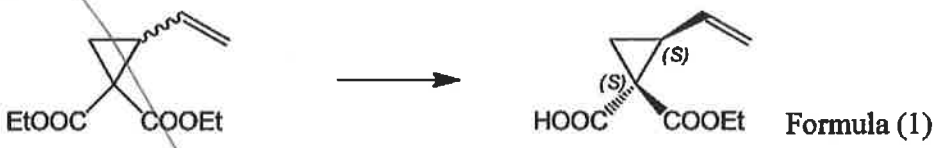
Claims

[Claim 1] A hydrolase protein, which consists of the amino acid sequence shown in any one of SEQ ID NOS. 2 to 5, and which has activity of catalyzing, at higher selectivity than the protein consisting of the amino acid sequence shown in SEQ ID NO. 1, the reaction represented by the following formula (1):

[Formula 1]



Formula (1)

[Claim 2] A hydrolase protein, which consists of an amino acid sequence wherein, in the amino acid sequence shown in any one of SEQ ID NOS. 1 to 5, one or more amino acids at amino acid numbers 70, 106, 107, 108, 219, 270, 271, 272, 273, 274, 275, 276, and 313 are substituted with other amino acids whose side chains have lower bulkiness than that of wild-type amino acids, and which has activity of catalyzing, at higher selectivity than the protein consisting of the amino acid sequence shown in SEQ ID NO. 1, the reaction represented by the following formula (1):

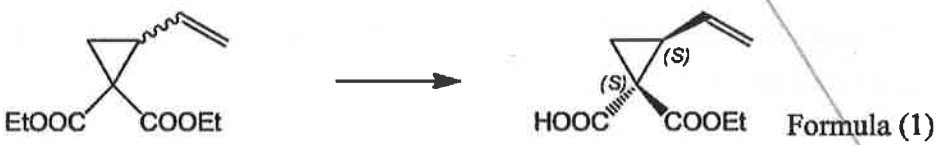
[Formula 2]



Formula (1)

[Claim 3] A hydrolase protein, which consists of an amino acid sequence wherein, in the amino acid sequence shown in any one of SEQ ID NOS. 1 to 5, one or more amino acids at amino acid numbers 188, 190, 193, 215, 216, 217, 314, 358, 362, and 363 are substituted with other amino acids whose side chains have higher bulkiness than that of wild-type amino acids, and which has activity of catalyzing, at higher selectivity than the protein consisting of the amino acid sequence shown in SEQ ID NO. 1, the reaction represented by the following formula (1):

[Formula 3]



Formula (1)

[Claim 4] A hydrolase protein, which consists of an amino acid sequence wherein, in the amino acid sequences shown in any one of SEQ ID NOS. 1 to 5, one or more amino acids at

amino acid numbers 70, 106, 107, 108, 219, 270, 271, 272, 273, 274, 275, 276, and 313 are substituted with other amino acids whose side chains have lower bulkiness than that of wild-type amino acids, and one or more amino acids at amino acid numbers 188, 190, 193, 215, 216, 217, 314, 358, 362, and 363 are substituted with other amino acids whose side chains have higher bulkiness than that of wild-type amino acids, and which has activity of catalyzing, at higher selectivity than the protein consisting of the amino acid sequence shown in SEQ ID NO. 1, the reaction represented by the following formula (1):

[Formula 4]



Formula (1)

[Claim 5] The hydrolase protein according to claim 2 or 4, wherein, in the amino acid sequence shown in any one of SEQ ID NOS. 1 to 5, at least one of amino acids at amino acid numbers 70, 270, 273, and 313 is substituted with another amino acid.

[Claim 6] The hydrolase protein according to claim 5, wherein, in the amino acid sequence shown in any one of SEQ ID NOS. 1 to 5, at least the leucine at amino acid number 70 is substituted with any one of aspartic acid, asparagine, serine, threonine, and glycine.

[Claim 7] The hydrolase protein according to claim 5 or 6, wherein, in the amino acid sequence shown in SEQ ID NO. 3 or 4, the leucine at amino acid number 270 is substituted with any one of serine, glutamine, glutamic acid, and alanine.

[Claim 8] The hydrolase protein according to claim 5 or 6, wherein, in the amino acid sequence shown in any one of SEQ ID NOS. 1, 2, and 5, the isoleucine at amino acid number 270 is substituted with any one of serine, glutamine, glutamic acid, and alanine.

[Claim 9] The hydrolase protein according to any one of claims 5 to 8, wherein, in the amino acid sequence shown in any one of SEQ ID NOS. 1 to 5, the leucine at amino acid number 273 is substituted with either arginine or histidine.

[Claim 10] The hydrolase protein according to any one of claims 5 to 9, wherein, in the amino acid sequence shown in any one of SEQ ID NOS. 1 to 5, the leucine at amino acid number 313 is substituted with any one of methionine, alanine, and proline.

[Claim 11] A hydrolase protein, which consists of an amino acid sequence wherein, in the amino acid sequence shown in SEQ ID NO. 4, the leucine at amino acid number 70 is substituted with aspartic acid, the leucine at amino acid number 270 is substituted with any one of glutamine, glutamic acid and alanine, the leucine at amino acid number 273 is substituted with arginine, and the leucine at amino acid number 313 is substituted with methionine, and which has activity of catalyzing, at higher selectivity than the protein consisting of the amino acid sequence shown in SEQ ID NO. 1, the reaction represented by the following formula (1):

[Formula 5]



Formula (1)

[Claim 12] A hydrolase protein, which consists of an amino acid sequence having homology of 90% or more with the amino acid sequence of the hydrolase protein according to claims 1 to 11, or an amino acid sequence comprising a substitution, deletion, and/or addition of one or several amino acids with respect to the amino acid sequence of the hydrolase protein according to claims 1 to 11, and which has activity of catalyzing, at higher selectivity than the protein consisting of the amino acid sequence shown in SEQ ID NO. 1, the reaction represented by the following formula (1):

[Formula 6]



Formula (1)

[Claim 13] A DNA encoding the hydrolase protein according to any one of claims 1 to 12.

[Claim 14] The DNA according to claim 13, which consists of the following nucleotide sequence:

(a) the nucleotide sequence shown in any one of SEQ ID NOS. 7 to 10;

(b) a nucleotide sequence of a DNA capable of hybridizing under stringent conditions with the DNA consisting of the nucleotide sequence shown in any one of SEQ ID NOS. 7 to 10, wherein the nucleotide sequence encodes a hydrolase protein having activity of catalyzing, at higher selectivity than the protein consisting of the amino acid sequence shown in SEQ ID NO. 1, the reaction represented by the following formula (1); or

(c) a nucleotide sequence comprising a substitution, deletion, and/or addition of one or several nucleotides with respect to the nucleotide sequence shown in any one of SEQ ID NOS. 7 to 10, wherein the nucleotide sequence encodes a hydrolase protein having activity of catalyzing, at higher selectivity than the protein consisting of the amino acid sequence shown in SEQ ID NO. 1, the reaction represented by the following formula (1):

[Formula 7]



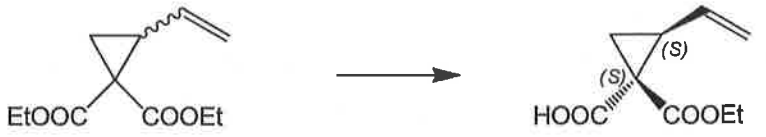
Formula (1)

[Claim 15] A method for producing (1S,2S)-1-alkoxycarbonyl-2-vinylcyclopropanecarboxylic acid, which comprises allowing the hydrolase protein according to any one of claims 1 to 12 to act on dialkyl 2-vinylcyclopropane-1,1-dicarboxylate to produce (1S,2S)-1-alkoxycarbonyl-2-vinylcyclopropanecarboxylic acid.

[Claims]

[Claim 1] A hydrolase protein, which consists of the amino acid sequence shown in any one of SEQ ID NOS. 2 to 5, and which has activity of catalyzing, at higher selectivity than the protein consisting of the amino acid sequence shown in SEQ ID NO. 1, the reaction represented by the following formula (1):

[Formula 1]



Formula (1)

[Claim 2] A hydrolase protein, which consists of an amino acid sequence wherein, in the amino acid sequence shown in any one of SEQ ID NOS. 1 to 5, one or more amino acids at amino acid numbers 70, 270, 273, and 313 are substituted with other amino acids whose side chains have lower bulkiness than that of wild-type amino acids, and which has activity of catalyzing, at higher selectivity than the protein consisting of the amino acid sequence shown in SEQ ID NO. 1, the reaction represented by the following formula (1):

[Formula 2]



Formula (1)

[Claim 3] The hydrolase protein according to claim 2, wherein, in the amino acid sequence shown in any one of SEQ ID NOS. 1 to 5, at least the leucine at amino acid number 70 is substituted with any one of aspartic acid, asparagine, serine, threonine, and glycine.

[Claim 4] The hydrolase protein according to claim 3, wherein, in the amino acid sequence shown in SEQ ID NO. 3 or 4, the leucine at amino acid number 270 is substituted with any one of serine, glutamine, glutamic acid, and alanine.

[Claim 5] The hydrolase protein according to claim 3, wherein, in the amino acid sequence shown in any one of SEQ ID NOS. 1, 2, and 5, the isoleucine at amino acid number 270 is substituted with any one of serine, glutamine, glutamic acid, and alanine.

[Claim 6] The hydrolase protein according to any one of claims 3 to 5, wherein, in the amino acid sequence shown in any one of SEQ ID NOS. 1 to 5, the leucine at amino acid number 273 is substituted with either arginine or histidine.

[Claim 7] The hydrolase protein according to any one of claims 3 to 6, wherein, in the amino acid sequence shown in any one of SEQ ID NOS. 1 to 5, the leucine at amino acid number 313 is substituted with any one of methionine, alanine, and proline.

[Claim 8] A hydrolase protein, which consists of an amino acid sequence wherein, in the amino acid sequence shown in SEQ ID NO. 4, the leucine at amino acid number 70 is substituted with aspartic acid, the leucine at amino acid number 270 is substituted with any one of glutamine, glutamic acid and alanine, the leucine at amino acid number 273 is substituted with arginine, and the leucine at amino acid number 313 is substituted with methionine, and which has activity of catalyzing, at higher selectivity than the protein consisting of the amino acid sequence shown in SEQ ID NO. 1, the reaction represented by the following formula (1):

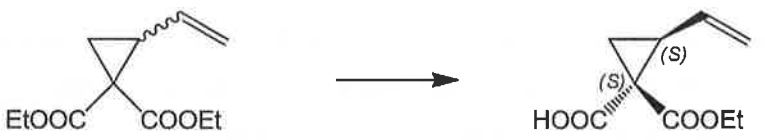
[Formula 3]



Formula (1)

[Claim 9] A hydrolase protein, which consists of an amino acid sequence having homology of 90% or more with the amino acid sequence of the hydrolase protein according to any one of claims 1 to 8, or an amino acid sequence comprising a substitution, deletion, and/or addition of one or several amino acids with respect to the amino acid sequence of the hydrolase protein according to any one of claims 1 to 8, wherein one or more amino acids which correspond to amino acid numbers 70, 270, 273, and 313 in the amino acid sequence shown in any one of SEQ ID NOS. 1 to 5 are substituted with other amino acids whose side chains have lower bulkiness than that of wild-type amino acids, and which has activity of catalyzing, at higher selectivity than the protein consisting of the amino acid sequence shown in SEQ ID NO. 1, the reaction represented by the following formula (1):

[Formula 4]



Formula (1)

[Claim 10] A DNA encoding the hydrolase protein according to any one of claims 1 to 9.

[Claim 11] The DNA according to claim 10, which consists of the following nucleotide sequence:

(a) the nucleotide sequence shown in any one of SEQ ID NOS. 7 to 10;

(b) a nucleotide sequence of a DNA capable of hybridizing under stringent conditions with the DNA consisting of a complementary strand of the nucleotide sequence shown in any one of SEQ ID NOS. 7 to 10,

wherein the nucleotide sequence encodes an amino acid sequence wherein one or more amino acids which correspond to amino acid numbers 70, 270, 273, and 313 in the amino acid sequence shown in any one of SEQ ID NOS. 1 to 5 are substituted with other amino acids whose side chains have lower bulkiness than that of wild-type amino acids, and

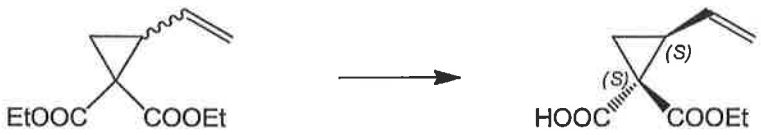
wherein the nucleotide sequence encodes a hydrolase protein having activity of catalyzing, at higher selectivity than the protein consisting of the amino acid sequence shown in SEQ ID NO. 1, the reaction represented by the following formula (1); or

(c) a nucleotide sequence comprising a substitution, deletion, and/or addition of one or several nucleotides with respect to the nucleotide sequence shown in any one of SEQ ID NOS. 7 to 10,

wherein the nucleotide sequence encodes an amino acid sequence wherein one or more amino acids which correspond to amino acid numbers 70, 270, 273, and 313 in the amino acid sequence shown in any one of SEQ ID NOS. 1 to 5 are substituted with other amino acids whose side chains have lower bulkiness than that of wild-type amino acids, and

wherein the nucleotide sequence encodes a hydrolase protein having activity of catalyzing, at higher selectivity than the protein consisting of the amino acid sequence shown in SEQ ID NO. 1, the reaction represented by the following formula (1):

[Formula 5]



Formula (1)

[Claim 12] A method for producing (1S,2S)-1-alkoxycarbonyl-2-vinylcyclopropanecarboxylic acid, which comprises allowing the hydrolase protein according to any one of claims 1 to 9 to act on dialkyl 2-vinylcyclopropane-1,1-dicarboxylate to produce (1S,2S)-1-alkoxycarbonyl-2-vinylcyclopropanecarboxylic acid.