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Baker's Yeast Reduction of Arylidenecycloalkanones

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Abstract: The baker's yeast reduction of arylidene cycloalkanones **5**, **6** and **7** occurs initially through the catalysis of different enzymes to give the saturation of the double bond leading to the saturated ketones **9**, **15** and **22** and the carbonyl reduction to the (S) allylic alcohols **8a**, **14** and **23**, possessing 0.99, 0.85 and 0.66 *ee*, respectively. The latter act as inhibitors for the carbonyl reducing enzyme thus preventing the further reduction of the saturated ketone. These compounds in the absence of allylic alcohols are further reduced to a mixture of diastereomeric saturated (S) alcohols of high-moderate enantiomeric purity. Reduction experiments in D_2O indicate that saturation of the double bond of **5** occurs by β re face addition of hydrogen, as shown by the obtainment of **10'**.

The reduction of carbonyl compounds is a very common operation encountered in enzymatic systems of microbial and human origin. Isolated enzymes and whole cells systems have been employed extensively in the reduction of carbonyl compounds. While microorganisms usually show a rather broad substrate specificity taking advantage of constitutive or inducible oxidoreductases and mixtures of them, the substrate specificity of isolated enzymes is usually rather narrow. Indeed yeast alcohol dehydrogenases (YADH) only reduce aldehydes and short chain ketones[1], *Thermoanaerobium brockii* alcohol dehydrogenase (TADH) is specific for methyl or ethyl ketones mainly[2], horse liver alcohol dehydrogenase (HLADH), one of the most extensively studied, accepts alkyl substituted cyclohexanones and cyclopentanones as substrates. A wider substrate specificity towards polycyclic compounds is observed in the *Pseudomonas testosteroni* alcohol dehydrogenases (PTADH)[1]. Different hydroxy steroid dehydrogenases (HSDH) selectively recognise one of the carbonyl groups in a steroid molecule[3] (Figure 1).

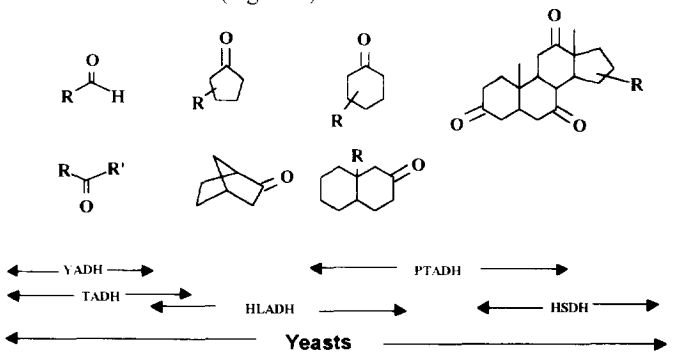
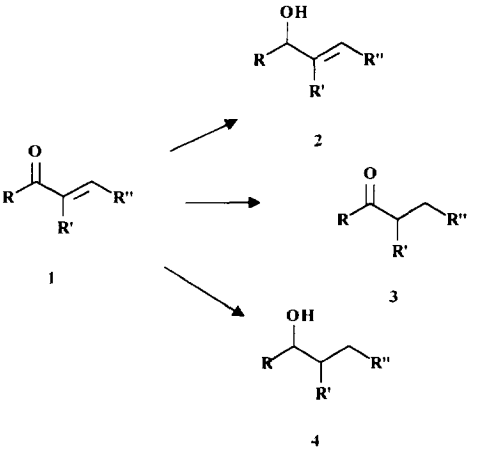


Figure 1

The catalytic properties of these enzymes have been exploited in biocatalysis both in the oxidative and the reductive direction.

Microorganisms for their nature of multienzymatic systems are able to catalyse a wide variety of reaction, but their use is particularly advantageous in reductions/oxidations, not requiring the addition and regeneration of cofactors.

We found of interest the reduction of α,β-unsaturated carbonyl compounds that we have been studying for some times now. The outcome of this reaction on substrates of type **1** in fermenting yeast due to the presence of several enzymes in the microbial system gives as products allylic alcohols **2** , saturated ketone **3**, or saturated alcohol **4** (Scheme 1) with different degree of selectivity dictated by the nature of the substrate or by the experimental conditions[4,5].



Scheme 1

As an extension of this work we have recently considered as substrates the arylidene ketones **5-7** depicted in Figure 2[6]. Enantioselective reduction of these compounds eventually leeds to valuable chiral products in enantiomerically enriched form of use in synthesis. The structural features of Michael acceptors of these arylidene ketones make them similar to the methylvinyl ketone and related substrates, whose properties as specific dehydrogenases inhibitors are well known[7]. Since the nature of the enzymes active in fermenting yeast is very poorly defined, a correlation through the substrate specificity and their inhibition properties can be useful in understanding the real catalytic property at work[8].

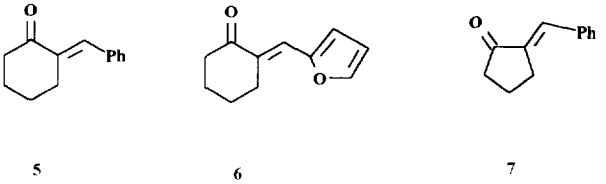
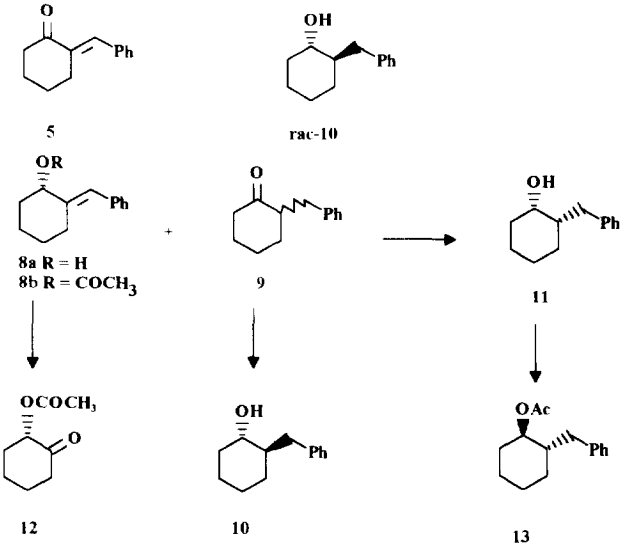


Figure 2

Our investigation in the baker's yeast transformation of structurally related cycloalkanones **5-7** shows that the product distribution observed is quite similar, but the corresponding products obtained from the three ketones differ considerably as far as the enantiomeric purity is concerned. Scheme 2 shows the products obtainable from the ketone **5**.

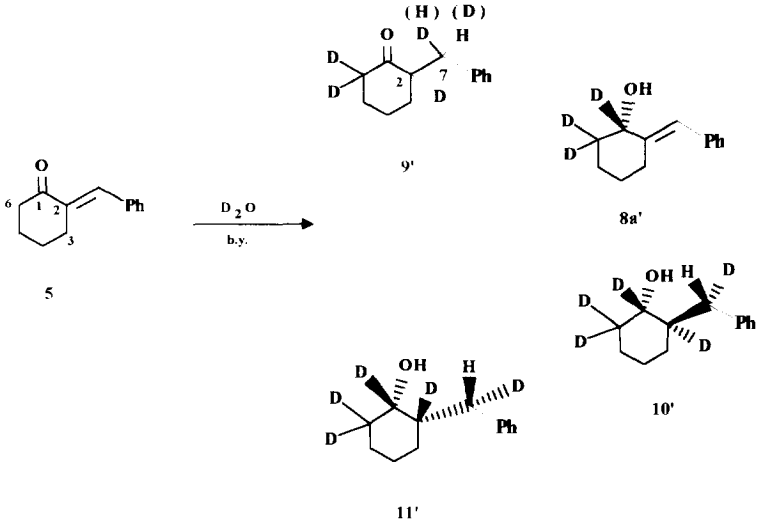
The baker's yeast reduction of the arylidene cyclohexanone **5** gives a 1: 1.5 mixture of the allylic alcohol **8a** and of the saturated ketone **9**. Compound **8a** is enantiomerically pure and its absolute configuration has been assigned through the conversion into (S)-2-acetoxy cyclohexanone **12** *via* **8b**. Through the known *face*-selective $LiAlH_4$ reduction[9], the allylic alcohol **5** is transformed into the corresponding saturated alkanol **10** with *trans* realative configuration as depicted in Scheme 2. The ketone **9**, $[\alpha]_D^{20}$ = 9.1, showed no enantiomeric enrichment from ^1H NMR measurements in the presence of chiral shift reagents. The same compound as recovered after purification from yeast reduction, or prepared from **5** *via* catalytic hydrogenation, was again introduced into fermenting yeast and rapidly reduced to a 1:1 mixture of the two carbinols **10** and **11** in enantiomerically pure form. The alcohol **11** was transformed into the tosylate. The tosyl group was displaced with acetate ion to give the acetate **13** enantiomer of the acetate of **10**.

This unusual sequence in the baker's yeast reduction can be explaned by assuming the mixed inhibition due to **5** and/or **8a** toward some of the enzymes involved in the reducing sequence. In fact the ketone **9** is effectively reduced. The enantioselectivity of the steps leading to **8a**, **10** and **11** is complete. Moreover, the reduction step from **9** to equal amounts of **10** and **11** proceeds in a stereospecific manner with respect to the carbonyl face with no influence by the stereochemistry of the near chiral carbon, as it is often the case (See for instance the non selective reduction of 2-alkyl substituted-3-keto esters[5]).



Scheme 2

In order to gain information about the stereochemistry of the B.Y. double bond saturation process we have performed reduction experiments of **5** in D_2O. The reactions afforded products **8'a, 9', 10'** and **11'** variously labelled with deuterium atoms (Scheme 3).



Scheme 3

The position and the stereochemistry of the deuterium atoms can be determined from the ^2H NMR spectra provided that the hydrogen spectra of the full protonated compounds are assigned.

The (E) stereochemistry of the double bond for the starting material **5** was obtained from NOE experiments performed on **8a**. The irradiation of the vinylic proton at 6.5 ppm enhanced (*ca.* 6%) the H-1 signal at 4.23 ppm and the saturation of the aromatic protons caused the enhancement of the CH_2-3 methylene hydrogens at 2.7 and 2.1 ppm (*ca.* 2%). These observations allow to establish unequivocally that the phenyl substituent and the CH_2-3 group are cis for both products **5** and **8a**.

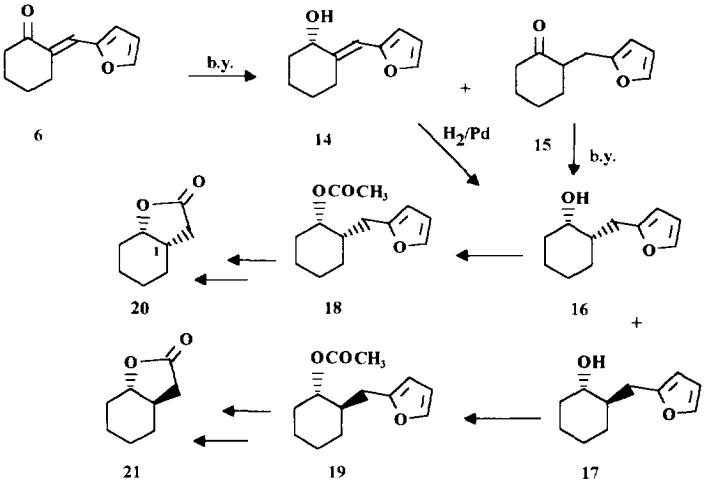
The ^2H spectrum of the saturated ketone **9'** obtained from **5** in D_2O shows the presence of five signals which can be assigned to deuterium atoms in the positions 2, 6, 6', 7 and 7'. The deuterium labelling at carbon 6 of the molecule indicates that fast hydrogen-deuterium exchange occurs in solution due to the keto-enolic equilibrium. This is in agreement with the fact that product **9'** is recovered racemic from the reaction mixture.

The spectrum of the unsaturated carbinol **8'a** displays three deuterium signals of comparable intensity which can be assigned to the atoms in position 1 (4.24 ppm), 6 and 6' (1.98 and 1.61 ppm). The occurrence of deuterium nuclei at carbon C-6 of **8'a** indicates that the rate of the keto-enolic equilibrium for **5** is faster than that of the carbonyl reduction. Moreover the presence of deuterium at the hydroxyl bearing carbon C-1 suggests that the reduction is mediated by an enzyme cofactor exchanging with water the hydrogen which eventually is delivered to the carbonyl carbon of the substrate[10].

The trans isomer **10'** contains deuterium at positions 1 (3.29 ppm), 2 (1.48 ppm), 6 (1.98 ppm), 6' (1.26 ppm) and 7' (3.18 ppm) while the position 7 (2.96 ppm) is not labelled. The distinction between H-7 and H-7' was performed on the full protonated compound **10** with the combined use of coupling constants and NOE effects. In fact the value of J(2.7) by 9.9 Hz suggests that H-2 and H-7 are preferentially *anti* oriented; in addition, the irradiation of the aromatic protons caused the enhancement of the H-2 (*ca.* 3%) and CH_2-3 (*ca.* 1%) signals, while the saturation of H-7' produced NOE only for H-2 (*ca.* 3%), thus suggesting that the phenyl group is preferentially *syn* to CH_2-3. The S stereochemistry of the deuterium atom at C-7 found for **10'** shows that the formal hydrogen addition to the double bond of the α,β-unsaturated ketone **5** takes place from the β *re* face of the molecule as observed in other cases[11].

The deuterium signals present in **11'**, relative to position 7 (2.54 ppm) has been recognized, while the signal at position 7' (2.65 ppm) is absent. This behavior suggests that the same reduction mechanism described above for **10'** holds also for **11'**. However in this case the assignement of the absolute stereochemistry of the hydrogen addition cannot be made unequivocally since free rotation occurs around C_2-C_7 bond as indicated by the values of J(2,7) (7.2 Hz) and J(2,7') (7.4 Hz), thus precluding the assignment of H-7 vs. H-7'.

The products obtained in the B.Y. reduction of furylidene cyclohexanone **6** are reported in Scheme 4, together with the chemical correlations which allowed the assignement of their absolute configuration.



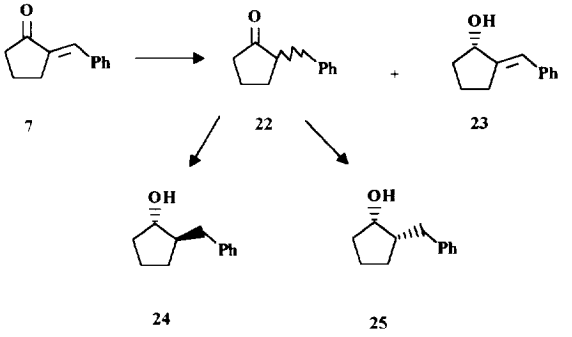
Scheme 4

The allylic alcohol **14**, $[\alpha]_D^{20}$ = 33.3, obtained close to racemic **15**, was assigned 0.85 *ee* on the basis of ^1H NMR studies on the ester with (+)-MTPA and comparison with the product obtained from the racemic carbinol of $NaBH_4$ reduction of **6**. Carbinol **14**, at variance with **8a**, resisted the $LiAlH_4$ reduction to the *anti*

carbinol **17**[9]. However, this material, together with the *syn* material **16**, was obtained either from **14**, upon catalytic hydrogenation or by B.Y.reduction of the racemic ketone **15**, which accompanied **14**, in the B.Y. treatment of **6**. Product **16** obtained under these circumstances was converted, upon ozonolysis, into lactone **20**. This material resulted to be the enantiomer of the material described by Corey[12] and of 83% optical purity. The *anti* carbinol **17**,which accompained **16**, was shown from ^{1}H NMR studies in the presence of Eu $(hfc)_3$ to be enantiomerically pure. Similarly to **16**, **17** affords the *anti* lactone **21**. Finally, the S carbinol **16**, upon conversion into tosylate and acetate displacement, gave rise eventually to a product which resulted to be the enantiomer of **17**. These studies allow the assignment of the S configuration to **14**, **16** and **17**.

The behavior of compound **6** is therefore very similar to the one previously observed with the arylidene cyclohexanone **5** in terms of product distribution, but the *ee* values are usually lower.

Scheme 5 summarises the outcome of the reduction of the cyclopentanone derivative 7. A mixture of two compounds was again obtained: the product of carbonyl reduction **23** of only 0.63 *ee* was present close to the racemic cyclopentanone **22** in a 1:2 ratio. Further reduction of the latter gave a mixture of isomeric saturated cyclopentanols **24** and **25** in 18% and 4% yields respectively.



Scheme 5

Their enantiomeric excess determined through HPLC studies on a chiral column was higher than 0.90. The unreacted ketone was recovered (78%) and resulted racemic from ^{1}H NMR experiments with chiral shift reagents. The assignement of the S configuration to this set of materials is based only on analogy with the above products obtained from **5** and **6**.

The above experiments thus indicate the dual behavior of B.Y. towards ketones **5**, **6** and **7**, represented by the capacity of either reducing the carbonyl group to give the unsatured alcohols **8a**, **14** and **23** or to saturate the double bond leading to the ketones **9**, **15** and **22**. While the former products show S absolute configuration and decreasing *ee* values on going from **8a** to **23**, the second set of materials shows a modest optical rotation, but appears substantially racemic at our studies. In the absence of inhibitory effect, expressed from the unsaturated ketones or by the allylic alcohols, the saturated ketones **9**, **15** and **22** are reduced to mixtures of *syn* and *anti* carbinols.

In the case of cyclohexanones **9** and **15** the reduction is fast, leading to S configurated carbinols of high-moderate *ee* values. Under similar conditions α-benzyl cyclopentanone **22** is reduced instead only to a

moderate extent. Combined together, these results show that, despite the fact that baker's yeast reductions are on the chemical scenario since a long time, there are still modes of transformations of unsaturated carbonyl compounds which are unexpected.

Experimental

Unsaturated ketones **5, 6, 7.** These compounds were obtained as described in the literature for analogous derivatives[13].Compound **5**, yellow crystalline material, m.p.=51°C, (hexane); δH ($CDCl_3$) 1.75 (2H,CH_2, m), 1.95 (2H, CH_2, m), 2.5 (2H, CH2, t), 2.85 (2H, CH_2, m), 7.3-7.4 (5H, ArH, m), 7.5 (1H, vinyl, s). Anal. Calcd for $C_{13}H_{14}O$: C, 83.83; H, 7.58. Found: C, 84.02; H, 7.6.

Compound **6**, yellow crystalline material, m.p.= 44 °C, (hexane); δH ($CDCl_3$) 1.76 (2H, CH_2, m), 1.98 (2H, CH_2, m), 2.5 (2H, CH_2, t), 2.92 (2H, CH_2, m), 6.5 (1H, CH, dd), 6.64 (1H, CH, d), 7.4 (1H, vinyl, t),7.55 (1H, CHO, d). Anal. Calcd for $C_{11}H_{12}O_2$: C, 74.78; H, 6.86. Found: C, 74.65; H, 6.92.

Compound **7**, yellow crystalline material, m.p.=65°C, (hexane); δH ($CDCl_3$) 2.05 (2H, CH_2, m), 2.4 (2H, CH_2, t), 3.0 (2H, CH_2, t), 7.4 (5H, ArH, m), 7.55 (1H, vinyl, m). Anal. Calcd for $C_{12}H_{12}O$: C, 83.69; H, 7.02. Found: C, 84.02; H, 6.97.

General Procedure for the Bioconversion of Substrates **5, 6, 7, 9, 15** *and* **22.** To a stirred solution of D-glucose (100g) and Baker's Yeast (500 g) in water (2 l) at 36- 40°C, the substrate (10 g) dissolved in the minimum amount of EtOH was added dropwise; the reaction mixture was kept under stirring for 2 days. At the end of this period, 1 l of AcOEt was added and the crude reaction mixture was filtered on a Buchner funnel through a Celite pad. The filtrate was extracted with AcOEt (2 x 1 l), the organic phase was dried over Na_2SO_4 and the solvent was evaporated under reduced pressure to dryness.The residue, 8-9 g, was purified by SiO_2 column chromatography to afford with increasing amounts of AcOEt in hexane the products indicated below.

(S)-α-benzylidencyclohexanol **8a**.The unsaturated carbinol **8a** was isolated from the treatment of **5** as white crystalline solid, m.p.= 76-79°C, (pentane); δH ($CDCl_3$) 1.5-3 (9H, 4 CH_2+OH, m), 4.2 (1H, OCH, m), 6.5 (1H, H vinyl, s), 7.1-7.4 (5H, ArH, m); $[\alpha]_D^{20}$ = -35.2 (c 1.2, $CHCl_3$).When the reduction was performed in the presence of D_2O, the deuterated analog **8'a** was obtained. **8'a**, δH ($CDCl_3$) 1.40-2.05 (7H, 3 CH_2 + OH, m), 2.12 (1H, H-3, m), 2.72 (1H, H-3', m), 4.23 (1H,C*H*OH, m), 6.5 (1H, CH vinyl, s), 7.15-7.35 (5H, C_6H_5, m); δ_D ($CHCl_3$) 4.23 (D-1), 1.61 (D-6), 1.98 (D-6') **8a**: Anal. Calcd for $C_{13}H_{16}O$: C, 82.94; H, 8.57. Found: C, 82.76; H, 8.56. This material was present in 1:1.5 ration with **9**.

α-benzylcyclohexanone **9**. The saturated ketone **9**, obtained in the experiment giving **8a**, was a colourless oil; δH ($CDCl_3$) 1.2-2.6 (10H, 5 CH_2, m), 3.2 (1H, CH, dd), 7.1-7.4 (5H, ArH, m); $[\alpha]_D^{20}$ = -9.1 (c 1.3, $CHCl_3$).^{1}H NMR studies on this material with Eu (hfc)$_3$ showed not noticeable enantiomeric enrichment. **9'**,δH ($CDCl_3$) 0.90-1.70 (6H, 3 CH_2, m), 1.80 (1H, H-6, m), 2.16 (1H, H-6', m), 2.23 (1H, H-2, m), 2.38 (1H, H-7, dd, J(H_7,$H_{7'}$) 14.0, J(H_7,H_2) 5.0 Hz), 7.05-7.25 (5H, C_6H_5, m); δ_D (C_6H_6) 1.78 (D.6), 2.17 (D-6,), 2.21 (D-2), 2.35 (D-7), 3.26 (D-7'). **9**: Anal. Calcd for $C_{13}H_{16}O$: C, 82.94; H, 8.57. Found: C, 92.98, H, 8.67.

Saturated carbinols **10** *and* **11**. (1S, 2R)-α–benzylcyclohexanol **10** and (1S, 2S)-α–benzylcyclohexanol **11** were obtained by fermentation of **9** in about 1:1 ratio in 70% overall yield. **10**, white crystals, m.p.= 46-48°C, (hexane); δH ($CDCl_3$) 1-2 (10 H, 4CH_2+CH+OH, m), 2.3 (1H, CH-7a, dd), 3.1 (1H, CH-7b, dd), 3.2 (1H,

OCH, m), 7.0-7.3 (5H, ArH, m); $[\alpha]_D^{20}$ = 49.2 (c 1, $CHCl_3$) and **11**,white crystals,m.p.= 67-70°C, (hexane); δH ($CDCl_3$) 1-2 (10H, $4CH_2$+CH+OH), 2.5 (1H, CH-7a, dd), 2.6 (1H, CH-7b, dd), 3.7 (1H, OCH, s), 7.0-7.3 (5H, ArH, m); $[\alpha]_D^{20}$ = 28.2 (c 1, $CHCl_3$). Anal. Calcd for $C_{13}H_{18}O$: C, 82.06; H, 9.54. Found: C, 81.96; H, 9.54 **10'**, δH ($CDCl_3$) 0.91 (1H, H-3ax, m), 1.09 (1H, H-4 (or H-5), m), 1.25 (2H, H-6ax + H-5 (or H-4), m), 1.50 (1H, H-2, m), 1.60 (2H, H-4' + H-5', m), 1.70 (1H, H-3eq, m),1.90 (1H, H-6eq, m), 2.36 (1H, H-7, dd, $J(H_7,H_{7'})$ 13.0, $J(H_7,H_2)$ 9.9 Hz), 3.17 (1H, H-7', dd, $J(H_{7'},H_2)$ 3.9 Hz), 3.30 (1H, CHOH, m), 7.15-7.30 (5H, C_6H_5, m); δ_D ($CHCl_3$) 1.26 (D-6ax), 1.48 (D-2), 1.98 (D-6eq), 3.18 (D-7'), 3.29 (D-1). **11'**, δH ($CDCl_3$) 1.15-1.70 (9H, 4 CH_2 + OH, m), 2.48 (1H, H-7, dd, $J(H_7,H_{7'})$ 13.0, $J(H_7,H_2)$ 7.5 Hz), 2.65 (1H, H-7', dd, $J(H_{7'},H_2)$ 7.2 Hz), 3.74 (1H, H-1, m), 7.10-7.25 (5H, C_6H_5, m); $[\alpha]_D^{20}$ = 28.2 (c 1, $CHCl_3$). δ_D ($CHCl_3$) 3.79 (D-1), 2.52 (D-7).

(S)-α-furylidenecyclohexanol **14**. The unsatured carbinol **14**, obtained by fermentation of **6** in 1:1 ratio with **15**, colourless oil; δH ($CDCl_3$) 1.5-2.1 (8H, $4CH_2$, m), 4.26 (1H, OCH, m), 6.24-6.28 (2H, H vinyl + CH Ar, m), 6.38 (1H, CH Ar, dd), 7.36 (1H, OCH, d); $[\alpha]_D^{20}$ =33.3 (c 1.23, $CHCl_3$). Anal. Calcd for $C_{11}H_{14}O_2$: C, 74.13; H, 7.92. Found: C, 73.92; H, 8.04. It was treated with (+)-MTPA-chloride in pyridine to give the corresponding ester. Similarly, the (+)-MPTA ester of the racemic alcohol obtained upon $NaBH_4$ reduction of the unsatured ketone **6** was prepared. Comparison of the relative intensity of the CH signals at 6.37 ppm (enantiomer S) and at 6.39 ppm (enantiomer R) allowed to assign 0.85 *ee* to **14**. The catalitic hydrogenation of **14** in AcOEt in the presence of 10% Pd/C (10:1 ratio) affords quantitatively a *ca.* 2:1 mixture of **16**, and **17**, separated by SiO_2 column chromatography. **16**, yellow oil, $[\alpha]_D^{20}$ = 19.3 (c 1, CH_3OH). **17**, yellow oil, $[\alpha]_D^{20}$ = 23 (c 1, CH_3OH).

Carbinols **16** *and* **17** *from the B.Y. reduction of α-furylmethylcyclohexanone*.α-furylmethylcyclohexanone **15**, a colourless oil was recovered in the racemic form from the B.Y. reduction of **6**, δH ($CDCl_3$) 1.25-2.72 (10H, $5CH_2$, m), 3.17 (1H, CH, dd), 6.0 (1H, CH Ar, d), 6.25 (1H, CH Ar, dd), 7.3 (1H, OCH, d). Anal. Calcd for $C_{11}H_{14}O_2$: C, 74.13; H, 7.92. Found: C, 74.13; H, 8.10. When **15** was submitted to the B.Y. reduction, it gave a *ca.* 1:1 mixture of **16** and **17**. **16**, yellow oil, δH ($CDCl_3$) 1.2-1.7 (8H, $4CH_2$, m), 1.73-1.83 (1H, CH, m), 2.58 (1H, CH-7a, dd), 2.74 (1H, CH-7b, dd), 3.83 (1H, OCH, m), 6.02 (1H, CH Ar, d), 6.28 (1H, CH Ar, dd), 7.31 (1H, OCH, d); $[\alpha]_D^{20}$ = 18.6 (c 1.09, CH_3OH). Anal. Calcd for $C_{11}H_{16}O_2$: C, 73.30; H, 8.95. Found: C, 73.32; H, 8.88. **17**, yellow oil, δH ($CDCl_3$) 1.1-1.3 (4H, $2CH_2$, m), 1.6-1.8 (4H, $2CH_2$, m), 2.0 (1H, CH, m), 2.58 (1H, CH_{7a}, dd), 3.0 (1H, CH_{7b}, dd), 3.25 (1H, OCH, m), 5.98 (1H, CH Ar, d), 6.28 (1H, CH Ar, dd), 7.3 (1H, OCH, d); $[\alpha]_D^{20}$ = 37 (c 0.8, CH_3OH). Anal. Calcd for $C_{11}H_{16}O_2$: C, 73.30; H, 8.95. Found: C, 73.22; H, 8.82. ^1H NMR studies on **17** in the presence of Eu $(hfc)_3$ and comparison with the material prepared from racemic **14**, indicated that this material is enantiomerically pure. The relative signals to CH-7a (dd) at 2.58 ppm and CH-7b (dd) at 3.0 ppm, in ^1H NMR of the racemic material **17** appear divided into a ddd in the presence of Eu $(hfc)_3$, while in the ^1H NMR of the enantiomerical pure form **17** remained a dd.

Lactones **20** *and* **21**. Product **16** obtained in the latter experiment, 4 g, was treated overnight with 10 ml of Ac_2O and 10 ml of pyridine at room temperature. The reaction mixture was poured into ice-water to give after extractive work-up with CH_2Cl_2 and SiO_2 column chromatography purification, the acetate **18**, oil, $[\alpha]_D^{20}$ = 91.5 in 85% yield. δH ($CDCl_3$) 1.22-1.96 (9H, $4CH_2$+CH, m), 2.08 (3H, $OCOCH_3$, s), 2.5 (1H, CH-7a, dd), 2.62 (1H, CH-7b, dd), 4.91 (1H, OCH, m), 5.95 (1H, CHAr, d), 6.26 (1H, CHAr, dd), 7.26 (1H, OCH, d). 2 g of **18** in 80 ml of CH_2Cl_2/MeOH 9:1 at -78°C was submitted to a stream of ozonized oxygen

until permanence of blue color. The reaction mixture was carefully treated under stirring at -78°C with 1 ml of 60% H_2O_2. The temperature was raised slowly and the reaction mixture was then refluxed for 1.5 h. The cooled mixture was treated with 100 mg of 10% Pd/C refluxed for 1 h and filtered. The mixture was diluted with MeOH and treated with an excess of ethereal diazomethane. The reaction mixture was evaporated and the major material isolated by SiO_2 chromatography was treated with excess 10% NaOH in H_2O/MeOH 1:1 at reflux for 3 h. The reaction mixture was concentrated, acidified with 6 N HCl and extracted with AcOEt (3 x 50 ml). The residue obtained upon evaporation of the solvent was submitted to bulb-to-bulb vacuum distillation (3 mm/Hg, oven temp.:100°C) to give lactone **20**, in 40% yield, oil, $[\alpha]_D^{20}$= -37.7 (c 0.52, MeOH) (lit.[12] for the 1S, 2R material +45.5°); δH ($CDCl_3$) 1.22-1.78 (8H, $4CH_2$, m), 2.02-2-12 (1H, CH, m),2.24 (1H, COCH, dd), 2.62 (1H, COCH, dd), 4.52 (1H, CHCO, m). Similarly, from **17** lactone **21**, oil, $[\alpha]_D^{20}$ = - 43.3 (c 0.5, CH_3OH), was obtained. δH ($CDCl_3$) 1.22-1.78 (8H, 4 CH_2, m), 2.02-2.12 (1H, CH, m), 2.25 (1H, COCH, dd), 2.64 (1H, COCH, dd), 4.51 (1H, CHCO, m). Anal. Calcd for $C_8H_{12}O_2$: C, 68.55; H, 8.63. Found: C, 68.42; H, 8.71.

Conversion of **16** *into the enantiomer of* **17**. Product **16**, 250 mg, in 4 ml of dry pyridine, was treated with 1 g of TsCl at 0°C overnight. The reaction mixture was poured into ice-water and extracted with CH_2Cl_2 (3 x 50 ml).The crude tosylate obtained upon evaporation of the organic extract, which was washed with HCl and $NaHCO_3$ soln., was dissolved in 15 ml of dry DMF and treated at reflux with 3 g of dry CH_3COONa. After 3 h, most of the solvent was evaporated under reduced pressure. The residue was taken up with 5 ml of MeOH and treated at reflux for 3 h with 15 ml of 10% NaOH. The reaction mixture was diluted with ice-water and extracted with CH_2Cl_2 (3 x 25 ml). Upon SiO_2 column chromatography of the residue obtained upon evaporation of the solvent a material identical in every respect to **17**, but showing $[\alpha]_D^{20}$ = -21 (c 1, MeOH), was obtained in 30% overall yield.

(S)-α–benzylidencyclopentanol **23**. The material was obtained by fermentation of 7 as an oil; δH ($CDCl_3$) 1.7-2.6 (7H, $3CH_2$+OH, m), 4.6 (1H, OCH, m), 6.55 (1H, H vinyl, s), 7.2-7.35 (5H, ArH, m) ; $[\alpha]_D^{20}$ = 34.3 (c 0.8, $CHCl_3$); *ee* = 0.63 by HPLC analisys (CHIRACEL OD, Daicel, hexane/isopropanol 95:5, 0.6 ml min^{-1}, UV 254 nm, t_r = 19.40 min enantiomer 1R, t_r = 22 min enantiomer 1S). Anal calcd for $C_{12}H_{14}O$: C, 82.72; H, 8.10. Found: C, 82.22; H, 8.1.

α–benzylcyclopentanone **22**. Obtained, close to **23,** from **7,** (2:1 ratio) as an oil; δH ($CDCl_3$) 1.6-2.5 (7H, $3CH_2$+CH, m), 2.55 (1H, H-6a, dd), 3.12 (1H, H-6b, dd), 7.15-7.30 (5H, ArH, m) . Anal Calcd for $C_{12}H_{14}O$: C, 82.72; H, 8.10. Found: C, 82.22; H, 8.12. The material is devoid of optical activity.

Saturated carbinols **24** and **25**. (1S, 2S)-α–benzylcyclopentanol **25** and (1S, 2R)-α–benzylcyclopentanol **24** were obtained by fermentation of **22** in 18 and 4% yield, respectively, and purified by column chromatography. **25**, colourless oil; δH ($CDCl_3$) 1.2-2.1 (8H, $3CH_2$+CH+OH, m), 2.55 (1H, H-6a, dd), 2.75 (1H, H-6b, dd), 3.9 (1H, OCH, m), 7.2 -7.4 (5H, ArH, m); $[\alpha]_D^{20}$ = 25.0 (c 1, $CHCl_3$); *ee* = 0.94. Anal. Calcd for $C_{12}H_{16}O$: C, 81.77; H, 9.15. Found: C, 81.87; H, 9.12. **24,** colourless oil; δH ($CDCl_3$) 1.2-2.1 (8H, $3CH_2$+CH+OH, m), 2.65 (1H, H-6a, dd), 2.85 (1H, H-6b, dd), 4.1 (1H, OCH, m), 7.2-7.4 (5H, ArH, m); $[\alpha]_D^{20}$ = 24.4 (c 1.1, $CHCl_3$); *ee* = 0.90. Anal. Calcd for $C_{12}H_{16}O$: C, 81.77; H, 9.15. Found: C, 81.92; H, 9.02. The *ee* was determined by HPLC analisys (CHIRACEL OD, Daicel, hexane/isopropanol 95:5, 0.6 ml min^{-1}, UV 254 nm, t_r = 16.56 min enantiomer *syn*, t_r = 25.44 min enantiomer *anti*).

(S)-α–acetoxycyclohexanone **12**. 2 g di (S)-α–benzylidencyclohexanol **8a** was treated with acetic anhydride (1.1eq) and pyridine (1.1eq).The reaction mixture was mantained at r.t. for 16 hours. The crude reaction

mixture was poured into ice water and extracted with CH_2Cl_2 (3 x 25 ml).The organic phase was washed with dil. HCl , 3% $NaHCO_3$, dried over Na_2SO_4 and evaporated to dryness to give **8b,** colourless oil, in quantitative yield; δH ($CDCl_3$) 1.5-2 (6H, $3CH_2$, m), 2.1 (3H, Me, s), 2.3-2.7 (2H, CH_2, m), 5.35 (1H, OCH, m), 6.4 (1H, H vinyl, s), 7.1-7.4 (5H, ArH, m); $[\alpha]_D^{20}$ = -13.5 (c 0.9, $CHCl_3$). Anal. Calcd for $C_{15}H_{18}O_2$: C, 78.23; H, 7.88. Found: C, 78.22; H, 7.86. 2 g of **8b** in CH_2Cl_2/MeOH 9:1 (30 ml), at -78°C were submitted to O_3 bubbling to complete saturation.The reaction mixture was mantained under nitrogen for 10 min, then the ozonide at the same temperature was treated with 1.1 eq of triphenylphosphine. The solution was evaporated under reduced pressure, and the Ph_3PO was precipitated with a ether-hexane mixture. The residue was purified by column chromatography to give compound **12** as a white crystalline material (m.p.= 70°C, hexane), 70% yield; δH ($CDCl_3$) 1.5-2.6 (11H, $4CH_2$+Me, m+s), 5.2 (1H, CH, m); $[\alpha]_D^{20}$ = -69.7 (c 1.1, $CHCl_3$) (lit[14] for the R enantiomer, $[\alpha]_D^{20}$ = 65.7 c 1.1, $CHCl_3$). Anal Calcd for $C_8H_{12}O_3$: C, 61.52; H 7.74. Found: C, 61.84; H, 7.78.

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