served at ~390 nm for the trans CuS_2N_2 chromophore of $Cu(H_2NCH_2CH_2SCH_3)_2 \cdot 2ClO_4$.[20]

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(8) Program MULTAN, P. Main, M. M. Woolfson, and G. Germain, Department of Physics, University of York, England.
(9) The discrepancy between d_{obsd} and d_{calcd} has not been resolved by our attempts to minimize sample dehydration;[7] the presence of additional H_2O, DMF, or other lattice species is not indicated by elemental analyses.[6]
(10) $R_F = \sum||F_o| - |F_c||/\sum|F_o|$; $R_{wF} = [\sum w(|F_o| - |F_c|)^2/\sum w|F_o|^2]^{1/2}$.
(11) This formulation agrees with the results reported[4] for complex II and is supported by the special chemical role of Cl^- in the formation of II,[2,12] An alternate formulation with S^{2-} as the central ion and Cl^- as a disordered lattice species cannot be ruled out entirely.
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SN2-Like Transition State for Methyl Transfer Catalyzed by Catechol-*O*-methyltransferase[1]

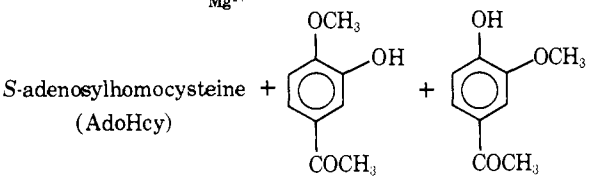
Sir:

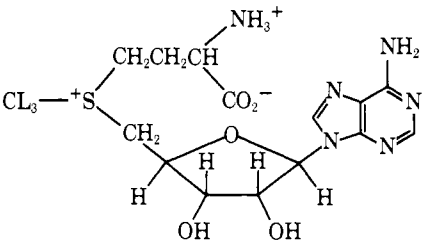
The velocity of methyl transfer (eq 1) from *S*-adenosylmethionine (AdoMet or AdoHcy-CL_3, **1**, L = H or D) to 3,4-dihydroxyacetophenone (DHA, **2**), catalyzed by rat-liver catechol-*O*-methyltransferase (COMT),[2] is increased substantially by trideuteration at the transferred methyl group ($V_H/V_D = 0.832 \pm 0.045$ at 37.00 ± 0.05°). This inverse α-deuterium secondary isotope effect is exactly what is observed for many classical SN2 reactions in organic-reaction systems,[3] and constitutes a strong indication that COMT catalysis involves rate-limiting SN2 methyl transfer with a trigonal-bipyramidal transition state of the type shown in structure **3**. This information should prove critical for efforts now in progress[4] to design transition-state-analogue inhibitors, in part for use as drugs, of this important enzyme and closely related enzymes of the liver and central nervous system.[5]

Table I.[a] Velocities of Methyl Transfer to DHA from AdoHcy-CH_3 and AdoHcy-CD_3, Catalyzed by COMT

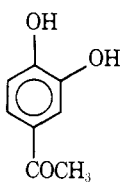
10^5[AdoMet], M	10^9V (M min^{-1})[b] AdoHcy-CH_3	AdoHcy-CD_3
3.86	1335 ± 7, 977 ± 11	—
7.73	1922 ± 7, 1854 ± 7, 1823 ± 6	2049 ± 6, 2021 ± 6, 1957 ± 8
11.59	2011 ± 5, 2024 ± 5	2386 ± 5, 2290 ± 5
15.45	2282 ± 5, 2307 ± 5, 2280 ± 4	2659 ± 4, 2597 ± 5, 2597 ± 5
38.63	2436 ± 6, 2545 ± 6	2813 ± 7, 2838 ± 9
103.0	2728 ± 11, 2361 ± 8	3024 ± 12, 3155 ± 11

[a] Rates measured at 360 nm, 37.00 ± 0.05°, phosphate buffer (0.125 M), pH 7.6, $[Mg^{2+}] = 1.5 \times 10^{-3}$ M, [DHA] = 2.5×10^{-4} M, [dithiothreitol] = 4.5×10^{-3} M, protein = 0.839 mg/ml. Velocities in M min^{-1} were calculated from d(absorbance)/dt using $\Delta_{360}^{eff} = 2877$. [b] Error limits are standard deviations within a single run.

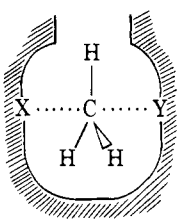
AdoMet + DHA $\xrightarrow[Mg^{2+}]{COMT}$ *S*-adenosylhomocysteine (AdoHcy) +  (1)



1, AdoMet or AdoHcy-CL_3



2, DHA



3

Table I shows velocities of methyl transfer by COMT at various concentrations of AdoHcy-CH_3 and AdoHcy-CD_3. The data for the CH_3 cofactor generate the Michaelis–Menten expression of eq 2, while that of eq 3 is produced by the rates for the CD_3 cofactor.

$$10^9V_H\ (\text{M min}^{-1}) = (2760 \pm 90)[\text{AdoMet}]/\{[\text{AdoMet}] + (4.1 \pm 0.6) \times 10^{-5}\} \quad (2)$$

$$10^9V_D\ (\text{M min}^{-1}) = (3220 \pm 60)[\text{AdoMet}]/\{[\text{AdoMet}] + (4.3 \pm 0.4) \times 10^{-5}\} \quad (3)$$

Absorbance changes at [AdoMet] $\ll K_m$ were too small to permit an accurate determination of K_m. Although the mean values of K_m^H and K_m^D are essentially equal, the large experimental error precludes definite exclusion of a

Table II.[a] Velocities and Isotope Effects for COMT-Catalyzed Methylation of DHA by AdoHcy-CH_3 and AdoHcy-CD_3

Cofactor[b]	10^9V(M min^{-1})[c]	V_H/V_D[d]
AdoHcy-CD_3-I	3045 ± 4	0.823
AdoHcy-CH_3-I	2507 ± 3	0.803
AdoHcy-CD_3-I	3123 ± 4	0.844
AdoHcy-CH_3-I	2636 ± 3	0.861
AdoHcy-CD_3-I	3063 ± 2	0.865
AdoHcy-CH_3-I	2651 ± 4	0.870
AdoHcy-CD_3-I	3047 ± 3	0.789
AdoHcy-CH_3-I	2403 ± 3	0.798
AdoHcy-CD_3-I	3011 ± 3	0.875
AdoHcy-CH_3-I	2634 ± 7	
AdoHcy-CD_3-II	2817 ± 4	0.772
AdoHcy-CH_3-II	2172 ± 5	0.795
AdoHcy-CD_3-I	2732 ± 6	
AdoHcy-CD_3-II	2780 ± 4	0.789
AdoHcy-CH_3-II	2192 ± 5	0.778
AdoHcy-CD_3-I	2819 ± 5	
AdoHcy-CD_3-II	2865 ± 5	0.920
AdoHcy-CH_3-I	2636 ± 5	0.919
AdoHcy-CD_3-II	2869 ± 4	0.831
AdoHcy-CH_3-I	2384 ± 4	0.836
AdoHcy-CD_3-II	2851 ± 5	0.810
AdoHcy-CH_3-I	2309 ± 6	
	Mean 0.832 ± 0.045	

[a] [AdoMet] = 10^{-3} M. Experimental conditions other than [AdoMet] are same as in Table I. [b] The suffixes I and II refer to completely independent biological preparations of the labeled and unlabeled cofactors. [c] Error limits are standard deviations within the single run. The three data sets were obtained on separate days. Differences in rates may reflect slight changes in enzyme activity. [d] Calculated as the ratio of adjacent measurements.

binding isotope effect. A substantial isotope effect is, however, observable for the maximum-velocity term: $V^H_{max}/V^D_{max} = 0.86 \pm 0.04$. For confirmation and further definition of this effect, carefully matched sets of velocities were obtained for CH_3 and CD_3 cofactors at [AdoMet] = 10^{-3} M $\simeq 20K_m$. These are shown in Table II, and yield a mean value $V_H/V_D = 0.832 \pm 0.045$.

For these experiments, AdoHcy-CD_3 was prepared by biological adenosylation of [methyl-$^2H_3$]-L-methionine (made from [methyl-$^2H_3$]methyl iodide and *S*-benzyl-L-homocysteine in sodium–liquid ammonia;[6] extent of deuteration (NMR): 90 ± 5% in cofactor), using a preparation of the yeast *Saccharomyces cerevisiae*.[7] Protiated AdoMet (AdoHcy-CH_3) was prepared in the same way and two completely independent preparations of AdoHcy-CH_3 gave indistinguishable velocities, while two completely independent preparations of AdoHcy-CD_3 gave identical velocities, quite distinct from those for the AdoHcy-CH_3 preparations (Table II).

Although the results strongly imply a trigonal-bipyramidal transition-state structure (as in **3**), they cannot indicate the nature of the methyl donor and acceptor structures X and Y. Our data are consistent with (1) rate-determining methyl transfer directly from AdoMet to DHA, or (2) methyl transfer from AdoMet to enzyme followed by enzyme-to-DHA transfer, with either or both steps determining the rate. Kinetic and inhibition studies are currently in conflict as to the likely involvement of a methylated-enzyme intermediate.[8] If two or more sequential steps or parallel pathways (as in meta and para methylation of DHA) contribute to rate limitation, the observed isotope effect will be a weighted average. The highest free-energy activated complex will be weighted most heavily for sequential processes and the lowest free-energy activated complex will be weighted most heavily for parallel processes. The large magnitude of the isotope effect observed here strongly suggests a "tight" SN2 character[3] for all contributing transition states.

The reasonably high precision within each set of enzymatic rates obtained here is due in part to the excellent stability of the COMT preparation and in part to the use of an automated spectrophotometric data-acquisition system. During each kinetic run, this system collects 1000 kinetic points (absorbances at 360 nm, determined by direct observation of the thermostated reaction mixture) each at least 15-fold time-averaged and in the current work 900-fold time-averaged, by direct digitization of the photomultiplier signal of the Cary 16 spectrophotometer. The data are stored in a Hewlett-Packard 2100A computer and fit to the appropriate rate law by a general least-squares procedure.

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The Synthesis of Zoanthoxanthins

Sir:

Zoanthoxanthins[1-5] are highly fluorescent metabolites of colonial anthozoans, marine animals belonging to the order of Zoanthidae. The pigments thus far identified belong to either the parazoanthoxanthin(1,3,5,7-tetrazacyclopent[*f*]azulene) or the pseudozoanthoxanthin(1,3,7,9-tetrazacyclo-