Mode of Enzymic Oxygenation at Primary Carbon Atoms: Stereochemistry of Hydroxylation of C-1 Chiral Octanes by *Pseudomonas oleovorans*†

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Summary It was shown, using (1*R*)- and (1*S*)- [1-^{3}H,^{2}H,-^{1}H;^{14}C]octanes, that C-1 hydroxylation by *P. oleovorans* strain TF4-1L proceeds with retention of configuration.

IN contrast to numerous reports on the stereochemistry of enzymic hydroxylation of unactivated secondary[1] and tertiary[2] carbon atoms, no published information is available on the stereochemistry of hydroxylation of primary carbon atoms.[3] The starting materials (1*R*)- and (1*S*)-[1-^{3}H,^{1}H;^{14}C]octan-1-ols, were synthesized essentially as previously described.[4] The (1*R*)-alcohol was at least 95—96% pure, since on oxidation with horse liver alcohol dehydrogenase [HLAD, EC.1.1.1.1] and NAD it lost 95—96% of tritium. By implication the parent[4] (1*S*)-octanol must contain at least 95—96% of (1*S*)-alcohol. The (1*R*)- and (1*S*)-octanols were mesylated, then hydrogenolysed (lithium triethylborodeuteride in diglyme) to yield (1*S*)-[1-^{3}H,^{2}H,-^{1}H;^{14}C]octane {spec. act. *ca.* 45 μCi ^{3}H μl^{-1} (7·3 mCi ^{3}H

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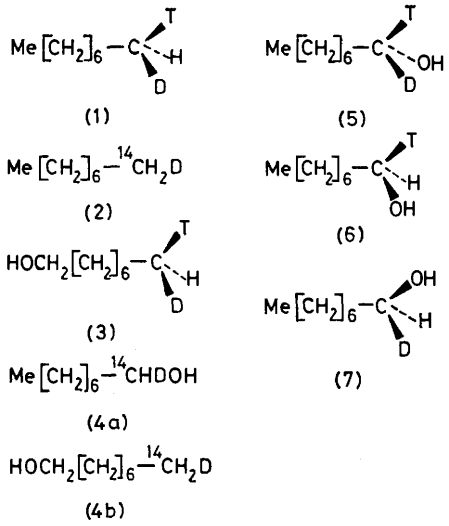
mmol^{-1}), [^{3}H : ^{14}C] ratio 8·47:1} and (1*R*)-[1-^{3}H,^{2}H,^{1}H;^{14}C]-octane {spec. act. *ca.* 45 μCi ^{3}H μl^{-1} (7·3 mCi ^{3}H mmol^{-1}), [^{3}H : ^{14}C] ratio 8·96 : 1}, respectively.‡

We found that the method of determining the stereochemical purity of (1*S*)-octanol by oxidation with horse liver, yeast, or *P. oleovorans* strain TF4-1L alcohol dehydrogenase and measuring the [^{3}H : ^{14}C] ratio of the resulting aldehyde is not applicable. Apparently the [1-^{3}H]octanal produced (RC^{3}HO) is oxidized enzymically to octanoic acid[5] at a considerably *slower rate* than is R^{14}CHO. Consequently, the [^{3}H : ^{14}C] ratio of the aldehydes isolated at a given point in time (from 7 min to 24 h) was higher (up to 40%) than that of the substrate (1*S*)-alcohol.



We then investigated the Günther, Simon *et al.*[6] equilibration method, which is presumed to exchange the (1*R*)-hydrogen atom exclusively.[6,7] In our hands, both the (1*R*)- and (1*S*)-hydrogen atoms of [1-^{3}H]octanols were exchanged, although the (1*S*)-hydrogen was exchanged at a considerably slower rate. Under the conditions employed by us the (1*R*)-octanol lost all the tritium within 24 h, while the (1*S*)-octanol consistently and reproducibly lost 30—40% of tritium, and (1*RS*)-[1-^{3}H; ^{14}C]octanol lost 65—70% of tritium. Based on these observations, determination of the overall chirality of the C-1 alcohols obtained by enzymic hydroxylation of C-1 chiral octanes was possible.

Assuming that hydroxylation involves a significant isotope effect $k_H > k_D > k_T$ and proceeds with retention of configuration, then (1*R*)-octane (**1**) should yield mainly (**3**) and lesser amounts of products of oxygenation of the chiral methyl [(**5**) > (**6**) > (**7**)]. For determination of the stereochemistry of the hydroxylation reaction, only the chirality of the major component of the mixture of C-1 *tritiated* alcohols (**5**) and (**6**) need be considered. Should hydroxylation of (1*R*)-octane proceed with inversion of configuration, then (1*R*)-octanol will be the major product. Similarly, hydroxylation of (1*S*)-octane with retention of configuration will yield mainly (1*R*)-octanol, whereas hydroxylation with inversion will yield mainly (1*S*)-octanol.

Chiral [1-^{3}H,^{2}H,^{1}H]octanes containing the [1-^{14}C]octane (**2**) were incubated with homogenates of *P. oleovorans* strain TF4-1L,[8] and the octanols from each incubation were recovered and purified to yield *ca.* 0·75 μCi of [^{3}H]octanol per incubation (*ca.* 1·7% ^{3}H recovered as octanol) admixed with [1-^{14}C]octanols (**4**). The extent of hydroxylation of the chiral methyls was determined by oxidizing aliquots of the biosynthesized alcohols with Jones' reagent and counting the obtained acids (**8**) as their *p*-toluidides.

$Me[CH_2]_6CO_2H$ (**8a**)

$TDHC[CH_2]_6CO_2H$ (**8b**)

$DH_2{}^{14}C[CH_2]_6CO_2H$ (**8c**)

$Me[CH_2]_6{}^{14}CO_2H$ (**8d**)

The Günther, Simon *et al.* exchange reactions were carried out in parallel using octanols derived enzymically from (1*R*)- and (1*S*)-octanes and synthetic (1*RS*)-octanol. The tritium lost in the equilibrations of the biosynthesized alcohols was corrected for the accompanying loss of (1*S*) tritium.§ From the difference between the tritium content at C-1 before equilibration and the *corrected* amount of tritium abstracted during equilibration, the fraction of (1*R*)-octanol in the biosynthetic mixtures was calculated.

The results are summarized in the Table. It is evident that hydroxylation of the octanes occurred mainly (70—80%) at the achiral methyl (column 3, Table). This indicates that enzymic hydroxylation involves a normal hydrogen isotope effect.

The hydroxylations of (1*R*)-octane gave mixtures of C-1 tritiated octanols. Equilibration of these mixtures of alcohols proceeded with the (corrected) loss of 37—38% of the tritium at C-1. It follows that 37—38% of the octanol in the mixtures was (1*R*)-octanol and, hence, the major

TABLE. Hydroxylation of (1*R*)- and (1*S*)-[1-^{3}H,^{2}H,^{1}H; ^{14}C]octanes by homogenates of *Pseudomonas oleovorans* strain TF4-1L.

Experiment	Chirality of octane	% ^{3}H at C-1 of octanols	% of (1*R*)-octanol[a]	C-1 chirality of the major octanol	Stereochemistry of hydroxylation
1	1*R*	27	38	1*S*	Retention
	1*S*	21	82	1*R*	Retention
2	1*R*	29	37	1*S*	Retention
	1*S*	29	74	1*R*	Retention

[a] The average deviation in the [^{3}H : ^{14}C] ratios from successive crystallizations of the acid and alcohol derivatives is ±0·05. The resulting relative error in the calculated (ref. 9) % of (1*R*)-octanol determination is ±7% (*e.g.* 38 ± 2·7%).

‡ The assignment of the configuration of the chiral octanes rests on the known inversion of configuration in the hydrogenolysis of methanesulphonyl esters.

§ The correction was computed on the basis of the amount of tritium lost from (1*S*)-octanol in the control equilibration of (1*RS*)-octanol.

product of hydroxylation of the chiral methyl terminus of (1*R*)-octane is (1*S*)-octanol. In contrast, hydroxylations of (1*S*)-octane gave octanols which on equilibration lost most (74—82%) of the tritium present at C-1. Therefore, the major product of hydroxylation of the chiral terminus of (1*S*)-octane is (1*R*)-*octanol*. These results are consistent with the view that the hydroxylation proceeds with retention of configuration in which mainly a hydrogen atom and, to a lesser extent, a deuterium atom is displaced.

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