Biocatalytic preparation of natural flavours and fragrances

Stefano Serra[1], Claudio Fuganti[2] and Elisabetta Brenna[2]

[1]C.N.R. Consiglio Nazionale delle Richerche, Istituto di Chimica del Riconoscimento Molecolare,
Sezione A. Quilico' (Institute of Chemistry of Molecular Recognition, A. Quilico section), Via Mancinelli 7, I-20133 Milano, Italy
[2]Dipartimento di Chimica, Materiali ed Ingegneria Chimica 'Giulio Natta' del Politecnico (G. Natta Department of Chemistry,
Materials and Chemical Engineering),Via Mancinelli 7, I-20133 Milano, Italy

During the past years biocatalytic production of fine chemicals has been expanding rapidly. Flavours and fragrances belong to many different structural classes and therefore represent a challenging target for academic and industrial research. Here, we present a condensed overview of the potential offered by biocatalysis for the synthesis of natural and natural-identical odorants, highlighting relevant biotransformations using microorganisms and isolated enzymes. The industrial processes based on biocatalytic methods are discussed in terms of their advantages over classical chemical synthesis and extraction from natural sources. Recent applications of the biocatalytic approach to the preparation of the most important fine odorants are comprehensively covered.

Introduction

The preparation of flavours and fragrances by isolating them from natural resources began in ancient times. Concurrently, the production of fermented foods (beer, wine and others) allowed the generation of new aromas and formed the roots of modern biotechnology. For many centuries these were the only methods for obtaining this type of compound, albeit in complex mixtures. Rapid progress began with the development of synthetic organic chemistry. More than a century ago the preparation of coumarin (1868) and vanillin (1874) provided the first fragrance and flavour compounds available by synthesis [1]. From the very beginnings of chemistry the improvement of analytical and synthetic knowledge allowed the isolation and preparation of an impressive number of aromas at the industrial scale [2]. Moreover, the non-natural fragrances were created both by emulation of natural structures – by systematic studies on the relationship between odour and chemical structure – and by serendipity [3]. Until recently all these compounds found widespread application in food, beverages, cosmetics, detergents and pharmaceutical products with a world-wide industrial size estimated at US$ 16 billion in 2003 (http://www.leffingwell.com/top_10.htm). Although the majority of these products were prepared by chemical synthesis or by extraction from plants, the employment of new biotechnological processes has increased considerably

in the past decades [4–8]. Chiral flavours often occur in nature as single enantiomers. Because different enantiomers or regioisomers could show different sensorial properties, their specific synthesis is beneficial [9]. Biocatalysis represents a useful tool in this field catalysing a large number of stereo- and regioselective chemical manipulations that are not easily achieved by the less selective classical synthetic procedures. Furthermore, the increasing sensitivity of the ecological systems supports the choice of environmentally friendly processes and consumers have developed a preference for 'natural' or 'organic' products, thus developing a market for flavours of biotechnological origin [10].

'Natural' flavours

Recent US [11] and European [12] 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 'nature-identical' and are less appreciated by consumers. These differences have stimulated much research aimed at developing new biotechnological processes for these valuable compounds. 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 chemist's point of view there is no difference between a compound synthesized in nature and the identical molecule produced in the laboratory, the price of a flavour sold as natural is often significantly higher than a similar one prepared by chemical synthesis. For example, vanillin (**1**) [13] is the most important flavour in terms of consumption levels (Figure 1). 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. The value of vanillin extracted from pods is variously calculated as being between US$1200/kg and US$4000/kg, whereas the price of synthetic vanillin, that is vanillin prepared mainly from guaiacol, is less than US$15/kg. Therefore, several

Corresponding author: Serra, S. (stefano.serra@icrm.cnr.it).
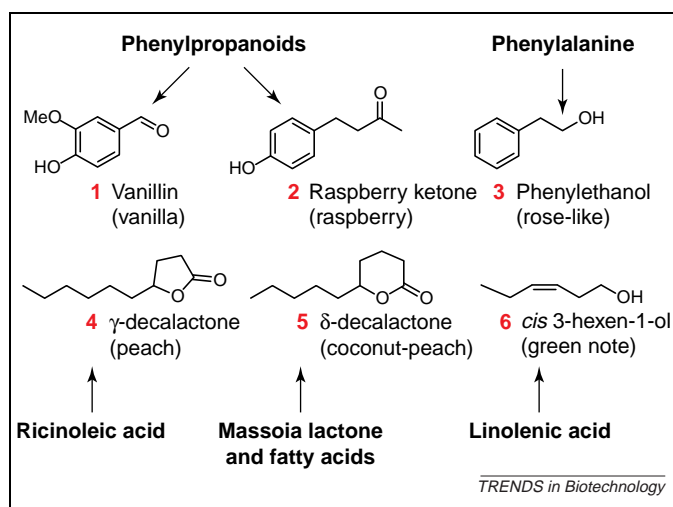
Figure 1. Examples of some relevant natural flavours prepared by biotransformation.

biotechnological processes for natural vanillin production have recently been developed [14] including the bioconversion of lignin, phenylpropanoids (ferulic acid, eugenol, isoeugenol) and phenolic stilbenes (isorhapontin) in addition to the *de novo* biosynthesis.

Similarly, raspberry ketone (Figure 1) (**2**) and 2-phenylethanol (**3**) are phenylpropanoids used in industries as flavours and/or fragrance ingredients. Compound **2** is the key flavour molecule of raspberries in which it occurs in trace amounts (<4 mg of ketone from 1 kg of berries). Compound **3** has a rose-like odour and occurs in fermented foodstuffs and in many essential oils. For both compounds extraction is unsuitable [5] and their main mode of production is the bioconversion of some natural precursors. 4-(4-hydroxyphenyl)butan-2-ol (betuligenol), its O-glucoside (betuloside) and 4-hydroxybenzalacetone are possible precursors for biotechnological production of raspberry ketone performed by oxidation of the secondary alcohol of the first two compounds and by double bond saturation of the third, using different microbial systems [15,16]. In the context of biogeneration of raspberry ketone in the fungus *Beauveria bassiana*, it emerged that odour inactivation of compound **2** occurs through Baeyer-Villiger oxidation to tyrosol [17]. Moreover, 2-phenylethanol (**3**) and its acetate are currently produced by yeast degradation of natural L-phenylalanine [18].

Lactones (**4**, **5**) and *cis*-3-hexenol (**6**) are also natural flavours produced at the industrial scale. **4**, **5** and analogues with up to twelve carbon atoms are widespread in fermented food, milk products and in a variety of fruits in minute amounts. Some of these materials are manufactured by degradation, via β-oxidation, of natural hydroxy-fatty acids [19,20]. Specifically, the γ-decalactone (**4**) is obtained by chain shortening of C-18 ricinoleic acid (from castor oil) by different microorganisms. Improvement of the processes caused the selling price of compound **4** to decrease from US$ 12000/kg in 1986 to US$ 500/kg in 1998 [19]. Similarly, some precious γ-lactones containing an odd number of carbon atoms are accessible by degradation of natural hydroxy acids [21]. Interestingly, δ-decalactone (**5**) can be obtained by natural modification

either by oxidation of hydroxy-fatty acids or by enzymic reduction of the α,β-unsaturated compound (massoia lactone) the main component of massoi bark oil. [22]. Linolenic acid is the natural precursor of *cis*-3-hexen-1-ol (leaf alcohol) (**6**). This compound has an odour of freshly cut grass and is essential for obtaining the 'green' organoleptic note in many formulations. The 'green notes' obtainable by distilling plant oil are expensive and different biotransformations were developed. The lipoxygenase- and hydroperoxide lyase-mediated oxidation of linolenic and linoleic acid produce *cis*-3-hexen-1-al and hexen-1-al, which can be reduced by yeast to the corresponding alcohols [23]. Additionally, *n*-hexanol is easily accessible by microbial reduction of the carboxylic group of extractive C-6 caproic acid [24].

Many biocatalytic processes for other attractive flavours have recently been described. In spite of this the number of industrial applications is limited and the cases illustrated above are the more promising ones. Moreover, an additional problem in this area is the occurrence of adulterations with readily available 'nature-identical' products. The achievement of new analytical methods for discriminating between natural and nature-identical flavours has become essential [25]. Different studies based on stable isotope characterisation of aromas have showed promising results and are now applied by specialized laboratories to prove authenticity [26–30].

Nature-identical flavours

The method of production of the nature-identical flavours and fragrances is determined by stringent economic considerations. Although the biocatalytic approaches to these compounds are often expensive, different applications have been described. Environmentally friendly conditions and high chemical selectivity make biocatalytic approaches attractive. Two separate fields should be examined: (i) industrial production and (ii) academic synthesis (synthesis not used for industrial production but mainly for scientific interest) of fine flavours. Few applications are related to the first case in which isolated enzymes were mainly used. Lipases were the favourite catalyst because they show remarkable chemoselectivity, regioselectivity and enantioselectivity. Moreover, they are easily available on a large scale and remain active in organic solvents [31,32].

Menthol: an outstanding industrial case

Menthol is one of the most important flavour compounds and it is used extensively as a food additive, in pharmaceuticals, cosmetics, toothpastes and chewing gum. 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 [33].

The majority of (−)-menthol is still obtained by freezing the oil of *Mentha arvensis* to crystallise the menthol present (Figure 2). Although many efforts have been devoted to the production of (−)-menthol from other readily available raw materials, only the Haarmann and Reimer (H&R) and the Takasago processes are commercial
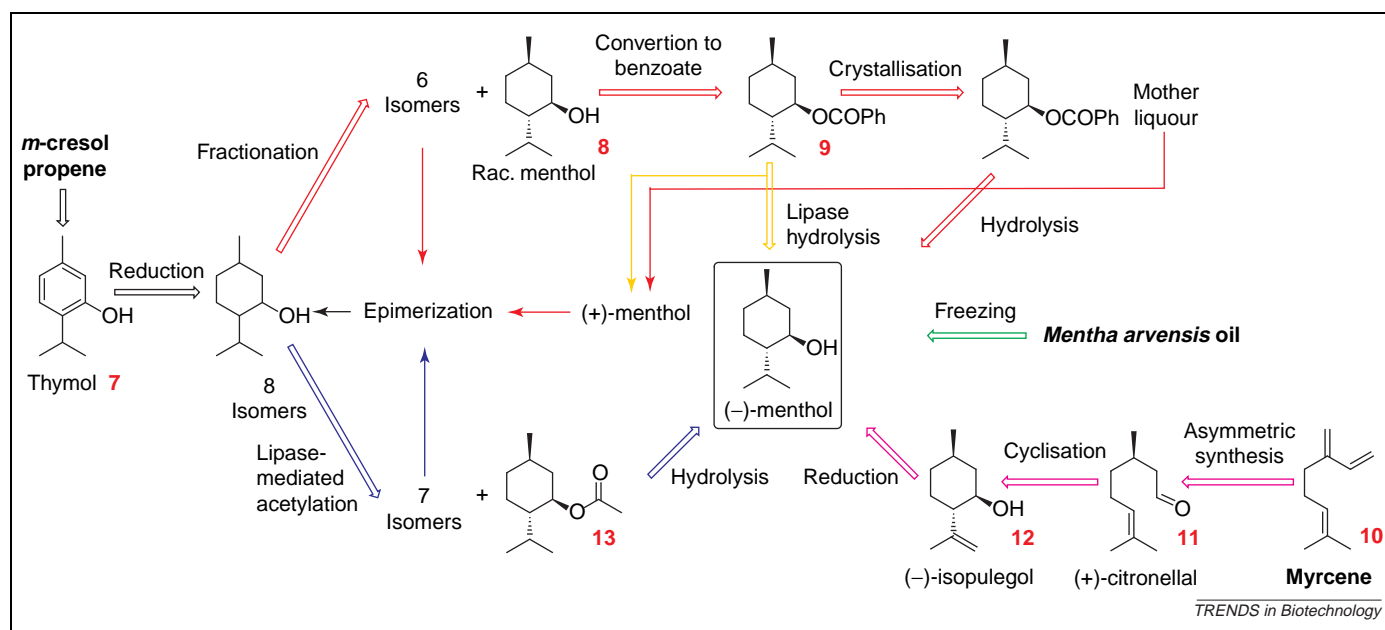
Figure 2. Industrial production of (−)-menthol. Red, green and violet arrows indicate Haarmann and Reimer, extractive and Takasago processes, respectively. Yellow and blue arrows indicate the new biocatalytic processes of Haarmann and Reimer and AECI, respectively.

synthetic sources of this flavour [34]. The first is based on a classical resolution procedure and starts from inexpensive *m*-cresol and propylene to produce thymol (**7**). This compound is hydrogenated to give the mixture of the eight isomers of menthol. Fractional distillation gives racemic menthol (**8**) (two isomers) which is converted to the corresponding benzoate (**9**) and resolved by fractional crystallization. Saponification produces (−)-menthol whereas the mother liquour gives (+)-menthol. The (+)-menthol and the other six isomers are recycled in a separate racemization step. Takasago uses asymmetric synthesis [35] in the key step of its process. Myrcene (**10**) is converted in diethygeranylamine, which is isomerised to (+)-citronellal (**11**) in the presence of a chiral rhodiun phosphine catalyst (RhI-(*S*)-BINAP). The transformation of citronellal into (−)-menthol is performed through cyclisation to (−)-isopulegol (**12**) followed by hydrogenation. Two new processes similar to the first described route based on lipase resolution have been proposed recently. Haarmann and Reimer (H&R) accomplished the resolution of racemic menthol benzoate (**9**) by lipase-mediated (e.g. *Candida rugosa* lipase) enantioselective hydrolysis to provide (−)-menthol with essentially complete enantioselectivity [36,37]. Furthermore, the AECI Ltd (http://www.aeci.co.za/) process starts directly from the mixture of the eight isomers of menthol [38]. The enantio- and diastereoselective acylation of this mixture using lipases yields menthyl acetate (**13**) in at least 96% enantiomeric excess. The ester is separated from the unreacted isomers by distillation and then hydrolysed to yield (−)-menthol. In both processes the undesired isomers are recycled by isomerisation. Although neither the H and R or AECI process have yet been commercialised, these means are based on the well-established route of racemic menthol preparation and will certainly be developed further.

p-Menthane monoterpenes: academic studies of industrial interest

The monoterpenes of the *p*-menthane family are widespread in nature and are well-known as flavouring ingredients and as valuable synthetic intermediates. Many industrial processes depend on this class of compounds because of the high commercial requirement for (−)-menthol. In addition, some new findings on the peculiar organoleptic properties of different *p*-menthane alcohols, lactones and ethers have prompted studies on their synthesis. For example, the mixture of the eight isomers of isopulegol (**12**) is conventionally used as a perfume ingredient whereas recent investigations have shown that its main component (−)-isopulegol is odourless and can be used as a cooling agent [39] (Figure 3). Furthermore, the lactone (**18**) [40], (−)-mint lactone (**19**) and (+)-isomint lactone (**20**) [41] were found to be minor components in the essential oil of peppermint and are used in commercial flavours for their much-appreciated coumarin-like and mint-like olfactive properties. The (−)-wine lactone (**21**) was recognized as a key flavour compound of different white wines and synthetic studies revealed [42] that natural **21** is the most powerful isomer with an odour threshold <0.04pg/L, whereas the weakest isomer shows a threshold $>10^6$ pg/L. A similar case is that of (+)-dill ether (**22**), which is the most important constituent of dill essential oil. The evaluation of its isomeric forms established that **22** shows a high odour-activity value and is the character-impact compound of dill flavour (the compound responsible for the features of the odour) [43]. These studies show that both the quality and intensity of these odorants are related to relative and absolute configurations. Taking advantage of the known selectivity of biocatalysis many specific preparations of these monoterpenes have been developed. (−)-Isopulegol (**12**) was prepared in optically pure form by lipase
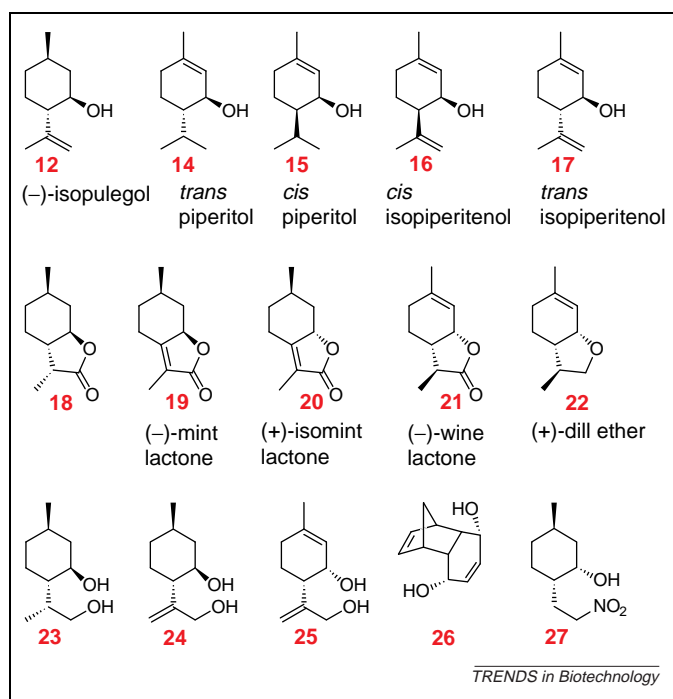
Figure 3. The alcohols **12**, **14**, **15**, **16** and **17**, esters **18**, **19**, **20** and **21** and ether **22** are *p*-menthane monoterpenes found in nature that can be prepared in enantiopure form by the use of the biocatalysis. Compounds **23-27** are key intermediates in their synthesis.

PS-mediated (lipase from *Pseudomonas* sp.) enantio- and diastereoselective acetylation of the commercial mixture of its eight isomers [44]. *Trans* and *cis* piperitol and isopiperitenol (**14**-**17**) (Figure 3) are valuable intermediates in the synthesis of menthol, whereas diols (**23**-**25**) are useful precursors of the *p*-menthane lactones. The diols were prepared in a single diastereoisomeric form and were resolved by lipase-mediated acetylation of the corresponding racemic materials [44,45]. The enantiopure diols obtained (**23** and **24**) were converted by chemical manipulation to lactone **18** and mint lactone **19**, respectively [45]. Diol **25** is more versatile and was transformed into either the wine lactone **21** or the dill ether **22** [46]. Lipase PS was also used for the resolution of the *meso* tricyclic diol **26** [47] whereas the alcohol **27** was obtained by stereoselective baker's yeast-mediated reduction of the corresponding ketone [48]. These two enantiopure materials were converted into the mint and isomint lactone, respectively.

Baker's yeast: another useful tool in biocatalysis
Many species of yeast have been used in biocatalysis, particularly for flavour preparation. Baker's yeast is the most commonly used microorganism in organic syntheses because it is easy available, inexpensive and versatile. Although it has been used for producing small chiral building blocks of general interest [49], several recent applications to flavour chemistry should be noted. For example, the baker's yeast-mediated reduction of the prochiral double bond of the alcohol **28** produced (*S*)-(+)-3-(*p*-tolyl)-butanol (**29**) in high enantiomeric purity [50] (Figure 4). This was used in the preparation of the natural bisabolane sesquiterpenes (+)-curcumene (**30**), (+)-turmerone (**31**), (+)-dehydrocurcumene (**32**) and (+)-nuciferal (**33**), which are flavour components of many essential

oils. Moreover, baker's yeast allowed the diastereo- and enantioselective reduction of the γ-keto-acids of type **34**. The enantiopure materials obtained were used as starting material for the preparation of (−)-*cis* **35** and (+)-*trans* **36** whisky lactones, (-)-*cis* **37** and (+)-*trans* **38** cognac lactones [51] and *cis*-aerangis lactone **39** [52]. The first four compounds are the key flavours of aged alcoholic beverages, such as whisky, brandy and cognac whereas lactone **39** is the main odour component of the flowers of orchid *Aerangis confusa*.

Valuable fragrances: violet, amber and jasmine
Iris essential oil, ambergris and jasmine absolute are considered the historically most important flavour and fragrance compounds. Although these natural products are expensive, they are still used in fine formulations because they give better results compared with the corresponding synthetic materials. This superiority is due to the complexity of the natural isomeric mixture in which each component might show different olfactory features. For example, until the end of the 19th century the only source of violet fragrance was violet flower oil and iris essential oil. The odorous principles components of these raw materials are the norterpenoid ionones and irones (Figure 5). Ionones have been found in several plants, whereas irones were formed during ageing and manufacturing of the iris rhizomes. These compounds occur in nature as a mixture of regioisomers (α, β and γ) and enantiomers. Overall, five ionone and ten irone stereoisomers are possible. Thanks to the use of chemical synthesis and enzyme-mediated reactions, all these isomers were prepared [53,54] and submitted to olfactory evaluation. The key steps in these syntheses were the enatioselective lipase-mediated acetylation of suitable
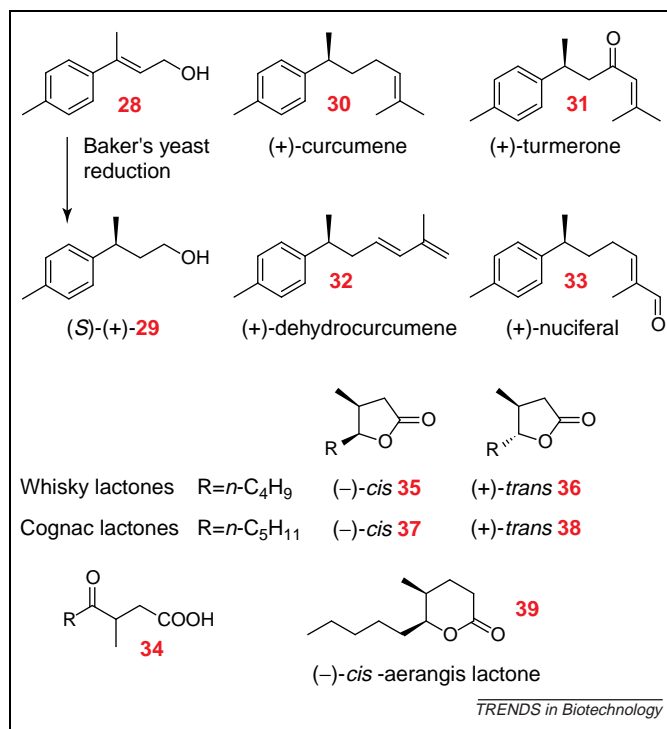

Figure 4. Compounds **30-33** and **35-39** are flavours and fragrances that can be prepared by baker's yeast-mediated biotransformations.
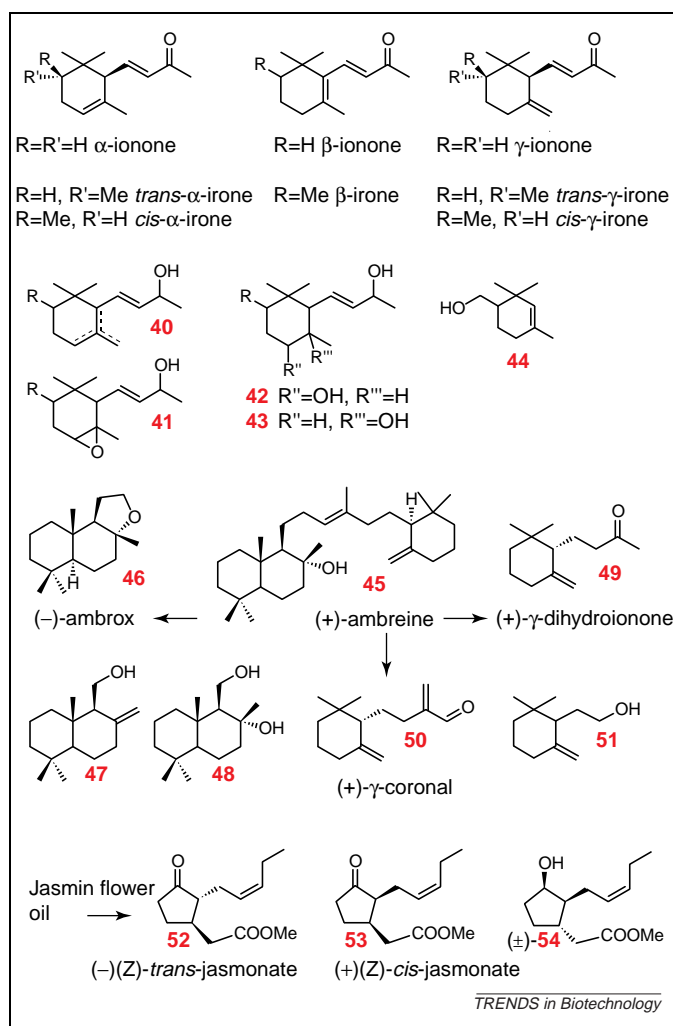
Figure 5. Violet, amber and jasmine. Examples of fine fragrances that can be prepared in enantiopure form by aid of the biocatalysis.

alcohols. Racemic ionols and irols **40** (Figure 5; R=H and Me) their corresponding α-epoxy-derivatives **41** and the diols **42** and **43**, were resolved by lipase PS [53–55]. Moreover enantiopure alcohol **44** was used in irone synthesis and was prepared from the racemic alcohol by sequential porcine pancreatic lipase (PPL)-mediated acetylation and hydrolysis [56].

Similar biotechnological approaches have been studied for amber and jasmine fragrances. The first class of compounds derives its name from the compound amber-gris [57], which is a secretion found in the intestinal tract of the sperm whale. 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. The most appreciated one is the tricyclic ether (−)-ambrox (**46**), which is currently produced by semi-synthesis from sclareol, a diterpene present in clary sage. Recently, **46** was obtained in several chemical transform-ations from enantiopure (+)-albicanol **47** and diol (+)-**48**. These compounds were prepared by kinetic resolution of the corresponding racemic materials mediated by lipase PL-266 (lipase from *Alcaligenes* sp.) [58] and lipase PS [59].

Other flavour components of ambergris are (+)-γ-Dihydroionone (**49**) and (+)-γ-coronal (**50**). The first was prepared in optically pure form by regioselective reduction of (+)-γ-ionone that was obtained by lipase PS-mediated resolution of the racemic γ-ionol [60]. Kinetic acetylation of racemic γ-cyclohomogeraniol (**51**) was catalysed by lipase AK (lipase from *Pseudomonas* AK) [61]. The (S) enantiomer obtained was used as a building block in the (+)-γ-coronal synthesis.

When in enantiopure form and in diastereoisomeric ratio of 93:3 (−)-*trans*-jasmonate (**52**) and (+)-*cis*-jasmon-ate (**53**) are the key components of jasmine oil fragrance. The synthetic *trans* **52** is commercially available in racemic form and its resolution has recently been reported. The key steps were the reduction of ketone functionality, the separation of the alcohol **54** as a single diastereoisomer and its resolution by lipase PS-mediated acetylation [62].

Concluding remarks

The examples presented here exemplify the power of biocatalysis in the production of flavours and fragrances although there is considerable variability in the different methods used. Well-established processes have been described both to point out their actual relevance and to outline their future perspectives. The new outstanding possibilities offered by biocatalysis have been illustrated by description of some methods of industrial and academic interest with particular attention to the legal differen-tiation of flavours. Natural and nature-identical com-pounds show different future prospects. New strategies for natural flavour biogeneration will take advantage of the current studies on biotechnology, biochemical pathways and microbiology and the preference of consumers for natural compounds will support their production. The preparation of nature-identical flavours using biocatalysis will enhance the possibilities offered by chemical synth-eses rather that compete with them. In this field, the most promising biocatalysts are certainly lipases because of their versatility and selectivity.

References

1 Pybus, D.H. (1999) The history of aroma chemistry and perfume. In *The chemistry of fragrances* (Pybus, D.H. and Sell, C.S., eds), pp. 3–23, RSC
2 Bauer, K. *et al*. (2001) *Common fragrance and flavor materials*, WILEY-VCH Verlag GmbH
3 Fráter, G. *et al*. (1998) Fragrance chemistry. *Tetrahedron* 54, 7633–7703
4 Schrader, J. *et al*. (2004) Applied biocatalysis for the synthesis of natural flavour compounds-current industrial processes and future prospects. *Biotechnol. Lett.* 26, 463–472
5 Vandamme, E.J. and Soetaert, W. (2002) Bioflavours and fragrances via fermentation and biocatalysis. *J. Chem. Technol. Biotechnol.* 77, 1323–1332
6 Krings, U. and Berger, R.G. (1998) Biotechnological production of flavours and fragrances. *Appl. Microbiol. Biotechnol.* 49, 1–8
7 Cheetham, P.S.J. (1993) The use of biotransformation for the production of flavours and fragrances. *Trends Biotechnol.* 11, 478–488
8 Brenna, E. (2003) Enzyme-mediated syntheses of chiral communi-cation substances: fragrances for perfumery applications. *Current Org. Chem.* 7, 1347–1367

9 Brenna, E. *et al*. (2003) Enantioselective perception of chiral odorants. *Tetrahedron Asymmetry* 14, 1–42

10 Cheetham, P.S.J. (1997) Combining the technical push and the business pull for natural flavours. *Adv. Biochem. Eng. Biotech.* 55, 1–49

11 US Code of Federal Regulations (1985) *21*, 101.22a.3. Food and Drug Administration, Washington, D.C.

12 The Council of the European Communities (1988) Council Directive 88/388/EEC of 22 June 1988

13 Walton, N.J. *et al*. (2003) Molecules of interest – Vanillin. *Phytochemistry* 63, 505–515

14 Priefert, H. *et al*. (2001) Biotechnological production of vanillin. *Appl. Microbiol. Biotechnol.* 56, 296–314

15 Kosjek, B. *et al*. (2003) Efficient production of raspberry ketone via 'green' biocatalytic oxidation. *Tetrahedron* 59, 9517–9521

16 Fuganti, C. and Zucchi, G. (1998) Product distribution in the microbial biogeneration of raspberry ketone from 4-hydroxybenzalacetone. *J. Mol. Catal., B Enzym.* 4, 289–293

17 Grogan, G.J. and Holland, H.L. (2000) The biocatalytic reactions of *Beauveria* spp. *J. Mol. Catal., B Enzym.* 9, 1–32

18 Etschmann, M.M.W. *et al*. (2002) Biotechnological production of 2-phenylethanol. *Appl. Microbiol. Biotechnol.* 59, 1–8

19 Waché, Y. *et al*. (2003) Catabolism of hydroxyacids and biotechnological production of lactones by *Yarrowia lipolytica*. *Appl. Microbiol. Biotechnol.* 61, 393–404

20 Gill, I. and Valivety, R. (1997) Polyunsaturated fatty acids, part 2: Biotransformations and biotechnological applications. *Trends Biotechnol.* 15, 470–478

21 Villa, M. *et al*. (1997) Process for the production of gamma nonalactones in natural form. *United States pat.* US 5646022

22 Fronza, G. *et al*. (1996) Stereochemical aspects of flavour biogeneration through baker's yeast mediated reduction of carbonyl-activated double bonds. *Pure Appl. Chem.* 68, 2065–2071

23 Muller, B. *et al*. (1996) Process for the enzymatic preparation of aliphatic alcohols and aldehydes from linoleic acid, linolenic acid, or a natural precursor. *United States pat.* US 5464761

24 Fronza, G. *et al*. (1995) Reduction of carboxylates to alkanols catalysed by *Colletotrichum gloeosporoides*. *Chem. Commun.*, 439–440

25 Dennis, M.J. (1997) Establishing food authenticity. *Chem. Ind.*, 997–1000

26 Fronza, G. *et al*. (2001) The positional δ(^{18}O) values of extracted and synthetic vanillin. *Helv. Chim. Acta* 84, 351–359

27 Fronza, G. *et al*. (1998) Natural abundance ^{2}H nuclear magnetic resonance study of the origin of raspberry ketone. *J. Agric. Food Chem.* 46, 248–254

28 Fronza, G. *et al*. (1999) Stable isotope characterization of raspberry ketone extracted from *Taxus baccata* and obtained by oxidation of the accompanying alcohol (Betuligenol). *J. Agric. Food Chem.* 47, 1150–1155

29 Aleu, J. *et al*. (2002) Differentiation of natural and synthetic phenylacetic acids by ^{2}H NMR of the derived benzoic acids. *Eur. Food Res. Technol.* 214, 63–66

30 Barbeni, M. *et al*. (1997) Natural abundance ^{2}H nuclear magnetic resonance study of the origin of (Z)-3-hexenol. *J. Agric. Food Chem.* 45, 237–241

31 Jaeger, K.E. and Eggert, T. (2002) Lipases for biotechnology. *Curr. Opin. Biotechnol.* 13, 390–397

32 Schmid, R.D. and Verger, R. (1998) Lipases: interfacial enzymes with attractive applications. *Angew. Chem. Int. Ed. Engl.* 37, 1608–1633

33 Clark, G.S. (1998) Menthol. *Perfumer & Flavorist* 23, 33–46

34 Sell, C. (1999) Ingredients for modern perfumery industry. In *The chemistry of fragrances* (Pybus, D.H. and Sell, C.S., eds), pp. 70–76, RSC

35 Akutagawa, S. (1992) A pratical synthesis of ()-menthol with the Rh-BINAP catalyst. In *Chirality in industry* (Collins, A.N. *et al.*, eds), pp. 313–323

36 Gatfield, I-L. *et al*. (2002) Verfahren zur Herstellung von D- oder L-menthol. *Eur. Pat.* EP 1223223 A1

37 Vorlová, S. *et al*. (2002) Enantioselective hydrolysis of D,L-menthyl benzoate to L-(−)-menthol by recombinant *Candida rugosa* lipase LIP1. *Adv. Synth. Catal.* 344, 1152–1155

38 Chaplin, J.A. *et al*. (2002) Process for preparing (−)-menthol and similar compounds. *PCT Int. Appl.* WO 02/36795 A2

39 Yamamoto, T. (1998) Method for purifying (−)-n-isopulegol and citrus perfume compositions containing purified (−)-n-isopulegol obtained by the method. *United States pat.* US 5773410

40 Gaudin, J-M. (2000) Synthesis and organoleptic properties of *p*-menthane lactones. *Tetrahedron* 56, 4769–4776

41 Ferraz, H.M.C. *et al*. (2002) Syntheses of mintlactone and isomintlactone. *Synthesis*, 2155–2164

42 Guth, H. (1996) Determination of the configuration of wine lactone. *Helv. Chim. Acta* 79, 1559–1571

43 Wüst, M. and Mosandl, A. (1999) Important chiral monoterpenoid ethers in flavours and essential oil-enantioselective analysis and biogenesis. *Eur. Food Res. Technol.* 209, 3–11

44 Serra, S. *et al*. (2003) Lipase-catalyzed resolution of *p*-menthan-3-ols monoterpenes: preparation of the enantiomer-enriched forms of menthol, isopulegol, *trans*- and *cis*-piperitol, and *cis*-isopiperitenol. *Tetrahedron Asymmetry* 14, 3313–3319

45 Serra, S. and Fuganti, C. (2002) Enzyme-mediated preparation of enantiomerically pure *p*-menthan-3,9-diols and their use for the synthesis of natural *p*-menthane lactones and ethers. *Helv. Chim. Acta* 85, 2489–2502

46 Serra, S. and Fuganti, C. (2004) Natural *p*-menthene monoterpenes: synthesis of the enantiomeric forms of wine lactone, epi-wine lactone, dill ether and epi-dill ether starting from a common intermediate. *Helv. Chim. Acta* 87, 2100–2109

47 Shimizu, M. *et al*. (1998) An enantio- and diastereocontrolled route to mintlactone and isomintlactone using a chiral cyclohexadienone equivalent. *Synlett*, 655–657

48 Forzato, C. *et al*. (1995) Synthesis of optically active condensed γ-lactones from 2-(2-nitroethyl)cyclohexanols. Part II. *Gazz. Chim. Ital.* 125, 223–231

49 D'Arrigo, P. *et al*. (1997) Old and new synthetic capacities of baker's yeast. *Adv. Appl. Microbiol.* 44, 81–123

50 Fuganti, C. *et al*. (1999) Baker's yeast mediated enantioselective synthesis of the bisabolane sesquiterpenes curcumene, turmerone, dehydrocurcumene and nuciferal. *J. Chem. Soc. Perkin Trans.* 1, 279–282

51 Benedetti, F. *et al*. (2001) Synthesis of all stereoisomers of cognac lactones via microbial reduction and enzymatic resolution strategies. *Tetrahedron Asymmetry* 12, 505–511

52 Brenna, E. *et al*. (2001) Baker's yeast-mediated approach to (−)-*cis*- and (+)-*trans*-Aerangis lactones. *Tetrahedron Asymmetry* 12, 1871–1879

53 Brenna, E. *et al*. (2002) Optically active ionones and derivatives: preparation and olfactory properties. *Eur. J. Org. Chem.*, 967–978

54 Brenna, E. *et al*. (2003) From commercial racemic fragrances to odour active enantiopure compounds: the ten isomers of irone. *C.R. Chimie* 6, 529–546

55 Brenna, E. *et al*. (2004) Changing the odor properties of commercial mixtures of alpha-irones by simple chemical transformations. *J. Essent. Oil Res.* 16, 339–341

56 Inoue, T. *et al*. (2000) Synthesis of both enantiomers of *cis*-α-irone and *cis*-γ-irone, principal constituents of iris oil, via resolution of (±)-2,2,4-trimethyl-3-cyclohexene-1-carboxylic acid. *Tetrahedron Asymmetry* 11, 3807–3818

57 Sell, C. (1990) The chemistry of ambergris. *Chem. Ind.*, 516–520

58 Akita, H. *et al*. (2000) A convenient synthesis of (+)-albicanol based on enzymatic function: total syntheses of (+)-albicanyl acetate, (−)-albicanyl 3,4-dihydroxycinnamate, (−)-drimenol, (−)-drimenin and (−)-ambrox. *Tetrahedron Asymmetry* 11, 1375–1388

59 Tanimoto, H. and Oritani, T. (1996) Pratical synthesis of ambrox® from farnesyl acetate involving lipase catalyzed resolution. *Tetrahedron Asymmetry* 7, 1695–1704

60 Fuganti, C. *et al*. (2000) Synthesis and olfactory evaluation of (+)- and (−)-γ-ionone. *Helv. Chim. Acta* 83, 2761–2768

61 Horiuchi, S. *et al*. (1999) Enzymatic resolution of (±)-γ-cyclohomogeraniol and conversion of its (*S*)-isomer to (*S*)-γ-coronal, the ambergris odorant. *Bioorg. Med. Chem.* 7, 723–726

62 Kiyota, H. *et al*. (2001) Lipase-catalyzed preparation of both enantiomers of methyl jasmonate. *Tetrahedron Asymmetry* 12, 1035–1038