, $^{13}CH_3$-$SC_{12}H_8^+$, and $^{12}CD_3$-$SC_{12}H_8^+$) tetrafluoroborates with methoxide ion in methanol at 25 °C shows $k_{3H}/k_{3D} = 0.97 \pm 0.02$ and $k_{12}/k_{13} = 1.08 \pm 0.02$. The large carbon isotope effect is similar to that for enzymic transmethylation and is consistent with a central, roughly planar methyl group in the transition state. The α-D effect is compared with others for enzymic and nonenzymic transmethylation and transalkylation reactions by estimating the transition-state fractionation factor ϕ_T relative to ethane, using the calculated factors of Hartshorn and Shiner. For 22 transmethylation reactions, ϕ_T is closely described in terms of reactant (ϕ_R) and product (ϕ_P) factors by $\phi_T = 0.99(\phi_R\phi_P)^{0.70}$. Transmethylation transition states appear structurally implastic with a roughly constant, probably high valency to methyl. Transalkylation transition states appear to be looser, relative to reactants and products, than transmethylation transition states and also far more structurally plastic. The enzymic transition state has an unusually large fractionation factor, consistent with transition-state compression as a mechanism of enzymic catalysis.

In the accompanying papers, we have reported the α-deuterium (α-D) isotope effects in transmethylation from the sulfonium methyl donor *S*-adenosylmethionine to the oxygen of the catechol acceptor 3,4-dihydroxyacetophenone, catalyzed by the rat-liver enzyme catechol *O*-methyltransferase[2] (COMT), and in sulfur-to-oxygen transalkylation in the intramolecular model reaction[3] devised by Coward, Lok, and Takagi.[4] These studies were intended to illuminate the origins of the catalytic power of COMT and similar transmethylases by producing information about the structure of the transmethylation transition state in both enzymic and nonenzymic reactions. Together with other information, these structural data could suggest how the enzyme liberates the free energy released upon its combination with the transmethylation transition state and thus how it catalyzes the reaction. The enzymic α-D isotope effect ($k_H/k_D = 0.83 \pm 0.05$) was the most inverse (largest in the direction $k_D > k_H$) of all the effects determined, indicating that the methyl hydrogens experience a greater increase in force constant upon formation of the enzymic transition state than in the model reactions. This is

0002-7863/79/1501-4351$01.00/0