signals indicate absorptions of microwave at any times and at any temperatures. Thus we observe the radicals produced from the triplet states of the peroxides. Two kinds of signals, **1**-**4**, are observed for each system; radicals **1** and **3** appear immediately after the laser irradiation and radicals **2** and **4** are produced at later stages. These four radicals were assigned in reference to the g values and hyperfine coupling constants (hfcc) of the known species. Radical **1** (g = 2.0123, $\Delta H_{1/2} \sim 2.8$ G) and radical **3** (g = 2.0121, $\Delta H_{1/2} \sim 2.2$ G) were assigned as the benzoyloxyl and the 4-methoxybenzoyloxyl radicals by referring to the EPR spectra of $C_6H_5COO\cdot$ (g = 2.0117) and $DOOCCH{=}CHCOO\cdot$ (g = 2.0119) produced by UV and X-ray irradiation of crystals of acetylbenzoyl peroxide[4b,c] and maleic acid.[7] Both radicals **1** and **3** provide relatively large g values and small hfcc ($a_H < 1$ G), which clearly indicates that the benzoyloxyl radicals observed are not π radicals but σ radicals.[7-11] Radicals **2** and **4** were assigned as the trichloromethyl (g = 2.0096, $a_{Cl} \sim 6.2$ G)[12] and the phenoxymethyl-type radicals $\cdot CH_2OC_6H_4X$ (g = 2.0033, $a_H \sim 17.5$ G),[13,14] respectively. The radicals observed for Cl-BPO were assigned as the 4-chlorobenzoyloxyl and the trichlomethyl radicals as in the case of BPO.

The decays of the EPR signals were observed at low microwave powers (≤0.1 mW) to avoid complication due to spin-spin relaxation.[15] The important result obtained is that the decay time of radical **1** is in good agreement with the rise time of radical **2**. A similar relation was also found for radicals **3** and **4**. These results suggest that the stepwise processes occur between radicals **1** and **2** and radicals **3** and **4**. We obtained the decay times (τ) of 0.25, 0.72, and 1.6 μs at 20 °C for the benzoyloxyl radicals produced from BPO, Cl-BPO, and MeO-BPO, respectively. These decay times are considered to be the lifetimes of these radicals for the following reasons. First, the values are much shorter than those (several microseconds) usually obtained for spin-lattice relaxation (SLR) times in solution. Second, the relation of $\tau_{MeO\text{-}BPO} > \tau_{BPO}$ is not expected for the SLR process but is consistent with the lifetimes from the thermal decomposition of BPO and MeO-BPO.[16,17] The decay times of radicals **2** and **4** are much longer (36 and 3-4 μs at 20 °C, respectively) and cannot be easily identified as the lifetimes or the SLR times.

We also examined temperature dependence of the lifetime of benzoyloxyl radical. The result is shown in Figure 2. When we assume that an Arrhenius type equation, $k(T) = k_0 \exp(-E_a/kT)$, holds in this case, we obtain $k_0 \sim 2 \times 10^{10}$ s^{-1} and $E_a \sim 5$ kcal/mol from the straight line of Figure 2. The rate of decarboxylation extrapolated to 130 °C ($\sim 4 \times 10^7$ s^{-1}) is in order of magnitude agreement with the value ($\sim 1 \times 10^8$ s^{-1}) estimated by CIDNP[18a] and spin trapping techniques[18b] for the thermal decomposition of BPO. However, the present values of k and E_a are quite different from the often quoted values ($k \sim 10^4$ s^{-1} and $E_a \sim 14$ kcal/mol).[19]

Finally, we summarize the reaction paths to explain the observations; the phenyl radical has not been observed yet.[20]

$$(C_6H_5COO)_2 \rightarrow C_6H_5COO\cdot \rightarrow [C_6H_5\cdot] + CO_2$$

$$[C_6H_5\cdot] + CCl_4 \rightarrow C_6H_5Cl + \cdot CCl_3 \quad (1)$$

$$(CH_3OC_6H_4COO)_2 \rightarrow CH_3OC_6H_4COO\cdot$$

$$CH_3OC_6H_4COO\cdot + (CH_3OC_6H_4COO)_2 \rightarrow CH_3OC_6H_4COOH + \cdot CH_2OC_6H_4COOOCOC_6H_4OCH_3 \quad (2)$$

(20) The phenyl radical has been reported to react very fast ($k \sim 10^6$ M^{-1} s^{-1}) with carbon tetrachloride.[3c]

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Mechanism-Based Inhibitors of Dopamine β-Hydroxylase Containing Acetylenic or Cyclopropyl Groups

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The hydroxylation of dopamine at the benzylic position to form norepinephrine catalyzed by the copper-containing protein dopamine β-hydroxylase is unusual in that it involves the hydroxylation of an aliphatic carbon-hydrogen bond.[1] Advantage has been taken of the broad substrate specificity of this enzyme[2] to develop mechanism-based inhibitors as probes of the mechanism of C-H bond activation.[2b,e,3] We have recently shown that variously substituted 3-phenylpropenes are mechanism-based inhibitors of dopamine β-hydroxylase and have proposed that the enzyme-bound intermediate which partitions between hydroxylation and inactivation is a benzylic radical, as shown in Scheme I.[3] This was based on a ρ value of −1.2 for the effect of ring substitution upon the rate of inactivation. However, such a result would not be inconsistent with a mechanism in which a second electron is removed from the benzylic radical intermediate to form a benzylic carbonium ion which then partitions between inactivation and hydroxylation. Such a mechanism would involve nucleophilic attack by an enzyme group on a Michael-type acceptor. In order to distinguish between these two possibilities, we have determined the effect upon inactivation of replacing the allylic side chain of the phenylpropenes with a propargyl group. In addition, we have also tested benzylcyclopropanes as inhibitors as a further test of a radical mechanism.

3-Phenylpropynes[4] and benzylcyclopropanes[5] containing either

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(4) 3-(*p*-Methoxyphenyl)propyne was synthesized by a Grignard coupling of anisylmagnesium bromide to propargyl bromide. The 3-(*p*-methoxyphenyl)allene formed as a side product was converted to the propyne by treatment with *n*-butyllithium as described in: Mulvaney, J. E.; Folk, T. L.; Newton, D. J.; *J. Org. Chem.* **1967**, *32*, 1674-1675. Demethylation to give 3-(*p*-hydroxyphenyl)propyne with BBr_3 was by the method of: McOmie, J. F. W.; Watts, M. L.; West, D. E. *Tetrahedron* **1968**, *24*, 2289-2292.
(5) Benzylcyclopropanes were synthesized from the respective 3-phenylpropenes by reflux at 120 °C for 3-4 days with excess copper and diiodomethane as described by: Kawabata, N.; Kamemura, I.; Naka, M. *J. Am. Chem. Soc.* **1979**, *101*, 2139-2145.

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Table I. Kinetic Parameters for Mechanism-Based Inhibitors of Dopamine β-Hydroxylase[a]

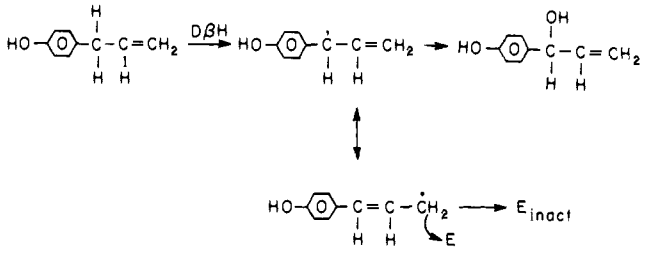
inhibitor	$k_i \times 100$, min^{-1}	K_I, mM	partition ratio[b]
3-(*p*-methoxyphenyl)propene	37	2.8	
3-(*p*-hydroxyphenyl)propene	170	0.9	84 (13)[c]
1-(*p*-methoxybenzyl)cyclopropane	1.8	0.24	
1-(*p*-hydroxybenzyl)cyclopropane	180	12	100 (25)
3-(*p*-methoxyphenyl)propyne	1.7	0.58	
3-(*p*-hydroxyphenyl)propyne	9.1	0.46	660 (60)

[a] Conditions: 1–3 μM dopamine β-hydroxylase, 12 mM ascorbate, 1.21 mM oxygen, 0.5 mg/mL catalase, 14% DMF, 0.1 M sodium acetate, 0.1 M MES, pH 5.5, 25 °C. [b] Products were separated and quantitated by HPLC as described in ref 3. [c] Standard deviation.

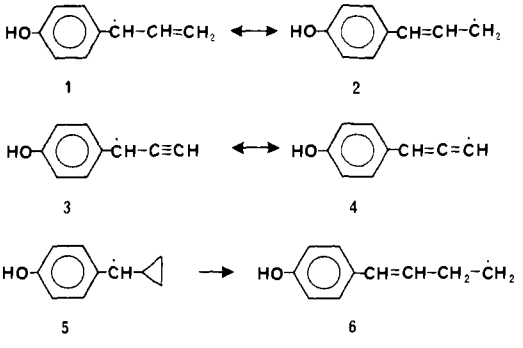
HO- or CH_3O- at the para position were tested as mechanism-based inhibitors of dopamine β-hydroxylase purified from bovine adrenal medulla.[2e,3] In all cases the inactivation was first order, the inactivation showed saturation kinetics, both oxygen and ascorbate were required for inactivation, and substantial protection from inactivation was afforded in the presence of the substrate tyramine. These compounds therefore fulfill the minimal criteria for mechanism-based inhibitors.[6] The kinetic parameters for inactivation for phenylpropenes, phenylpropynes, and benzylcyclopropanes at a single oxygen concentration are given in Table I. With both the benzylcyclopropanes and the 3-phenylpropynes, the *p*-HO-substituted compound inactivated more rapidly than the *p*-CH_3O-substituted compound.[7] This is consistent with the results found for a series of ring-substituted 3-phenylpropenes.[3] 3-Hydroxy-3-(*p*-methoxyphenyl)propene, 3-hydroxy-3-(*p*-methoxyphenyl)propyne, and α-cyclopropyl-*p*-methoxybenzyl alcohol did not inactivate dopamine β-hydroxylase, either in the presence or absence of ascorbate, ruling out the possibility that inactivation was occurring after hydroxylation was complete.[8]

Partition ratios were determined by measuring the amount of hydroxylated product present after complete inactivation of the enzyme by the three *p*-HO-substituted compounds.[3,9] Mass spectral analysis of the isolated enzymatic products established that all three compounds are substrates for dopamine β-hydroxylase, being hydroxylated at the benzylic position. As shown in Table I, replacement of the vinylic moiety with an acetylenic moiety results in an eightfold increase in the partition ratio. Since triple bonds are much more susceptible to nucleophilic attack than double bonds,[10] a mechanism involving attack upon a carbonium ion intermediate should have resulted in a much lower partition ratio for the 3-phenylpropynes. The intermediates involved in activation by a radical mechanism are shown in Scheme II, as well as the relevant resonance structures. Inactivation by the mechanism proposed in Scheme I involves reaction at the terminal carbon of the side chain, while hydroxylation of all substrates for

Scheme I



Scheme II



dopamine β-hydroxylase involves reaction at the benzylic position. With 3-(*p*-hydroxyphenyl)propene the terminal carbon of the radical species **2** resembles a primary carbon radical, a more reactive species than the vinylic radical formed in the case of 3-(*p*-hydroxyphenyl)propyne (**4**).[11] Thus, a radical mechanism predicts a lower partition ratio for phenylpropenes than for phenylpropynes. Further, propargyl radicals preferentially add at the propargyl position (**3**);[12] this would result in hydroxylation instead of inactivation in the case of dopamine β-hydroxylase and contribute to an increased partition rato for the phenylpropyne. In contrast, allylic radicals do not show such a preference, so that the likelihood of inactivation is much higher for phenylpropenes than for phenylpropynes.

Inactivation by (*p*-hydroxybenzyl)cyclopropane is further evidence for the proposed radical mechanism. Double bonds are more reactive to both electrophilic and nucleophilic attack than cyclopropyl rings,[13] so that a much higher partition ratio is expected for benzylcyclopropanes than for phenylpropenes if inactivation involves either type of mechanism. However, a mechanism involving a radical intermediate does predict a relatively low partition ratio, as is found, since such an intermediate could ring open as shown in Scheme II.[14] This would generate a highly reactive radical (**6**). If such a species is generated, it must react quite efficiently with the enzyme, since no 1-(*p*-hydroxyphenyl)butene or 4-(*p*-hydroxyphenyl)-3-butenol was detected.

In conclusion, we have demonstrated that two new types of compounds, 3-phenylpropynes and benzylcyclopropanes, are mechanism-based inhibitors of dopamine β-hydroxylase. In addition, the partition ratios for these compounds lend further support to the concept that the mechanism of the reaction catalyzed by dopamine β-hydroxylase involves a benzylic radical intermediate.

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