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Stereochemistry of the Yeast - Mediated Conversion of Delta 2 - Decenolide into Delta Decanolide

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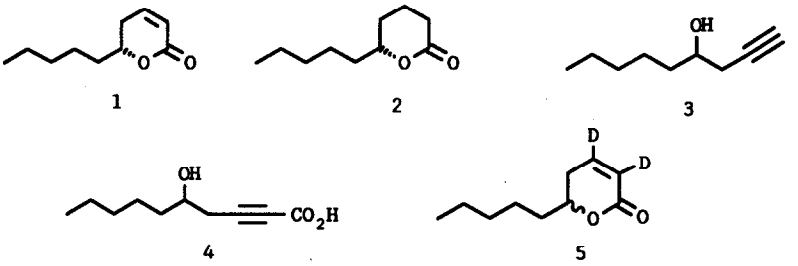
NMR studies on δ-decanolide samples obtained from 1 and 5 in reduction experiments with baker's yeast in D_2O and H_2O, respectively, show that the double bond saturation involves beta re-face trans formal addition of hydrogen atoms, arising to a large extent from water. The reduction does not depend on the absolute configuration at position 5 and shows a kinetic preference for the (*R*) enantiomer.

A recent report[1] on the capacity of baker's yeast to convert (*R*)-2-decenolide 1 into (*R*)-decanolide 2 prompted us to disclose the results of the stereochemical studies we were performing on this subject. These studies concerned the origin and the stereochemistry of the hydrogen atoms formally added onto the (*Z*) double bond of 1, the influence of the absolute configuration of the substrate on the mode of double bond saturation and, finally, the enantioselectivity of the transformation. To this end, a series of 2,3-dideuterated forms of δ-decanolide were obtained in experiments in which either 1 was reduced with baker's yeast in D_2O/H_2O mixtures as solvent or racemic, regiospecifically 2,3-dideuterated 2-decenolide 5 was used as substrate in H_2O. NMR and multidimensional GLC studies on these materials allowed the obtainment of the required stereochemical informations on which we report now.

The reduction of 1 to 2 is complete within 70 h at 1 g/l substrate concentration using 25 g of baker's yeast. The product recovery was 80%. However, the rate of the reduction decreased significantly when deuterared water was used as solvent under identical conditions. Only 8% reduction was observed in 80% D_2O after 24 h incubation, whereas the conversion of 1 into 2 raised to 60% in 50% D_2O. ^2H NMR studies (Figure 1, A) on the material obtained under the latter conditions indicated that two deuterium atoms are incorporated at positions 2 and 3 of the saturated lactone with a signal intensity ratio of ca. 6:4. The extent of monodeuteration at positions 2 and 3, when the reduction is perfomed in 80% D_2O, resulted ca. 70% and 45%, respectively. The proton spectrum of δ-decanolide was analyzed and completely assigned previously.[2] All protons display

different chemical shifts in a diluted C_6D_6 solution. The hydrogens, located at carbons C-2 and C-3, cis to the alkyl chain at C-5 resonate at 1.97 and 1.10 ppm, while the hydrogens trans to the substituent were found at 2.06 and 1.00 ppm, respectively. In the present case the deuterium atoms of δ-decanolide obtained in deuterated water show chemical shifts at 1.95 and 0.98 ppm and thus a trans stereochemistry as depicted in the structural formula 6.



1 2 3 4 5

Table. Product distribution in the baker's yeast reduction of 2-δ-decenolide 5, determined through GLC and NMR analysis.

Entry	incubation (h)	2-decenolide (GLC)		decanolide (GLC)		(NMR)
		S	*R*	*S*	*R*	(7)/(8)
1	30	45	32	5	18	75/25
2	48	27	1	23	49	63/37
3	70	------		50	50	50/50

Confirmation of the observed anti, beta re-face mode of double bond saturation arose also from reduction experiments of the racemic dideuterated lactone 5. This material was prepared from 3 via 4 and triple bond partial saturation (Lindlar catalyst) using deuterium gas. The enantiomeric composition of the lactones present in the mixture extracted at different incubation times, determined through multidimensional GLC analysis,[3] indicates (Table) that the (*R*) enantiomer is reduced at higher rate. Examination of the ^2H NMR spectra (Figure 2) of these materials allowed to assign structural formulas 7 and 8, respectively, to the (*R*) and (*S*) enantiomeric forms of δ-lactone formed from 5. In fact the chemical shifts of the deuterium atoms located at carbons C-2 and C-3 of the (*R*) enantiomer (the major enantiomer at short incubation times) occur at 2.04 and 1.08 ppm, while that of the (*S*) enantiomer are found at 1.95 and 0.98 ppm, respectively. The ratio of the enantiomers determined from the relative NMR signal intensities is in agreement with those obtained by GLC (Table). Thus, the baker's yeast saturation of the double bond of 2-δ-decenolide occurs by formal trans addition of hydrogen atoms from the beta re face, irrespective of the stereochemistry at position 5, with kinetic preference for the (*R*) enantiomer. Recently,[4] in studies on the mechanism of

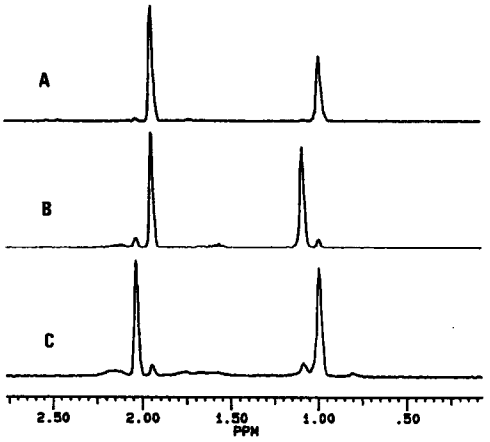


Figure 1. Expanded region of the ^2H NMR spectra in C_6D_6 of δ-decanolide obtained by reduction of A) 1 with baker's yeast in D_2O (structure 6) B) 5 by catalytic hydrogenation (structures 9) and C) 1 by catalytic deuteration (structure 10).

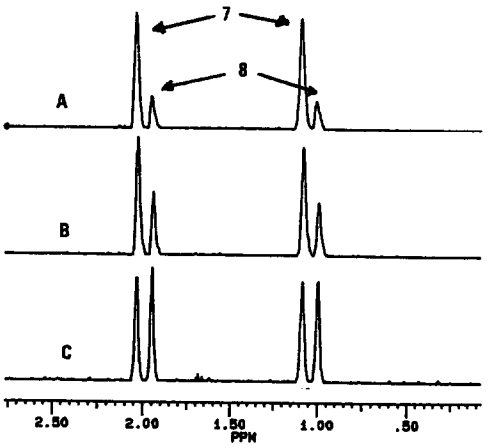
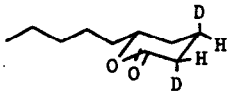


Figure 2. Expanded region of the ^2H NMR spectra in C_6D_6 of δ-decanolide obtained from the reduction of racemic 5 with baker's yeast at different incubation times A) after 30 h B) after 48 h and C) after 70 h. Arrows indicate the resonances corresponding to the structures 7 and 8.



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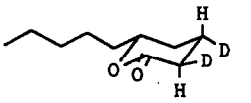

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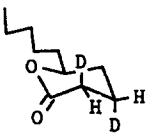

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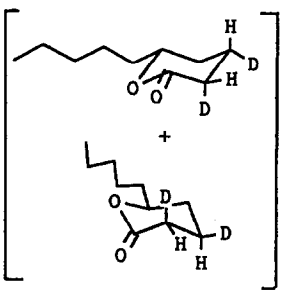

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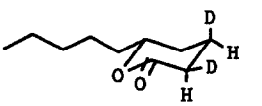

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the microbial biogeneration of γ- and δ-lactones it has been established that 2,3-dideuterated (*S*)-δ-undecanolide, obtained in *Cladosporium suaveolens* from [8,9-$^2H_2$] 11-hydroxy-heptadec-8*E*-enoic acid, bears the deuterium atoms in syn, (2*R*,3*R*) configuration. Since in that process the unsaturated lactone was envisaged as possible intermediate, the present results would suggest that, if this is true, the mode of double bond saturation in *C. suaveolens* differs from that observed in baker's yeast.

However, it is interesting to note that the saturation of the double bond of (*Z*)-configurated 1 and of cinnamaldehyde proceeds with the same anti stereochemistry[5] in baker's yeast. Moreover, the steric course of the reduction of carboxyl-activated double bonds catalyzed by enoyl-CoA reductases from various sources has been previously studied.[6] In all latter instances the stereochemistry of the reduction is different from that reported above. However, in these cases, the hydrogen atom added in position 2 arises from water, whereas NADPH delivers the hydrogen atom in position 3. The nucleotide specificity determines the stereospecificity of the double bond reduction. The nature of the enzymic system(s) participating, amongst those present in baker's yeast showing reducing capacity,[7] to the saturation of the double bond of 1 is unknown. However, the observation that the hydrogen atom added in position 3 arises to a large extent from water suggests the possible involvement in this process of reduced pyridine nucleotide coenzyme, which in baker's yeast exchanges the hydrogen atom with water under the catalysis of diaphorase.[5,8]

Finally, it is worthwhile to note that catalytic hydrogenation of 2-δ-decenolide proceeds in syn fashion, as shown by NMR spectra (Figure 1, B and C) of rac-9 and 10 obtained from 5 and 1, performing the reduction with deuterium and hydrogen gas, respectively, in the presence of 10% Pd/C. The saturation of the double bond occurs trans with respect to the substituent at C-5 with over 90% stereochemical control. We will report in due course on substrate specificity and steric outcome of the baker's yeast reduction of unsaturated racemic γ-and δ-lactones bearing different substituents in the ring and in positions 4 and 5, respectively.

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