Steric Course of Hydroxylation at Primary Carbon Atoms. Biosynthesis of 1-Octanol from (1*R*)- and (1*S*)-[1-^{3}H,^{2}H,^{1}H;1-^{14}C]Octane by Rat Liver Microsomes

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Abstract: Incubations of (1*R*)- and (1*S*)-[1-^{3}H,^{2}H,^{1}H;1-^{14}C]octane with rat liver microsomes gave mixtures of 1-octanols. It was shown that hydroxylation at the chiral termini involved a normal hydrogen isotope effect and proceeded with retention; i.e., the incoming hydroxyl assumed the orientation of the displaced hydrogen (or isotopic hydrogen) atom. The ramifications of these observations in the context of proposed mechanisms of enzymic hydroxylation are briefly discussed.

Hydroxylations involving the fixation of one atom of molecular oxygen into a hydroxyl moiety ($RH + O_2 + 2H \rightarrow ROH + H_2O$) are ubiquitous biochemical reactions,[3] the mechanism or mechanisms of which remain obscure.[4] In contrast to numerous reports on the stereochemistry of enzymic hydroxylation at unactivated secondary[5] and tertiary[6] carbon atoms, the steric course of hydroxylation at a primary carbon atom has been documented for only one biochemical system. Previously, we showed[7] that the biosynthesis of 1-octanol from (1*R*)- and (1*S*)-[1-^{3}H,^{2}H,^{1}H;1-^{14}C]octane by cell-free extracts of the bacterium *Pseudomonas oleovorans* strain TF4-1L proceeds with a normal kinetic hydrogen isotope effect and with net retention; i.e., the incoming hydroxyl group assumes the steric orientation of the displaced hydrogen atom.

Hydroxylation appears to be a mandatory first step in the metabolism and disposition of very many xenobiotic substances.[8] Oxidation of these substances occurs mainly in the liver,[9] rendering them more polar and water soluble so that their excretion via the kidney will be more favorable.[10] One route of *n*-alkane metabolism by mammalian liver involves terminal hydroxylation to the homologous *n*-alcohol, which in turn can be further metabolized.[11]

The alkane hydroxylases of *Ps. oleovorans* and rat liver microsomes are structurally quite distinct. These monooxygenases are both sideroproteins; however, whereas P_{450} is a *b*-type cytochrome[12] containing iron protoporphyrin IX at the active site,[13] the prosthetic group of the bacterial enzyme contains nonheme iron.[14] It was thus of considerable interest to compare the steric course of reaction catalyzed by these two different enzymes. In this paper we report the results of our investigation of the stereochemistry of hydroxylation of (1*R*)- and (1*S*)-[1-^{3}H,^{2}H,^{1}H;1-^{14}C]octane by rat liver microsomes.

Experimental Section

Materials. Horse liver alcohol dehydrogenase (HLAD) (E.C. 1.1.1.1, lot no. 40F-8015, 2 U/mg of protein), ovalbumin (grade V), bovine serum albumin (fraction V powder), NAD (grade III), NADH (grade III), NADP (98–100%), NADPH (type I), glucose 6-phosphate (disodium salt), Folin–Ciocalteu reagent (2 N), and DL-α-lipoamide were purchased from Sigma Chemical Co. Porcine heart diaphorase (E.C. 1.6.4.3, grade I, control no. 1360124/Oct 1981) and yeast glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49, grade II, control no. 6473240) were obtained from Boehringer Mannheim GmbH (Mannheim, West Germany). Lithium triethylborodeuteride ($LiEt_3B^2H$; Super-Deuteride, 1 M solution in tetrahydrofuran) was purchased from Aldrich. Silica gel 60 HF 254 + 366 and silica gel 60 (70–230 mesh) (both purchased from E. Merck A.G., Darmstadt, West Germany) were used for thin-layer chromatography and column chromatography, respectively.

Liquid scintillation counting was performed in a Mark II liquid scintillation system (Nuclear-Chicago) with 15 mL of Liquifluor (New England Nuclear). Preparative GC of 1-octanol was carried out with a Varian Aerograph model 920 gas chromatograph equipped with a Supelco stainless steel 10% QF-1 Chromosorb W-HP column (100–120 mesh, $^1/_4$-in. diameter × 6-ft length) and a thermal conductivity detector. Melting points were determined with a Monoscop IV hot stage apparatus (H. Bock, Frankfurt/M, West Germany) and are corrected.

(1*R*)- and (1*S*)-[1-^{3}H,^{2}H,^{1}H;1-^{14}C]octane. The previously described substrates[7] (1*R*)- and (1*S*)-[1-^{3}H,^{2}H,^{1}H]octane admixed with [1-^{14}C]-octane, were used. (1*R*)-[1-^{3}H,^{2}H,^{1}H;1-^{14}C]octane, specific activity ca. 45 μCi of ^{3}H μL^{-1} (7.3 mCi of ^{3}H mmol^{-1}), [^{3}H]:[^{14}C] ratio 8.96; (1*S*)-[1-^{3}H,^{2}H,^{1}H]octane, specific activity ca. 45 μCi of ^{3}H μL^{-1} (7.3 mCi of ^{3}H mmol^{-1}), [^{3}H]:[^{14}C] ratio 8.47.

Rat Liver Microsomes.[15] Microsomes were prepared from uninduced,[16] unstarved Sprague–Dawley CD male albino rats (150–160 g)

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(Charles River Breeding Laboratories). Each animal was killed by a blow on the head, and the liver was immediately excised and rinsed in ice-cold distilled water. The liver was blotted on filter paper, weighed, minced, and homogenized in 0.25 M sucrose (5 mL/g) with a motor-driven Potter–Elvehjem glass–Teflon tissue grinder. The homogenate was centrifuged at 10000*g* (4 °C, 20 min) and the supernate spun at 105000*g* in a Beckman Model L5-65 ultracentrifuge fitted with a 50Ti fixed-angle rotor (4 °C, 1 h). The supernate was discarded, and the reddish transparent pellets were washed by resuspending them in a few milliliters of ice-cold 1.15% KCl and recentrifuging at 105000*g* (4 °C, 1 h). Supernates were discarded, and the pellets (ca. 15 mg of protein[17] each) were resuspended in 100 mM potassium phosphate, pH 7.4 (5 mL).

Octane Hydroxylation by Rat Liver Microsomes. Hydroxylation experiments with (1*R*)-[1-^{3}H,^{2}H,^{1}H;1-^{14}C]octane and (1*S*)-[1-^{3}H,^{2}H,^{1}H;-1-^{14}C]octane were conducted in parallel with liver microsomes obtained from the same rat. Microsomes (ca. 15 mg of protein in 5 mL of 100 mM potassium phosphate, pH 7.4), glucose 6-phosphate (0.07 mmol), and glucose-6-phosphate dehydrogenase (12 μL, 12 μg, 0.2 U[18]) were shaken at room temperature (10 min), NADP and NADPH (6 μmol each in 1 mL of 100 mM postassium phosphate, pH 7.4) were added, and the reaction was initiated by addition of chiral octane (1 μL, 6 μmol, 45 μCi of ^{3}H) in 1-octanol (20 μL). After the mixture was shaken at 30 °C for 4 h in tightly sealed (25 mL) Corex centrifuge tubes, incubations were terminated by chilling (ice bath) the reaction mixtures and saturating them with NaCl. Unlabeled 1-octanol (100 μL) was added to each reaction vessel, and the mixtures were extracted with CH_2Cl_2 (2 × 10 mL). The aqueous phase was then acidified with 10% H_2SO_4 (0.5 mL) and reextracted with CH_2Cl_2 (2 × 10 mL). The CH_2Cl_2 extracts from each incubation were pooled, dried (Na_2SO_4), and distilled to 1–2 mL through a 20-cm Vigreux column. 1-Octanol was recovered by chromatography on a column of silica gel (11 g)[7b] and purified by preparative GC (column temperature, 85 °C; injection port temperature, 200 °C; detector temperature 220 °C; He flow rate, 60 mL/min; R_t of 1-octanol, 13 min). [^{3}H]:[^{14}C] Ratios of the recovered 1-octanols were determined on the *p*-tolylurethane derivatives.

Analysis of Biosynthesized Octanols. Exchange reactions were carried out in parallel with 1-octanol (20 μL) derived enzymically from (1*R*)- and (1*S*)-[1-^{3}H,^{2}H,^{1}H;1-^{14}C]octane and synthetic (1*RS*)-[1-^{3}H,^{1}H;1-^{14}C]octanol (20 μL) in the system HLAD–NAD/NADH–diaphorase.[7,19] The mixtures were vigorously shaken in the dark at 35 °C (24 h), and the reactions were terminated by immersion in an ice bath and addition of NaCl (3 g). Mixtures were extracted with CH_2Cl_2 (2 × 10 mL); the aqueous phase was then acidified with 10% H_2SO_4 (0.5 mL) and reextracted with CH_2Cl_2 (2 × 10 mL). The CH_2Cl_2 extracts from each reaction were pooled, dried (Na_2SO_4), and distilled through a 20-cm Vigreux column. The residue was taken up in dry benzene (3 mL), to which *p*-tolyl isocyanate (2 drops) and pyridine (3 drops) were added. The solution was refluxed (90 min) and the product recovered with ether (30 mL). The ether solution was washed with 1 N HCl (2 × 10 mL), H_2O (10 mL), and saturated aqueous NaCl (10 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was fractionated by TLC (20 cm × 20 cm plate; 99:1 benzene–ethyl acetate), and octyl *p*-tolylurethane was recovered (ether) and crystallized from 9:1 95% ethanol–H_2O as tiny white needles, mp 62 °C.

Oxidation of Octanols to Octanoic Acids. Jones' reagent[20] (0.4 mL) was added dropwise to a chilled, stirring solution of biosynthetic octanol (30 μL, 0.19 mmol) in acetone (1.0 mL). After 5 min the reaction was terminated with 2-propanol, and the products were extracted with pentane (4 × 5 mL). The pentane solution was washed with saturated aqueous NaCl (2 × 1 mL), and acids were extracted with 1 N NaOH (4 × 1 mL). The alkaline solution was chilled in an ice bath, acidified (concentrated HCl) and extracted with pentane (3 × 3 mL). The pentane solution was dried (Na_2SO_4) and distilled. The residue was taken up in a solution of dicyclohexylcarbodiimide (250 mg, 1.21 mmol) and *p*-toluidine (70 mg, 0.65 mmol) in dry CH_2Cl_2 (10 mL) and shaken overnight at room temperature. The reaction mixture was washed with 1 N HCl (2 × 10 mL), H_2O (10 mL), and saturated aqueous NaCl (10 mL) and dried (Na_2SO_4). The filtrate was rotary evaporated to an oily residue, and 1:1 pentane–ether was added dropwise until the mixture solidified. The solid was stirred with ether (3 × 15 mL), and the combined extract was filtered and concentrated under reduced pressure. The residue was fractionated by TLC (20 cm × 20 cm plate; 9:1 benzene–ethyl acetate). Octanoyl *p*-toluidide was eluted with ether, crystallized from *n*-hexane as tiny white needles (mp 69 °C; lit.[21] mp 70 °C), and counted.

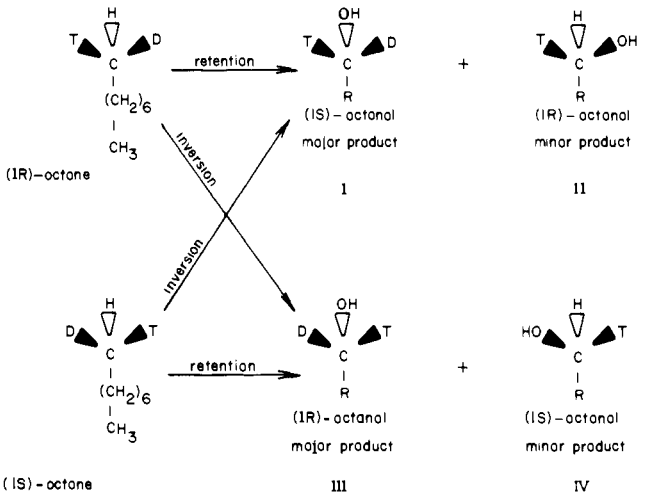

Figure 1.

Results and Discussion

As previously reported,[7] our synthesis of (1*R*)- and (1*S*)-[1-^{3}H,^{2}H,^{1}H;1-^{14}C]octane proceeded through the intermediacy of (1*S*)- and (1*R*)-[1-^{3}H,^{1}H]octanol. The tritiated chiral alcohols were mixed with [1-^{1}H$_2$,1-^{14}C]octanol, and the mixtures were converted to the corresponding mesyl esters. (1*R*)- and (1*S*)-[1-^{3}H,^{1}H;1-^{14}C]octyl mesylate were treated with lithium triethylborodeuteride to yield (1*S*)- and (1*R*)-[1-^{3}H,^{2}H,^{1}H;1-^{14}C]-octane, respectively. The tritium specific activity of the hydrocarbons was ca. 7.3 mCi mmol^{-1}, and their [^{3}H]:[^{14}C] ratio was ca. 8.50. Assuming that $LiEt_3B^2H$ was fully deuterated (~100% ^{2}H), the mode of synthesis of the octanes requires that ~100% of the molecules be monodeuterated. Given that the radioactivity of tritium at 100% isotopic abundance is 29 Ci mmol^{-1} and that of carbon-14 is 62.4 mCi mmol^{-1} the percentages of chiral [1-^{3}H,^{2}H,^{1}H]octane and [1-^{3}H,^{2}H,^{1}H;1-^{14}C]octane in the hydrocarbon mixtures are calculated to be ca. 0.025% and ca. 1.4%, respectively. The composition of the resultant octane mixtures used for enzymic hydroxylation is as in **1**.

1.

a	$H_3C-(CH_2)_6-CH_2D$	(ca. 98.5%)
b	$H_3C-(CH_2)_6-{}^{14}CH_2D$	(ca. 1.4%)
c	$H_3C-(CH_2)_6-CHDT$	(ca. 0.025%)

It was anticipated that hydroxylation of octane would proceed with a normal kinetic hydrogen isotope effect and stereospecifically in either the retention or inversion mode (Figure 1). It follows that, irrespective of the chirality of the substrate, mixtures of (1*R*)-[1-^{3}H]octanol and (1*S*)-[1-^{3}H]octanol will be formed. Depending upon whether hydroxylation at a chiral terminus (1*R* or 1*S*) proceeds with retention or inversion, mixtures of (1*R*)- and (1*S*)-[1-^{3}H]octanol will be produced in different proportions (Figure 1).[22] The chiral composition of the mixtures of [1-

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Table I

expt	source of octanol	$[^3H]:[^{14}C]$ ratio of octanol[a]	octanoic acid $[^3H]:[^{14}C]$ ratio[b]	octanoic acid % of ^3H lost on oxid
1	(1*R*)-[1-^{3}H,^{2}H,^{1}H;1-^{14}C] octane	6.84	5.13	25
	(1*S*)-[1-^{3}H,^{2}H,^{1}H;1-^{14}C] octane	7.86	5.58	29
2	(1*R*)-[1-^{3}H,^{2}H,^{1}H;1-^{14}C] octane	7.68	5.24	32
	(1*S*)-[1-^{3}H,^{2}H,^{1}H;1-^{14}C] octane	8.29	5.87	29

[a] Counted as the *p*-tolylurethane in 15 mL of Liquifluor. [b] Counted as the *p*-toluidide in 15 mL of Liquifluor.

Table II

expt	source of octanol	$[^3H]:[^{14}C]$ ratio[a] of octanol	$[^3H]:[^{14}C]$ ratio of octanol[a] recovd after 24-h incub
1	(1*R*)-[1-^{3}H,^{2}H,^{1}H;1-^{14}C] octane	6.84	5.95
	(1*S*)-[1-^{3}H,^{2}H,^{1}H;1-^{14}C] octane	7.86	5.80
	synthetic (1*RS*)-[1-^{3}H,^{1}H;1-^{14}C] octanol	5.98	2.02
2	(1*R*)-[1-^{3}H,^{2}H,^{1}H;1-^{14}C] octane	7.68	6.21
	(1*S*)-[1-^{3}H,^{2}H,^{1}H;1-^{14}C] octane	8.29	6.35
	synthetic (1*RS*)-[1-^{3}H,^{1}H;1-^{14}C] octanol	6.39	1.73

[a] Counted as the *p*-tolylurethane in 15 mL of Liquifluor.

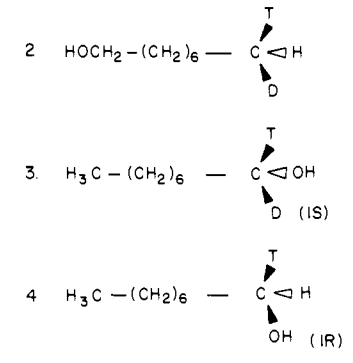
Table III. Hydroxylation of (1*R*)- and (1*S*)-[1-^{3}H,^{2}H,^{1}H;1-^{14}C] Octanes by Rat Liver Microsomes

expt	chirality of octane	% ^3H at C-1 of octanols	% of (1*R*)-[1-^{3}H;^{14}C] octanol	C-1 chirality of the major octanol	stereochemistry of hydroxylation
1	1*R*	25	24	1*S*	retention
	1*S*	29	85	1*R*	retention
2	1*R*	32	26	1*S*	retention
	1*S*	29	63	1*R*	retention

[a] The average deviation in the $[^3H]:[^{14}C]$ ratios from successive crystallizations of the acid and alcohol derivatives is ±0.05. The resulting relative error[24] in the calculated percentages of (1*R*)-[1-^{3}H;^{14}C]octanol is ±7% (e.g., 24 ± 2%).

^{3}H]octanols derived from octanes of opposite chirality must be complementary. Were hydroxylation to proceed nonstereospecifically, or stereospecifically in the absence of an isotope effect, equal, amounts of (1*R*)- and (1*S*)-[1-^{3}H]octanol will be produced regardless of the chirality of the substrate octane.

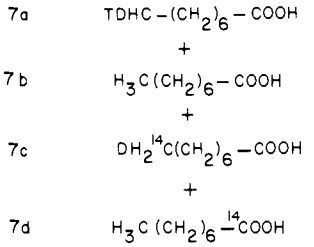
Hydroxylation of (1*R*)-[1-^{3}H,^{2}H,^{1}H;1-^{14}C]octane (**1c** = 1*R*) with a normal hydrogen isotope effect in the retention mode will yield radiolabeled octanols **2**–**6** (**2** > **3** > **4**). The stereochemistry



of hydroxylation can be deduced from the relative amounts of (1*R*)- and (1*S*)-[1-^{3}H]octanols derived from oxygenation at the chiral termini of (1*R*)- and (1*S*)-[1-^{3}H,^{2}H,^{1}H]octane (Figure 1). The simultaneously biosynthesized [^{14}C]octanols **5** and **6** serve as internal references against which changes in tritium content can be gauged.

As indicated earlier, the substrate octanes contained small amounts of C-1 chiral molecules (0.025%), and their enzymic oxygenation gave minute amounts of mixtures of chiral [1-^{3}H]-octanols. The sensitivity of available physicochemical methods of analysis was insufficient for the determination of the chiral composition of the products obtained, and therefore enzymic methods were employed.

Samples of (1*R*)- and (1*S*)[1-^{3}H,^{2}H,^{1}H]octane admixed with [1-^{14}C]octane were incubated with rat liver microsomes and the biosynthesized 1-octanols recovered and analyzed. For determination of the extent of oxygenation at the chiral termini, aliquots of biosynthetic 1-octanol were oxidized (Jones' reagent) to octanoic acids (**7**) and the accompanying change in $[^3H]:[^{14}C]$ ratio was



determined (Table I). On oxidation to acids, tritium was lost only from alcohols obtained by oxygenation at the chiral terminus (e.g., **3** and **4** give **7b**). The difference in $[^3H]:[^{14}C]$ ratio between biosynthesized octanol and derived acid also defines the maximal amount of tritium at C-1 of octanol that is available for exchange in the equilibration procedure.[7,19]

Exchange reactions were carried out in the system HLAD–NAD/NADH–diaphorase. It should be noted that in the absence of HLAD, the exchange reaction does not proceed (to be published). Equilibrated alcohols were recovered and converted to *p*-tolylurethanes, which were extensively purified by TLC and multiple crystallization for $[^3H]:[^{14}C]$ ratio determination (Table II). The amount of (1*R*)-[1-^{3}H]octanol in the biosynthetic mixtures was then calculated by correcting the total amount of tritium exchanged for the amount of tritium lost from the 1*S* alcohol.[7,19] The results are summarized in Table III.

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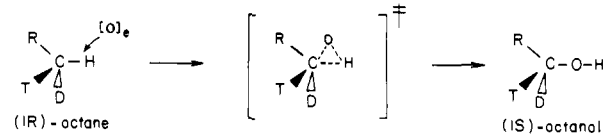

Figure 2.

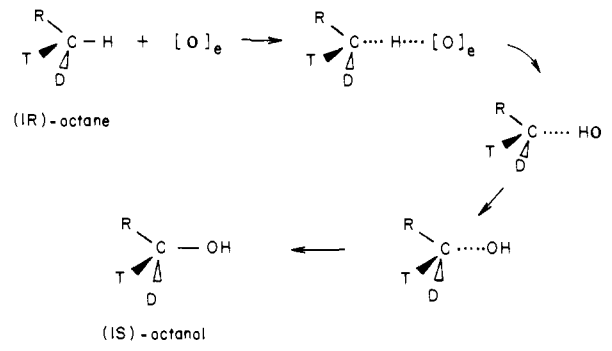

Figure 3.

Hydroxylation of the octanes was observed to occur mainly (ca. 70%) at the achiral methyl terminus; e.g., the main product of oxygenation of (1*R*)-[1-^{3}H,^{2}H,^{1}H]octane is **2**. This indicates that hydroxylation of the methyl groups by rat liver microsomes proceeds with a normal hydrogen isotope effect. Our findings are contrary to those of Hamberg and Björkhem, who reported[25] the formation of 10-hydroxydecanoate from [10-^{2}H$_3$]decanoate by rat liver microsomes without an isotope effect.

Oxygenation of the 1*R* terminus produced mixtures of 1*R*- and 1*S*-tritiated octanols. Kinetic configurational assay[7,19] showed that 24–26% of the octanol in the mixtures was (1*R*)-[1-^{3}H]octanol, and hence the major product of hydroxylation at the chiral methyl terminus of (1*R*)-[1-^{3}H,^{2}H,^{1}H]octane is (1*S*)-[1-^{3}H]octanol (**3**). In contrast, hydroxylation of the 1*S* terminus gave C-1 octanols containing mostly (63–85%) (1*R*)-[1-^{3}H]octanol. These results are consistent with the view that the hydroxylation proceeds with retention, in which a protium atom, and to a lesser extent a deuterium atom, is displaced.

Our results show that oxygenation of octane to 1-octanol by *Ps. oleovorans* strain TF4-1L and by rat liver microsomes proceeds with a normal hydrogen isotope effect and with net retention. Any proposed mechanism(s) of hydroxylation at aliphatic carbons must therefore satisfy these two requirements.

Hamilton postulated[26] that monooxygenases interact with O_2 to form "oxenoid" species capable of inserting a singlet oxygen atom into a C–H bond to produce an alcohol. Oxenoid transfer was envisioned as proceeding by attack of electrophilic oxygen, $[O]_e$, at a σ bond to generate a triangular "Skell–Doering" transition state[27] which collapses to product with retention of configuration (Figure 2). A recent quantum-chemical study of model P_{450} hydroxylations by Pudzianowski and Loew[4b] has suggested that, rather than simple insertion, oxygenation proceeds via a concerted attachment–rearrangement with retention of configuration (Figure 3). Either of these two mechanisms would account for the observed stereochemistry of enzymic hydroxylation. However, since no well-defined or distinct bond-breaking or bond-forming steps are involved in these mechanisms, it is not clear whether hydroxylation would proceed with a primary hydrogen isotope effect,[28] as required by our results.

Similarities between hydroxylations at aliphatic carbons by Fenton's reagent and P_{450}-catalyzed monooxygenations led Groves

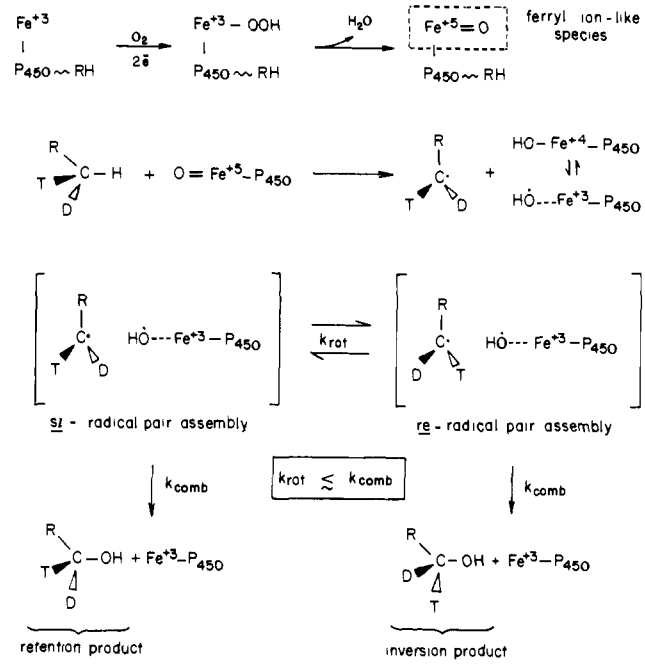

Figure 4.

and his collaborators[29,30] to suggest that $[O]_e$ is a ferryl ion-like species containing pentavalent iron (Fe^{5+}=O) obtained by dehydration of Fe^{3+}–OOH (Figure 4). The position to be hydroxylated is "selected" through its proximity and geometry vis-à-vis the ferryl oxygen atom. Abstraction of a substrate hydrogen by ferryl oxygen results in the formation of Fe^{4+}–OH and a substrate carbon radical. The carbon–hydrogen bond cleavage is expected to involve a primary isotope effect.[28] An electronic rearrangement of Fe^{4+}–OH yields Fe^{3+}---OH, recombination of which with the carbon radical yields oxygenated product and regenerates ferric P_{450}, ready to commence another round of catalysis.

Homolytic cleavage of a carbon–hydrogen bond in a chiral methyl moiety will give rise to a chiral radical pair assembly. This is illustrated for (1*R*)-[1-^{3}H,^{2}H,^{1}H]octane in Figure 4. Homolysis of the C–H bond at the chiral terminus of (1*R*)-[1-^{3}H,^{2}H,^{1}H]-octane gives rise to a radical pair assembly in which the *si* face of the (planar) 1-octanyl radical is oriented toward the hydroxyl radical. Recombination of this assembly will yield the retention product, (1*S*)[1-^{3}H,^{2}H]octanol. Just as a methyl moiety is expected to rotate freely within the active site of an enzyme,[31] so will a primary carbon radical also be torsiosymmetric.[32] An out-of-plane 180° rotation about the R–C· axis generates the enantiometric assembly, in which the *re* face of the 1-octanyl radical is directed toward the hydroxyl radical. Recombination of this assembly will yield the inversion product, (1*R*)-[1-^{3}H,^{2}H]octanol.

The stereochemical outcome of a reaction proceeding through a chiral pair assembly will depend upon the relative rates of rotation (k_{rot}) and combination (k_{comb}). If hydroxylation indeed proceeds via abstraction–recombination as proposed by Groves et al.,[30] then the fact that the reaction occurs with net retention means that $k_{rot} \lesssim k_{comb}$. This is consistent with the observation that "cage" reactions initiated by single-bond homolyses proceed

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with a high degree of retention of configuration.[33]

Our observations on the steric course of hydroxylation at the methyl termini of *n*-octane can be accommodated by either of the discussed mechanisms, each with its own restrictions. The Groves et al.[29,30] mechanism should involve a normal hydrogen isotope effect.[28] Assuming that k_{rot} of the hypothetical trigonal 1-octanyl radical is not greater than k_{comb} with the hydroxyl radical, hydroxylation will proceed with retention as required by our results. Alternatively, simple insertion (Figure 2) or concerted attachment–rearrangement (Figure 3) would require that substitution of an oxenoid species for a hydrogen atom proceed with a primary kinetic isotope effect.

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Registry No. 2, 81133-14-4; **3**, 81133-15-5; **4**, 62012-45-7; (1*R*)-[1-^3H,^2H,^1H]octane, 81133-16-6; (1*S*)-[1-^3H,^2H,^1H]octane, 81133-17-7; (1*RS*)-[1-^3H,^1H]octanol, 80446-73-7; (1*S*)-[1-^3H,^1H]octanol, 62012-44-6; (1*R*)-[1-^3H,^2H]octanol, 81133-18-8; [1-^{14}C]octane, 81133-19-9.

Communications to the Editor

Chemistry of Higher Order Mixed Organocuprates. 2.[1] Reactions of Epoxides†

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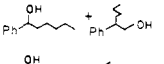
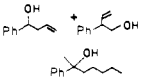
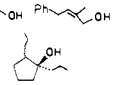
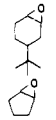
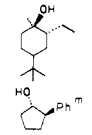
The formation of carbon–carbon bonds via substitution reactions is one of the characteristic modes in which organocopper reagents continue to play an important role in organic synthesis.[3] We have recently described the first general procedure, to our knowledge, for effecting such a process at a secondary, unactivated center. This occurs without significant competition from reduction and/or elimination, through the agency of higher order mixed organocuprates of the general formula $R_2Cu(CN)Li_2$, **1**.[1] As epoxide

$$CuCN + 2RLi \longrightarrow \underset{\mathbf{1}}{R_2Cu(CN)Li_2}$$

cleavage is formally a substitution process,[3a] we were interested in evaluating the reactivity and efficacy of these newer species (i.e., **1**) for effecting ring opening. We now report our preliminary results which indicate that intermediates **1** are perhaps among the mildest and most efficient means available for generating carbon–carbon bonds by way of oxirane cleavage by using organocopper chemistry.

Literature reports of earlier efforts clearly demonstrate that, in general, reactions of homocuprates (i.e., R_2CuLi) with disubstituted epoxides oftentimes lead to mixtures of products resulting from rearrangement or elimination in addition to substitution.[4] Thus, while the efficiency of reactions in monosubstituted systems is high, 1,2-disubstituted cases afford product(s) in low to moderate yields. Two equivalents or more of cuprate are necessary, and reaction temperatures of 0 °C and higher are typical. Mixed Gilman reagents, RCu(CN)Li, are reported[5] to give good reactions with monosubstituted epoxides by using stoichiometric amounts of reagent, and yet in 1,2-disubstituted cases the efficiency drops considerably. Trisubstituted oxiranes, likewise, afford moderate yields of product(s) under these conditions.

Table I. Reactions of Epoxides with $R_2Cu(CN)Li_2$

Entry	Epoxide	RLi	Product(s)[a]	Ratio	Yield, %[b]
1		n-BuLi[c]			95[d]
2		n-BuLi[e]		85.8[f]	93
3		 Li[g]		57.35[f]	92
4		n-BuLi[h]			96
5		PhLi[i]		77.19[n]	96
6		n-PrLi[j]			86[d]
7		EtLi[k]			98
8		PhLi[l]			98

[a] All compounds gave satisfactory IR, NMR, and mass spectral data. [b] Isolated yields unless noted otherwise. [c] Reaction run at −20 °C for 2 h with 1.1 equiv of reagent. [d] By quantitative VPC using a 6 ft × 1/8 in. column (20% SE-30 on Chromosorb W). [e] Reaction was conducted at −40 °C over 2 h with 1.3 equiv of reagent. [f] Determined by isolation. [g] Product formed over 2.5 h at −10 °C using 2.3 equiv of reagent. [h] Starting material consumed in 2 h at −20 °C using 1.3 equiv of reagent. [i] A total of 1.1 equiv of reagent was sufficient at −45 °C over 1.5 h. [j] Run at 0 °C over 8 h with 2 equiv of cuprate. [k] Carried out at 0 °C for 6 h with 2 equiv of reagent. [l] Required room temperature overnight (10 h) with 2 equiv of reagent. [m] No cyclopentanone was detected by VPC. [n] Determined by NMR analysis.

In contrast to reactions of RR′CuLi (R = alkyl, R′ = alkyl, CN), mono-, di-, and trisubstituted epoxides reacted with **1** to afford excellent yields of products(s) in all examples investigated. The results are summarized in Table I. Several noteworthy features of this method deserve comment. As is the case with RCu(CN)Li, ca. 1.1–1.3 equiv of reagent is sufficient for complete consumption of starting material (entries 1, 2, 4). Not surprisingly, the less reactive reagent derived from vinyllithium (i.e., **1**, R = vinyl) required 2.3 equiv, while the fused-ring systems (entries 6–8) gave good reactions with 2 equiv of the corresponding mixed

† Dedicated to Professor H. H. Wasserman on the occasion of his 60th birthday.

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