pyridyl)ethanimine *N*-oxide (first step) and its reaction with phenyl vinyl sulfoxide followed by rearrangement to 1 (second step) were carried out in 80 and 29% yields, respectively.

In summary, we have demonstrated that phenyl vinyl sulfoxide may be used successfully as a masked equivalent of acetylene dipolarophile in 1,3-dipolar cycloaddition reactions. As an example, the synthesis of α- and β-nicotyrines was accomplished in only two steps by using a Δ^4-isoxazoline → pyrrole rearrangement. This new synthetic approach should allow for the convenient preparation of other biologically active ring-substituted congeners of the insecticidal β-nicotyrine. Previous synthetic approaches toward β-nicotyrine were limited to the use of nicotine only as starting material.

Experimental Section

Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. The infrared (IR) spectra were obtained on a Nicolet MX-1 FT spectrometer as KBr disks. The proton nuclear magnetic resonance (^{1}H NMR) spectra were taken on a Varian EM-360A (60 MHz) spectrometer with tetramethylsilane as an internal standard; the 200-MHz ^{1}H NMR spectra were recorded on a Bruker-IBM 200 SY Fourier transform spectrometer with the same internal standard. All spectra were consistent with the assigned structures. Elemental analyses were within the acceptable limits of 0.4% of theory.

***N*-Methyl-1-(3-pyridyl)ethanimine *N*-Oxide (4).** A suspension of 16.20 g (0.134 mol) of 3-acetylpyridine (3), 13.0 g (0.156 mol) of *N*-methylhydroxylamine hydrochloride, and 25.61 g (0.312 mol) of sodium acetate in 80 mL of ethanol was stirred at ambient temperature for 24 h under a nitrogen atmosphere. The suspension was diluted with 500 mL of water, basified with potassium carbonate, and extracted with chloroform (4 × 150 mL). The combined organic extract was dried ($MgSO_4$) and concentrated. The residual oil crystallized from ethyl acetate–hexane (1:1), yielding 16.83 g (84%) of nitrone 4, mp 77–80 °C. IR (KBr, cm^{-1}): 3032 (m), 1578 (m), 1417 (m), 1254 (s), 1128 (m), 1084 (m), 1059 (m), 1025 (m), 894 (m), 826 (m), 719 (m). ^{1}H NMR (200 MHz, $CDCl_3$): 2.45 (d, 3 H, J = 1.3 Hz, CCH_3), 3.69 (d, 3 H, J = 1.3 Hz, NCH_3), 7.37–7.43 (m, 1 H), 7.58–7.64 (m, 1 H), 8.56–8.58 (m, 1 H), 8.64–8.67 (1, 1 H) ppm. Anal. Calcd for $C_8H_{10}N_2O$: C, 63.98; H, 6.71; N, 18.65. Found: C, 63.85; H, 6.67; N, 18.57.

***N*-Methyl-1-(2-pyridyl)ethanimine *N*-oxide** was prepared from 2-acetylpyridine by a procedure similar to that described for 4. Yield 80%, mp 61-64 °C (ether). IR (KBr, cm^{-1}): 1584 (s), 1565 (m), 1473 (m), 1436 (m), 1290 (m), 1255 (s), 1237 (s), 1100 (m), 1084 (m), 989 (m), 788 (m). ^{1}H NMR (200 MHz, $CDCl_3$): 2.47 (d, 3 H, J = 1.3 Hz, CCH_3), 3.83 (d, 3 H, J = 1.3 Hz, NCH_3), 6.71–6.82 (m, 2 H), 7.73–7.82 (m, 1 H), 8.66–8.69 (m, 1 H) ppm. Anal. Calcd for $C_8H_{10}N_2O$: C, 63.98; H, 6.71; N, 18.65. Found: C, 63.48; H, 6.73; N, 18.49.

3-(1-Methylpyrrol-2-yl)pyridine (β-Nicotyrine) (2). Under a nitrogen atmosphere, a solution of 4.59 g (30.6 mmol) of *N*-methyl-1-(3-pyridyl)ethanimine *N*-oxide (4) and 5.25 g (34.6 mmol) of phenyl vinyl sulfoxide in 50 mL of toluene was heated to reflux and stirred for 6 h. Upon cooling to ambient temperature, the reaction mixture was filtered through a short column of neutral silica gel with ethyl acetate as eluent. The filtrate was concentrated, and the residual oil was flash chromatographed on neutral silica gel with a 2:3 mixture of ethyl acetate–hexane as eluent; 0.66 g (14%) of 2 was obtained after bulb-to-bulb distillation, bp 78–80 °C (0.15 mm).

The tartrate dihydrate salt of 2 was also prepared, mp 101–104 °C (water) (lit[21] mp 105–106 °C). IR (KBr, cm^{-1}): 3320 (s), 3270 (s), 2800–2560 (br, m), 1736 (m), 1563 (s), 1415 (m), 1306 (s), 1263 (s), 1215 (m), 1136 (m), 1076 (m), 1068 (m), 738 (m), 682 (s). ^{1}H NMR (200 MHz, DMSO-d_6): 3.22–3.59 (m, 4 H, 2 H_2O), 3.68 (s, 3 H, NCH_3), 4.32 (s, 2 H, 2 CH), 4.84–5.47 (m, 2 H, 2 OH), 6.12–6.30 (m, 2 H), 6.91–6.95 (m, 1 H), 7.44–7.51 (m, 1 H), 7.85–7.93 (m, 1 H), 8.49–8.68 (m, 2 H), 12.31–13.33 (m, 2 H, 2 CO_2H) ppm. Anal. Calcd for $C_{14}H_{20}N_2O_8$: C, 48.84; H, 5.85; N, 8.14. Found: C, 48.81; H, 5.79; N, 8.12.

2-(1-Methylpyrrol-2-yl)pyridine (α-nicotyrine) (1) was obtained from *N*-methyl-1-(2-pyridyl)ethanimine *N*-oxide and phenyl vinyl sulfoxide. Yield 29%, bp 106–110 °C (1.1 mm) [lit.[21] bp 150 °C (22 mm)]. IR (NaCl plate, cm^{-1}): 1589 (s), 1560 (m), 1542 (m), 1490 (s), 1455 (s,) 1437 (m), 1318 (m), 773 (m), 720 (m). ^{1}H NMR (200 MHz, $CDCl_3$): 3.98 (s, 3 H, NCH_3), 6.15–6.18 (m, 1 H), 6.54–6.57 (m, 1 H), 6.70–6.72 (m, 1 H), 7.00–7.07 (m, 1 H), 7.48–7.65 (m, 2 H), 8.53 (d, 1 H, J = 4.4 Hz) ppm. Anal. Calcd for $C_{10}H_{10}N_2$: C, 75.92; H, 6.37; N, 17.71. Found: C, 75.98; H, 6.43; N, 17.71.

Registry No. 1, 525-75-7; 2, 487-19-4; 2·tartrate, 4315-37-1; 3, 350-03-8; 4, 119908-57-5; 5, 20451-53-0; 2-acetylpyridine, 1122-62-9; *N*-methyl-1-(2-pyridyl)ethanimine *N*-oxide, 119908-58-6.

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Microbial Transformations. 10. Evidence for a Carbon-Radical Intermediate in the Biohydroxylations Achieved by the Fungus *Beauveria sulfurescens*

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The ability of cytochrome P-450 type enzymes to achieve selective hydroxylation of nonactivated carbon atoms is one of the most fascinating aspects of enzymatic reactions that the organic chemist can use to perform chemical synthesis. These reactions are, up to now, almost impossible to achieve with acceptable selectivities and yields by using chemical methods.[1] However, the mechanism involved in these reactions is still not unambiguously established. In particular, a widespread belief exists that the cytochrome P-450 enzymes perform their hydroxylation reactions with retention of configuration at the hydroxylated carbon, perhaps by way of a direct insertion into a C–H bond achieved by a so-called "oxenoid" species. However, some apparently puzzling results have been obtained recently as far as the stereochemical outcome of these processes is concerned. Starting from prochiral or enantiomerically pure compounds, four possibilities can be selected out of these results: (a) The observed reactions are highly stereoselective, as far as the abstracted hydrogen is concerned, and lead to one single stereoisomeric alcohol with retention of configuration. (b) The hydrogen abstraction is stereoselective, but the reaction leads to a mixture of stereoisomeric alcohols. (c) The reaction involves nonstereoselective hydrogen abstraction but leads to one single stereoisomeric alcohol. (d) No stereoselectivity is observed in either the hydrogen abstraction or the formation of the resulting alcohols. Case a has been observed in numerous studies[2] as, for instance, by Corey et

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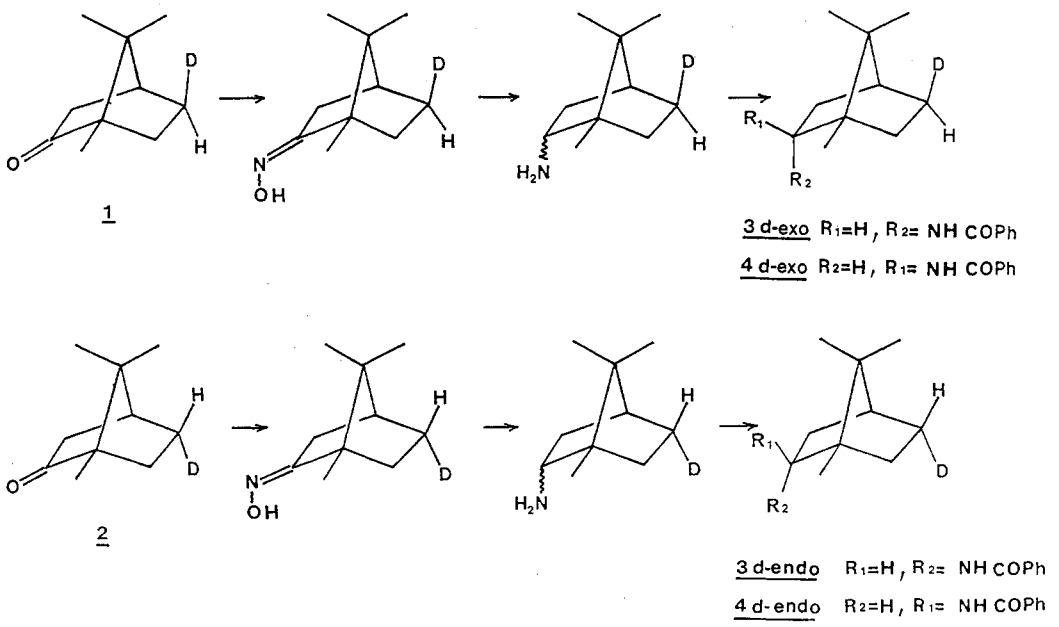
Scheme I. Synthesis of C(5)-Deuterated Isotopomers of Bornylamides 3 and 4



al., who have examined some hydroxylation processes of steroids,[3] or more recently by Englard et al., who have studied the stereochemical course of the hydroxylation of γ-butyrobetaine,[4] or by Ortiz de Montellano et al.[5] as well as by Sligar et al. during their study on stereospecific deuterated norcamphor models.[6] Case b has been observed for instance by Leak et al., in the course of their work on various cyclohexane derivatives, and by Holland et al., who have studied the hydroxylation of ethylbenzene by the fungus *Mortierella isabellina*.[7] Case c has been reported by Sligar et al., who have determined the stereochemistry and deuterium isotope effects in camphor isotopomer hydroxylations, using the cytochrome P-450$_{cam}$ monooxygenase system.[8] Finally, case d has been observed by Groves et al., in the case of some norbornane isotopomer hydroxylations[9] as well as, much more recently, by White et al., in a very elegant and complete study of the hydroxylation stereochemistry of isotopically substituted phenylethane substrates achieved with a single isozyme of rabbit liver microsomal cytochrome P-450.[10]

As far as the mechanism of these hydroxylations is concerned, it appears that, whereas case a may be perfectly consistent with a discrete "oxene" intermediate leading to direct insertion into the C–H bond,[11] cases b and c indicate that a more complex mechanism must be considered. Thus, a two-step process involving the formation of an intermediate radical species has been proposed by Groves et al.[9] and has also been invoked by some other groups, on the basis of results obtained by using essentially purified and/or reconstituted P-450 hemoproteins.[5-10] As stated by White et al., this mechanism is particularly interesting since it allows a unified view of the biological hydroxylation process. Indeed, the first step involves abstraction of the hydrogen atom by an oxo-heme species, thus leading to a carbon-radical intermediate, the second step being the delivery of a hydroxyl moiety from the enzyme system to this radical. This mechanism allows all the various previously mentioned possibilities (a–d) to be explained, and the observed stereoselectivities will essentially depend upon sterical constraints of the substrate binding site provided by the protein environment.

In this context we now describe the results we have obtained studying the hydroxylations achieved by the fungus *Beauveria sulfurescens* which, to our best knowledge, have never been studied from the mechanistic point of view.

Results

In a previous work, we have studied the biohydroxylation of various bridged bicyclic amides and used these models as molecular "probes" to explore the topology of the hydroxylating enzyme(s) of *B. sulfurescens*. In particular, we have observed that, in the case of camphor derivatives bearing an amide function in the C(2) position, a C(5)-hydroxylated product was obtained.[12] Moreover, we had noticed that, whatever the configuration (endo or

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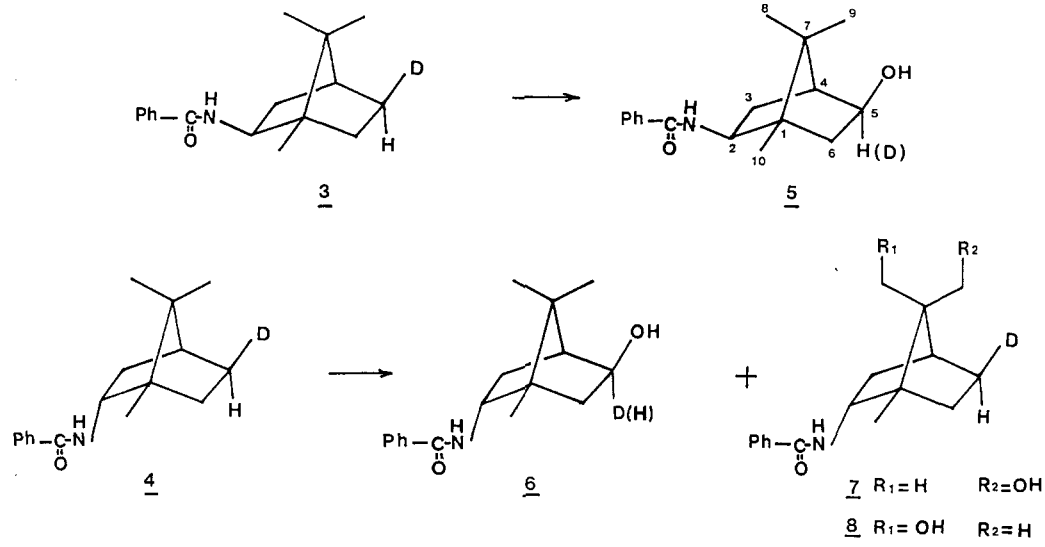
Scheme II. Biohydroxylation Products of Bornylamides 3 and 4



exo) of the amide side chain of the starting substrate was, the obtained hydroxyl function exclusively showed the exo configuration. This was quite a surprising result as far as the enzymatic mechanism was concerned. Indeed, it is very likely that the electron-rich oxygen atom of the amide moiety is used as an anchoring site in order to achieve positioning of the substrate into the enzymatic active site. This has been postulated on the basis of various results[13] and has been recently demonstrated by X-ray crystallographic studies of a cytochrome P-450 camphor complex.[14] Owing to this fact, it was reasonable to predict that a different positioning of the substrate into the site, governed by the exo or endo configuration of the amide side chain, would induce a different stereoselectivity of the hydroxylation process, thus leading to either the exo or the endo C(5) alcohol. As this appeared not to be the case, we thought that one explanation could be that a two-step mechanism, involving a discrete carbon-radical intermediate, could be operative. In order to check this hypothesis, we decided to study the biohydroxylation of stereospecifically deuterium labeled substrates.

The racemic camphor derivatives **1** and **2**, bearing an exo or endo deuterium atom in the C(5) position, have been prepared by following the procedure previously described by Sligar et al.[8] Starting from these isotopomers, we have synthesized the mixture of epimeric **3**-*d*-*exo* and **4**-*d*-*exo* as well as that of the **3**-*d*-*endo* and **4**-*d*-*endo*, following the straightforward method indicated in Scheme I. Thus, the camphor oximes are reduced to the corresponding epimeric amines as previously described by Paquette et al.[15] These are directly benzoylated to yield a 40/60 mixture of **3**-*d*-*exo* and **4**-*d*-*exo* (or **3**-*d*-*endo* and **4**-*d*-*endo*). The deuterium content of each one of the **3**-*d*-*exo*, **3**-*d*-*endo*, **4**-*d*-*exo*, and **4**-*d*-*endo*, as well as that of the resulting hydroxylated products, has been determined by using the coupled mass spectrometry–gas chromatography technique. The respective proportions of the $M^{\bullet+}$ and $(M + 1)^{\bullet+}$ peaks have been measured, and the deuterium content has been deduced from these measurements, taking into account the value of the $(M + 1)^{\bullet+}$ peak present in the nondeuterated substrates. It thus appears that our starting substrates exhibit a deuterium content higher than 95%, whereas different values are obtained for the C(5)-hydroxylated products.

We have previously described hydroxylation of the nondeuterated isomeric substrates **3** and **4** and shown that, whereas racemic **3** leads to one single racemic alcohol **5**, endo amide **4** affords a mixture of three products **6**, **7**, and **8** (respective proportions 36%, 20%, and 44%)[12] which show some optical activity. Although these proportions slightly depend upon the absolute configuration of the substrates, the hydroxylated carbon atoms are identical from one enantiomer to the other, thus permitting further studies on racemic material. Hydroxylation of each one of the mixtures of exo (or endo) deuterated benzoyl amides **3** and **4** affords the same products **5**, **6**, **7**, and **8** (respective proportions 43%, 20%, 12%, and 25%) (Scheme II).

Starting from the mixture of the C(5) exo deuterated epimers **3** and **4**, we observe values of respectively 59% and 67% deuterium content for **5** and **6**, whereas starting from the C(5) endo deuterated substrates **3** and **4**, values of 65% and 83% deuterium content are obtained for **5** and **6**. As expected, the deuterium contents of alcohols **7** and **8** are shown to be unchanged (>95%) relative to the starting substrates. This does rule out a very improbable, but still not impossible, hydrogen shift from C(9) to C(5).

For comparison, we have performed quantitative analysis of the 200-MHz ^1H NMR spectra of products **6** prepared from the mixture of **3**-*d*-*endo* and **4**-*d*-*endo*. The thus-obtained value of deuterium content is 81% (against 83% measured by the GC–MS technique).

Discussion

Interestingly enough, it appears that, starting from both exo-deuterated substrates **3** and **4**, *we do observe a high deuterium retention ratio*, as shown by the relatively low values of d_1/d_0 obtained for alcohol **5** ($d_1/d_0 = 1.5$) as well as for alcohol **6** ($d_1/d_0 = 2$). This is quite a surprising fact owing to the exclusive formation of the C(5) exo alcohols **5** and **6**. Similarly, as far as the result of the hydroxylation of **3**-*d*-*endo* and **4**-*d*-*endo* mixture is concerned, a low value of d_1/d_0 is obtained for compound **5** formed from **3** ($d_1/d_0 = 1.9$), whereas a much higher value of 4.9 is measured for **6** formed from **4**.

The more meaningful result thus obtained is the important ratio of deuterium retention observed when starting from either **3**-*d*-*exo* or **4**-*d*-*exo*. *This implies an inversion of the C(5) stereochemistry* and thus leads un-

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Table I. Deuterium Contents of the Hydroxylation Products Obtained from C(5) Isotopomers of 3 and 4

substrate	product	mol % d_1	% C(5)-endo abstraction	d_1/d_0
3-*d-exo*	5	59	59	1.5
3-*d-endo*	5	65	35	1.9
4-*d-exo*	6	67	67	2
4-*d-endo*	6	83	17	4.9

ambiguously to the conclusion that *a stepwise mechanism has to be involved* in this process. Thus, as a first step, abstraction of a C(5) hydrogen atom involves either the exo or the endo hydrogen (or deuterium). Furthermore, one must notice that, when the C(5) deuterium is placed in the endo position, the endo configuration of the amide moiety induces, presumably because of a different positioning into the active site, a much higher stereoselectivity for the exo hydrogen abstraction. Thus it appears that, in either case, this first step is *not* stereoselective.

In the case of amide **3**, it also appears that there is not much of an isotope effect (k_H/k_D) at either exo or endo position. Such low values have been previously observed, for instance, for deuterated camphor isotopomers[8] as well as for hydroxylation of bicyclo[2.1.0]pentane.[16] This can be due to various factors, i.e., the fact that hydrogen abstraction is not the rate-determining step, and/or the geometrical nonlinearity of the transition state involved in this process.[17] An alternate explanation would be that each different H abstraction proceeds from a unique initial enzyme–substrate complex and that the interconversion among these complexes is slower than forward progress to products. However, this would also imply (1) a very high regio- and stereoselective hydrogen abstraction process in each one of these complexes and (2) that the positioning of the substrate into the active site, i.e., the unambiguous formation of each one of these slightly different enzyme–substrate complexes, would be in part governed by the C(5) endo or exo configuration of the *deuterium* atom. As we feel these hypotheses not very plausible, we consider this explanation as being highly improbable.

As far as the results obtained from **4** are concerned, it appears that a relatively small, yet significant, primary isotope effect does appear. Indeed, deuterium substitution at the C(5) exo or C(5) endo position considerably alters the proportion of H(5) endo abstraction, a fact that can be considered as being a type of metabolic switching. This has been visualized in Table I, where the percentage of C(5) endo abstraction indicates that, whereas the variation of this value is only by a factor of 2 in the case of exo amide **3**, its value varies by a factor of 4 in the case of endo amide **4**. Another type of metabolic switching would have been the significant increase of C(8) and C(9) hydrogen abstraction upon C(5) exo deuteration. Interestingly, however, such an effect, which would lead to formation of higher proportions of **7** and **8** from C(5) exo deuterated **4**, has not been observed. This is quite surprising as compared to the previously described metabolic switching described for various stereospecifically deuterated norcamphor models[6] or octane,[18] but is consistent with the rather low observed isotope effect. It suggests that substrate positioning into the active site does not allow, because of steric interactions with the surrounding apoprotein, easy variation of the hydroxylation regioselectivity. Similar results have been reported previously with camphor as substrate,[8] and it has been shown that altered regioselectivity is observed only when a large kinetic barrier to hydroxylation has been presented, as was demonstrated with 5,5-*gem*-difluorocamphor metabolism to 9-hydroxycamphor.[19] The lack of such an effect may be due also to the fact that H(5) endo abstraction is still energetically favored compared to H(8) or H(9) abstraction. Finally, an alternate quite plausible explanation would be that **4** binds predominantly in two different Michaelis complexes, one in which C(5) hydrogens but not the methyl ones are easily available for attack, and one in which both *gem*-dimethyls but not C(5) hydrogens are available for hydrogen abstraction.

It is also interesting to compare the results obtained for both isomers **3** bearing an exo or endo deuterium at C(5) with those obtained for the isotopomers **4**. It appears that, in the case of **3**, the configuration of the deuterium atom has very little influence on the d_1/d_0 ratio, leading to the conclusion that the stereoselectivity of the hydrogen abstraction is essentially governed by the primary isotope effect. Thus, the hydroxylation of **3**-*d-exo* occurs predominantly by abstraction of the H(5) endo hydrogen, whereas abstraction of the H(5) exo hydrogen is favored for amide **3**-*d-endo*. On the other hand, in the case of **4**-*d-exo*, abstraction of H(5) endo is favored by a factor of 2 ($d_1/d_0 = 2$) whereas for **4**-*d-endo*, abstraction of H(5) exo is much more highly favored ($d_1/d_0 = 4.9$). These observations lead to the conclusion that, in the case of amides **3**, the positioning of the substrate into the enzymatic active site places the hydrogen-abstracting entity "in between" the two C(5) hydrogen atoms, whereas in the case of endo amides **4**, this entity is located much more favorably for abstraction of H(5) exo. In other words, this is an additional argument to the previous hypothesis that this amide moiety plays an "anchoring" role in the enzyme–substrate complex formation, and that the configuration of the amide moiety partly governs the stereochemistry of the hydrogen (or deuterium) abstraction. It is quite interesting to emphasize the fact that this conclusion is consistent with the hydroxylation *regioselectivities* we observe and perfectly fits with our model of the hydroxylation site previously proposed.[13] Indeed, in the case of exo amides **3**, the anchoring of the amide moiety will lead to a positioning of the C(5) hydrogen atoms so as to make them equivalent for the abstracting species (Scheme III). On the other hand, the endo amide isomer **4** will be forced into a position where the carbon skeleton is completely rocked over, leading to a much higher proximity of the hydrogen-abstracting species with the H(5) (or D(5)) exo atom.

It is also worth noting that the experiments reported herein utilized racemic substrates, similarly to the study previously described by Sligar et al. on racemic norcamphor.[6] Although we have shown previously[12] that the enantiomers of these substrates are processed with different kinetic parameters, we feel that, owing to the relatively small differences thus observed, our actual results should not be noticeably different for the pure enantiomers and thus should not affect our overall results and the above discussion.

At this point of the discussion, the problem remains to determine the real nature of the intermediate formed in the first step of the mechanism. A priori, two hypotheses may be valid: the formation of a C(5) carbocation or the formation of a carbon-centered radical. However, the best evidence presently available suggests that the reactive

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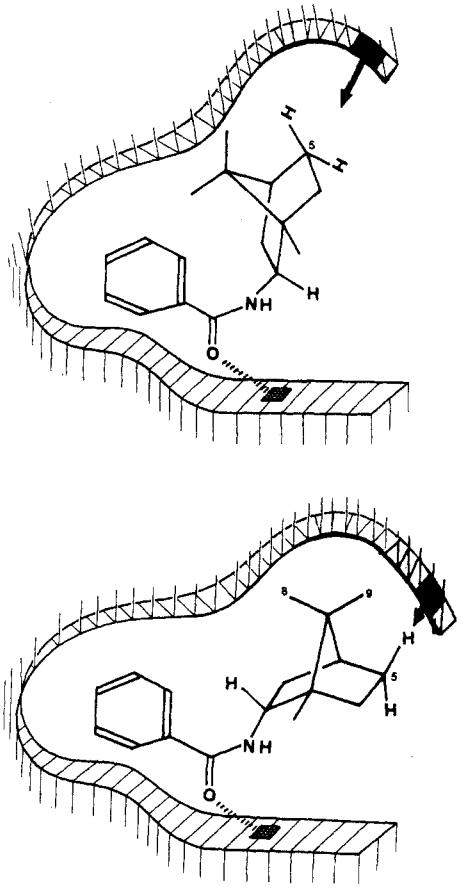
Scheme III. Positioning of *exo*- and *endo*-Bornylamides 3 and 4 into the Model Postulated for the Hydroxylating Site of *B. sulfurescens*



oxygen intermediate of the cytochrome P-450 enzymes is an oxo-iron species that is two oxidation equivalents above the ferric state, formally equivalent to peroxidase compound I.[20] This is expected to bear unpaired electron density in the Fe–O orbitals, which are perpendicular to the heme plane.[21] Thus, if this intermediate is formed in the hydroxylation process, it is highly probable that a carbon radical will be formed. This radical will subsequently be trapped by a homolytic hydroxyl transfer from the enzymatic active site, the stereochemistry of this transfer being governed by the location of this entity relative to the radical and, also, by the intrinsic reactivity of this radical. However, as it is known that camphor C(5)-centered carbon radicals can react from either the exo or the endo side,[22] we must conclude that the exclusive exo configuration of the obtained alcohol essentially results from the localization of the hydroxyl entity. Therefore, our results would be more consistent with a more recent mechanism proposal which involves, as a first step, the homolytic release of a hydroxyl radical, which would be able to abstract either the exo or the endo C(5) hydrogen atom, followed by a stereospecific delivery of an iron-bound activated oxygen atom.[23]

As a conclusion, we have shown that, in the case of the biohydroxylations of amides achieved by the fungus *B. sulfurescens*, a stepwise mechanism involving very likely a carbon-centered radical as an intermediate must be operative. We also have shown that the configuration of the amide moiety is an important factor which partly governs the stereochemistry of the hydrogen abstraction occurring in these processes. This confirms the "anchoring" role previously attributed to this function and is, to our best knowledge, the first time that such an influence of a remote-substituent configuration on the hydrogen-abstraction selectivity is observed.

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Experimental Section

General Procedures. The strain used in the present work is *B. sulfurescens* ATCC 7159, originally purchased as *Sporotrichum sulfurescens*. The culture conditions used have been described previously (see, for instance, ref 7c). The ^1H and ^{13}C NMR spectra have been realized on a Bruker AM 200 apparatus using $CDCl_3$ as solvent. Chemical shifts (δ) are given in parts per million relative to TMS as internal standard. IR spectra were recorded by using a Beckman Acculab 4 spectrometer using chloroform as solvent. Elemental analyses of C, H, N were performed by the Service Central d'Analyse du CNRS (Vernaison, France).

Synthesis of 5-*endo*- and 5-*exo*-Deuteriocamphor (1 and 2). These two key intermediates have been obtained according to the procedure described in ref 8. The intermediate 5-deuterio pericyclo camphanone was carefully purified and submitted to NMR analysis: ^1H NMR ($CDCl_3$) 0.8, 0.9, 0.96 (3 s, 3 CH_3, nonattributed), 1.43 (d, 1 H, J = 5.4 Hz, C(3)-H or C(4)-H), 1.7 (d, 1 H, J = 10.8 Hz, C(6)-H_{endo} or C(6)-H_{exo}), 1.94, (d, J = 10.8 Hz, C(6)-H_{endo} or C(6)-H_{exo}), 1.97 (d, 1 H, J = 5.4 Hz, C(3)-H or C(4)-H). Irradiation at 1.43 ppm leads to a singlet at 1.97.

Synthesis of 5-Deuterio-*N*-benzoyl-2-aminobornane (3 and 4). The two mixtures of 5-deuterio benzamides were obtained by reduction of the corresponding oximes prepared from 1 and 2 according to ref 15. The reduction is not stereoselective, and the mixture of epimeric amines is benzoylated without separation by using benzoyl chloride. The 40/60 mixture of exo and endo benzamides 3 and 4 is purified by silica gel chromatography. The products are conveniently analyzed by capillary gas chromatography using a 25-m OV 1701 column.

Incubation Experiments and Product Analysis. The two mixtures were used for incubation experiments according to the already described procedure.[12] The bioreactions were performed in 500-mL Erlenmeyer flasks containing 100 mL of culture medium and 400 mg/L of substrate. Two flasks were used for the D-exo benzamides and five for the D-endo mixture. The crude extracts were analyzed by liquid chromatography with an analytical column (5-μm silica gel, 100 × 4 mm i.d.) eluted with a 70/30 ethyl acetate–hexane mixture. Under these conditions, four peaks corresponding to the products **5**, **6**, **7**, and **8** were observed.

Analysis of the crude extracts was also performed by using gas chromatography on an OV 1701, 25-m capillary column at 220 °C. No separation is observed for products **7** and **8**.

Products **5** and **6**, prepared from the less tedious to obtain D-endo benzamides, were isolated by using preparative HPLC (7-μm silica gel, 250 × 9 mm i.d. column) eluted with the same mixture as above. The ^1H NMR spectra were recorded in $CDCl_3$ by using a 200-MHz Bruker AM 200 instrument. Comparison of the integrations of the signals corresponding to the hydrogen atom α to the nitrogen atom and to the one geminal to the hydroxyl group allowed us to determine the deuterium content as being 81%. The crude extracts were analyzed by gas chromatography/mass spectra with a Ribermag apparatus using a 50-m OV 1701 column at 200–280 °C, 4 deg/min. The results were as follows (m/e (intensity)).

3: 257 (20), 258 (4.2). **4**: 257 (21), 258 (4.1). **5**: 273 (6.5), 274 (1.3). **6**: 273 (3.8), 274 (0.6).

3-*d*-*endo*: 257 (0.1), 258 (21), 259 (4.0). **4-*d*-*endo***: 257 (0.2), 258 (22), 259 (4.2). **5**: 273 (2.6), 274 (5.4), 275 (1.4). **6**: 273 (1.4), 274 (6.1), 275 (1.7).

3-*d*-*exo*: 257 (0.5), 258 (20.6), 259 (4.1). **4-*d*-*exo***: 257 (1.1), 258 (22.4), 259 (4.4). **5**: 273 (3.1), 274 (4.6), 275 (0.8). **6**: 273 (1.6), 274 (3.1), 275 (0.6).

Calculation of deuterium percentage K in **5** and **6** is made by using the formula

$$K = \frac{I_{274} - kI_{273}}{I_{274} - kI_{273} + I_{273}} \times 100$$

I_{274} is the intensity of the corresponding peak, and $k = I_{274}/I_{273}$ for the nondeuterated compound.

Catalysis of the Enolization of Acetone by Mono- and Dicarboxylate Bases. The Role of Hydrogen Bonding

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The enolization of acetone catalyzed by a series of aliphatic carboxylate bases (eq 1) was studied by Bell and Lidwell,[1] who reported a Brønsted exponent β of 0.88 for the four bases that comprised the series. As a part of a study of steric and other effects in proton-transfer reactions,[2] we have reexamined and greatly extended their series and have included in it a considerable number of dicarboxylic acid dianions.

$$CH_3CCH_3 + RCO_2^- \longrightarrow CH_3C{=}CH_2 + RCO_2H \quad (1)$$

In Tables I and II, the rate constants for the reaction shown in eq 1 are given for the anions of 12 monocarboxylic acids and the dianions of 13 dicarboxylic acids, together with the corresponding pK values. The Brønsted plot for these bases is shown in Figure 1 and includes appropriate statistical corrections.[3,4] (The number of basic sites in the catalyst and the number of proton sites in its conjugate acid are given by q and p, respectively; q and p are deemed to be 2 and 1 for monocarboxylate catalysts and 4 and 1 for dicarboxylate catalysts.) The monocarboxylate ions fall on the straight line on the left side of the figure, which is governed by eq 2 (correlation coefficient 0.9974).

$$\log (k_{A^-}/q) = -10.90\ (\pm 0.10) + 0.885\ (\pm 0.020)(pK + \log p/q) \quad (2)$$

The Brønsted exponent for this line, 0.89, is virtually identical with that reported by Bell and Lidwell (0.88) for their much smaller set. Such high values of β are generally accepted as being indications of "late" transition states.[5,6]

The behavior of dicarboxylate bases (Table II), which appear on the right side of Figure 1, is not so straightforward. There is a group of eight of these bases that fall on a straight line that is displaced from that of the monocarboxylate bases, but is almost parallel to it; it is governed by eq 3 (correlation coefficient 0.9926).

$$\log (k_{A^{2-}}/q) = -11.24\ (\pm 0.19) + 0.865\ (\pm 0.043)(pK_2 + \log p/q) \quad (3)$$

As can be seen, the slopes of the two lines, which represent the respective Brønsted exponents, are quite close, 0.89 and 0.87 (neglecting the five deviating points), suggesting that the presence of an extra unit of negative charge in the base does not have a great effect on the extent of proton transfer in the transition state. What is the cause, then, of the lateral displacement of the line (by roughly 0.5 pK units) in the direction that suggests that a dianion is *less* effective in removing a proton from acetone than is a monoanion of the same equilibrium base strength? Such an effect has been observed previously in other systems,[7,8] without being satisfactorily explained. It

Table I. Data for the Enolization of Acetone Catalyzed by Monocarboxylate Anions in Water at 25 °C, Ionic Strength 0.1

acid	pK^a	$10^7\ k_{A^-}$, $M^{-1}\ s^{-1}$
$C_6H_5CHOHCO_2H$	3.41	0.143 ± 0.058
$CH_3OCH_2CO_2H$	3.57	0.243 ± 0.039
$HOCH_2CO_2H$	3.83	0.339 ± 0.031
$C_6H_5CH_2CO_2H$	4.31	1.19 ± 0.09
CH_3CO_2H	4.76	2.45 ± 0.06
CD_3CO_2H	4.77[b]	2.31 ± 0.13
$CH_3CH_2CH_2CO_2H$	4.82	2.79 ± 0.05
$(CH_3)_2CHCO_2H$	4.86	3.03 ± 0.41
$CH_3CH_2CO_2H$	4.87	3.22 ± 0.02
c-$C_6H_{11}CO_2H$	4.91	3.19 ± 0.41
$(CH_3)_3CCH_2CO_2H$	5.01[c]	4.24 ± 0.05
$(CH_3)_3CCO_2H$	5.03	4.02 ± 0.09

[a] Values from ref 15 unless otherwise noted. [b] Reference 16. [c] Determined titrimetrically in this work.

Table II. Data for the Enolization of Acetone Catalyzed by Dicarboxylate Anions in Water at 25 °C

no.	acid	pK_2	$10^7\ k_{A^{2-}}$,[a] $M^{-1}\ s^{-1}$	$K_1/2K_E$
1	2-methoxyisophthalic	4.32[b]	0.319 ± 0.057	
2	5-bromoisophthalic	4.36[c]	0.383 ± 0.004	
3	5-iodoisophthalic	4.41[b]	0.512 ± 0.039	
4	isophthalic	4.75[b]	0.985 ± 0.020	0.93[b]
5	5-methylisophthalic	4.82[d]	1.09 ± 0.05	0.91[b]
6	phthalic	5.41[b,e]	2.78 ± 0.11	1.1[f]
7	3-methylglutaric	5.44[g]	4.04 ± 0.34	1.0[b]
8	succinic	5.63[h]	4.96 ± 0.32[i]	1.1[d,j]
9	3,3-dimethylglutaric	6.45[g]	7.74 ± 0.02	5.0[b]
10	*meso*-2,3-diethylsuccinic	6.46[e]	13.5 ± 0.07	2.4[d,k,l]
11	(±)-2,3-diethylsuccinic	6.60[e]	7.29 ± 0.18	8.9[d,kl]
12	diethylmalonic	7.29[e]	15.9 ± 1.5	16.0[f]
13	tetramethylsuccinic	7.41[e]	23.5 ± 0.52	13.5[d,k]

[a] Rate constants measured at 0.05 M ionic strength except where noted. [b] Value determined in this work. [c] Determined from a Hammett plot. [d] Reference 4, p 35. [e] Reference 15. [f] Reference 10. [g] Reference 17. [h] Reference 18. [i] Ionic strength 0.10 M. [j] Reference 19. [k] Reference 20. [l] Water–ethanol, 1:1.

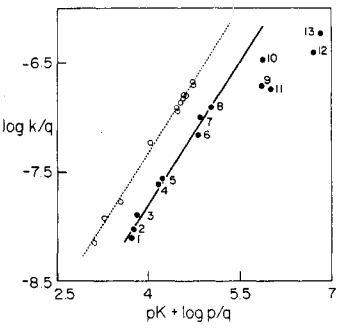

Figure 1. Brønsted plot for enolization of acetone catalyzed by monocarboxylate anions (open circles) and dicarboxylate dianions (closed circles). The numbers that identify the dicarboxylic acids refer to Table II.

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