thermal parameters, bond lengths, bond angles, anisotropic thermal parameters, and hydrogen atom coordinates for **3** (11 pages); tables of observed and calculated structure factors for **3** (36 pages). Ordering information is given on any current masthead page.

Facile Stereoselective Allylic Hydroxylation by Dopamine β-Monooxygenase

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The copper protein, dopamine β-monooxygenase (DBM, E. C.1.14.17.1) catalyzes hydroxylation at the pro-R hydrogen of dopamine to form norepinephrine in a variety of mammalian tissues.[1-4] We and others have shown that DBM also readily catalyzes benzylic oxygenation of functionalities such as carbon (saturated or unsaturated), carbinol, sulfur, selenium, or nitrogen in a variety of substrate analogues,[5-17] and the mechanism of DBM catalysis has been the subject of much recent interest.[9,12,18-22] We now report the first example of allylic oxygenation by DBM, and we demonstrate that this reaction is highly facile and stereoselective, with the absolute configuration of the product corresponding to that previously established for benzylic hydroxylation[3] and sulfoxidation[5] by DBM.

In view of the well-known physico-chemical similarities of allylic and benzylic systems,[23] 2-(1-cyclohexenyl)ethylamine (CyHEA) was chosen as the prototypical substrate for allylic hydroxylation, since it shares a number of structural similarities with 2-phenethylamine. DBM was isolated from bovine adrenal medullae and purified as described previously.[9,24] Kinetic parameters for CyHEA turnover were found to be k_{cat} = 90 s^{-1} and K_M = 6.1 mM under standard turnover conditions. These values represent highly facile turnover, comparable to those for the most highly active DBM substrates known to date.[25]

Preparative scale enzymatic reaction allowed product isolation by preparative TLC after derivatizing enzymatic reaction mixtures with succinimidyl-4-nitrophenylacetate (SNPA),[9] and the product was identified as 1-(1-cyclohexenyl)-2-[(4-nitrophenyl)acetamido]ethanol on the basis of FT NMR and mass spectrometry [^{1}H NMR (δ, $CDCl_3$) 7.83 (dd, 4 H), 5.8 (br s, 1 H), 5.65 (m, 1 H), 4.04 (br s, 1 H), 3.45 (s, 2 H), 3.2-3.6 (m, 2 H), 1.4-2.1 (m, 8 H); mass spectrum (EI) M^+ 304]. An oxygen/ascorbate/product stoichiometry of 1:1.2:1.1 was determined for CyHEA oxygenation by quantitative comparison of oxygen con-

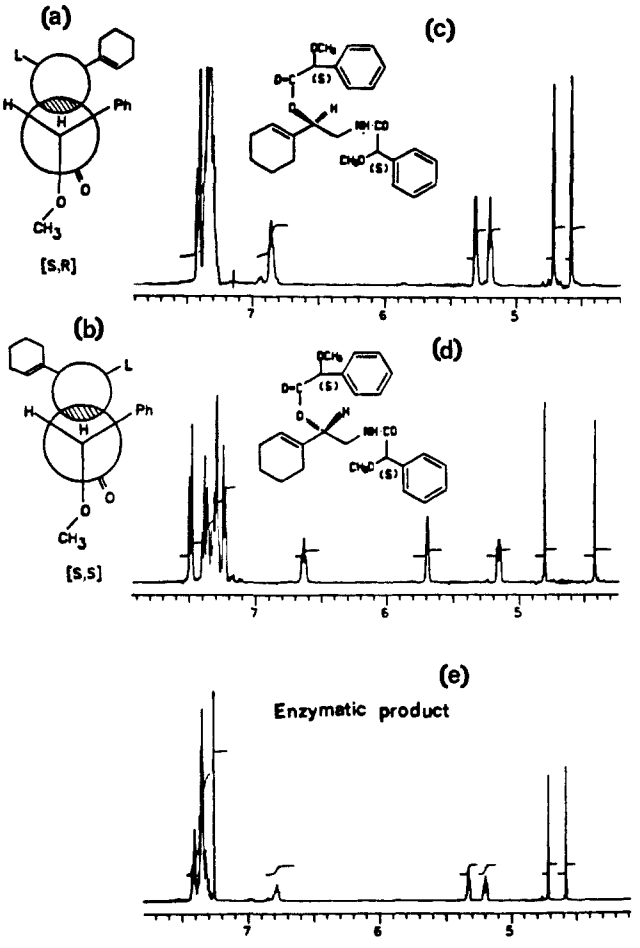

Figure 1. Configuration correlation models and FT NMR spectra of (*S*)-*O*-methylmandelic acid esters of (*R*)- and (*S*)-1-(1-cyclohexenyl)-2-aminoethanols. The proton resonance for the vinyl proton was identified by NMR decoupling. (a) The extended Newman projection for diacylated (*R*)-1-(1-cyclohexenyl)-2-aminoethanol. (b) The extended Newman projection for diacylated (*S*)-1-(1-cyclohexenyl)-2-aminoethanol. (c) FT NMR spectrum of *N*-[(*S*)-α-methoxyphenylacetyl]-*O*-[(*S*)-α-methoxyphenylacetyl]-1-(1-cyclohexenyl)-2-aminoethanol (slow eluting diastereomer). (d) FT NMR spectrum of *N*-[(*S*)-α-methoxyphenylacetyl]-*O*-[(*S*)-α-methoxyphenylacetyl]-1-(1-cyclohexenyl)-2-aminoethanol (fast eluting diastereomer). (e) FT NMR spectrum of *N*-[(*S*)-α-methoxyphenylacetyl]-*O*-[(*S*)-α-methoxyphenylacetyl]-1-(1-cyclohexenyl)-2-aminoethanol (enzymatic product).

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sumption [measured polarographically[9]], ascorbate consumption [measured by HPLC/EC[10]], and allylic alcohol product formation. These quantitative experiments confirm that 2-amino-1-(1-cyclohexenyl)ethanol (CyHEA-OH) is the *only* product formed during enzymatic turnover. Similarly, trapping experiments with the epoxide trapping agent 4-(*p*-nitrobenzyl)pyridine (NBP)[9] confirmed the absence of epoxide products. Thus, DBM processing of CyHEA gives rise only to allylic hydroxylation at C-2, and neither epoxidation nor allylic rearrangement is observed.[26]

The absolute configuration of the enzymatic product was determined with the elegant method of Mosher et al.[27a,b] Upon derivatization of racemic synthetic CyHEA-OH[28] with (*S*)-*O*-methylmandelic acid, two diastereomeric derivatives were obtained which were separated by preparative TLC by using 45% ethyl-acetate in hexane. HPLC analyses on silica gel showed that the similarly derivatized enzymatic product yielded only material which co-eluted with the slow eluting diastereomer of synthetic CyHEA-OH. The (*S*)-*O*-methylmandelate derivatives were then examined by NMR, and the signal arising from the vinyl proton was identified by NMR decoupling experiments. The vinyl proton of the fast eluting diastereomer appeared at 5.69 δ, while that of the slow eluting diastereomer appeared at 5.32 δ with an upfield shift of 0.375 ppm. Using the Mosher model (Figure 1), the slow eluting isomer having the upfield vinyl NMR signal was assigned an absolute configuration of *R*, while the other isomer was assigned the *S* configuration. Thus, enzymatically produced CyHEA-OH is assigned an absolute configuration of *R*, and no (*S*)-CyHEA-OH is detectable by HPLC, TLC, or NMR.

There is evidence that DBM oxygenation of benzylic olefinic and heteroatom functionalities proceeds via initial electron abstraction to give a substrate radical cation.[9,10,12] Thus, the absence of epoxide products from oxygenation of CyHEA by DBM—implying an inability of the activated copper–oxygen species to effect radical cation formation from a *non*conjugated olefinic moiety—is consistent with the well-known difference in oxidation potential and reactivity between conjugated and nonconjugated olefins.[29] On the other hand, it is well known that allylic and benzylic C–H bonds exhibit strikingly similar chemical reactivities.[23] This suggests that the probable mechanism for allylic hydroxylation of CyHEA is analogous to that proposed for benzylic hydroxylation of phenethylamines, i.e., hydrogen atom abstraction to form a resonance stabilized benzylic (or allylic) radical.[19]

Groves and co-workers[30] found that cytochrome P-450 catalyzes both epoxidation and allylic hydroxylation of cyclohexene, and they propose that epoxidation by P-450 proceeds via an olefinic cation radical whereas allylic oxygenation proceeds via an allylic radical. However, they observed that allylic hydroxylation of cyclohexene is accompanied by allylic rearrangement and that methylenecyclohexane gives rise to both 2-methylenecyclohexanol and 1-cyclohexenylmethanol. In contrast, we find that allylic hydroxylation of CyHEA by DBM proceeds without rearrangement. It is possible that geometric constraints at the DBM binding site prevent interaction of the activated oxygen species with carbon centers in the cyclohexene ring. However, we have also observed that DBM catalyzes allylic hydroxylation of *cis*-2-hexenylamine with hydroxylation occurring cleanly at C-4 and without any detectable allylic rearrangement.[31] These results may be suggestive of a mechanism in which copper interacts with the olefin moiety during the catalysis, thus precluding rearrangement of the double bond. Such copper–olefin interactions have been proposed to account for the lack of allylic rearrangement in oxidation by peroxides in the presence of copper salts.[32]

The demonstration of facile, stereoselective allylic hydroxylation by DBM suggests new possibilities for the design of inhibitors and pseudo-substrates for this enzyme, a goal which is being actively pursued in a number of laboratories.[33-37]

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(26) As with many DBM substrates[9,12,14] examination of the time course of CyHEA oxygenation reveals that time-dependent enzyme inactivation occurs with apparent kinetic constants k_{inact} = 0.33 min^{-1} K_I = 82.5 mM.

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(29) The $E_{1/2}$ for the oxidation of cyclohexene is 0.33 V higher than that of α-methylstyrene. $E_{1/2}$ values were calculated from ionization potentials (IP) for cyclohexene and α-methylstyrene (*Hand Book of Physics and Chemistry*; 58th ed.; Weast, R. C., Ed.; CRC Press: E74, with $E_{1/2}$ = 0.89 (IP) - 6.04 (Miller, L. L.; Nordblom, G. D.; Mayeda, E. A. *J. Org. Chem.* **1972**, *37*, 916).

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Proton-Transfer Reactions of Sulfonium Ylides: Unit Brønsted Slopes Do Not Require Diffusion-Controlled Proton Transfer[†,1]

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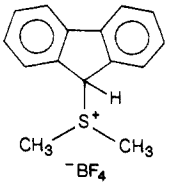
We report here evidence that the Brønsted β coefficient of 1.0 for deprotonation of a sulfonium salt overestimates the amount of bond formation to the base catalyst in the transition state; in the reverse protonation direction α = 0 underestimates the degree of proton transfer from the acid catalyst. Proton transfers to and from carbon occur directly[2-4] so that an acid catalyst must break a hydrogen bond to water before it can protonate a carbanion. We suggest that a value of $\alpha_d \approx -0.2$ for desolvation offsets α = 0.2 for protonation to give α_{obsd} = 0; β_{obsd} is then 1.0. This estimate is supported by the dependence on phenol acidity of ΔG for transfer of phenols and substituted benzenes from the gas phase to water. Similar effects of hydrogen bonding on the nucleophilic reactivity of bases have been reported.[5,6]

Figure 1 shows that the Brønsted plot for ^1H and ^3H exchange of dimethyl-9-fluorenylsulfonium tetrafluoroborate (**1**) in D_2O[7]



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