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On the Mode of Baker's Yeast Reduction of C-7---C-10 2-Alken-4-Olides

Giovanni Fronza, Claudio Fuganti, Piero Grasselli, Andrea Mele, Antonella Sarra

Dipartimento di Chimica del Politecnico, Centro CNR per la Chimica delle Sostanze Organiche Naturali, 20133 Milano, Italy

Gianna Allegrone, Massimo Barbeni

San Giorgio Flavors, 10147 Torino, Italy

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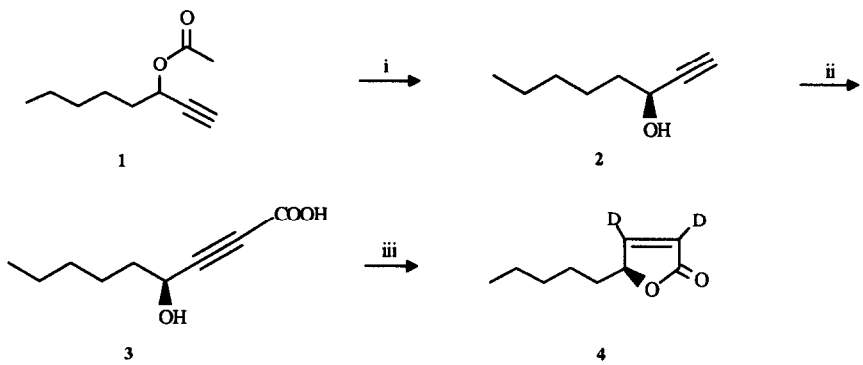
Abstract: Baker's yeast reduction of lactones **8, a-d** proceeds under kinetic resolution to give (S) **11, a-d** of low *ee* values, increasing with the length of the side chain, occurring the double bond saturation in *anti* fashion on the beta *re* face, as indicated by the obtainment from **4** and **6** of **5** and **7**, respectively.

A current area of application of enzymes in organic synthesis is the biogeneration of flavours, *i.e.* the production from natural precursors through enzymic procedures of aroma substances not available in quantities by extractive manipulation of plant materials.[1] Increased value is indeed added upon the aroma components produced by these means, because they can be labelled 'natural'[2] thus receiving consumer preferences. However, one of the major problem faced in this field concerns the steric outcome of the transformations, due to the dependence of the sensory response of chiral flavour materials from the enantiomeric composition.[3]

In this context, for some time now we have been studying the steric course of the microbial biogeneration of C-6--C-12 γ- and δ-lactones, obtained either by microbial β-oxidation of suitably oxidized forms of C-18 fatty acids[4] or by enzymic saturation of the double bond of easily available, naturally occurring, unsaturated lactones, *i.e.* 2-decen-5-olide (*Massoia* lactone).[5] In the latter instance, baker's yeast (b. y.) saturation of the double bond to give (R) decan-5-olide takes place readily onto the beta *re* face, with formal *anti* addition of hydrogen, and when a racemic material is used as substrate in the reduction, a kinetic preference for the (R) enantiomer is observed. In a study designed to extend the above procedure to the production of 'natural' nonan-4-olide from 2-nonen-4-olide, which is present in *Dipteryx odorata* Willd. (Tonka beans)[6] as 46:54 mixture of (R) and (S) enantiomers we observed that b. y. reduction of racemic 2-nonen-4-olide **8c** proceeded

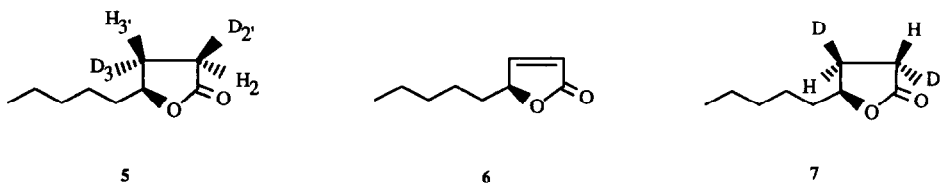
rather slowly to give nonan-4-olide containing an excess of the (S) enantiomer **11c**, as shown by multidimensional GLC analysis. The inversion of the stereochemical preference respect to 2-decen-5-olide,[5] induced us to verify whether there is also an inversion of the steric course of the double bond saturation. To this end, the required substrate (4S) [2,3-$^2H_2$] **4** was obtained (Scheme 1) through unexceptional steps from (3S) 1-octyn-3-ol (ca. 0.85 *ee*) **2**, prepared, in turn, by b. y. hydrolysis of the racemic acetate **1**.[7] B. y. reduction of **4** gave a saturated material shown by ^2H NMR studies (Figure, A) to possess the stereochemistry depicted in structural formula **5**. The chemical shifts of the two deuterium atoms (1.88 and 1.28 ppm) compare well with that of the hydrogens H-2' (trans to H-4) and H-3 (cis to H-4) determined from the analysis of the ring protons of the fully protonated compound.[8] Thus, double bond saturation in **4** occurred in *anti* fashion onto the beta *re* face of the molecule. Confirmation of these results arose from reduction experiments of **6** in deuterated water. The ^2H NMR spectrum of the reduced lactone **7** is reported in the Figure, B.



i) baker's yeast, pH 7.5 (40%); ii) 2 mol.eq. n-BuLi, THF/-40 °C, then dry CO_2 (75%); iii) deuterium gas, Lindlar catalyst (90%)

Scheme 1

The *ee* values of (S) nonan-4-olide obtained in b. y. from the corresponding unsaturated racemic product are considerably lower that those of (R) decan-5-olide (which bears a side chain of the same length of nonan-4-olide) obtained under similar conditions from racemic *Massoia* lactone.[5] In order to gain more information onto the structural factors governing the enantioselectivity of the process we submitted to b. y. reduction the racemic C-7--C-10 lactones **8, a-d**.

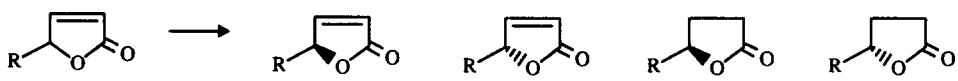


Scheme 2

The distribution of products **9-12** in the incubation mixture, determined at similar conversions in different experiments, is reported in the Table.

Table. Product distribution in the b. y. reduction of **8, a-d.**

entry		R in **8**	(S) **9**	(R) **10**	(S) **11**	(R) **12**
1	a	C_3H_7	37	39.3	12.7	11.0
2	b	C_4H_9	39.2	41.7	10.4	8.7
3	c	C_5H_{11}	33.3	37.8	16.8	12.1
4	d	C_6H_{13}	31.2	38.5	18.5	11.8



8 a-d **9** **10** **11** **12**

R = C_3H_7 (a), C_4H_9 (b), C_5H_{11} (c), C_6H_{13} (d)

These observations clearly show that the dimension of the side chain at position 4 of unsaturated γ-lactones influences the rate of transformation of the two enantiomers by the enzyme(s) presiding over the double bond saturation. Indeed, at similar conversions (entries 1-4), the *ee* values of the reduced products increase with the chain length.

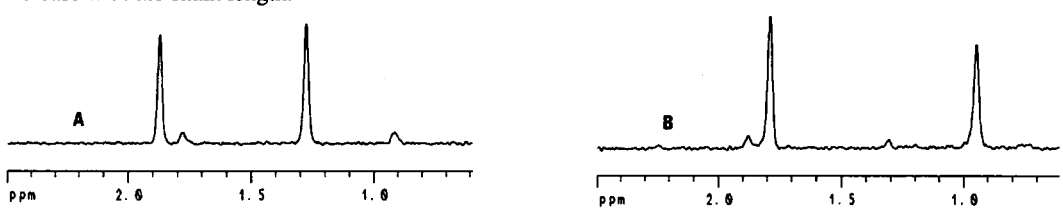


Figure. ^2H NMR spectra (C_6H_6) of A) **5** obtained by b. y. treatment of **4** in H_2O and B) **7** obtained by b. y. reduction of **6** in D_2O.

The above results, seen together, require some comments. In plants[9] alkan-4-olides usually occur in the (R) absolute configuration. However, in *D. odorata*[6] nonan-4-olide which accompanies nearly racemic 2-nonen-4-olide holds the (S) configuration (0.2 *ee*). At present, the nature of the biosynthetic link between the unsaturated and saturated γ-lactones occurring in *D. odorata* is unknown. However, if the second arises in the plant from the former, it is interesting to note the correspondence of the mode of reduction now observed in b. y. with that of the plant. Furthermore, in the mode of b. y. reduction of 2-nonen-4-olide and 2-decen-5-olide there is identity in the mode of hydrogen addition onto the double bond (beta *re*, *anti* addition), while the

enantiomeric discrimination at the chain-bearing chiral center is (S) and (R), respectively. A similar phenomenon has already been observed in *Cladosporium suaveolens*.[4] In this microorganism, degradation of racemic ricinoleic acid and of structural analogs provides a set of (R) γ-lactones, whereas when isomeric materials in which the hydroxyl group is located at odd position are used as substrates (S) δ-lactones are obtained. Moreover, in that occasion it was observed that the *ee* values of the lactones produced by β-oxidation resulted increasing with the length of the side chain and higher for the δ-lactones than for the γ-ones. Last but not least, in the b. y. reduction of **6** there is incorporation of deuterium from the solvent at positions 2 and 3 of **7** in nearly 100:85 ratio, higher than the 100:65 ratio observed at the corresponding positions in the reduction of *Massoia* lactone.[5] It might be possible that the lower rate of reduction of **6** favours a more extended enzyme-assisted exchange with the solvent of the hydrogen atom of the reduced nicotine cofactor to be delivered in β position during the reduction, at variance with what usually occurs in the enzymic saturation of carbonyl-activated double bonds.[10]

Apart from the preparative significance in the field of natural flavours, the present study might be of some mechanistic interest since it poses questions regarding the structural factors dictating the inversion of stereochemical preference in the b. y. transformation on going from γ- to δ-lactones, while there is the same mode of hydrogen addition across the double bond.

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8. ^{1}H NMR of **5** (C_6D_6, protio compound), δ: 3.71 (m, 1 H, H-4, J(3,4) 6.3, J(3',4) 8.0 Hz), 1.78 (ddd, 1 H, H-2, J(2,2') 17.5, J(2,3) 9.4, J(2,3') 9.4 Hz), 1.90 (dt, 1 H, H-2', J(2',3?) 4.0, J(2',3') 9.5 Hz), 1.29 (dddd, 1 H, H-3, J(3,4) 6.3, J(3,3') 12.5 Hz), 0.93 (dddd, 1 H, H-3', J(3',4) 8.0 Hz), 0.95-1.30 (m, 8 H, 4 CH_2), 0.85 (t, 3 H, CH_3). ^{2}H NMR of **5** (C_6H_6), δ: 1.88 (D-2'), 1.28 (D-3) (major diastereoisomer), 1.78 (D-2), 0.92 (D-3') (minor diastereoisomer). ^{2}H NMR of **7** (C_6H_6) δ: 1.88 (D-2'), 1.29 (D-3) (minor distereoisomer), 1.79 (D-2), 0.94 (D-3') (major diastereoisomer).
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