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STEREOCHEMISTRY OF THE BIOGENERATION OF C-10 AND C-12 GAMMA LACTONES IN *YARROWIA LIPOLYTICA* AND *PICHIA OHMERI*

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Summary. Degradation of the C-16 and C-18 racemic hydroxy acids **1-4** to C-10 and C-12 γ-lactones **5-8** proceeds in *Yarrowia lipolytica* and *Pichia ohmeri* with opposite stereochemistry

There is a current interest for the production of γ- and δ-lactones useful as flavours through microbial procedures (Armstrong, 1989). In this context, the endogenous production in *Fusarium poae* of the C-12 unsaturated γ-lactone **7** seemed to follow a pathway reasonably different from that of the biogeneration in *Cladosporium suaveolens* from the C-18 hydroxyacid **3**, since lactone **7** isolated in the two experiments shows opposite absolute configuration (Guichard et al., 1991; Allegrone et al., 1991). More recently, it has been observed (Cardillo et al., 1991; Albrect et al., 1992) that (S) 13-hydroxy-(Z,E)-9,11-octadecadienoic acid ((S)-coriolic acid) provides (S) δ-decanolide in *Cladosporium suaveolens*, but the (R)-enantiomer in *Sporobolomyces odorus*. This result thus indicate that even the degradation of the same substrate can follow two different pathways in the two microorganisms. In the specific case, the formal inversion of configuration occurring in *S. odorus* at some stage of the sequence in order to account for the conversion of the C-18 (S)-configurated precursor into the C-10 (R)-lactone has been tentatively explained supposing the sequential participation within the biodegradation process of two oxidoreductases, the first of which converts the (13S) carbinol into a ketone, reduced by the second enzyme to the (13R) C-18 hydroxyacid, actual substrate for the β-oxidation to (R) δ-decanolide. The determination of the steric course of the microbial biogeneration of different lactones from racemic and optically active natural precursors becomes thus important not only for its mechanistic significance, but also for practical reasons. Indeed, different sensory response is sometime shown by the two enantiomers of an aroma component (Pickenhagen, 1989; Guichard et al., 1991) and, moreover, the enantiomeric composition has been proposed as criterion of 'naturality' of the food components (Nitz et al., 1989).

In this context, we would like to report now on the observation that the different mode of biodegradation of the same precursor by two microorganisms is not limited to the case of δ-decanolide reported above, but occurs also in the case of the set of C-10--C-12 δ–lactones **5-8**, produced in *Yarrowia lipolytica* and *Pichia ohmeri*, respectively, from **1-4** (**Scheme**) . As indicated in the **Table**, the absolute configuration of lactones **5-8**, biogenerated in the two microbial systems from the racemic hydroxy acids **1-4**, prepared from the unsaturated fatty acids palmitoleic, oleic and linoleic, respectively, by photooxidation/reduction, through procedures mimicking those occurring in nature (Fronza et al., 1991; Lebeque et al., 1987) is opposite, whereas the enantiomeric excess values (ee) are nearly the same.

Experimental

Feeding experiments: *Y. lipolytica* (CBS 2074) grown on MPGA is used to seed 50 mL of MPGB (20, 5, 20) in 300 mL conical flasks. After 48 h growth under shaking at 30 °C, the cultures served as inoculum (10:1) for 300 mL conical flasks containing 100 mL of 0.5% meat extract, 0.01% Tween 80 and 1 g of precursor mixture. After 48 h, the acidified mixture is extracted with dichloromethane (2 x 20 mL). The residue obtained upon careful evaporation of the dried solution is distilled bulb-to-bulb (130-150 °C at 0.2 mm/Hg) to give the desired lactone mixture. Identical procedure is used for *P.ohmeri* (CBS 5367).

Determination of the enantiomeric composition: Analyses were carried out on a Perkin-Elmer 8500 gas chromatograph equipped with a Megadex-1 (permethylated β-cyclodextrine coated fused silica capillary column, 25 m x 0.25 i.d.) and a flame ionization detector. Injector (split 1:50). Temperature: 240 °C. Detector temperature: 250 °C. Helium was used as carrier gas at a flow of 1 ml/min. Oven temperature program: 110 °C for 3 min, followed by an increase of 1 °C/min to 200 °C. The structural assignements for the enantiomeric forms of lactones **5-8** were made by comparison with authentic samples (Allegrone et al., 1991; Fronza et al, 1991).

Discussion

Y. lipolytica and *P. ohmeri* were used in this study because these microorganisms are particularly effective in converting by β-oxidation natural (R) ricinoleic acid (**4**, no double bond in position 13) into the (R) form of γ-decanolide **5**. To this end, C-16 and C-18 hydroxy acids **1** and **2** were fed in admixture with the isomeric 9-hydroxy derivatives, as obtained, by photoxidation and cysteine reduction of the intermediate hydroperoxides, from palmitoleic and oleic acids, respectively (entries 1, 5 and 8). Similarly, a 1:1 mixture of **3** and **4**, containing a minor amount of the 9 and 13-hydroxy isomers, prepared in analogous way from linoleic acid, was used as substrate. In the latter instance, since linoleic acid contained a small amount of oleic acid, also product **2** was present (entries 3, 4, 6, 7). Finally, in one experiment with *Y. lipolytica* was used the whole set of precursors (entry 2).

The steric course of the degradation is thus opposite in the two microorganisms. However, *P. ohmeri* seems to possess a capacity not apparent in *Y. lipolytica*. This regards the conversion of doubly unsaturated hydroxyacid **4** into γ-decanolide **5**, produced in (R) enantiomeric form and very high optical purity (entries 6 and 7). This operation requires formal saturation of the double bond originally present in position 13, which might formally occur before or after formation of **8**. The fact that in the experiment of entry 6 the ratio **5/8** changes from 1:4 to 1.5:1 from 24 to 48 h incubation, while the total amount of C-10 lactones remains nearly constant, likely indicates that double bond saturation takes place onto **8**. However, unexplained is the fact that **5** is nearly optically pure, whereas its unsaturated analog **8** is nearly racemic. Thus, the degradation in the two microorganisms of four different substrates **1-4** to lactones **5-8** proceeds in opposite way in all instances. It is worthwhile to remind that (R) **5** and (R) **6** show the same steric distribution of the substituents of (S) **8** and (S) **7**, respectively; saturation of the double bonds of the latter gives rise to γ-decanolide and γ-dodecanolide possessing, according to the Cahn-Ingold-Prelog rules, the (R) configuration.