Unique applications

Novel Oxidation Reactions

The role of the lipase from *Candida antarctica* in the synthesis of flavor compounds

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It has long been common knowledge that the lipase from *Candida antarctica* can convert long chain fatty acids into the corresponding peracids in the presence of hydrogen peroxide.[1] These peracids can bring about the oxidation of olefins, forming epoxides, which are useful synthetic intermediates.[2-4] However, if ethyl acetate is used as solvent and long chain fatty acids are not present, the lipase not only hydrolyzes the ester bond but also produces peracetic acid from the liberated acetic acid. The so-formed peracetic acid can further oxidize unsaturated organic molecules, whereby the corresponding epoxides are invariably the intermediates in the resulting oxidation step.[5] These epoxides can undergo further rearrangement reactions, thereby resulting in the formation of molecules with interesting flavor properties.

Under standard conditions, cinnamic aldehyde can be readily converted into benzaldehyde (F-1), the character impact compound of bitter almonds.[6] Oxidation of the aldehyde group also occurs quite readily under the reaction conditions, forming benzoic acid (16 percent), cinnamic acid (19 percent) and a small amount of phenylacetaldehyde (6 percent).

The use of the less reactive cinnamic alcohol as substrate influences the overall reaction such that very little C-C bond cleavage takes place. Thus, the main products are derivatives of cinnamic alcohol.

These derivatives arise from the enzymatic acetylation of alcohols that are either present from the start, i.e. cinnamic alcohol itself, or produced by ring opening of the epoxy intermediate (F-2). None of these products can be detected in the absence of the lipase, proving that this conversion reaction is enzyme catalyzed.

Surprisingly, this enzyme system is also able to convert isoeugenol into vanillin — not only in good yields (50 percent), but also under very mild conditions. The major byproducts formed are the corresponding diethyl acetal of vanillin (10 percent) and the methyl ketone (25 percent), as shown in F-3.
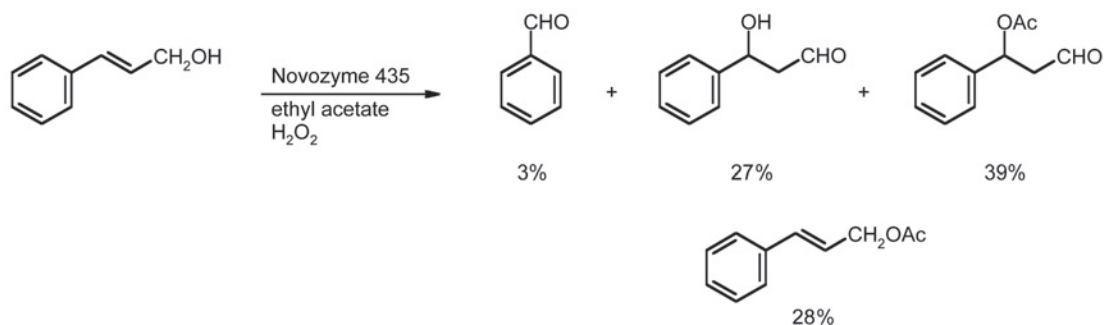
Screenings of other suitable precursors of vanillin, which could act as substrates for this enzymatic oxidation

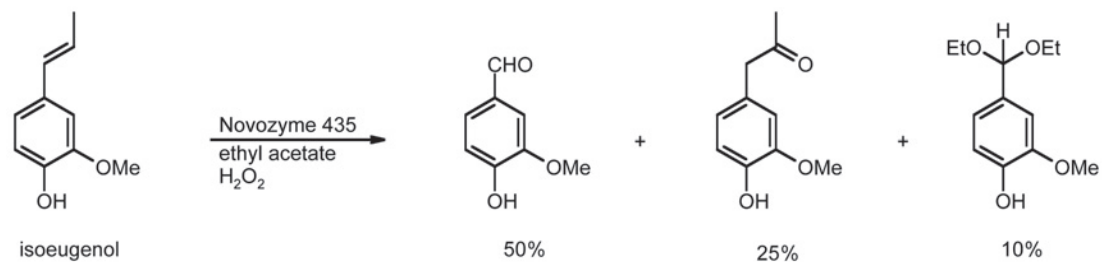**Reaction products from cinnamic aldehyde after treatment with Novozyme 435 under standard conditions**

F-1

F-2
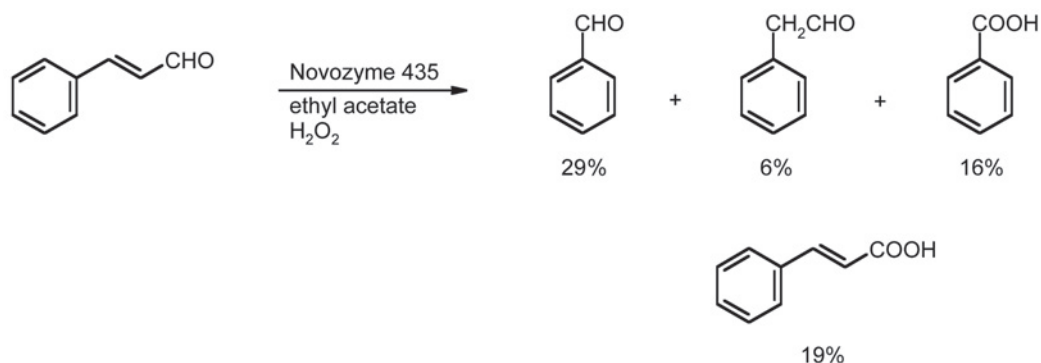
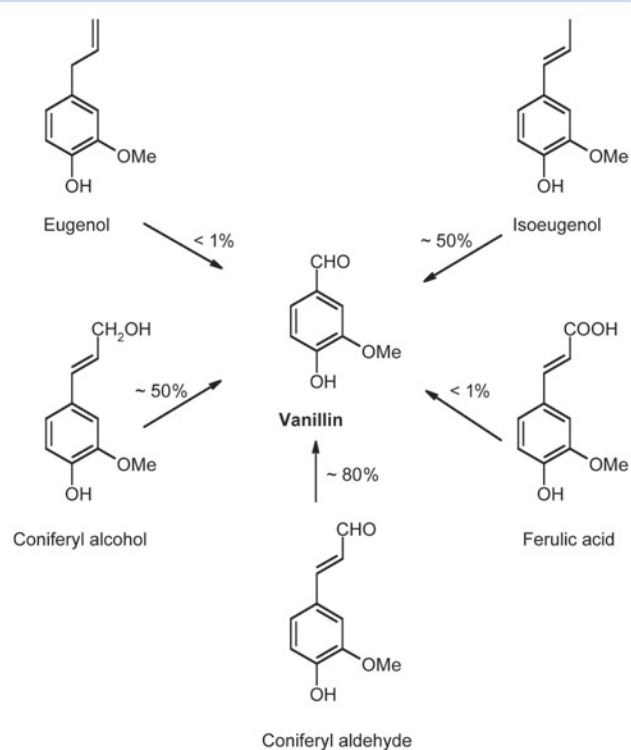### Reaction products from cinnamic alcohol

F-3

Conversion of isoeugenol to vanillin with Novozyme 435

F-4

Synthesis of vanillin from various precursors using Novozyme 435 under standard conditions


reaction, have shown that the nature of the C_3-side chain considerably influences the reactivity towards the enzyme system. Thus, isoeugenol, coniferyl alcohol and coniferyl aldehyde are readily converted into vanillin in yields > 50 percent (F-4). In contrast, eugenol only undergoes epoxidation and partial ring opening, resulting in less than 1 percent of vanillin being formed. Ferulic acid is more or less unsusceptible to oxidation by the enzyme system, and is thus recovered virtually unchanged. Only very small amounts of vanillin can be detected in the crude isolate.

When anethol is treated under standard conditions, a qualitative similarity with isoeugenol can be detected. Thus, the corresponding aldehyde (~ 5 percent) and methyl ketone (~ 2 percent) are formed (F-5), but the major product is the monoacetylated diol (~ 80 percent), arising through ring opening of the intermediate epoxide and enzymatic esterification of the resulting diol.

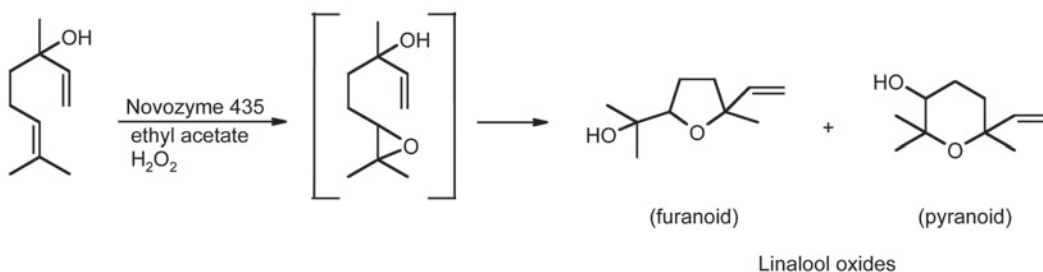The monoterpene alcohol linalool has two C-C double bonds; this mol-

Products obtained upon conversion of anethole using Novozyme 435 under standard conditions   **F-5**
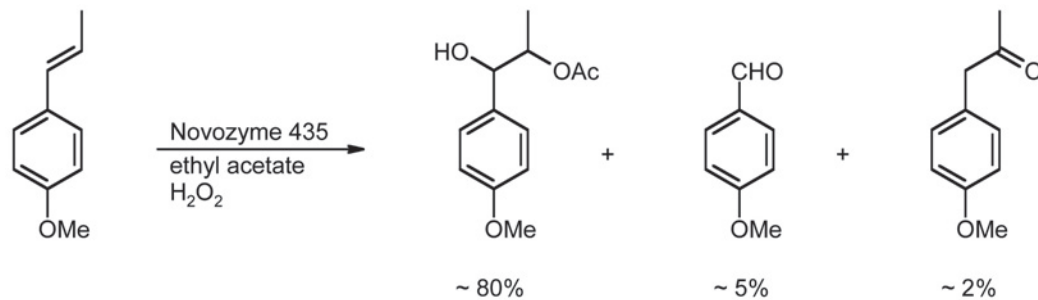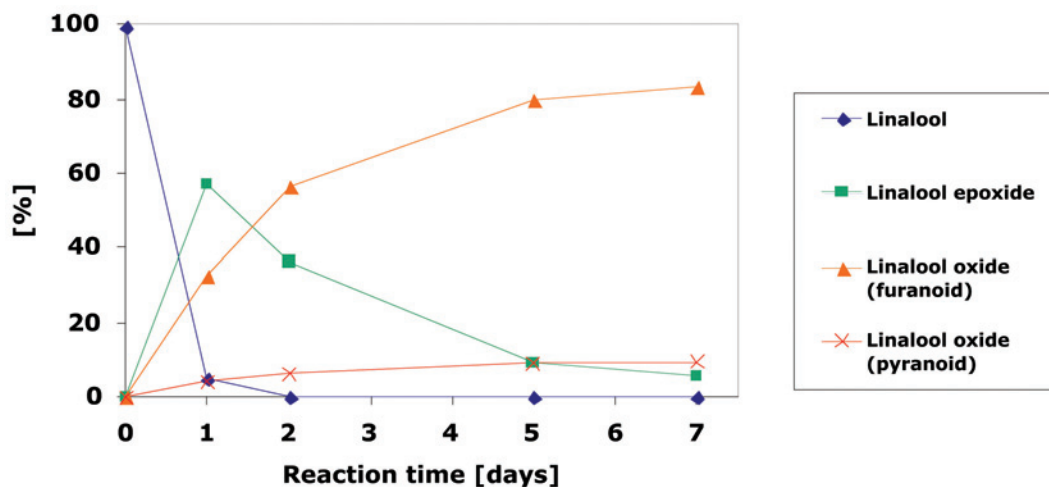

Conversion of linalool to linalool oxides using Novozyme 435   **F-6**

ecule also undergoes epoxidation via this enzyme system. One monoepoxide seems to be formed preferentially (F-6). This intermediate then rearranges in situ to form both the *cis-* and the *trans*-isomers of the furanoid and pyranoid forms of linalool oxide. The time profile of this conversion is shown in F-7, which also illustrates the formation and further conversion of the monoepoxide.

Linalool oxides, found in many types of fruit such as grapes and papaya, are among the main constituents of the aroma of black tea.[7-9] These compounds display a floral, sweet, citrus-like, slightly woody flavor character. When 2-methyl-2-hepten-6-ol is used as substrate, the major product of the enzymatic reaction is sulcatol oxide both in the furanoid (58 percent) and pyranoid (37 percent) forms. All four isomeric compounds formed display a sweet, fruity and roasty flavor character.

References

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3. A.W.P. Jarvie, N. Overton and C.B. St. Pourcain, Chem. Commun., 177 (1998).

4. F. Björkling, S.E. Godtfredsen and O. Kirk, Chem. Commun., 1301 (1990).

5. I.L. Gatfield, unpublished results.

6. Standard reaction conditions: 5 mL ethyl acetate, 0.7 mmol substrate, 0.5 mL 30 percent hydrogen peroxide, 125 mg Novozyme 435, gentle agitation at 20°C for 24 h; Novozyme 435 was obtained from Novozymes, Bagsvaerd, Denmark.

7. M. Kawakami, S.N. Ganguly, J. Banerjee and A. Kobayashi, J. Agric. Food Chem., **43**, 200 (1995).

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