CLAIMS

1. A process for preparing (-)-Ambrox or a mixture comprising (-)-Ambrox, the process comprising enzymatically converting (*3E,7E*)-homofarnesol (EEH) or a mixture of isomers of homofarnesol comprising EEH to (-)-Ambrox or a mixture comprising (-)-Ambrox using a SHC/HAC enzyme variant,

wherein the SHC/HAC enzyme variant has an amino acid sequence having at least about 70.0 % identity to SEQ ID NO: 1,

wherein the SHC/HAC enzyme variant amino acid sequence has amino acid alterations relative to SEQ ID NO: 1 at positions corresponding to positions 132, 224 and 432 of SEQ ID NO: 1 which are M132R, A224V and I432T respectively, and

wherein the SHC/HAC enzyme variant amino acid sequence has amino acid alterations relative to SEQ ID NO: 1 at a position corresponding to position 557 of SEQ ID NO: 1 and at least one position corresponding to position 81, 431 or 613 of SEQ ID NO: 1.

2. A process for preparing Ambra oxide or a mixture comprising Ambra oxide, the process comprising enzymatically converting (*E,E*)-Bishomofarnesol (BisEEH) or a mixture of isomers of bishomofarnesol comprising BisEEH to Ambra oxide or a mixture comprising Ambra oxide using a SHC/HAC enzyme variant,

wherein the SHC/HAC enzyme variant has an amino acid sequence having at least about 70.0 % identity to SEQ ID NO: 1,

wherein the SHC/HAC enzyme variant amino acid sequence has amino acid alterations relative to SEQ ID NO: 1 at positions corresponding to positions 132, 224 and 432 of SEQ ID NO: 1 which are M132R, A224V and I432T respectively, and

wherein the SHC/HAC enzyme variant amino acid sequence has amino acid alterations relative to SEQ ID NO: 1 at a position corresponding to position 557 of SEQ ID NO: 1 and at least one position corresponding to position 81, 431 or 613 of SEQ ID NO: 1.

3. An SHC/HAC enzyme variant having an amino acid sequence having at least about 70.0 % identity to SEQ ID NO: 1,

wherein the SHC/HAC enzyme variant amino acid sequence has amino acid alterations relative to SEQ ID NO: 1 at positions corresponding to positions 132, 224 and 432 of SEQ ID NO: 1 which are M132R, A224V and I432T respectively, and

wherein the SHC/HAC enzyme variant amino acid sequence has amino acid alterations relative to SEQ ID NO: 1 at a position corresponding to position 557 of SEQ ID NO: 1 and at least one position corresponding to position 81, 431 or 613 of SEQ ID NO: 1.

4. The process of claim 1 or 2 or the SHC/HAC enzyme variant of claim 3, wherein the SHC/HAC enzyme variant has an amino acid sequence having at least about 90.0 % identity to SEQ ID NO: 1.

5. The process or SHC/HAC enzyme variant of any preceding claim, wherein the SHC/HAC enzyme variant amino acid sequence has amino acid alterations relative to SEQ ID NO: 1 at positions corresponding to positions 557 and 431 of SEQ ID NO: 1.

6. The process or SHC/HAC enzyme variant of any preceding claim, wherein the SHC/HAC enzyme variant amino acid sequence has amino acid alterations relative to SEQ ID NO: 1 at positions corresponding to positions 557 and 613 of SEQ ID NO: 1.

7. The process or SHC/HAC enzyme variant of claim 5 or 6, wherein the SHC/HAC enzyme variant amino acid sequence has a further amino acid alteration relative to SEQ ID NO: 1 at a position corresponding to position 81 of SEQ ID NO: 1.

8. The process or SHC/HAC enzyme variant of any preceding claim, wherein one or more of the amino acid alterations at positions 81, 431, 557, or 613 are substitutions, for example non-conservative substitutions.

9. The process or SHC/HAC enzyme variant of any preceding claim, wherein:
- the amino acid alteration at a position corresponding to position 557 of SEQ ID NO: 1 substitutes the amino acid of SEQ ID NO: 1 for a neutral hydrophilic amino acid, for example threonine; and/or
- the amino acid alteration at a position corresponding to position 81 of SEQ ID NO: 1 substitutes the amino acid of SEQ ID NO: 1 for a basic amino acid, for example histidine; and/or
- the amino acid alteration at a position corresponding to position 431 of SEQ ID NO: 1 substitutes the amino acid of SEQ ID NO: 1 for a hydrophobic amino acid, for example leucine; and/or
- the amino acid alteration at a position corresponding to position 613 of SEQ ID NO: 1 substitutes the amino acid of SEQ ID NO: 1 for a neutral hydrophilic amino acid, for example serine.

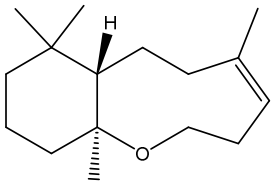
10. The process or SHC/HAC enzyme variant of any preceding claim, wherein the SHC/HAC enzyme variant has an amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, and SEQ ID NO: 5, for example an amino acid sequence selected from SEQ ID NO: 2, SEQ ID NO: 4 and SEQ ID NO: 5.

11. The process of any preceding claim, wherein the enzymatic conversion takes place at a temperature in the range of about 30ºC to about 50ºC, for example from about 40^0 to about 50^0C, and/or at a pH in the range of about 5 to about 6.

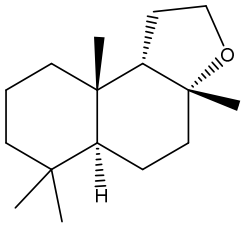
12. The process of any preceding claim, wherein the process comprises culturing recombinant host cell that produces the SHC/HAC enzyme variant.

13. The process of claim 12, wherein the recombinant host cells comprise a nucleic acid sequence selected from SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 or SEQ ID NO: 9, for example a nucleic acid sequence selected from SEQ ID NO: 6, SEQ ID NO: 8 or SEQ ID NO: 9.

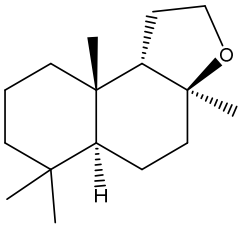
14. The process of any preceding claim, wherein (-)-Ambrox is produced in admixture with at least one or more of the by-products (II), (III), and (IV),



(II)



(III)



(IV)

15. The process or SHC/HAC enzyme variant of any preceding claim, wherein the SHC/HAC enzyme variant has an increased enzymatic activity relative to SEQ ID NO: 1 or wherein the SHC/HAC enzyme variant provides an increased rate of EEH conversion over the first 6 hours or over the first 8 hours or over the first 12 hours of the reaction compared to SEQ ID NO: 1 and/or SEQ ID NO: 10.

~~16. The process or SHC/HAC enzyme variant of any preceding claim, wherein the SHC/HAC enzyme variant provides an increased rate of EEH conversion over the first 6 hours or over the first 8 hours or over the first 12 hours of the reaction compared to SEQ ID NO: 1 and/or SEQ ID NO: 10.~~

~~17. (-) Ambrox obtained by or obtainable by the process of any preceding claim, for example in amorphous or crystalline form.~~

~~18. Use of (-)-Ambrox of claim 17 as part of a fragrance or a cosmetic or a consumer product such as fabric care, toiletry, beauty care, a cleaning product, a detergent product, and/or a soap product.~~

~~19. A fragrance or a cosmetic or a consumer product comprising (-)-Ambrox of claim 17.~~

~~20. A nucleic acid sequence encoding the SHC/HAC enzyme variant of any preceding claim.~~

~~21. The nucleic acid sequence of claim 20, wherein the nucleic acid sequence is selected from SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8 and SEQ ID NO: 9, for example a nucleic acid sequence selected from SEQ ID NO: 6, SEQ ID NO: 8 and SEQ ID NO: 9.~~

~~22. A construct comprising the nucleic acid sequence of claim 20 or 21.~~

~~23. A vector comprising the construct of claim 22.~~

~~24. A recombinant host cell comprising the nucleic acid sequence of claims 20 or 21, the construct of claim 22, or the vector of claim 23.~~

~~25. The recombinant host cell of claim 24, wherein the recombinant host cell is a bacteria having a genus selected from *Escherichia, Streptomyces, Bacillus, Pseudomonas, Lactobacillus* and *Lactococcus*, for example wherein the recombinant host cell is *E. coli*.~~

~~26. The process according to any one of claims 1- 16 wherein the mixture of isomers comprising EEH is an EE:EZ isomer mixture.~~

~~27. The process according to claim 26 wherein the homofarnesol EE:EZ isomer mixture is in a weight ratio is selected from the group consisting of: EE:EZ 90:10; EE:EZ 80: 20; EE:EZ 86:14; EE:EZ 70:30; EE:EZ 69:31; and EE:EZ 66:34 or the homofarnesol EE:EZ isomer mixture is in a weight ratio of EE:EZ 90:10; EE:EZ 80: 20; EE:EZ 86:14; EE:EZ 70:30; EE:EZ 69:31; or EE:EZ 66:34.~~

~~28. The process of claim 27 wherein the EE: EZ isomer mixture is in a weight ratio of 80:20.~~

~~29. The process of any one of claims 1-16 or 26-28 wherein the weight ratio of SHC/HAC biocatalyst to EEH or a mixture of isomers of homofarnesol comprising EEH is in the range of about 0.5-2:1 or about 0.25-2:1 or about 0.1-2:1 or about 1:1 or about 0.5:1.~~

~~30. The process according to claim 29 wherein the weight ratio of SHC/HAC biocatalyst to EEH or a mixture of isomers of homofarnesol comprising EEH in an EE:EZ isomer mixture in a weight ratio of 80:20 is about 1:1 or about 0.5:1 or about 0.1:1.~~