Chimia 47 (1993) 107–109

ISSN 0009-4293

Biogeneration of Aromas

Giovanni Fronza, Claudio Fuganti*, Piero Grasselli, and Stefano Servi

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 by the food industry and not available by extractive manipulation of plant materials by 'enzymolysis' of natural products, using either isolated enzymes or whole cells [2].

This circumstance offered new challenging possibilities of application of enzymes in organic synthesis. There is, however, a characteristic which makes this research more stimulating. At variance with usual organic transformations, where enzymes can be used quite often as an option to well established a-biological procedures, in the production of 'natural' flavours from natural precursors the involvement of enzymes becomes compulsory.

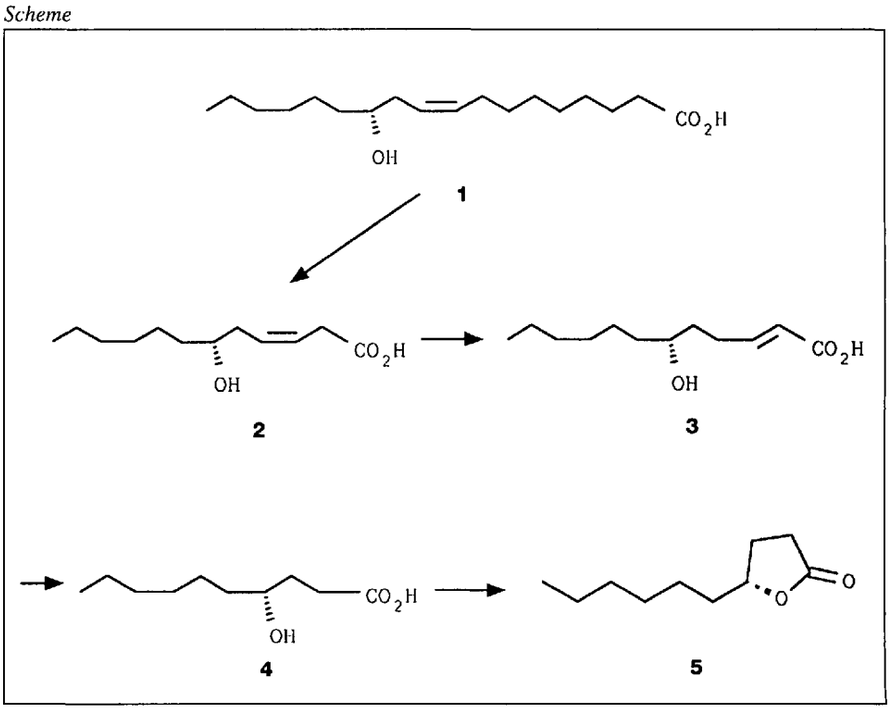
The extreme structural variety of the aroma substances to be obtained through these means would suggest that a great number of enzymes should be used. When the biosynthetic origin of the substance of interest is well defined, as in the case of the 'fruit esters', the lipase-mediated production from acids and alcohols appears unexceptional. Similarly, microbial oxidation of alcohols to acids is applied for the preparation of the acids required in the above process [3].

More difficult is the biogeneration from natural precursors of flavours whose biosynthetic origin is still unknown. This is the case, *e.g.*, of C_6–C_{12} γ- and δ-lactones, aroma components in, *e.g.*, peaches, apricots, strawberries, milk products, and fermented foods, where they are present in trace amounts [4]. Their generation in fruits usually takes place at the time of ripening, when catabolic processes are prevalent. However, the mechanism which activates their production is obscure. Simple structural considerations allowed to envisage [5] for this class of compounds a derivation from fatty acids. However, the circumstance that both optical purity and absolute configuration can vary for identical lactones isolated from different sources [6] supports the idea of the presence of different biosynthetic pathways, involving either anabolic or degradative processes. Degradative processes include β-oxidation of suitably oxygenated intermediates which can be either optically active, such as those involved in the lipoxygenation cascade or those formed in enzymic hydration of methylene-interrupted polyunsaturated fatty acids (or in the homoallylic hydroxylation of unsaturated fatty acids), or racemic as those formed in photo- or autoxidation. The former set of products is expected to lead to optically active lactones incorporating the chirality of the precursors. Conversely, racemic precursors are expected to generate educts whose optical purity and absolute configuration may depend upon the susceptibility of the degradative enzyme(s) to the configuration of the OH-bearing C-atom located in the molecule far away from the place where the β-oxidation starts.

In this context, we have been studying the ability of a variety of microorganisms to degrade 'natural' hydroxy fatty acids and synthetic structural analogs to γ- and δ-lactones, with particular emphasis on the stereochemical aspects of the processes. This is due to the relevance of the relationship between absolute configuration and sensory response of flavour materials [7]. The research originates from the early observation [8] that γ-decanolide (**5**) is obtained in *Yarrowia lipolytica* (*Scheme*) from ricinoleic acid (**1**) by β-oxidation due to the presence of an isomerase acting onto the C_{12} intermediate **2**, transformed into **3**. The latter, in turn, on C_2 degradation gives rise to (4*R*)-4-hydroxydecanoic acid (**4**), the open form of lactone **5**. Currently, γ-decanolide (**5**) is indeed manufactured on large scale from ricinoleic acid using the above and other microorganisms [9].

γ-Decanolide (**5**) obtained in the above processes incorporates at C(4) the chiral center originally present at C(12) of natural ricinoleic acid **1**. Quite different stereochemical course shows the degradation of the racemic C_{14}–C_{19} fatty acids **6**–**11**, all of which contain the structural unit (*Z*)-$CH{=}CHCH_2CH(OH)R$, to C_7–C_{11} lactones by growing cultures of *Cladospori-*

Scheme



**Correspondence*: Prof. C. Fuganti
Dipartimento di Chimica del Politecnico
Centro CNR per la Chimica delle Sostanze
Organiche Naturali
Via Mancinelli 7
I-20131 Milano

um suaveolens [10]. As expected, in agreement with C_2 degradation by β-oxidation the C_{14}, C_{17}, and C_{19} hydroxy acids **6**, **9**, and **11**, respectively, afforded C_8 and C_{11} δ-lactones, whereas the C_{15}, C_{16}, and C_{18} acids **7**, **8**, and **10**, respectively, gave rise to γ-lactones. However, the absolute configuration of the prevalent enantiomer in the two series differs, the (*S*)-δ-lactones **12** and **13** and the (*R*)-γ-analogs **14**, **15**, and **16** being obtained. Moreover, the optical purity (*Table 1*) is higher within the first set and decreases in each series on shortening the alkyl side chain. Furthermore, the *ee* values in *Entries 1* and *2* suggest operation, within the biodegradation, of kinetic resolution. In this instance, of particular interest is the observation that the inversion of configuration, associated with the lactone ring size, is a consequence of the shift of the γ-alkene moiety along the fatty-acid chain, and that the length of the alkyl side chain influences the optical purity of the educts.

Photo- and autoxidations are the most common ways followed by nature for the introduction of O functionalities into the unsaturated fatty-acid framework. Reduction of the intermediates hydroperoxides affords hydroxy fatty acids in racemic form. Simple structural considerations suggested a possible biosynthetic link between hydroxy acids **17–20** and **21–22**, accessible by photooxidation/reduction of linoleic and oleic acids, respectively, and lactones **23–28**. This has been verified in *Yarrowia lipolytica* and *Pichia ohmeri* [11]. Indeed, on feeding mixture **17–22** a complex mixture of lactones was obtained, in which **24**, **25**, and **27** appeared as the most abundant educts. The absolute configuration of the materials was determined, as well as that of γ-decanolide obtained on feeding the mixture of the C_{16} hydroxy acids **29** and **30**, shorter analogs of **21** and **22**, obtained by photooxidation/reduction of palmitoleic acid. The results of the study are reported in *Table 2*. It, thus, emerges that the lactones obtained in the two microbial systems possess opposite absolute configuration. Furthermore, in *P. ohmeri* saturation of the C=C bond of lactone **24** can occur, thus giving rise to the formation of γ-decanolide enriched in the (*R*)-enantiomer from racemic **18**, which differs from ricinoleic acid **1** for the presence of the (*E*)-double bond in position 13. Conversely, **29** provides in the two microorganisms γ-decanolide of opposite configuration. These results, seen together, indicate different ways of biogeneration from natural precursors of γ-decanolide in both the natural and unnatural enantiomeric forms.

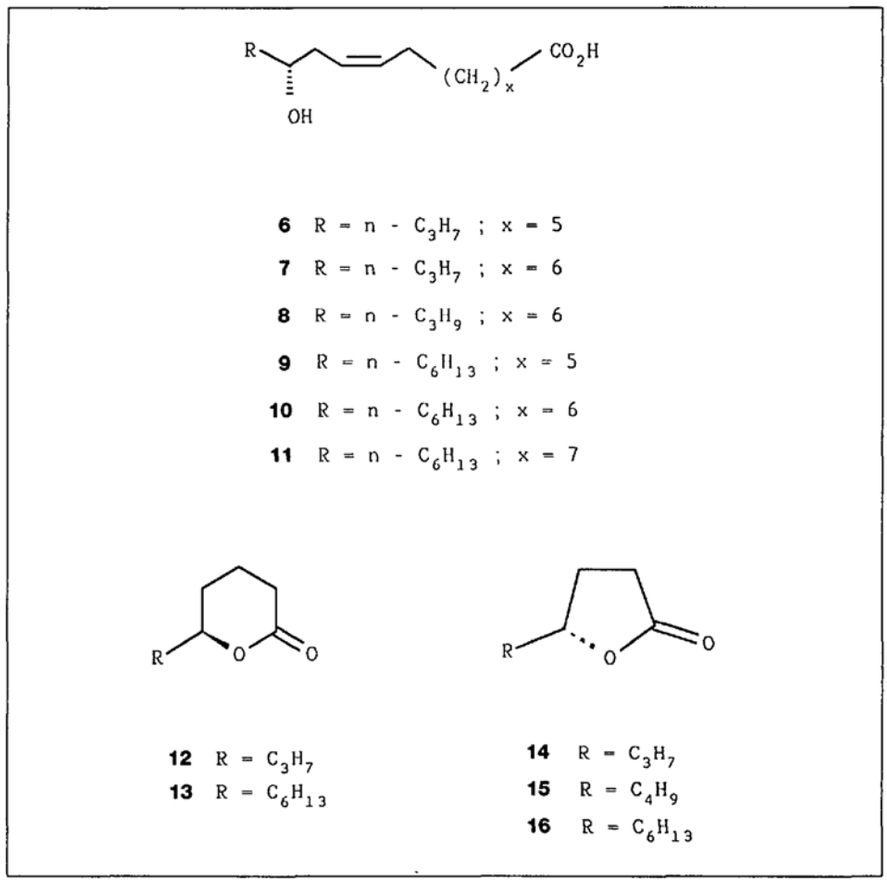


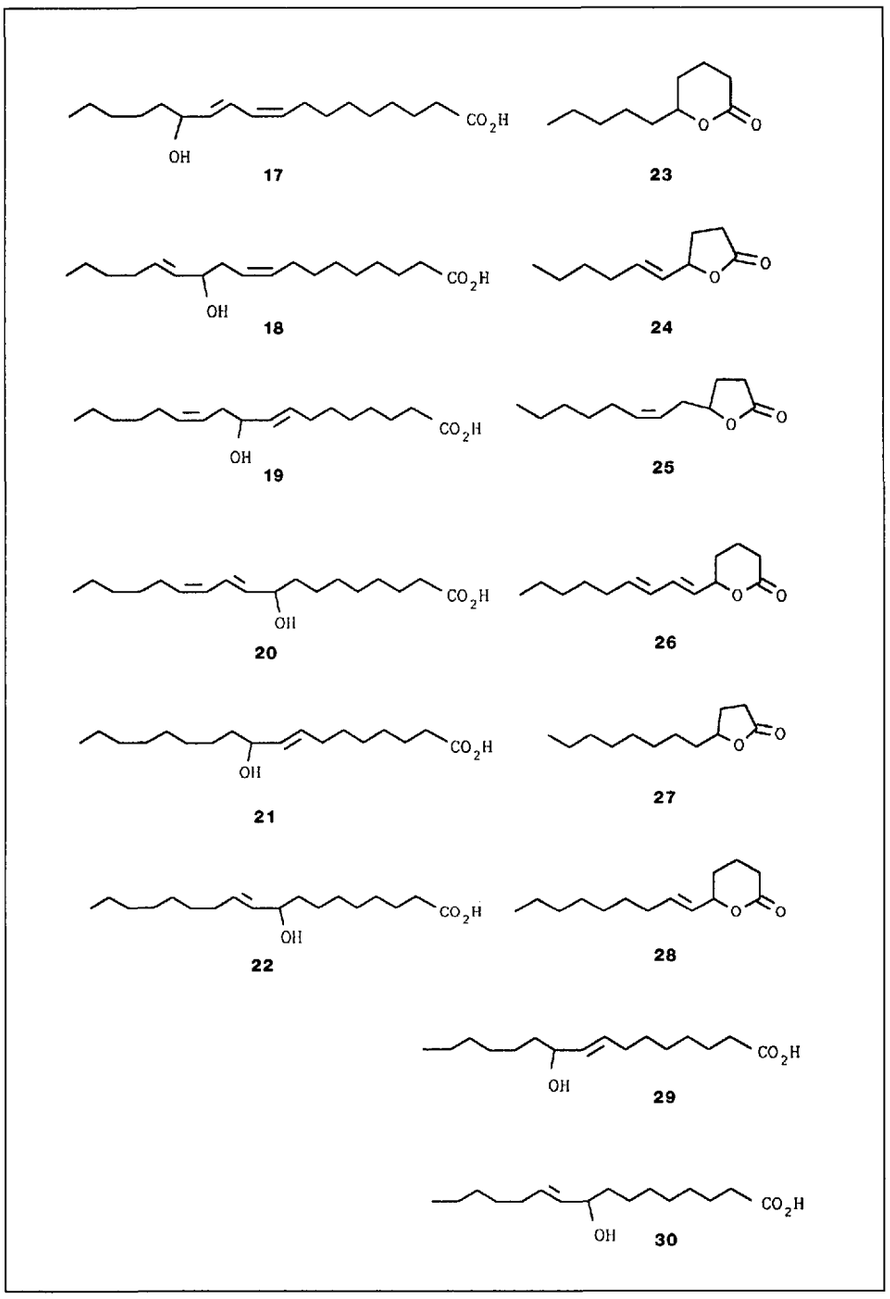
Table 1. *Enantiomeric Excess Values of Lactones* **12–16** *Obtained from Racemic* **6–11** *in Growing Cultures of* C. suaveolens

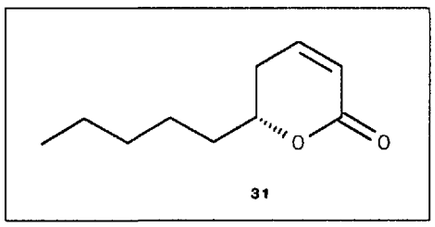
			ee Values			
Entry	Precursor	Lactone	24 h	48 h	120 h	198 h
1	**6**	(*S*)-12	0.58	0.50 0	0.38	
2	**7**	(*R*)-14		0.30 0	0.26	
3	**8**	(*R*)-15			0.22	
4	**9**	(*S*)-13		0.88		
5	**10**	(*R*)-16		0.54		
6	**11**	(*S*)-13				0.8

Table 2. *Enantiomeric Composition (%) of Lactones* **5**, **27**, **24**, *and* **25** *Biogenerated in* Y. lipolytica *and* P. ohmeri *from Hydroxy Acids* **18**, **19**, **21**, *and* **29**[a]). *Respectively*

		Lactone:	**5**		**27**		**25**		**24**	
			R	*S*	*R*	*S*	*R*	*S*	*R*	*S*
Entry	Precursor									
1	**29**	*Y.lipolytica*	6	4						
2	**18+19+21+29**	*Y.lipolytica*	3	97	20	80	72	28	97	3
3	**18+19+21**	*Y.lipolytica*			23	77	89	11	89	11
4	**18+19+21**	*Y.lipolytica*			17	83	92	8	85	15
5	**21**	*Y.lipolytica*			18	82				
6	**18+19+21**	*P.ohmeri*	93	7	78	22	20	80	51	49
7	**18+19+21**	*P.ohmeri*	96	4	79	21	39	61	42	58
8	**29**	*P.ohmeri*	73	27						

[a]) The amount of each lactone varies from *ca.* 50 to 150 mg/l.





Racemic **17** is in the above microorganisms a poor precursor of δ-decanolide (**23**). However, in *Cladosporium suaveolens* the (*S*)-enantiomer of **17**, obtained by lipoxygenation of linoleic acid, followed by cysteine reduction of the intermediate hydroperoxide, was converted into the (*S*)-enantiomer of δ-decanolide (**23**) [12]. Strange enough, when (*S*)-**17** was fed to *Sporobolomyces odorus* the (*R*)-enantiomer of δ-decanolide was obtained. This result has been explained supposing the participation within the biodegradation process of two oxidoreductases, the first of which converts the (13*S*)-carbinol to a ketone, reduced by the second enzyme to the (13*R*)-C_{18}-hydroxy acid, actual substrate for the β-oxidation to (*R*)-δ-decanolide [13].

A more direct access to natural (*R*)-δ-decanolide arises from the biological saturation of the C=C bond of (*R*)-δ-dec-2-enolide (**31**), extracted from the bark of the Massoi tree (*Cryptocaria massoia*) [14][15]. In baker's yeast, saturation of the C=C bond of **31** involves β-*re*-face *trans* formal addition of H-atoms, arising to a large extent from H_2O, as indicated from reduction experiments in deuterated water [15][16]. Furthermore, the mode of reduction does not depend upon the absolute configuration at C(5), as indicated from experiments using racemic **31** as substrate, and shows a kinetic preference for the (*R*)-enantiomer.

The microbial degradation of 'natural' hydroxy fatty acids provides γ- and δ-lactones meeting the requisites of 'naturality' dictated by the current rules. However, the stereochemical outcomes of the processes in different microorganisms suggest the participation within the biodegradation of a variety of enzymes, whose nature will be defined by further studies.

[1] *US Code of Federal Regulations*, 21:101.22.a.3.
[2] D.W. Armostrong, in 'Flavor Chemistry, Trends and Developments, Eds. R. Teranishi, R.G. Buttery, and F. Shahidi, ACS Symposium Series 388, American Chemical Society, Washington, DC, 1989, p. 105.
[3] L. Janssen, H.L. De Pooter, N.M. Schamp, E.J. Vandamme, *Process Biochem.* **1992**, *27*, 195.
[4] H.D. Belitz, W. Grosch, 'Food Chemistry', Springer Verlag, Berlin, 1987, p. 270.
[5] R. Tressl, in 'Biogeneration of Aromas', Eds. T.H. Parliment, and R. Croteau; ACS Symposium Series 317, American Chemical Society, Washington, DC, 1986, p. 114.
[6] A. Mosandl, C. Guenther, M. Gessner, W. Deger, G. Singer, G. Heusinger, in 'Bioflavor'87', Ed. P. Schreier; de Gruyter, Berlin, 1988, p. 55.
[7] W. Pickenhagen, in 'Flavor Chemistry, Trends and Developments', R. Eds. Teranishi, R.G. Buttery, and F. Shahidi; ACS Symposium Series 388, American Chemical Society, Washington, DC, 1989, p. 151.
[8] S. Okui, M. Uchiyama, M. Mizugaki, A. Sagawara, *Biochim. Biophys. Acta* **1963**, *70*, 346.
[9] US Patent 4,560,656 and Eur. Patent 0 258 993.
[10] R. Cardillo, G. Fronza, C. Fuganti, P. Grasselli, V. Nepoti, M. Barbeni, P.A. Guarda, *J. Org. Chem.* **1989**, *54*, 4979.
[11] B. Ercoli, C. Fuganti, P. Grasselli, S. Servi, G. Allegrone, M. Barbeni, A. Pisciotta, *Biotechnol. Lett.* **1992**, *14*, 665.
[12] R. Cardillo, G. Fronza, C. Fuganti, P. Grasselli, A. Mele, D. Pizzi, G. Allegrone, M. Barbeni, A. Pisciotta, *J. Org. Chem.* **1991**, *56*, 5237.
[13] W. Albrecht, M. Schwarz, J. Heidas, R. Tressl, *J. Org. Chem.* **1991**, *57*, 1954.
[14] P. van der Schaft, N. ter Burg, S. van der Bosch, A.M. Cohen, *Appl. Microbial. Biotechnol.* **1992**, *57*, 712.
[15] G. Fronza, C. Fuganti, P. Grasselli, M. Barbeni, *Tetrahedron Lett.* **1992**, *33*, 6375.
[16] G. Fronza, C. Fuganti, P. Grasselli, M. Barbeni, M. Cisero, *J. Agric. Food Chem.* **1993**.