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Chloroperoxidase-catalyzed oxidation of conjugated dienoic esters

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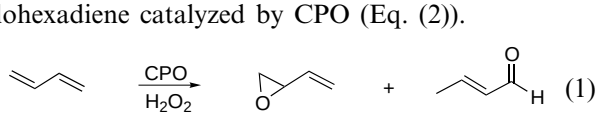
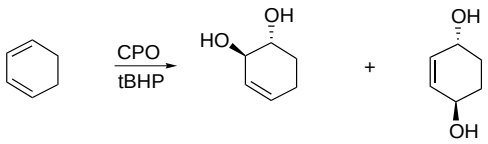
Abstract—The chloroperoxidase (CPO)-catalyzed oxidations of dienes conjugated to an ester group were studied using *tert*-butyl hydroperoxide as the terminal oxidant.

Chloroperoxidase first isolated in 1961 by Hager[1,2] from *Caldariomyces fumago* (CPO, EC 1.11.1.10), is one of the most versatile and promising of the heme enzymes for synthetic applications.[3–7] Various transformations typical of catalases and cytochrome P-450 like monooxygenases are catalyzed by CPO.[8] The enzyme shows broad substrate specificity catalyzing various halide-dependent as well as halide-independent reactions.[9] In the halide-independent oxidation reactions, CPO uses hydrogen peroxide or other organic peroxides as the source of oxygen without requiring cofactors.[10] Substrate specificity studies as well as mechanistic studies have shown that CPO catalyzes the epoxidation of a number of functionalized or unfunctionalized olefins with a high degree of enantio- and diastereoselectivity.[11–13] Epoxidation is often accompanied by the formation of aldehydes as well as by allylic hydroxylation.[4] The chain length of the alkene as well as the size and type of substituents adjacent to the double bond control the enantioselectivity of the epoxidation.[14–16]

Although CPO-catalyzed oxidations of a wide variety of simple and functionalized alkenes have been studied extensively, only two examples of conjugated dienes have been reported in the last few years. For example, Elfarra and his co-workers[17] reported the CPO-catalyzed epoxidation of 1,3-butadiene, which gave butadiene monoxide accompanied by the formation of a small amount of crotonaldehyde (Eq. (1)). Recently, Nicolosi and his co-workers reported[18] the asymmetric oxidation of 1,3-cyclohexadiene catalyzed by CPO (Eq. (2)).



(1)



(2)

In this communication, we report the first examples of CPO-catalyzed oxidations of dienes conjugated to an ester group, and we discuss possible mechanisms for these synthetically useful transformations.

The reactions of the isomeric conjugated dienoic esters methyl (2*E*,4*E*) (**1**), methyl (2*Z*,4*E*) (**2**), methyl (2*Z*,4*Z*) (**3**) and methyl-(2*E*,4*Z*)-hexadienoate (**4**) were investigated. The stereoisomers **2**, **3** and **4** were prepared by photoisomerization[19] of **1** by irradiation at 254 nm, followed by separation by flash column chromatography on silica gel enriched with 25% $AgNO_3$. *tert*-Butyl hydroperoxide (*t*BHP) was used as the terminal oxidant. None of the substrates used in this study isomerize under the enzymatic reaction conditions, as shown by control experiments. The enzymatic oxidation of each isomer was carried out in a phosphate buffer (100 mM, pH 6), by using 25 mg of substrate, 1000 units of commercially available CPO and 2 equiv. of 70% *t*BHP which was added in two aliquots to a total volume of 5 ml. All the reactions were monitored by gas chromatography, the products were identified by ^{1}H NMR and GC–MS, and all product yields are reported after 6 h reaction.

The CPO-catalyzed oxidation of (*E*,*E*)-**1**, under aerobic conditions gave the aldehydic ester **5** as the major product (83%), accompanied by small amounts of the epoxide **7** (4%) and a second aldehydic ester **6** (13%), as

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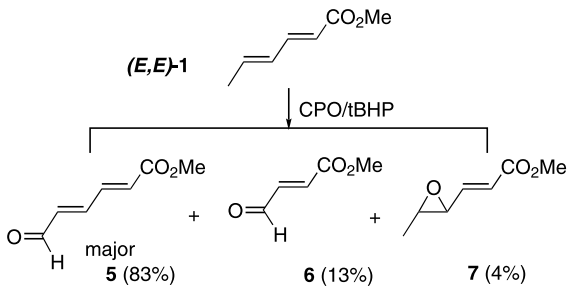
shown in Scheme 1. When the reaction was run under anaerobic conditions (nitrogen flow) only the aldehyde **5** and epoxide **7** were detected, in a 92:8 ratio.

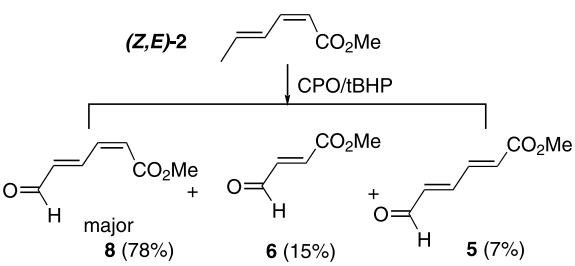
Similarly, from the catalytic oxidation of (*Z*,*E*)-**2**, (Scheme 2), under aerobic conditions, the aldehydic ester **8** was the major product (78%), accompanied by small amounts of **5** (7%) and **6** (15%).

The CPO-catalyzed oxidation of substrate (*Z*,*Z*)-**3**, under aerobic conditions gave the three products shown in Scheme 3; the aldehydic esters **6** (38%) and **8** (27%) and the *cis*-epoxide **9** (35%) with high enantioselectivity (96% ee).

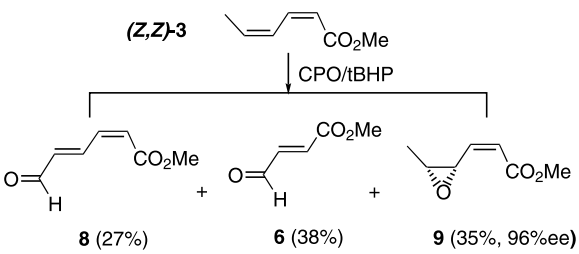
The (*E*,*Z*)-**4** dienoic ester was oxidized as a mixture with the isomer (*E*,*E*)-**1**, in a ratio 48/52 because efforts to separate the two were unsuccessful. As shown in Scheme 4, under anaerobic conditions, the major products were the aldehyde **5** (60%) and the *cis*-epoxide **10** (40%) with high enantioselectivity (93% ee). The ee values for epoxides **9** and **10** were determined by gas chromatography with a chiral column (HP-Chiral 20% permethylated-cyclodextrin, 30 m×0.25 mm). In a control experiment the racemic mixtures of the epoxides **9** and **10** were prepared separately by *m*CPBA



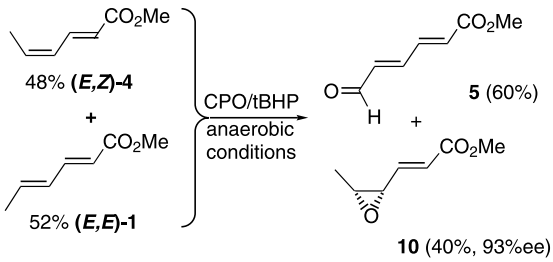
Scheme 1. CPO-catalyzed oxidation of (*E*,*E*)-**1**.



Scheme 2. CPO-catalyzed oxidation of (*Z*,*E*)-**2**.



Scheme 3. CPO-catalyzed oxidation of (*Z*,*Z*)-**3**.

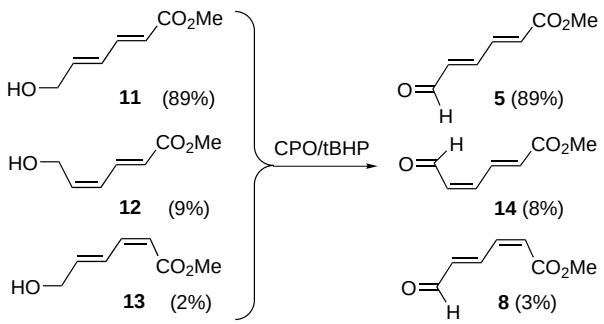


Scheme 4. CPO-catalyzed oxidation of (*E*,*Z*)-**4** and (*E*,*E*)-**1** mixture.

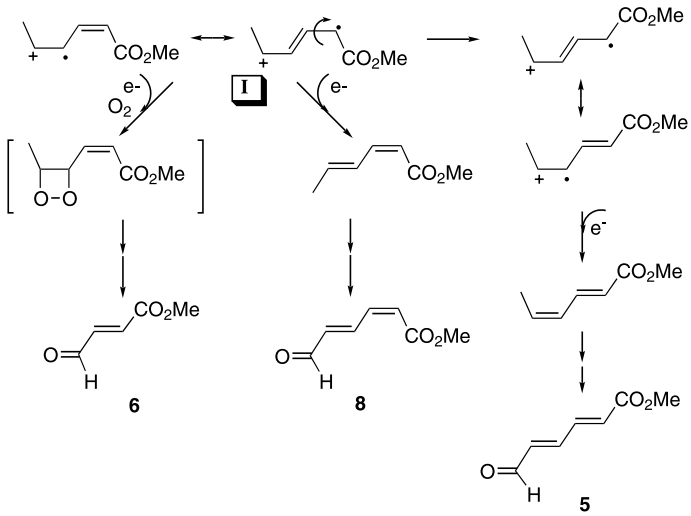
epoxidation[20] of their corresponding dienoic ester precursors. The absolute configuration of **9** and **10** was assigned to be *R*,*S* in analogy to that of related compounds.[21] The same stereochemistry *R*,*S* is also predicted by the model proposed by Sheldon.[22]

The above results show that the reaction mode of CPO-epoxidation versus allylic oxidation depends on the stereochemistry at the C4–C5 double bond. When the alkyl terminal double bond has the *trans* configuration, as in substrates **1** and **2**, allylic oxidation predominates over C4–C5 double bond epoxidation. This finding is consistent with previous results showing that in cases of *trans* alkenes, only limited, if any, epoxidation occurs.[4,13]

In a control experiment, a mixture of alcohols **11**, **12** and **13** was subjected to CPO oxidation (Scheme 5). We wish to point out the following observations: the starting ratio of alcohols **11**, **12** and **13** remained constant during the course of the reaction. In the absence of enzyme, none of the alcohols isomerized. The ratio of **5**, **14** and **8** remained constant in a phosphate buffer (pH 6) for 6 h. The relative ratio of the aldehydic esters produced by the enzymatic oxidation was the same as that of the reactant alcohols. Interpretation of these results indicated that the aldehydes **5** and **8** formed in the reactions of **1**–**4**, arise from the oxidation of their corresponding alcohols which are intermediates in these enzymatic reactions. Although we did not detect these alcohols during the course of the enzymatic reaction, we were not surprised. It is well established that the oxidation of alcohols to aldehydes is a much faster reaction than the initial hydroxylation.[23–27]



Scheme 5. CPO-catalyzed oxidation of alcoholic esters.



Scheme 6.

When the C4–C5 double bond has the *Z* configuration, as in substrates **3** and **4**, enantioselective epoxidation competes well with allylic oxidation, to give the epoxides **9** and **10** and the allylic aldehydes **8** and **5** in comparable amounts. It is worth pointing out again the high enantiomeric purity of the epoxides **9** (96% ee) and **10** (93% ee) which are of the highest observed in such reactions.

A reasonable explanation for the formation of all products, including aldehyde **6**, under aerobic but not anaerobic conditions, involves the intermediacy of the radical cation **I**, Scheme 6. This intermediate can lead to partial isomerization of the dienoic esters that competes with the oxidation of the terminal methyl group, and can react with oxygen to form the dioxetane intermediate that leads to the cleaved product aldehyde **6**. Similar C–C bond cleavage products have been observed in many cases.[28] Further studies on the mechanism of these reactions, including the competition between isomerization and hydrogen abstraction, are currently under investigation in our laboratories.

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