The use of enzymes in the context of flavors and fragrances

Compounds that find widespread application in food, beverages, cosmetics, detergents and pharmaceutical products

world-wide industrial size estimated at US$ 16 billion

ELSEVIER
Review
TRENDS in Biotechnology Vol.23 No.4 April 2005
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Biocatalytic preparation of natural flavours and fragrances

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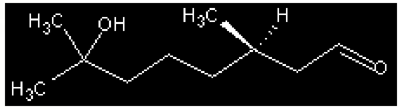
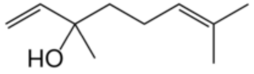
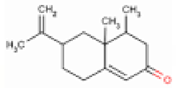
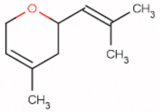
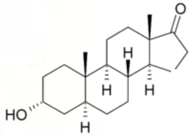
Chirality and odor

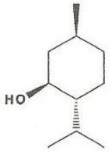
Compound	Odour description
7-Hydroxy-6,7-dihydro-citr onellal	(+): Lily of the valley with green minty notes (−): Sweet lily of the valley note
Linalool	(+): Sweet, petigrain (−): Woody, lavende
Nootkatone	(+): Grapefruit (−): Woody, spicy
Nerol oxide	(+): Green, floral (−): Green, spicy, geranium
Androstenone	(+): Odourless (−): Sweaty, urine, strong, musky
Menthol	(−): Sweet, fresh, minty, strong cooling effect (+): Dusty, vegetable, less minty, less cooling



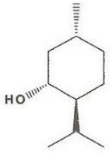






(+)-menthol



(-)-menthol

‘Natural’ flavours

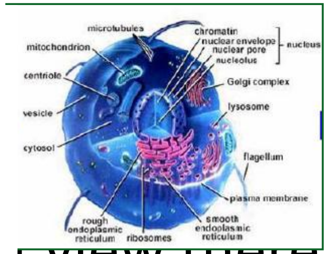
US and European legislations have meant that ‘natural’ flavour substances can only be prepared either by physical processes (extraction from natural sources) or by enzymatic or microbial processes, which involve precursors isolated from nature.
This classification created a dichotomy in the market because compounds labelled ‘natural’ become profitable products whereas other flavours that occur in nature but are produced by chemical methods must be called ‘natureidentical’ and are less appreciated by consumers.

These differences have stimulated much research aimed at developing new biotechnological processes for these valuable compounds.

CE 1334/2008
EC Flavor directive 88/388/EEC
US Code of Federal Regulation 21 CFR 101.22

The ‘natural’ routes for flavour production are:

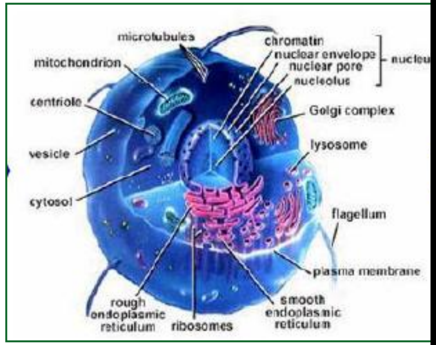
- the bioconversions of natural precursors using biocatalysis,
- de novo synthesis (fermentation) and isolation from plants and animals.

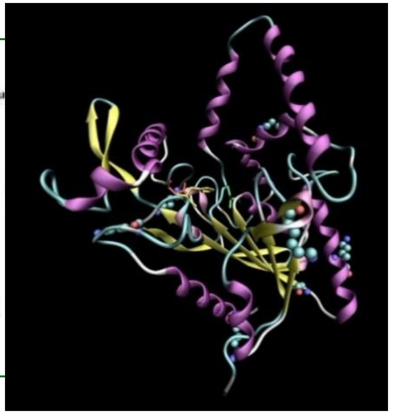


Although from the c […] view there is no difference between a compound synthesized in nature and the molecule produced in the laboratory (i.e. nature identical) the price of a flavour sold as natural is often significantly higher than a similar one prepared by chemical synthesis.

Biotechnological methods for the synthesis of natural fragrances and flavors

- Biocatalysis
  - Enzymes
  - Whole cells





- Biosynthesis and fermentation

-Fungi,

-moulds,

-bacteria

Biotechnological methods for the synthesis and production of flavors and fragrances

Some examples

- Vanillin

- Menthol

- Grey Amber (Ambergris)

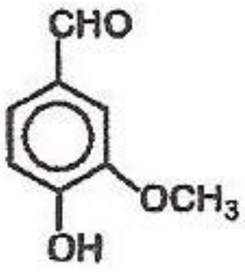
- Irones

Vanilla planifolia, Orchidacea

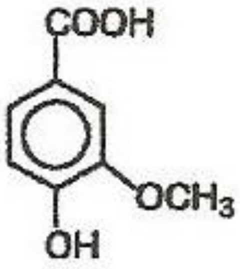
This compound occurs in the pods of tropical Vanilla orchids (mostly *Vanilla planifolia*) at levels of 2% by weight, but less than 1% of the global market is covered by the extracted compound.

Principal NATURAL COMPONENTS

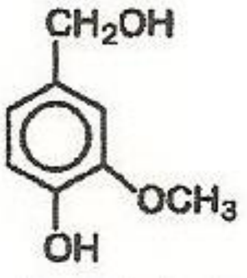
Vanillin



Vanillic acid



Vanillyl alcohol



Consumed: 12.000 t
Request: 15.000 t
Produced by extracton: 20 t → $1200-4000/kg
Produced by biotechnology: 1-10 t → $1000/kg
The rest obtained by chem. synthesis → $15/kg

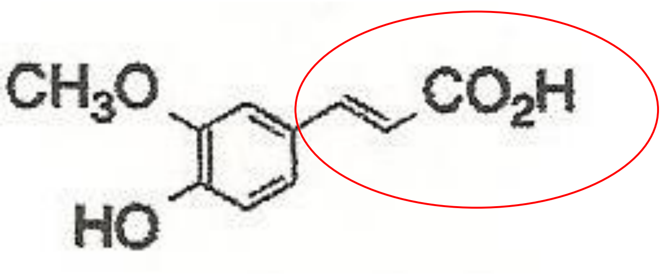
Cost

Examples of studies for the production of vanillin via biotechnological routes

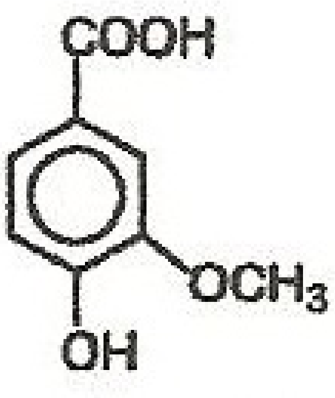
Micro-organism	Substrate	Product	Author
Pycnoporus cinnabarinus	Ferulic acid	Vanillin	Falconnier *et al* 1994
Aspergillus niger	Ferulic acid	Vanillin	Lesage-Meessen *et al* 1996
Pycnoporus cinnabarinus			
Pseudomonas acidovorans	Ferulic acid	Vanillin	Toms and Wood 1970
Corynebacterium glutamicum	Ferulic acid	Vanillin	Labuda *et al* 1993
Paecilomyces variotii	Ferulic acid	Vanillin	Rahouti *et al* 1989
Pestalotia palmarum			
Spirulina platensis	Ferulic acid	Vanillin	Ramachandra Rao *et al* 1996
Haematococcus pluvialis	Ferulic acid	Vanillin	Usha Tripathi *et al* 1999
Pseudomonas fluorescens	Ferulic acid	Vanillic acid	Andreoni *et al* 1995
Escherichia coli	Ferulic acid	Vanillin	Otuk 1985
Alcaligenes paradoxus	Ferulic acid	Vanillin	Krishnamohan and Khanna 1994
Streptomyces setonii	Ferulic acid	Vanillin	Sutherland *et al* 1983
Fomes fomentarius	Ferulic acid	Vanillin	Ishikawa *et al* 1963
Polyporus versicolor	Ferulic acid	Vanillin	Rosazza *et al* 1995
Rhodotorula rubra	Ferulic acid	Vanillic acid	Huang *et al* 1993
Corynebacterium glutamicum	Eugenol	Vanillin	Tadasa and Kayahara 1983
Pseudomonas spp	Eugenol	Vanillin	Rabenhorst 1996
Serratia spp	Eugenol &	Vanillin	Rabenhorst 1991
Enterobacter spp	Isoeugenol		
Arthobacter globiformis	Eugenol	Vanillin	Cooper 1987
Serratia marcescens	Vanillin	Vanillin acid	Prestelo *et al* 1989
Streptomyces viridosporus	Vanillin	Vanillic acid	Pomento and Crawford 1983
Aspergillus niger	Vanillylamine	Vanillin	Yoshida *et al* 1997
Escherichia coli	Vanillylamine	Vanillin	
Pycnoporus cinnabarinus	Vanillic acid	Vanillin	Lesage-Meessen *et al* 1997
Proteus vulgaris	*m*-Methoxytyrosine	Vanillin	Casey and Dobb 1992
Stilbene dioxygenase	Isorhaponin	Vanillin	Hagedorn and Kaphammer 1994
Lipoxygenase	Coniferyl aldehyde	Vanillin	Markus *et al* 1992
	Ferulic acid	Vanillin	Mane and Zucca 1992
Brettanomyces anomalus	Ferulic acid	Vanillin	Edlin *et al* 1995
	Caffeic acid		
	Coumaric acid		
Penicillium simplicissimum	Vanillyl alcohol	Vanillin	Fraaije *et al* 1997

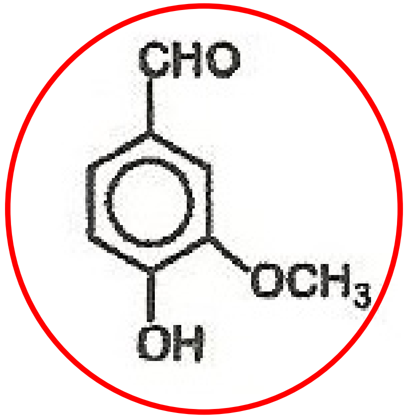
Production of vanillin through bioconversion of lignin components

Ferulic acid → (*P. cinnabarinus*) → vanillic acid → Vanillin



Building block of lignin

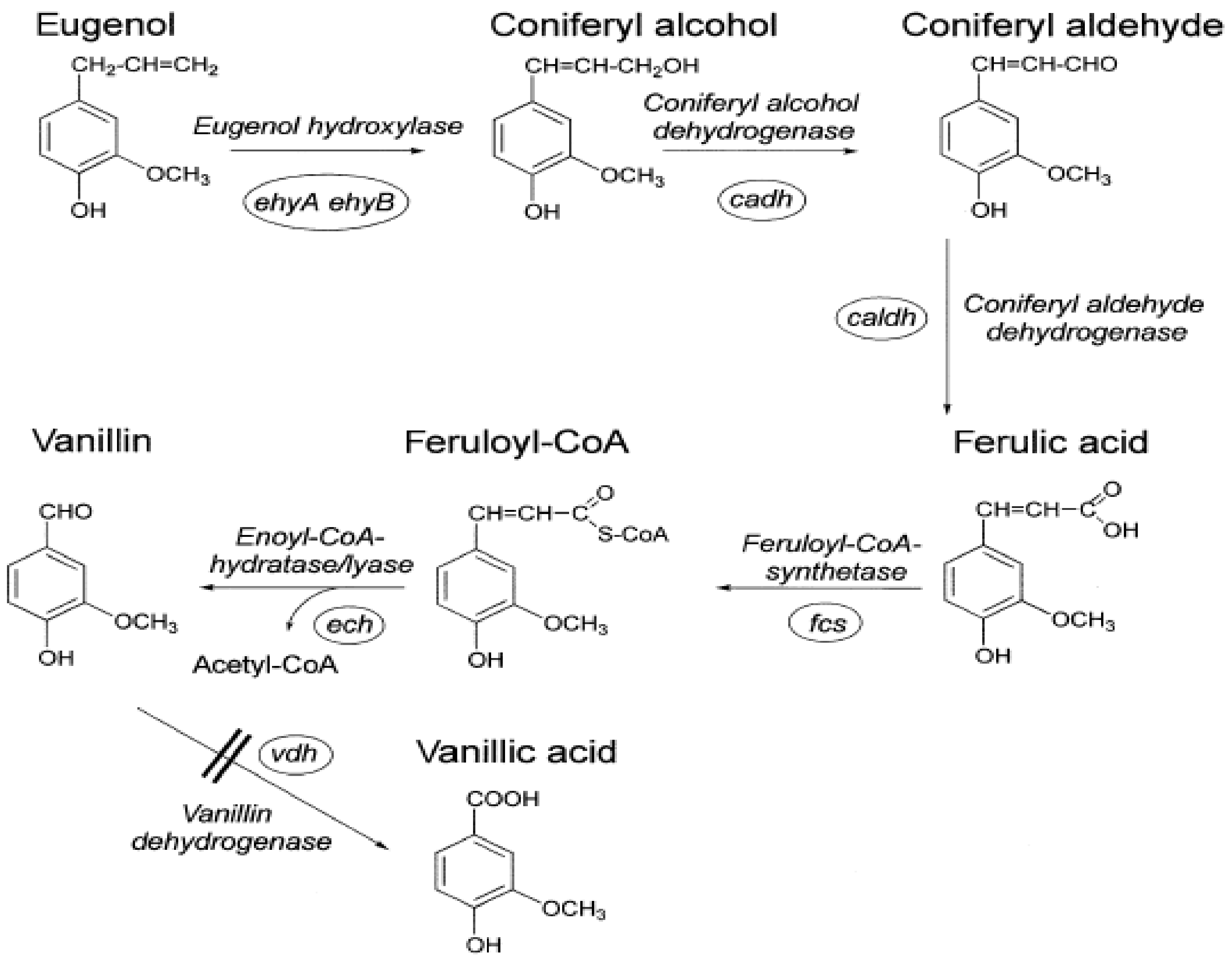


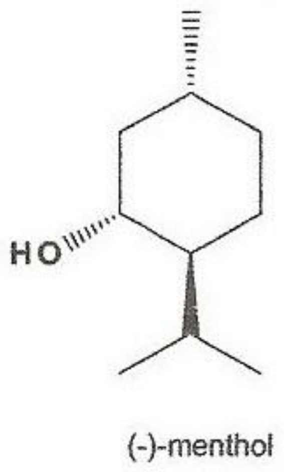


•*Pycnoporus cinnabarinus* degrades side chain of ferulic acid to obtain vanillic acid that is then reduced to vanillin

Production of vanillin by means of Pseudomonas sp. (engineered)

From essential oil of clove, nutmeg, cinnamon, basil



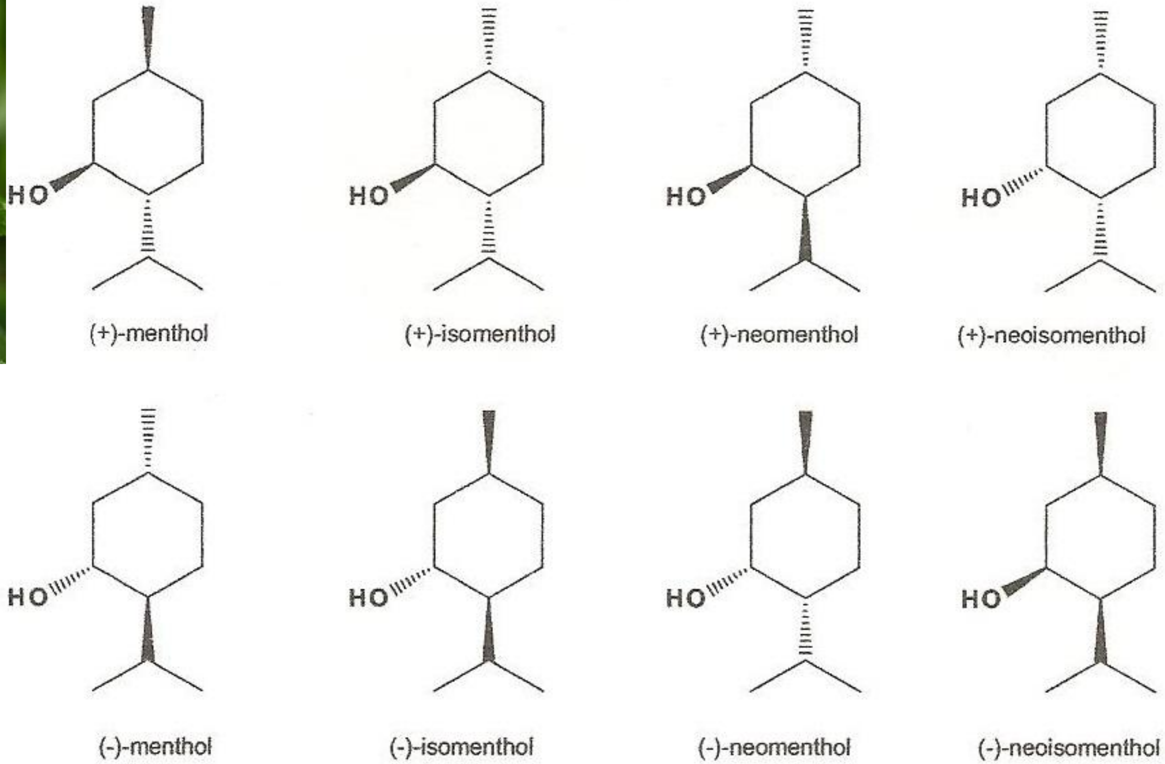


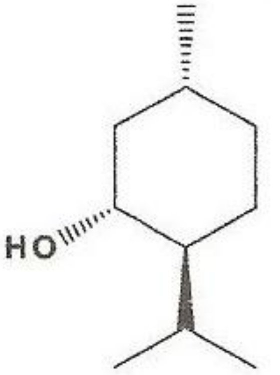
Menthol
Genus *Mentha, Lamiaceae*

Mentha piperita, *M. arvensis*, *M. spicata*

main specie used for extraction

estimated world production of menthol: 11 800 tons.



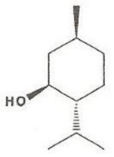


(-)-menthol

The desired organoleptic properties of this monoterpene are related to its absolute configuration and from the eight possible isomers, only the natural (-)-(1R,3R,4S) isomer is suitable as a flavourant. In 1998, the estimated world production of menthol was 11 800 tons.

Extraction and crystallization

The majority of (-)-menthol is still obtained by freezing the oil of *Mentha arvensis* to crystallise the menthol present.



(+)-menthol

(-)-menthol

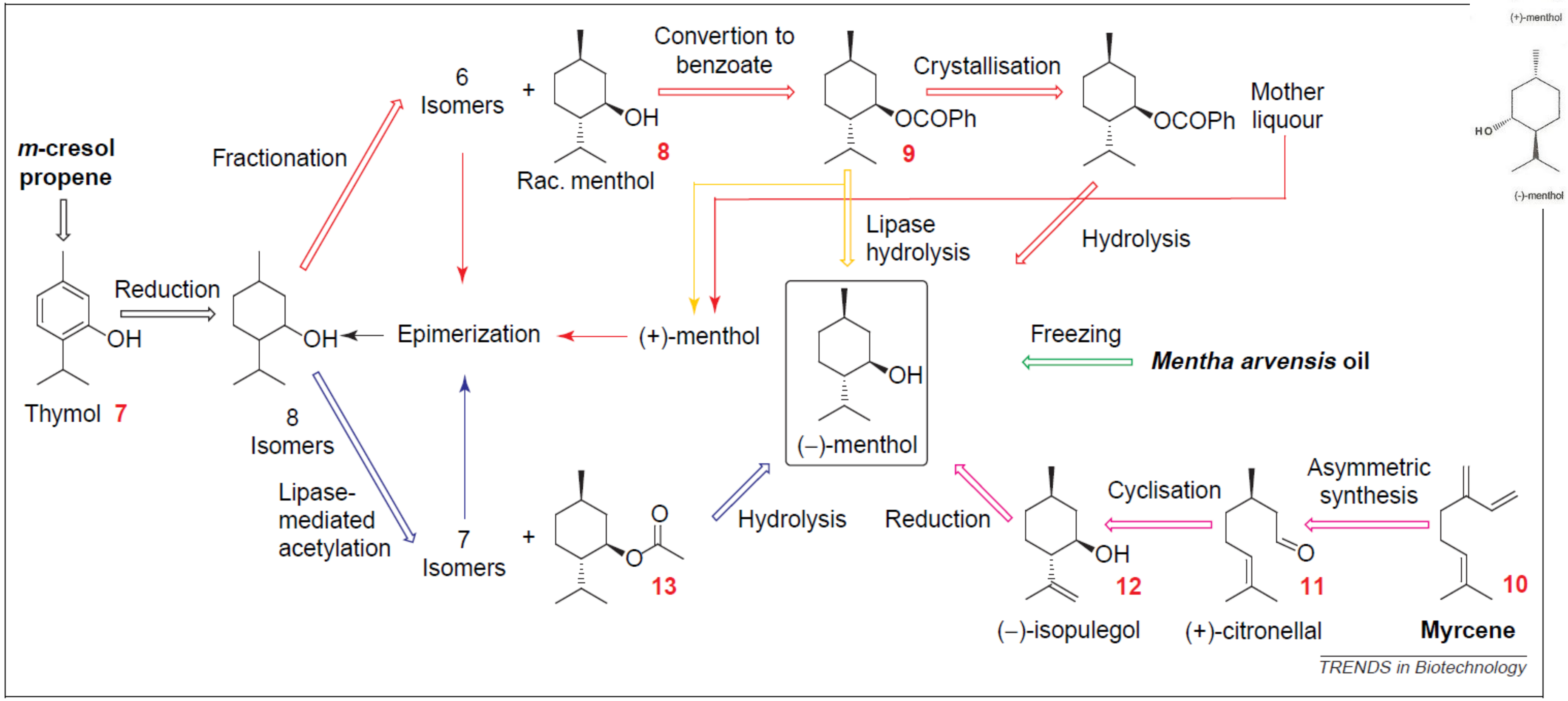


Figure 2. Industrial production of (−)-menthol. Red, green and violet arrows indicate Haarmann and Reimer, extractive and Takasago processes, respectively. Yellow and blue arrows indicate the new biocatalytic processes of Haarmann and Reimer and AECI, respectively.

Fragrances for parfumes

- Grey Amber (Ambergris)

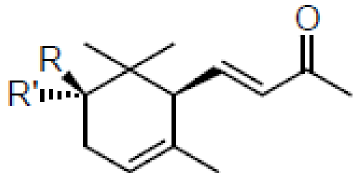
- Iris (Irones)




Although these natural products are expensive, they are still used in fine formulations because they give better results compared with the corresponding synthetic materials.

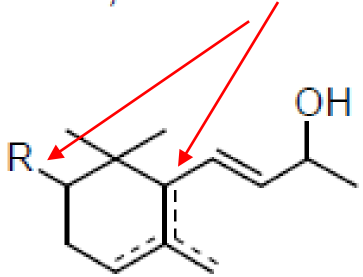
The odorous principles components of these raw materials are the norterpenoid ionones and irones.

This superiority is due to the complexity of the natural isomeric mixture in which each component might show different olfactory features.



R=R'=H α-ionone

R=Me, R'=H *cis*-α-irone

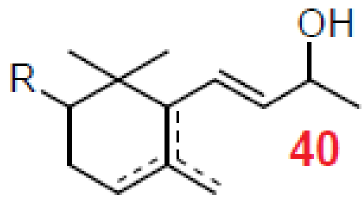


Iris, Iridaceae

(rhizomes: modified subterranean stem)

Ionones have been found in several plants, whereas **irones** were formed during ageing and manufacturing of the iris rhizomes.

R=Me, R'=H *cis*-α-irone



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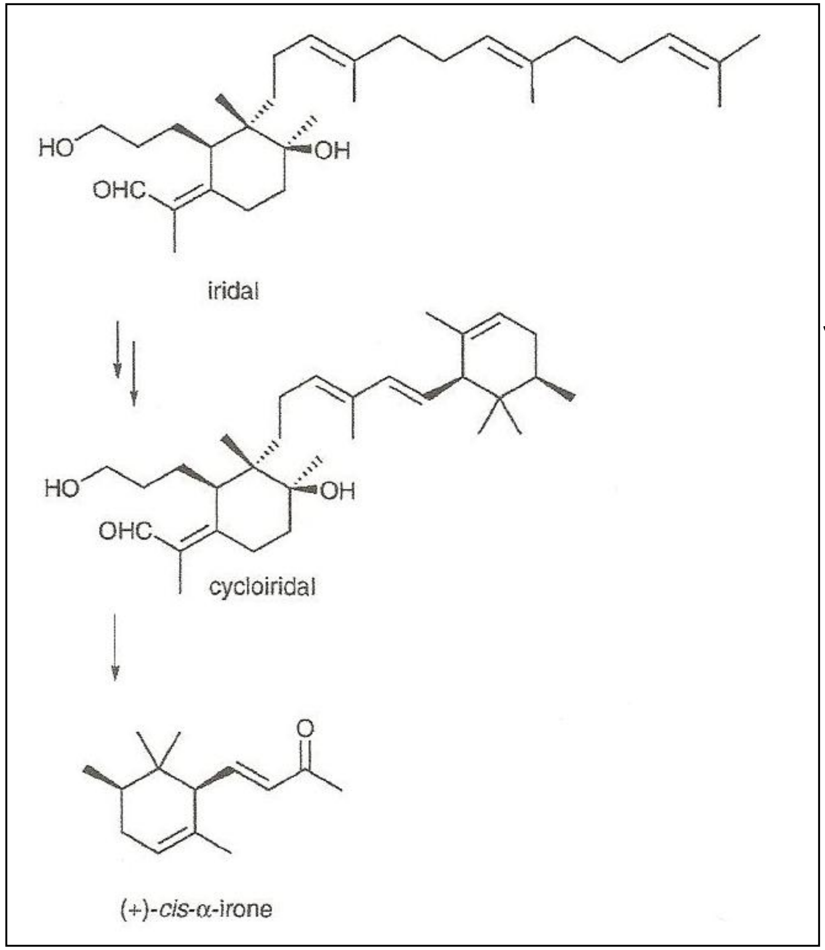
Iris absolute: 40000-50000 €/kg

Chanel No. 19

Traditional method: 400 mg/ kg after 3 years drying

Patented biotechnological method

Traditional method: 400 mg/ kg after 3 years drying



Iris rhizomes

↓

Serratia liquefaciens + Pseudomonas maltophilia

↓

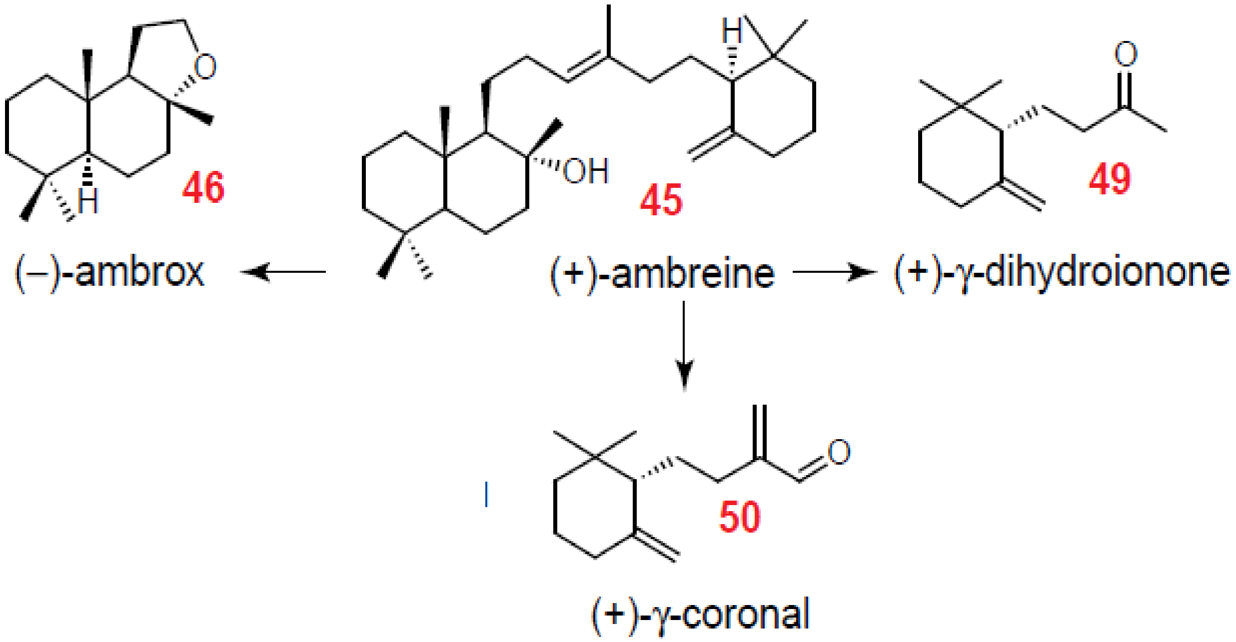
irones

1 g/kg after 8 days

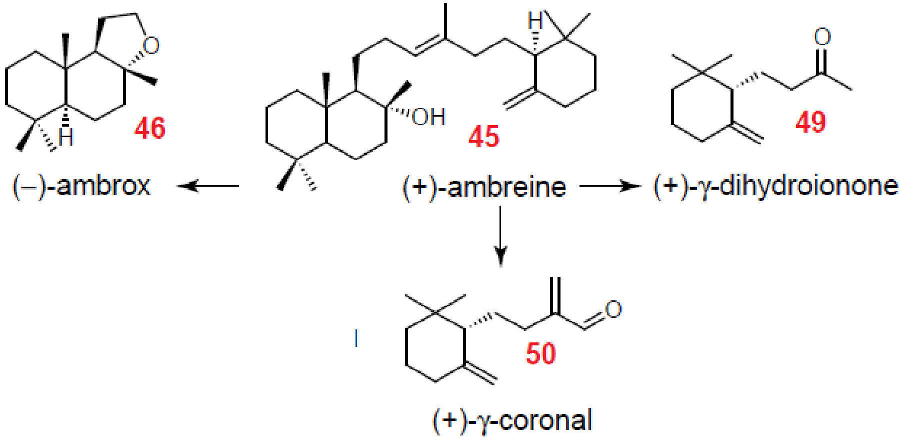
Grey amber (ambergris)

Ambergris is a secretion found in the intestinal tract of the sperm whale. (*Physeter catodon macrocephalus)*

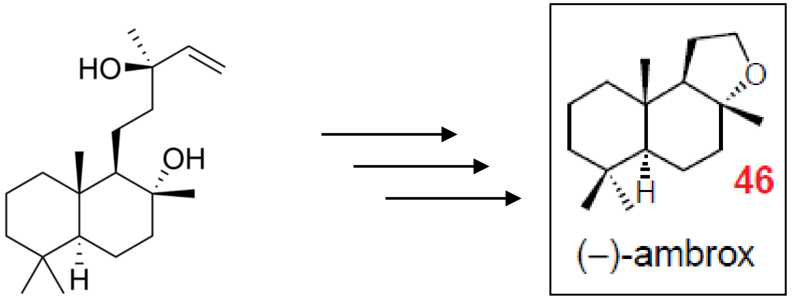
This secretion contains the odourless triterpene ambreine (45) that on exposure to sunlight, air and seawater, undergoes a degradative process deriving compounds that are responsible for the complex odour of ambergris.



Grey amber (ambergris)



The most appreciated one is the tricyclic ether (-)-ambrox (46), which is currently produced by semisynthesis from sclareol, a diterpene present in clary sage.



sclareol