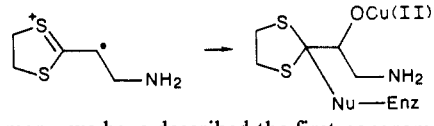
concentration of the substrate tyramine was varied in the range 0.25–5 mM at each concentration of inhibitor. The rate of turnover of saturated analogue **2** was again performed under standard assay conditions except that 640 μg of DBH and 10.5 μM $CuSO_4$ were required to obtain a measurable rate of O_2 consumption with use of a Clark oxygen electrode at 40 mM of **2**.

Figure 1 shows the psuedo-first-order decay on incubation of DBH with **1a** at concentrations in the range 0.1–10 μM. Inset is the Kitz and Wilson plot[14] from which the kinetic parameters, k_{inact} and K_I, can be estimated.[15] In addition, compounds **3** and **5a** also produced the expected pseudo-first-order loss of enzymatic activity (see Table I) and showed linear inactivation plots. In order to produce time-dependent inhibition, O_2, Cu^{2+}, and ascorbate were required and tyramine protected from inhibition by **1a**. Extensive dialysis did not result in reversal of the inhibition. These facts suggest that the inhibition is an enzyme-activated phenomenon. Fully saturated derivative **2** was not a time-dependent inhibitor but did exhibit good competitive inhibition (K_i = 11 μM versus tyramine), indicating that the ylidene moiety is essential for time-dependent inactivation but not for active site recognition. Compound **2** was also a poor substrate with a rate of turnover at 40 mM of about 6% of tyramine. Dithiane **3** inactivated DBH at a much lower rate than dithiolane **1a**. This indicates the importance of mimicking the planarity of an aromatic ring and is reinforced by the fact that the open chain analogue **4** was not an inhibitor. Of the two monosulfur analogues of **1a**, only isomer **5a**, with aminomethyl trans to sulfur, is a time-dependent inhibitor. The other isomer, **5b**, is neither time-dependent nor competitive. This result suggests that the spatial arrangement of the NH_2 relative to S is very important for recognition and indicates that the heterocyclic ring is not simply mimicking the planar aromatic ring of tyramine but implies some positive interaction between one of the S atoms and the active site.

One possible mechanism of inhibition by **1a**, analogous to those proposed for other unsaturated inhibitors,[9] proceeds by abstraction of an electron from **1a** by HOO-Cu(II) to form a resonance stabilized radical cation whose unpaired electron should combine with the unpaired electron of O=Cu(III) (eq 1). The resulting sulfur-stabilized cation can undergo attack by enzyme nucleophile. Consistent with this mechanism is the observation that prolonged dialysis of inactivated enzyme did not lead to recovery of activity.



(1)

In summary, we have described the first nonaromatic mechanism-based inhibitors of DBH based on the concept that a planar substrate analogue capable of forming a very stable radical cation should result in enzyme inactivation. Compound **1a** appears to have the largest value of k_{inact}/K_I for any mechanism-based inhibitor of DBH yet reported. Further work is ongoing to define the active site of the enzyme and to determine the potential therapeutic utility of the described inhibitors.

Acknowledgment. We thank Dr. Michael Whalon and Robert Barbuch of the Analytical Chemistry Department for aid with spectral interpretations and Dr. Perry Gordon of the Drug Metabolism Department for aid with the stability study of **1a** and **1b**. In addition, we thank Professor Michael Johnston for helpful discussions.

Supplementary Material Available: Experimental procedures for compounds **1a**, **1b**, **2**, **3**, **4**, **5a**, and **5b**, Schemes I–IV, which outline the syntheses of the compounds, and a brief discussion of the syntheses (18 pages). Ordering information is given on any current masthead page.

(14) Kitz, R.; Wilson, T. B. *J. Biol. Chem.* **1962**, *237*, 3245.

(15) Owing to the nonlinearity of the curves at 4 μM **1a** and above and the uncertainty whether the curvature is biphasic or triphasic, these data were not included when the estimates of the kinetic parameters given in Table I were calculated from the Kitz and Wilson plot.

A Revision of the Generally Accepted Hypothesis for the Biosynthesis of the Tropane Moiety of Cocaine

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It has been established[2] that the administration of [5-^{14}C]-ornithine (**1**) to *Erythroxylum coca* plants affords radioactive cocaine which is labeled equally at C-1 and C-5 of its tropane moiety. It was also shown that [1-^{14}C]acetate preferentially labeled C-3 (48%) and C-9 (38%).[3] These results are consistent with the generally accepted biosynthesis of cocaine which is illustrated in Scheme I. Ornithine is decarboxylated to yield putrescine (**2**) which is methylated to afford *N*-methylputrescine (**3**). Oxidation of the primary amino group of **3** affords 4-(methylamino)butanal (**4**), the cyclic form of which is the 1-methyl-Δ^1-pyrrolinium salt **7**. Condensation with acetoacetate (**8**) (possibly as the thioester with coenzyme A) affords **6**. Oxidation then yields a new iminium salt **5** which then undergoes another aldol condensation to yield the bicyclic tropane ring system **9**. This compound, 2-carbomethoxy-3-tropinone, is a direct precursor of cocaine.[4] Subsequent steps are presumably reduction to methyl ecgonine (**10**) and then benzoylation to yield cocaine (**11**).[5]

This hypothesis has now been further examined by feeding [2-^{13}C,^{14}C,^{15}N]-1-methyl-Δ^1-pyrrolinium chloride (**7**, X = Cl)[7] to *Erythroxylum coca* plants, by the leaf painting method.[2] Radioactive cocaine was isolated from the plants, harvested 3 weeks after the initial feeding. However, the specific incorporation (0.1%) was not high enough for us to observe a ^{13}C–^{15}N coupling in the signals for C-1 or C-5 in the ^{13}C NMR spectrum of the labeled cocaine. A chemical degradation[2] of the labeled cocaine indicated that negligible radioactivity was present at the C-1 position (activities of the degradation products are recorded in the Supplementary Material). This surprising result led us to carry out a second feeding, this time administering the diethyl acetal of [1-^{13}C,^{14}C,^{15}N]-4-(methylamino)butanal (**12**).[7] It was considered that this acetal would undergo hydrolysis to **4** in the acidic plant tissues.[8] In order to monitor whether cocaine was being synthesized at the time of feeding this acetal, the *N*-acetylcysteamine thioester of [4-^{3}H]benzoic acid was administered to the same coca plants 2 weeks after the initial feeding of **12**. After a total feeding time of 24 days labeled cocaine was isolated with specific incorporations of ^{14}C and ^{3}H of 0.32% and 8.5%, respectively.[9] Examination of the ^{13}C NMR spectrum of this labeled cocaine and natural cocaine, using exactly the same concentrations and instrument parameters, revealed an enhancement (27%) of the signal resulting from C-5. By using a narrow spectral window, the signal at C-5 (61.58 ppm from TMS)[10] showed an upfield satellite resulting from ^{13}C adjacent

(1) Contribution no. 206 from this laboratory. Presented in part at the 3rd International Symposium on Biosynthesis, Banff, Alberta, Canada, May 31–June 6, 1987, and at the 28th Annual meeting of the American Society of Pharmacognosy, Kingston, RI, July 19–22, 1987. Part 39 in the series "Chemistry of the Tropane Alkaloids and Related Compounds".

(2) Leete, E. *J. Am. Chem. Soc.* **1982**, *104*, 1403.

(3) Leete, E. *Phytochemistry* **1983**, *22*, 699.

(4) Leete, E. *J. Am. Chem. Soc.* **1983**, *105*, 6727.

(5) The benzoyl moiety of cocaine is derived from phenylalanine,[3] and the *N*-acetylcysteamine thioester of [*carbonyl*-^{13}C,^{14}C]benzoic acid is an excellent (10.5% specific inc.) precursor of cocaine.[6]

(6) Leete, E.; Bjorklund, J. A.; Kim, S. H. *Phytochemistry* **1988**, in press.

(7) Leete, E.; Kim, S. H.; Rana, J. *Phytochemistry* **1988**, *27*, 401.

(8) The pH of the aqueous solution squeezed from coca leaves was 4.6–4.8.

(9) Hydrolysis of this labeled cocaine (^{14}C: 2.30 × 10^5 dpm/mmol, ^{3}H: 8.29 × 10^5 dpm/mmol) afforded ecgonine (^{14}C: 2.30 × 10^5 dpm/mmol) and benzoic acid (^{3}H: 8.51 × 10^5 dpm/mmol).

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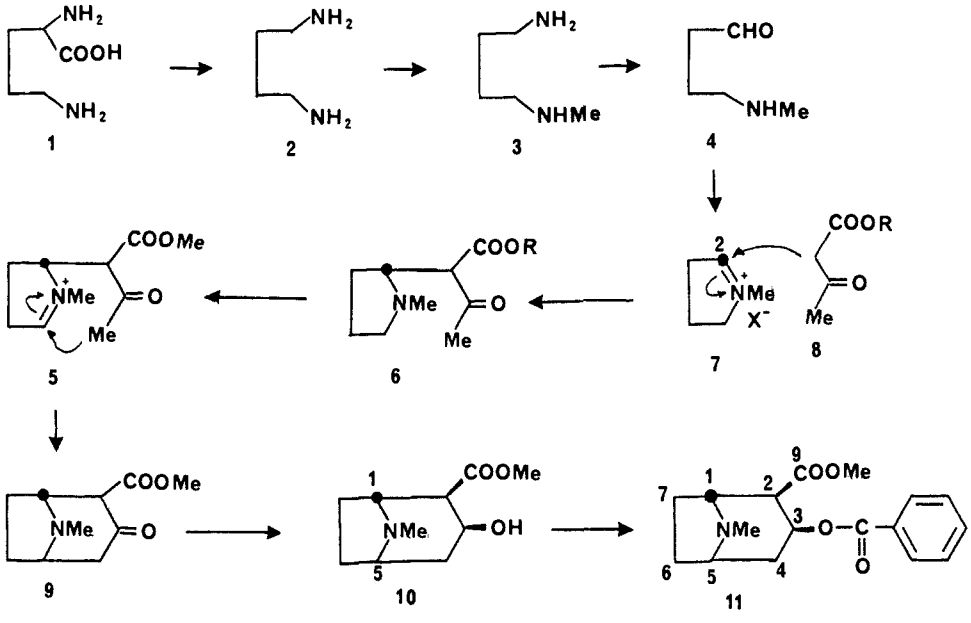
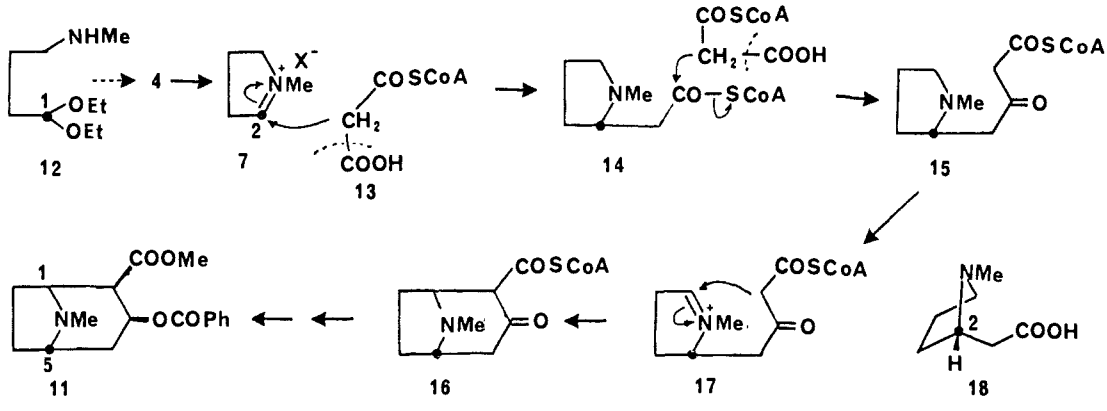
Scheme I. Old Hypothesis for the Biosynthesis of Cocaine



Scheme II. New Hypothesis for the Biosynthesis of Cocaine



to ^{15}N (2.0 Hz from the main signal due to ^{13}C adjacent to ^{14}N). The downfield satellite was apparently obscured by this main signal. No satellites were observed on the signal due to C-1 (64.90 ppm). Ecgonine (**10**, where the COOMe group is COOH), obtained by hydrolysis of this labeled cocaine, also exhibited enhancement of its C-5 position in its ^{13}C NMR spectrum.[11] An upfield satellite ($^1J_{^{13}C-^{15}N}$ 3.7 Hz) was also observed at the C-5 signal (65.42 ppm) but none at the C-1 signal (67.95 ppm).

A new hypothesis, illustrated in Scheme II, is now proposed to explain this unexpected mode of incorporation of the iminium salt **7** or its precursor **12** into the tropane moiety of cocaine. It is considered that **7** reacts with malonyl coenzyme A **13** to yield the coenzyme A ester of 1-methylpyrrolidine-2-acetic acid **14**. Subsequent addition of another two carbons from malonyl coenzyme A yields **15**. Oxidation of the pyrrolidine ring affords a new iminium salt **17** which undergoes an aldol condensation to give **16**. Subsequent steps, as in Scheme I, then yield cocaine, derived from the $^{13}C/^{14}C$ labeled precursors **7** or **12**, which will be labeled at C-5. One piece of circumstantial evidence in favor of this new scheme is the fact that the methyl ester of (*S*)-1-methylpyrrolidine-2-acetic acid (**18**) has been found in nature.[12] A trapping experiment to detect this amino acid in the coca plant was successful. Nonradioactive (*R,S*)-(**18**) was added to the aqueous alkaline extract of the coca plants which had been fed [2-^{14}C]-**7**. The reisolated amino acid, after extensive purification as its methyl ester, was radioactive (0.52% absolute incorporation from **7**). This ester was subjected to a degradation,[13] and all its ^{14}C was found at the C-2 position, a result consistent with its formation from the iminium salt **7** and a two-carbon unit derived from malonyl coenzyme A.

In summary, our results are consistent with the iminium salt **7** serving as a "starter unit" for a polyketide, where the two-carbon units are added one at a time, rather than the direct reaction with a four-carbon unit. We are currently investigating derivatives of **15** and **18** as precursors of the tropane moiety of cocaine and also other tropane alkaloids such as hyoscyamine and scopolamine.[14]

(10) There has been considerable controversy regarding the assignments of the signals in the ^{13}C NMR spectrum of cocaine. These problems have now been resolved, and the definitive publication is as follows: Carroll, F. I.; Coleman, M. L.; Lewin, A. H. *J. Org. Chem.* **1982**, *47*, 13. Our assignments of the ^{13}C NMR spectrum of cocaine by 2D COSY and 2D HETCOR NMR are in agreement with their assignments. Our work[6] with cocaine labeled with ^{13}C on the benzoyl carbonyl group indicates that the C=O groups were misassigned by Avdovich and Neville (Avdovich, H. W.; Neville, G. A. *Can. J. Spectrosc.* **1983**, *28*, 1).

(11) Our assignments for the ^{13}C NMR of ecgonine, based on 2D COSY and 2D HETCOR, are in agreement with the previous published data: Baker, J. K.; Borne, R. F. *J. Heterocycl. Chem.* **1978**, *15*, 165.

(12) Bremner, J. B.; Cannon, J. R.; Joshi, K. R. *Aust. J. Chem.* **1973**, *26*, 2559.

(13) This degradation, detailed in the Supplementary Material, involved the reduction of the methyl ester of **18** with $LiAlH_4$ to 2-(1-methyl-2-pyrrolidinyl)ethanol, conversion to the quaternary ammonium salt with methyl iodide, and a Hofmann degradation to yield 6-dimethylamino-2-(*E*)-hexen-1-ol. Hydrogenation of the double bond, followed by another Hofmann degradation yielded 5-hexen-1-ol which was hydrogenated to afford 1-hexanol, having the same specific activity as the starting **18**. A modified Kuhn–Roth oxidation on this alcohol yielded a mixture of the C-2 to C-6 carboxylic acids which were separated and assayed as their α-naphthylamides.

Acknowledgment. This investigation was supported by a research grant GM-13246-31 from the National Institutes of Health, U.S. Public Health Service.

Supplementary Material Available: ^{13}C NMR spectra of natural cocaine and ecgonine and the corresponding enriched compounds derived from [1-^{13}C,^{14}C,^{15}N]-4-(methylamino)butanal diethyl acetal and activities of the degradation products of ^{14}C-labeled cocaine and 1-methylpyrrolidine-2-acetic acid derived from [2-^{14}C]-7 (7 pages). Ordering information is given on any current masthead page. These data will also be provided with requests for a reprint.

(14) The biosynthesis of other tropane alkaloids in which acetoacetate has been proposed as an intermediate is reviewed: Leete, E. *Planta Medica* **1979**, *36*, 97.

Enhanced Kinetic Resolution and Enzyme-like Shape Selectivity

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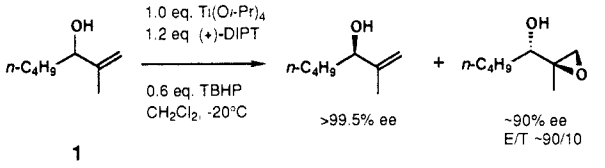
The kinetic resolution of secondary allylic alcohols[1] (Scheme I) has proven to be an important reaction, both from a synthetic standpoint and from the insights it has provided into asymmetric catalysis. The most efficiently resolved secondary allylic alcohol measured to date, **1**, has k_f/k_s = 138, where k_f and k_s are the epoxidation rates of the fast and slow enantiomers, respectively. Recently, Sato et al.[2] reported that (*E*)-1-trimethylsilyl-1-octen-3-ol, **2**, exhibits a very large rate difference for its two enantiomers, seemingly much greater than for substrate **1** mentioned above. The synthetic advantage of such enhanced kinetic resolution is that in one reaction both allylic alcohol and erythro epoxy alcohol are obtained in extremely high enantiomeric excess.[3] Reported herein is a new class of secondary allylic alcohols (of which **2** is a member) that are kinetically resolved with unprecedented efficiency and a discussion of the factors responsible for this phenomenon.

Our initial intuition was that the enhanced kinetic resolution observed by Sato was due to the presence of increased steric bulk in the substituent at the olefin terminus, since kinetic resolution efficiencies for such β-branched substrates had not been studied before. Thus several disubstituted secondary allylic alcohols bearing branched substituents at the trans position were synthesized, and their relative rates of epoxidation under four different epoxidation conditions were studied, as shown in Table I. The rates were measured by means of competition experiments[4] with

Scheme I



1

a substrate of known absolute reactivity[1a] having minimum steric bulk at the olefinic terminus—substrate **3**.[5,6] Knowing that k_f/k_s for **3** is 104 at −20 °C, one can then calculate $(k_f/k_s)_{substrate}$ = (104)rel k_f/rel k_s.

Before comparing reagent selectivities, it was necessary to attempt to decouple the electronic and steric properties of each substrate. Peracid epoxidation is known to be relatively insensitive to steric influences[7] and promises to be a good probe of substrate electronic differences. The values of rel $k_{m\text{-CPBA}}$ for substrates **3**, **4**, and **6** are quite similar and thus consistent with this observation. The slow relative rate for substrate **2** compared to **3**, **4**, and **6** is then probably due to an electronic effect,[8,9] since at the face of the olefin the trimethylsilyl group is less sterically demanding than *tert*-butyl, given the greater length of the C–Si bond.[10] Therefore, a distinction will be made between silicon and non-silicon-substituted olefins in the discussion of allylic alcohol reactivities below. The significant decrease in rate observed in changing from **2** to **9** indicates that even *m*-CPBA is sensitive to the presence of the extremely bulky triisopropylsilyl group.

Unlike rel $k_{m\text{-CPBA}}$, the rate of epoxidation by Ti(O-*i*-Pr)$_4$/TBHP decreases markedly with respect to increasing steric bulk at the olefinic terminus of the substrate. Note that the relative rates of epoxidation of the slow enantiomers by [Ti(DIPT)(O-*i*-Pr)$_2$]$_2$/TBHP are very similar to those of the racemates in the Ti(O-*i*-Pr)$_4$/TBHP manifold. Apparently, the steric effects encountered by the substrate in the transition states of these two distinct epoxidation systems must be very similar.[11]

In contrast to the Ti(O-*i*-Pr)$_4$/TBHP and the [Ti(DIPT)(O-*i*-Pr)$_2$]$_2$/TBHP/slow enantiomer manifold described above, the epoxidation of the fast enantiomers by [Ti(DIPT)(O-*i*-Pr)$_2$]$_2$/TBHP is *not slowed down* by increasing bulk at the olefin terminus. On the contrary, the rate of epoxidation *increases slightly* as the size of the olefinic substituent increases; substrate **4** is faster than **3**, **6** and **7** are faster than **5**. Unfortunately, there is no satisfactory unhindered reference compound for substrate **2**, so it is not possible to observe such a rate enhancement in the silicon series. The decrease in rate upon going from substrate **2** to **8** and **9** suggests that despite the initial rate-enhancing effects of increasing bulk of the olefinic substituent, too much bulk decreases the rate of epoxidation. The cause of this rate enhancement was

(1) (a) Martin, V. S.; Woodard, S. S.; Katsuki, T.; Yamada, Y.; Ikeda, M.; Sharpless, K. B. *J. Am. Chem. Soc.* **1981**, *103*, 6237–6240. (b) Finn, M. G.; Sharpless, K. B. In *Asymmetric Synthesis*; Morrison, J. D.; Ed.; Academic Press: New York, 1985; Vol. 5, Chapter 8. (c) Finn, M. G. Ph.D. Dissertation, Massachusetts Institute of Technology, Cambridge, MA, 1985. (d) Woodard, S. S. Ph.D. Dissertation, Stanford University, Stanford, CA, 1981. (e) Rossiter, B. E. In *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic Press: New York, 1985; Vol. 5, Chapter 7. (f) Gao, Y.; Hanson, R. M.; Klunder, J. M.; Ko, S. Y.; Masamune, H.; Sharpless, K. B. *J. Am. Chem. Soc.* **1987**, *109*, 5765.

(2) (a) Kitano, Y.; Matsumoto, T.; Sato, F. *J. Chem. Soc., Chem. Commun.* **1986**, 1323. (b) Kitano, Y.; Matsumoto, T.; Takeda, Y.; Sato, F. *J. Chem. Soc., Chem. Commun.* **1986**, 1732.

(3) In the normal kinetic resolution domain ($k_f/k_s \sim 100$) the epoxy alcohol is recovered having ~90% ee. Sato reported (ref 2a) that both the allylic and epoxy alcohols can be recovered having ≥99% ee.

(4) All kinetic experiments were carried out at −20 ± 2 °C in CH_2Cl_2 at substrate concentrations from 0.1 to 0.2 M. Only 5 mol% oxidant was added in each case, so that the observed product ratio accurately reflected the product of the relative stoichiometry and epoxidation rates. For the rel k_{ti}, rel k_s, and rel k_f experiments, 100% of the titanium catalyst was used.

(5) For the rel $k_{m\text{-CPBA}}$ and rel k_{ti} experiments, racemate was used. For the rel k_s experiments, enantiomerically pure slow enantiomer was used. For the measurement of rel k_f, racemate was used again, since during the first 5% of reaction, the products would overwhelmingly be derived from the fast enantiomer.

(6) The product ratios were measured by gas chromatography of the crude or acetylated product mixtures. In the rel k_f experiments the products are almost exclusively erythro; in the other experiments both erythro and threo are formed. The measured rates are in actuality the product of substrate binding constants and absolute oxygen transfer rates (see ref 1b and c). Unfortunately, independent determination of each of these terms by means of enzyme kinetic techniques has not been possible to this date.

(7) Newman, M. S.; Gill, N.; Thomson, D. W. *J. Am. Chem. Soc.* **1967**, *89*, 2059–2062.

(8) (a) Colvin, E. W. *Silicon in Organic Synthesis*; Buttersworths Monographs in Chemistry and Chemical Engineering; Buttersworths: London, 1980. (b) Eaborn, C. *Organosilicon Compounds*; Academic Press Inc.: New York, 1960. (c) Eisch, J. J.; Trainor, J. T. *J. Org. Chem.* **1963**, *28*, 487–492.

(9) A recent study reports that whereas replacement of a hydrogen substituent by trialkylsilyl activates olefins toward epoxidation, replacement of a carbon substituent by trialkylsilyl results in net electron withdrawal and deactivation of the olefin (Peterson, P. E.; Nelson, D. J.; Risener, R. *J. Org. Chem.* **1986**, *51*, 2381–2382).

(10) The difference in bond lengths is dramatically borne out in the relative *A* values of *t*-Bu (>4.5 kcal mol^{-1}) and SiMe$_3$ (2.4–2.6 kcal mol^{-1}) (Kitching, W.; Olszowy, H. A.; Drew, G. M.; Adcock, W. *J. Org. Chem.* **1982**, *47*, 5153).

(11) Also lending credence to this hypothesis is the observation that epoxidation diastereoselectivities (generally modest threo selectivity) in the two distinct systems are quite similar.

0002-7863/88/1510-2978$01.50/0