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Review

Rearrangement reactions catalyzed by cytochrome P450s

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ABSTRACT

Cytochrome P450s promote a variety of rearrangement reactions both as a consequence of the nature of the radical and other intermediates generated during catalysis, and of the neighboring structures in the substrate that can interact either with the initial radical intermediates or with further downstream products of the reactions. This article will review several kinds of previously published cytochrome P450-catalyzed rearrangement reactions, including changes in stereochemistry, radical clock reactions, allylic rearrangements, "NIH" and related shifts, ring contractions and expansions, and cyclizations that result from neighboring group interactions. Although most of these reactions can be carried out by many members of the cytochrome P450 superfamily, some have only been observed with select P450s, including some reactions that are catalyzed by specific endoperoxidases and cytochrome P450s found in plants.

Introduction

Among the reactions catalyzed by cytochrome P450s are several rearrangements that are a consequence both of the nature of the radical and other intermediates formed from the substrates, and of interacting structural elements in the substrates. Several of these reactions that involve short-lived intermediates have served as useful tools to help unravel the catalytic mechanisms of cytochrome P450 enzymes, while other rearrangement reactions that lead to longer-lived intermediates and products have revealed the complexity of reactions that can be initiated by these enzymes [1,2]. The purpose of this article is to review the variety of rearrangement reactions catalyzed by cytochrome P450s, which range from changes in stereochemistry and radical clock reactions to cyclizations that result from neighboring group interactions.

Stereochemistry of hydrocarbon hydroxylation

Retention of stereochemistry

Early work on cytochrome P450-catalyzed hydroxylation reactions suggested that insertion of the hydroxyl group into the substrate proceeded with retention of stereochemistry. Thus, the 7α-hydroxylation of cholesterol, the 11α-hydroxylation of pregnane-3,20-dione [3,4] and the hydroxylation of (1*R*)- and (1*S*)-[1-^{3}H,^{2}H,^{1}H:1-^{14}C]octane to 1-octanol by rat liver microsomes [5] were shown to occur with retention of stereochemistry. Likewise, NMR analysis demonstrated that the hydroxylation of (*R*)-(8-$^{2}H_1$)[8-$^{3}H_1$]- and (*S*)-(8-$^{2}H_1$)[8-$^{3}H_1$]geraniol by cytochrome P450 gave products in which both the stereochemistry and regiochemistry was preserved [6]. Many other examples are now known in which the stereochemistry is retained during the hydroxylation of a C–H bond.

Loss of stereochemistry

The hydroxylation of 2,3,5,6-tetradeuteronorbornane by liver microsomes was shown in a seminal study to yield the *exo*- and *endo*-2-hydroxylated metabolites in a 0.76:1 ratio, in contrast to undeuterated norbornane, which gave the same two (but undeuterated) products in a 3.4:1 ratio (Fig. 1) [7]. The product ratio was therefore subject to an isotope effect of $k_H/k_D = 11.5$. Even more importantly, approximately 25% of the *exo*-hydroxylated 2,3,5,6-tetradeuteronorbornane retained all four deuterium atoms. This requires that abstraction of the *endo*-hydrogen from a fraction of the substrate molecules by the P450 catalytic oxygen is followed by inversion of stereochemistry and delivery of the hydroxyl from the *exo*-side, yielding the *exo*-hydroxy product with an *endo*-deuterium retained on the hydroxylated carbon atom. An analogous loss of stereochemistry was observed in the 5-*exo*-hydroxylation of camphor by cytochrome $P450_{cam}$ (CYP101). Studies with camphor labeled either on the 5-*exo*- or 5-*endo*-position with deuterium showed that the hydrogen abstraction could occur from either face, but delivery of the hydroxyl only occurred to give the 5-*exo*-hydroxyl product [8]. Similar results were reported for hydroxylation by the fungus *Beauveria sulfurescens* of a camphor derivative in which the carbonyl was replaced by a benzamido (PhCONH-) function [9]. The *exo*-alcohol was formed exclusively with high retention of deuterium

Abbreviations: CYP, cytochrome P450; NIH, National Institute of Health.

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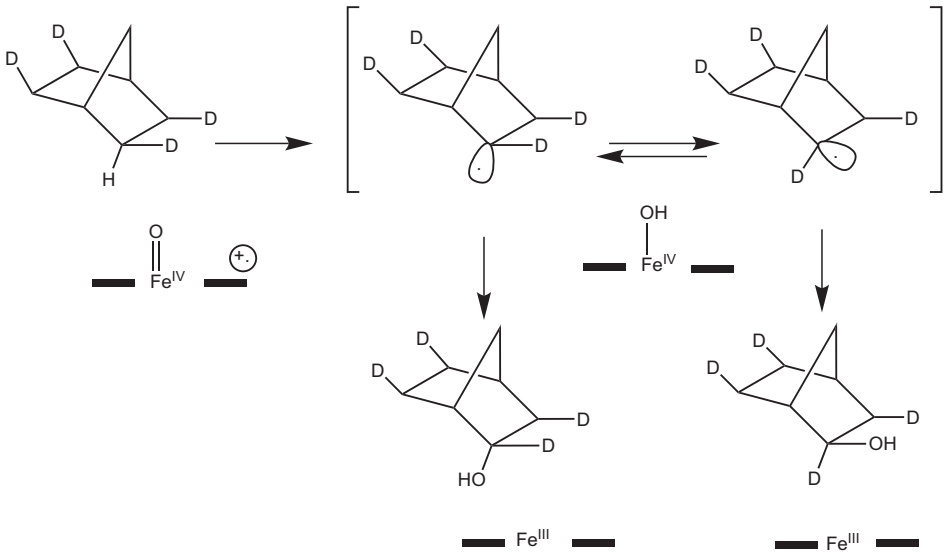

Fig. 1. Loss of stereochemistry in the oxidation of all-*exo*-2,3,5,6-tetradeuterated norbornane by microsomal cytochrome P450. The cytochrome P450 heme group is represented by the iron between two solid bars. The oxidation state of the iron is shown, as is a radical cation delocalized over the porphyrin ring of the heme. Only the distal ligand to the iron atom is shown.

whether the deuterium was initially in the *endo*- or *exo*-position. It was also reported that the hydroxylation of 1-[^{2}H]-ethylbenzene to 1-phenylethanol proceeds with partial loss of stereochemistry [10]. Thus, although carbon hydroxylation can proceed with retention of stereochemistry, the hydroxylation mechanism must be such that in appropriate circumstances an intermediate is formed that can undergo conformational inversion prior to addition of the oxygen atom.

Allylic rearrangements

Rearrangements also provide evidence for the involvement of an intermediate in cytochrome P450-catalyzed carbon hydroxylation, as illustrated by the oxidation of 3,4,5,6-tetrachlorocyclohexene by housefly and rat liver microsomes [11], 3,3,6,6-tetradeuterated cyclohexene, methylenecyclohexane and β-pinene by a purified P450 enzyme [12], and linoleic acid by liver microsomes [13] (Fig. 2). The oxidation of (*R*)-(+)-pulegone by rat liver microsomes is exceptional because there is extensive isomerization about the double bond during the hydroxylation process (Fig. 3) [14]. The reaction appears to proceed via hydrogen abstraction from the (*E*)-methyl group, allylic isomerization of the resulting radical, and collapse of the radical with the iron-bound hydroxyl radical equivalent on the enzyme to give the alcohol that subsequently closes to give the furan.

Radical clocks

Simple cyclopropyl probes

The term radical clocks refers to compounds that, when converted to a radical, undergo a radical rearrangement reaction at a

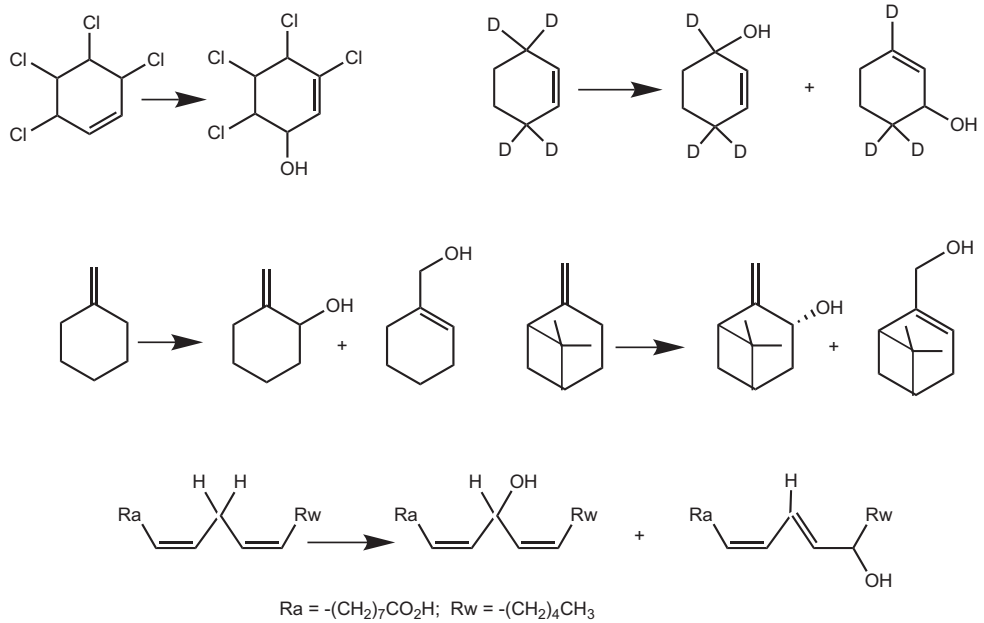

Fig. 2. Some of the allylic rearrangements observed in cytochrome P450-catalyzed hydroxylation reactions.