in that it is possible to determine the temperature profile of those reactions leading to chemiluminescence even if they are a very small fraction of the total reaction pathway. Thus, by determination of the instantaneous chemiluminescence intensity at several temperatures, the conglomerate activation energy of the sequence leading to chemiluminescence can be determined.[11,12] Results of application of this temperature jump method to the DPA and DBA activated chemiluminescence of **1** are shown in Table I. It is seen that the path leading to activated emission of the dyes requires *ca.* 4–6 kcal/mol more activation energy than the main pathway leading to reaction of **1**. This excess activation energy necessary to produce excited state products may account for the very low chemiluminescence yield from **1**.

An *upper* limit for the yield of acetophenone excited singlet can be estimated from the unquenched activated DPA chemiluminescence at 10^{-2} *M* diene. A conservative calculation indicates that the singlet yield is *below* 1% of the triplet yield, *i.e.*, the estimated upper limit for the singlet acetophenone yield is 3×10^{-4}%.

We have considered the possibility that, as in the case of 1,2-dioxetanes,[13] a catalytic pathway might be responsible for the low value of E_a as measured by the standard methods involving the disappearance of **1**. However, the experimental indistinguishability of E_a in CH_3CN and benzene (Table I) is good evidence against such a complication. Furthermore, the comparable excitation efficiencies of **1** and Dewar benzene itself argue against an unknown catalytic pathway. Finally, the activation entropy for disappearance of **1** is within experimental error of 0 eu, a value which is inconsistent with a bimolecular catalytic pathway for reaction.

In summary, the thermal rearrangement of Dewar acetophenone to acetophenone represents the first example of an electrocyclic reaction of a "Dewar" structure for which it is energetically feasible to populate both a singlet or triplet electronically excited state of the product. The system is of special theoretical interest because the key bond breaking and making processes are expected to be located mainly on the benzene moiety whereas the final excited states are localized on the carbonyl moiety. This dislocation of the incipient location of bond energy release may require more activation than that for the major path for rearrangement. Thus, the final location of electronic excitation starts off "π,π^*-like" but must finish "n, π^*-like;" however, the n, π^* states may not come into operation during the lifetime of the transition state of the chemiexcitation step. This molecular feature may be responsible for the extra activation energy and hence the low efficiency of excited state production from thermolysis of **1**.

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Isotope Effects in the Hydroxylation of Phenylethylamine by Dopamine β-Hydroxylase

Sir:

Dopamine β-hydroxylase (EC 1.14.17.1) (3,4-dihydroxyphenylethylamine, ascorbate:oxygen oxidoreductase (β-hydroxylating)) catalyzes the hydroxylation of phenylethylamine derivatives such as 3,4-dihydroxyphenylethylamine (Dopamine)[1] at the benzylic position. The reaction, which is the final step in the biosynthesis of norepinephrine, has been shown[2] to proceed according to eq 1.

$$\text{Dopamine} + O_2 + \text{ascorbate} \longrightarrow (R)\text{-norepinephrine} + \text{dehydroascorbate} + H_2O \quad (1)$$

The enzyme from bovine adrenal glands is a tetrameric protein[3,4] containing about 4% carbohydrate,[3] variable amounts of copper (4–7 mol per mole of enzyme), and a constant amount of Cu(II) (2 mol per mole of enzyme).[5] Although it is not known whether the carbohydrate is essential for enzymatic activity, it is known that the copper is required. Apoenzyme devoid of copper has no hydroxylase activity.[5] Furthermore, it has been shown that the first step[5] in the hydroxylation sequence is the reduction by ascorbate of most of the protein-bound Cu(II) to Cu(I).

There has been a great deal of speculation as to how the oxygen might be activated to bring about the hydroxylation in mixed function oxidases. The schemes usually invoke either an "oxenoid"[6] or an electrophilic species[7] as the active hydroxylating agent. Although inversion at the hydroxylated center has been reported, the hydroxylations normally proceed with a net retention at the hydroxylated center.[8] It has recently been demonstrated that the hydroxylation of *d*-amphetamine by Dopamine β-hydroxylase takes place with a net retention of configuration at the benzylic center.[9]

In order to evaluate various mechanistic speculations put forth concerning the mechanism of action of

(1) Abbreviations used in this paper: Dopamine, 3,4-dihydroxyphenylethylamine; Dopamine β-hydroxylase, 3,4-dihydroxyphenethylamine β-hydroxylase.

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Dopamine β-hydroxylase, we have prepared the (*R*)- and (*S*)-[β-^{2}H]phenethylamine and examined the absolute stereochemistry and primary and secondary isotope effects in its hydroxylation.

The (*R*)-[β-^{2}H]- and (*S*)-[β-^{2}H]-*N*-acetylphenylalanines were synthesized by the method of Kirby.[10] The proton magnetic resonance (pmr) spectra of these intermediate deuterated phenylalanines showed only an AX quartet, indicating the presence of only one diasteriomer. The enantiomers were esterified using diazomethane and the D,L-*N*-acetylphenylalanine methyl ester resolved using α-chymotrypsin.[11] This method of resolution offers a milder route to the enantiomeric deuterated compounds which is less likely to induce epimerization at the α center than the *N*-chloroacetyl method used earlier. After resolution, the free deuterated phenylalanines were generated by acid hydrolysis and decarboxylated using acetophenone.[12] Analysis of the final (*R*)- and (*S*)-[β-^{2}H]phenylethylamine by both pmr and mass spectroscopic techniques[13] showed greater than 92% deuterium at the one benzylic position. The Dopamine β-hydroxylase enzyme used was purified by the method of Kaufman through the calcium phosphate gel step.[4]

Hydroxylation of these enantiomeric deuterated substrates leads to complete loss of deuterium from the *R* isomer and complete retention of deuterium in the *S* isomer, demonstrating that the hydroxylation takes place with a net retention of configuration at the benzylic center.[13] This confirms the results of Taylor[9] obtained using a different substrate and an entirely different chemical approach to the creation of the isotopic asymmetry at the benzylic center.

A variety of anions stimulate the activity of Dopamine β-hydroxylase,[4] with fumarate being particularly effective. We examined the primary and secondary isotope effects on the rate of hydroxylation under saturating conditions both in the presence and absence of fumarate. In the presence of fumarate the k_H/k_D for the hydroxylation of the (*R*)-[β-^{2}H]-phenylethylamine is 2.0 and for the *S* isomer the secondary isotope effect is 1.0. In the absence of fumarate the primary isotope effect for the *R* isomer is 5.0 and the secondary isotope effect for the hydroxylation of the *S* isomer is 1.7.[14]

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(13) The retention and/or loss of deuterium was determined by mass spectrometric analysis of tne product of the enzymic reaction on a LKB-9000 combined gas chromatograph–mass spectrometer utilizing a 1% OV-17 column on Supelcoport 60/80. We are grateful to Dr. H. Fales, Laboratory of Chemistry, National Heart and Lung Institute, for access to this insrument and measurement of deuterium incorporation in the substrate by chemical ionization mass spectroscopy.

(14) For the hydroxylation in the presence of fumarate the reaction mixture contained the following: phosphate buffer pH 6.3, 1.8 mmol; fumaric acid, 150 μmol; catalase, 1350 units; phenylethylamine, 9.9 μmol; 0.475 mg of Dopamine β-hydroxylase; ascorbate 18 μmol, in a total volume of 3 ml of H_2O. For the hydroxylation in the absence of fumarate the conditions were the same except that fumaric acid was omitted and 0.950 mg of Dopamine β-hydroxylase was used. The reaction mixture was incubated at 35° in open shaking tubes. Samples were taken every 10 min for 50 min, the phenylethanolamine product separated on a Bio-Rex 70 cation exchange resin using borate buffer, pH 9.9, and assayed using ninhydrin. The effect of phosphate on the fumarate stimulation of the hydroxylation of phenethylamine is evidently quantitatively different from its effect on Dopamine.[4]

In an earlier kinetic study of this system Goldstein[15] suggested that the rate limiting step in the overall hydroxylation reaction was the interconversion of the central ternary complex. The isotope effects in the absence of fumarate are consistent with this. It appears that the effect of the fumarate is to reduce the activation energy of the breaking of the benzylic C–H bond to the point where that step is only partially rate determining.

A number of isotope effects have been reported for the reaction of nitrenes and carbenes with C–H bonds. In these cases the direct insertion of the singlet nitrene into a C–H bond is characterized by an isotope effect of 1.3–1.7.[16] For triplet nitrenes, which react *via* a pathway of hydrogen abstraction followed by C–N bond formation an isotope effect of 4.2 is observed.[16] For carbene reactions isotope effects of 1.1–1.4 have been reported;[17] while for "carbenoid" species values of 1.4–2.0 are observed.[18] A substantial secondary isotope effect indicates that there is a large change in the geometry of the reacting site between the ground state and the transition state. The isotope effects observed for the hydroxylation in the absence of fumarate are only consistent with a mechanism that involves the abstraction of the benzylic pro-R hydrogen followed by the subsequent formation of a C–O bond. They are not consistent with the direct insertion of an "oxenoid" species into the C–H bond.

Further studies of this system using other stereospecifically deuterated substrates are being pursued.

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Reversible Oxygen Adduct Formation in Cobalt(II) "Picket Fence Porphyrins"

Sir:

Replacement of Fe(II) by Co(II) in hemoglobin, to give "cobalglobin," CoHb,[1] results in an oxygen carrying protein remarkably similar to the natural iron protein, Hb.[2–4] Small differences in the degree of co-

(1) The abbreviations used in this paper are: CoHb, cobalt substituted hemoglobin or "cobalglobin;" Hb, hemoglobin; DMF, dimethylformamide; TPP, tetraphenylporphine; 1-MeIm, 1-methylimidazole; H_2PDME, protoporphyrin IX dimethyl ester; Co(*p*-OCH_3)TPP, *meso*-tetra(*o*-methoxyphenyl) porphyrincobalt(II).

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