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Stereochemical aspects of flavour biogeneration through baker's yeast mediated reduction of carbonyl-activated double bonds

Giovanni Fronza, Claudio Fuganti, Monica Mendozza, Romina Rigoni, Stefano Servi, Gioia Zucchi

Dipartimento di Chimica del Politecnico, CNR Centro di Studio per le Sostanze Organiche Naturali, Via Mancinelli 7, 20131 Milano, Italy

Abstract

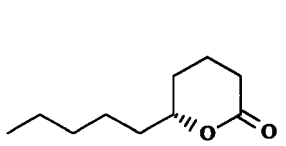
The baker's yeast mediated reduction of the unsaturated carbonyl compounds **4**, **5** and **6** has been studied in the context of the generation of the natural forms of the flavour materials **1**, **2** and **3**. The stereochemical aspects of these processes and of those regarding the reduction of the structurally related enones **18**, **19**, **30**, **31** and **32** have been elucidated also by means of deuterium labelling experiments.

Among the 6.000 chemically defined molecules identified up to now in trace amounts in food, whose function is aromatic rather than nutritional, nearly 1.000 are used by industry in formulations. About 85% of the industrially needed flavour materials are obtained by chemical synthesis, whereas the reminder are mainly obtained by extractive manipulation of plant materials or by fermentation.

Recently there has been legislative discrimination (1) between chemically identical food constituents of synthetic origin and those derived from natural sources. It has, therefore, become desirable to produce quantities of these flavour agents required from the food flavour industry and not available by extraction through *enzymolysis* (1) of natural products, using either isolated enzymes or whole cells.(2) Increased value is indeed added upon the aroma components produced through these means, because they can be labelled 'natural' thus receiving consumer preferences. This circumstance offered new challenging possibilities of application of enzymes in organic synthesis. There is, however, a peculiarity which makes this research more stimulating. At variance with usual organic transformations, where enzymes can be used as alternative to well established a-biological techniques, in the production of 'natural' flavours from natural precursors the involvement of enzymes becomes compulsory. Moreover, a major problem faced in this field concerns the steric outcome of the transformations, due to the dependence of the sensory response of chiral flavours materials from the enantiomeric composition.

Since our interest concerned principally the mode of formation of C-6--C-12 γ and δ-lactones(3) we planned the biogeneration of δ-decanolide **1** and γ-nonanolide **2**, two compounds of key importance in fruit aroma formulations occurring in Nature in trace amounts in many fruits, and of 4-(4-hydroxyphenyl)-butan-2-one (raspberry ketone) **3**, the impact flavour of raspberry fruit,(4) from the corresponding unsaturated compounds **4**, **5** and **6**, respectively, by means of baker's yeast mediated saturation of the carbonyl-activated double bond. Indeed, the unsaturated lactones **4** and **5** are obtained by extraction from *Cryptocaria massoia*(5) and from *Dipteryx odorata* Willd. (Tonka

beans),(6) respectively, where they occur in the (R) and in the (R,S) forms, respectively. The unsaturated ketone **6** is easily accessible by condensation of 4-hydroxybenzaldehyde isolated from botanical sources with acetone obtained by fermentation.



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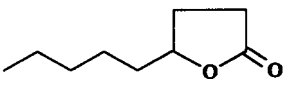

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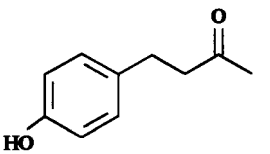

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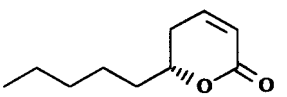

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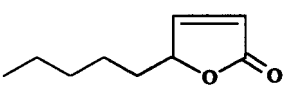

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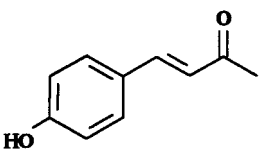

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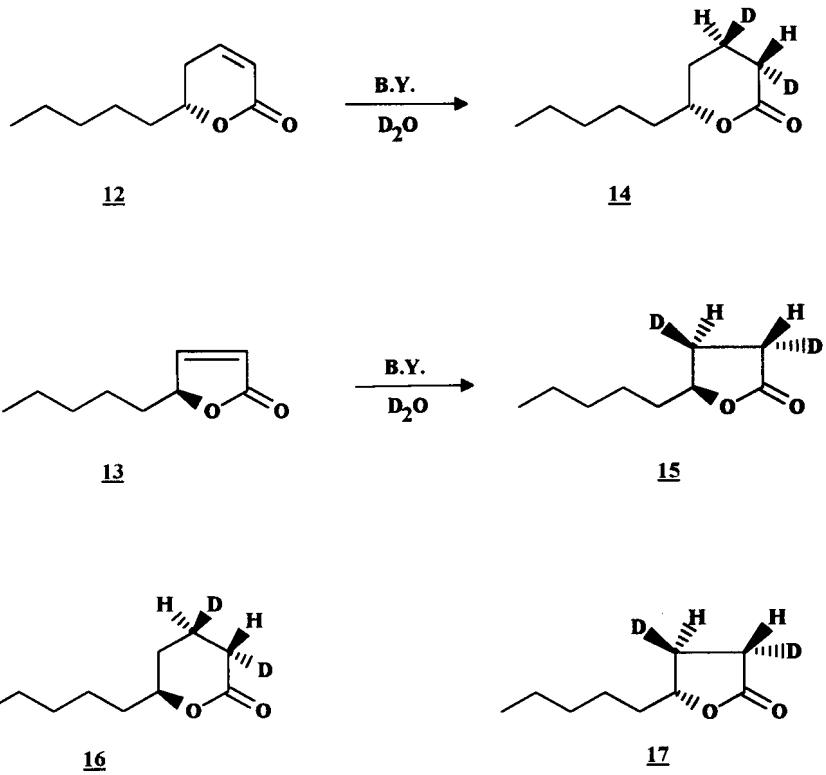
Experiments of incubation with baker's yeast of racemic **4** and **5** led to the production of the desired saturated lactones **1** and **2**. The rate of reduction was much higher in the first instance. Moreover, the reduction process occurs with kinetic resolution. The (R) enantiomer of **4** is transformed more rapidly, whereas the reverse is true for **5**. Also, at comparable conversions, the *ee* values are higher in the former case. In order to determine the influence of the length of the side chain on the mode of transformation, experiments of reduction of a series of racemic unsaturated γ-lactones with the side chain varying from C-3 to C-6 were performed. These indicated that the *ee* values moderately increase with the length of the side chain, with kinetic preference in all instances for the (S) enantiomer. (7,8)

The study of the mode of baker's yeast reduction of unsaturated lactones was extended to products **7**, **8** and **9**. In this instances, the *ee* values observed at ca. 50% conversion were considerably higher than in the case of **4**, but the stereochemical preference remained identical. Under suitable conditions, together with product **11** possessing 0.77 *ee*, (+)-(R) **10** of 0.99 *ee* was obtained. The latter material, goniothalamin, possessing CNS activity, occurs in *Goniothalamus* species, as well as its embriotoxic epoxide. (9) The above enzymic resolution represents an access to (R) **10** alternative to the one reported which utilizes, as starting materials, a component of the 'pool of chirality'. (10) The enantioselection observed in the yeast reduction of the diastereoisomeric epoxides **8** and **9** is slightly lower than in the case of **7** and is not influenced by the stereochemistry of the epoxide ring.

The change from the (R) to the (S) stereochemical preference in the baker's yeast mediated reduction of α-unsaturated δ and γ-lactones thus observed induced us to study the cryptic stereochemistry of the double bond saturation by means of deuterium labelling experiments. The baker's yeast reduction in deuterated water of enantiomerically pure (R) **12** and (S) **13** produced the dideuterated species **14** and **15**, formed by β-*re-face* trans formal addition of hydrogen atoms. The fact that in these reductions the β-hydrogen atom largely arises from the solvent water is explained by the

intervention of a diaphorase which equilibrates with water the hydrogen atom of the reduced nicotinamide cofactor expected to be delivered as hydride in the bioreduction process. (11,12) When the racemic materials **4** and **5** were submitted to the yeast reduction in deuterated water, together with (R) **14** and (S) **15**, increasing quantities of the saturated lactones (S) **16** and (R) **17** were formed, bearing identical deuterium labelling pattern. This result supports the view that the saturation of the double bond of δ and γ-lactones **4** and **5** is not influenced by the absolute configuration of the oxygen-bearing carbon atom. These labelling experiments were of crucial importance in the elaboration of a SNIF-NMR method enabling the authentication of the origin of δ-decanolide samples obtained from Massoia lactone **4** by chemical or biochemical saturation of the α-double bond. (13)

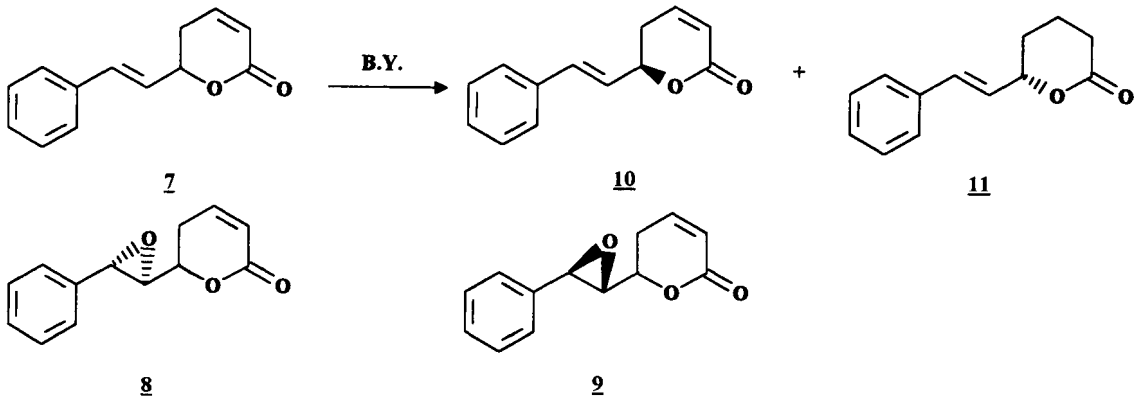


12 **14**

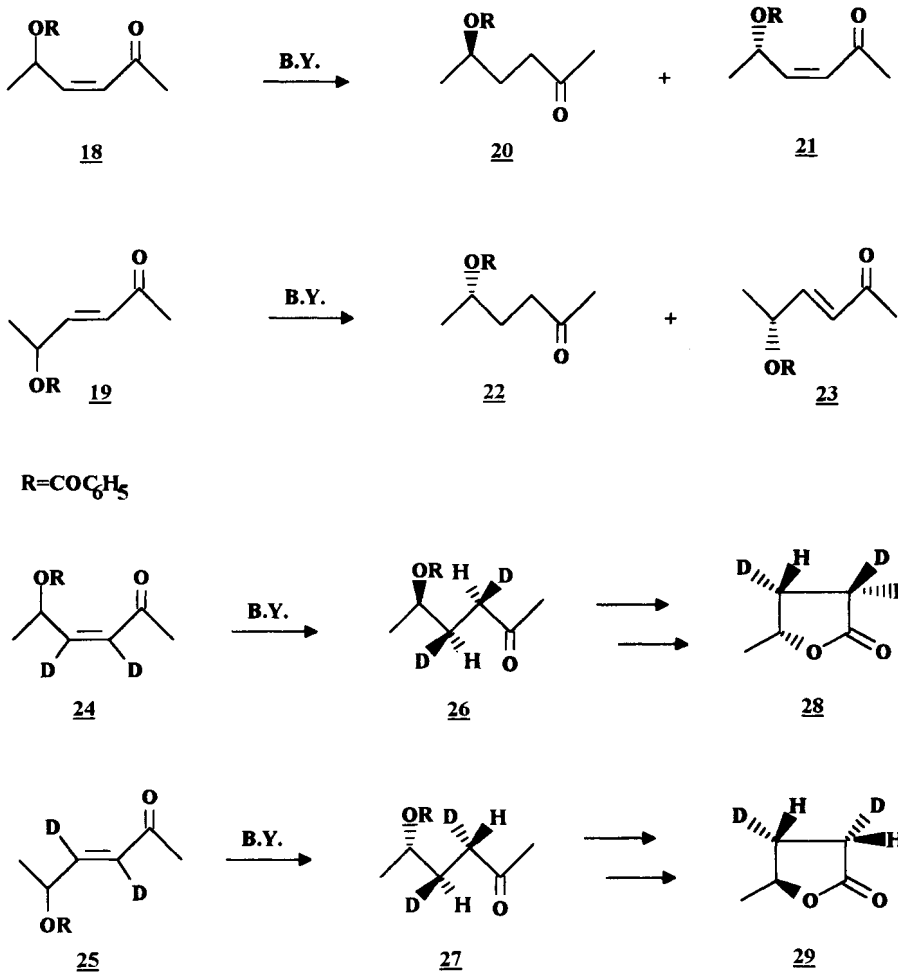
13 **15**

16 **17**

The stereochemical study was subsequently extended to the baker's yeast reduction of the (Z) and (E) γ-benzoyloxy α,β-unsaturated methyl ketones **18** and **19**, possessing some of the structural features of **5**. (14) Quite surprisingly, the only transformation observed when the above ketones were incubated with actively fermenting baker's yeast was the saturation of the double bond. No trace of the unsaturated or saturated carbinols was detected. The saturation of the double bond of **18** and **19** occurs with kinetic preference for the (R) and (S) enantiomers, respectively. In the two instances, the (R) and (S) forms **20** and **22** of 5-benzoyloxy hexan-2-one are obtained, together with survived (S) **21** and (R) **23**. At ca. 20% conversion, the *ee* values of the enantiomeric materials **20** and **22** formed from **18** and **19** resulted to be 0.62 and 0.48, respectively.

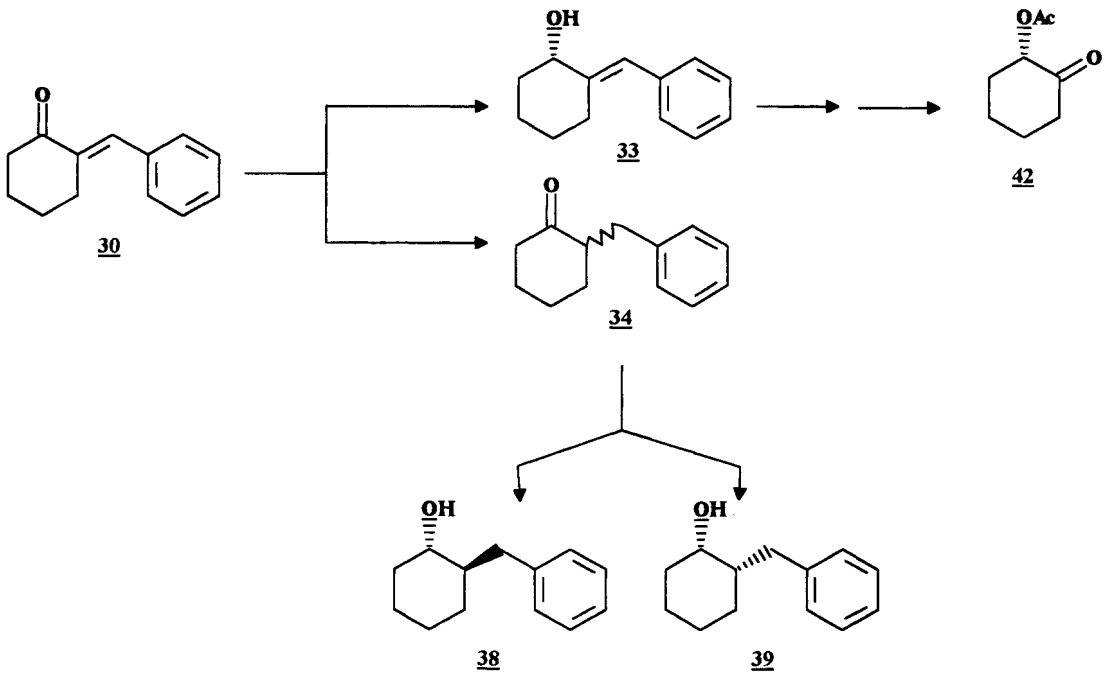


The mode of baker's yeast mediated saturation of the (Z) and (E) double bond of the above enones was studied using dideuterated **24** and **25** as substrates. At low conversion these products gave rise to **26** and **27** possessing high enantiomeric excess. The latter methyl ketones were chemically degraded to dideuterated (R) and (S) γ-valerolactones, respectively. The prevalent enantiomers present in the two samples were assigned by ^2H NMR studies structural formulas **28** and **29**, thus indicating that the reduction of the (Z) and (E) double bonds of **18** and **19** occurs as in the case of δ and γ-lactones **4** and **5**, *i.e.*, by β-*re-face* trans formal addition of hydrogen.

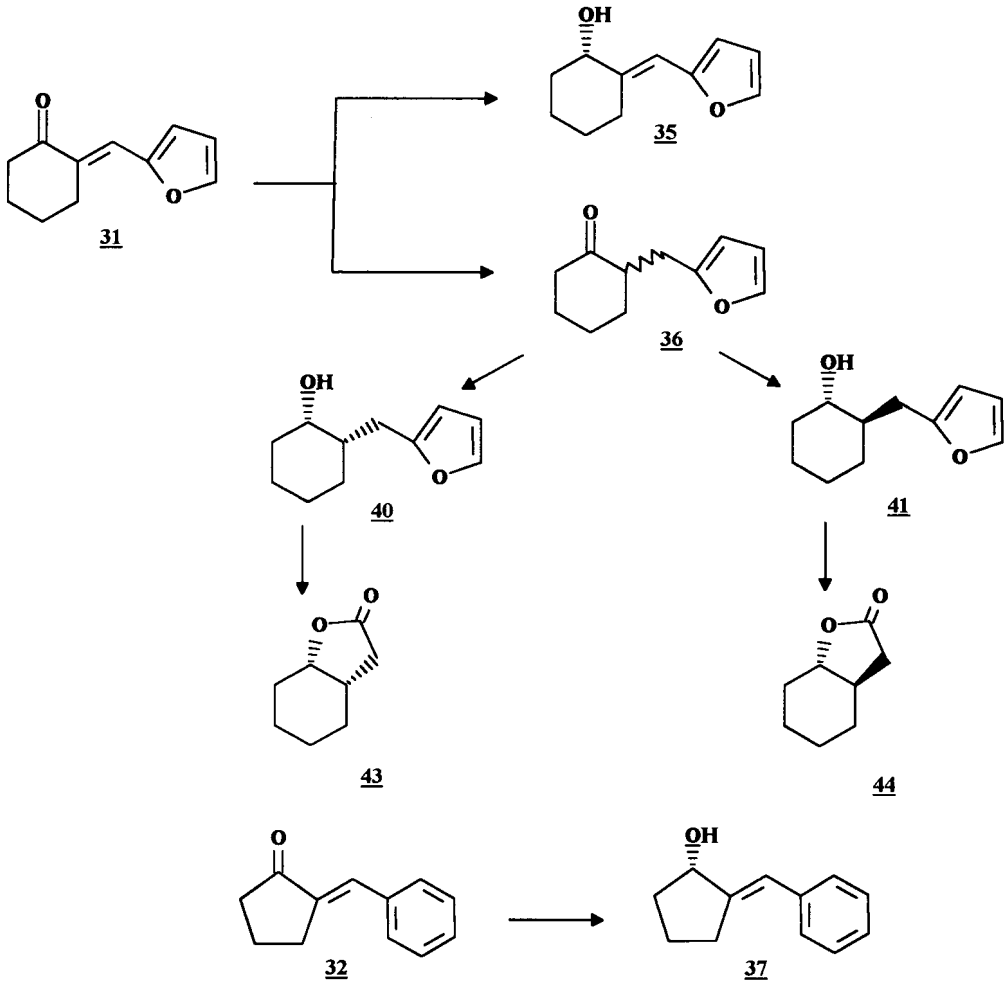


We then extended the study of the mode of baker's yeast mediated reduction of α,β-unsaturated carbonyl compounds to enones **30**-**32**, in which the carbonyl group is part of a carbocyclic moiety. (14) Whereas the cyclohexanone derivatives **30** and **31** afforded a mixture of the allylic alcohols **33** and **35**, possessing 100% and 85% enantiomeric purity, respectively, and of the racemic saturated ketones **34** and **36** (ratio **33**/**34** ca. 1:2), the cyclopentanone derivative **32** was a poor substrate. The allylic alcohol **37** was obtained in moderate yield and showed 0.63 *ee*.

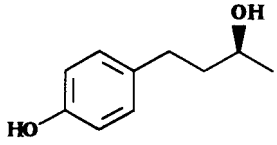
However, the saturated ketones **34** and **36** incubated in separate experiments with baker's yeast in the absence of the allylic alcohols **33** and **35** and of the enones **30** and **31** were readily reduced to enantiomerically pure **38**, **39** and **41**, whereas the *syn* carbinol **40** showed 0.83 *ee*. The (S) absolute configuration of the carbinols obtained from **30** was determined converting **33** into enantiomerically pure (S) α-acetoxy cyclohexanone **42** and chemically correlating **33** with **38** and **39**. In the case of the furan derivative **31**, the stereochemical assignment was made by correlation of **35**, **40** and **41** with the known γ-lactones **43** and **44**, respectively.



The inhibitory effect of the unsaturated ketones and of the allylic alcohols towards the carbonyl reduction thus observed in the baker's yeast incubation of **30**-**32** operates also in the case of the enone **6**. The latter material has been recently shown to be the intermediate in raspberry plant in the biosynthesis of **3**. Its C-6--C-4 framework is formed by condensation of C-6--C-3 *p*-coumaryl-CoA with malonyl-CoA, followed by hydrolysis and decarboxylation (16). Indeed, baker's yeast readily saturate the double bond of **6** affording **3** as substantially unique transformation product. Only when the amount of untransformed **6** present in the mixture decreases to 3-4% respect to the saturated ketone **3** starts the reduction of the latter to the (S) carbinol **45** of over 0.9 *ee*. Baker's yeast reduction of **6** in deuterated water affords the multiply labelled product **46** (17). The stereochemistry of the deuterium atoms incorporated at positions 3 and 4 of **46** during its generation from **6** was determined by ^2H NMR studies onto the diacetyl



derivative of the (S) carbinol **47**, obtained from **46** upon HLADH/NADH mediated reduction. The spectroscopic studies indicated that ca. 70% of the deuterium incorporated at position 4 of **46** holds the (S) configuration, as the majority of the deuterium at position 3, thus suggesting a prevalence of *syn* hydrogen addition in the double bond saturation process, with formal hydride delivery from the β *re-face*.
It is interesting to note that a variety of growing microorganisms reductively transforms the unsaturated ketone **6**, but in these instances, at variance with what is observed with baker's yeast, the saturated ketone is reduced readily to the ketone while the unsaturated precursor is still present.



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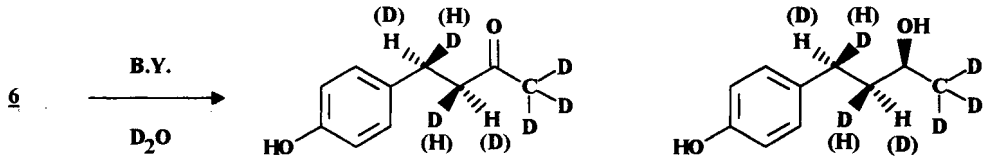
Yeast extract (Sigma) reduction experiments of **6** in deuterated water indicate that only the hydrogen atom incorporated α to the carbonyl group of **3** arises from water. Moreover, incubation of **6** with the extract in the presence of stereospecifically deuterated NADPH and NADH indicated that H_R from the former and H_S from the

second, respectively, are transferred at β position of the unsaturated substrate. These results, seen together with the outcome of the deuterium incorporation experiments using the whole cells system which led to **46** and **47**, seem to suggest the participation within the bioreduction process of two distinct enzymic activities adopting different cofactors and likely acting with opposite stereochemistry.



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The variety of synthetically useful transformations of enones mediated by fermenting baker's yeast here reported further supports the practical significance of this biological system, which although on the chemical *scenario* since a long time (18), offers, as in the present instances, easy solutions to new problems in the expanding field of the biogeneration of aroma substances.

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