s, $C(CH_3)_2$), 1.08–2.28 (6 H, m, H-5,6,6′,7,7′,8a), 2.38 (1 H, dd, J = 5 and 9 Hz, H-3), 2.77 (1 H, dt, J = 3 and 10 Hz, H-5′), 3.28–3.58 (2 H, m, H-3′,8), 3.67 (1 H, s, OH), 4.34–4.82 (2 H, m, H-1,2); high-resolution mass spectrum, calcd for $C_{11}H_{19}NO_3$ m/z 213.1364, found (M) 213.1369.

(1*S*,2*R*,8*S*,8a*S*)-Octahydro-1,2,8-indolizinetriol (8,8a-Di-*epi*-swainsonine, 6). A solution of **27** (35 mg, 0.16 mmol) in a 1 M aqueous HCl solution (1.5 mL) was refluxed for 30 min and concentrated. The residue was dissolved in a small amount of water and charged on a column of Amberlite IRA-400 (OH^-). The column was eluted with water, and the ninhydrin-positive fractions were concentrated. The residual solid was recrystallized from $CHCl_3$–hexane to give white crystals of **6** (24 mg, 84%), mp 130–131 °C dec. **6**: TLC R_f 0.32 (ammonia–water/butanol/CH_2Cl_2/EtOH 1:3:3:3, ninhydrin coloring); $[\alpha]^{22}_D$ −21.2° (c 0.78, MeOH); IR ν^{KBr}_{max} 3500–3200 (br), 2930, 2830, 2800, 1360, 1250 cm^{-1}; ^1H NMR (D_2O) δ 1.60–2.40 (6 H, m, H-5,6,6′,7,7′,8a), 2.22 (1 H, dd, J = 6 and 10 Hz, H-3), 2.73–3.02 (1 H, m, H-5′), 3.22–3.66 (2 H, m, H-3′,8), 3.72–4.34 (2 H, m, H-1,2); ^{13}C NMR δ 24.86, 34.67, 52.83, 61.94, 68.47, 72.63, 74.55, 75.26; high-resolution mass spectrum, calcd for $C_8H_{15}NO_3$ m/z 173.1050, found (M) 173.1042.

Enzyme Assays and Inhibition Experiments. Eleven glycosidase activities in a water-soluble extract of human liver were assayed at their pH-optima in the presence and absence of 1 mM (−)-2,8a- (**5**) and (−)-8,8a-di-*epi*-swainsonine (**6**) by using the appropriate fluorigenic (4-methylumbelliferyl) substrate (Koch-Light Ltd., Haverhill, Suffolk, U. K.) at a concentration of 0.5 mM as described previously.[19] The enzymes were α- and β-D-mannosidase, α- and β-D-glucosidase, α- and β-D-galactosidase, *N*-acetyl-β-D-hexosaminidase, α-L-fucosidase, β-D-xylosidase, α-L-arabinosidase, and β-D-glucunonidase. The percent activation or inhibition was calculated by comparing the activities in the presence and absence of the swainsonine analogues. The nature and the value of K_i for the inhibition of α-D-mannosidase by the (−)-8,8a-di-*epi*-swainsonine (**6**) were determined by the Dixon graphical procedure using a computer program to get the lines of best fit.

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(19) Burditt, L. J.; Chotai, K.; Hirani, S.; Nugent, P. G.; Winchester, B. *Biochem. J.* **1980**, *189*, 467.

Microbiological Reduction of Acyclic β-Diketones†

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Regio- and enantiospecificities of the biological reduction of various acyclic β-diketones by lower fungi were studied in order to obtain ketols of *R* configuration. Only 2-hydroxy compounds were obtained from 2,4-diketones as already observed for *S* ketols formed with bakers' yeast. Both 3-hydroxy and 5-hydroxy ketols were produced from 3,5-diones. All but 4-hydroxy-3-methylpentan-2-one had the *R* configuration expected.

Introduction

In previous work[1] we showed that bakers' yeast (*Saccharomyces cerevisiae*) reduces 2,4-diones to the corresponding ketols bearing a 2-hydroxy group of configuration 2*S* with high enantiomeric excess. The microbiological reduction of 2,4-diones by *S. cerevisiae* is both enantio- and regioselective, as confirmed in recent work by Ohta et al.[2] Such optically active ketols are of great interest in the chemistry of naturally occurring substances since this grouping is present in biologically active compounds[3] and chiral ketols make excellent synthons for the stereospecific synthesis of antibiotics[4] and pheromones.[5]

Having available a method for readily obtaining β-ketols of absolute configuration 2S, we set out to find biological systems that would enable us to obtain enantiomeric 2*R* ketols.

In the course of work on the synthesis of the two enantiomers of the pheromone sulcatol (6-methylhept-5-en-2-ol),[6] we found that certain microorganisms such as *Geotrichum candidum* and *Aspergillus niger* reduced a monoketone to the corresponding alcohol of *R* configuration. We report here the results obtained with these two fungi on a variety of 2,4-diones and 3,5-diones and, when not previously published, results obtained with bakers' yeast.

Results and Discussion

1. Reduction of 2,4-Diones. The 2,4-diones shown in Figure 1 were studied.

The microbiological reductions were carried out with washed resting cells suspended in water or glucose solution (see Experimental Section). The glucose was used not for fermentation or as a reducing agent, but simply to avoid metabolism of the reduced product, particularly by *G. candidum*. The results are collected in Table I. For comparison with our previous work,[1] the results obtained with bakers' yeast are also displayed in Table I.

† Use of Biological Systems for the Synthesis of Chiral Molecules. 5.

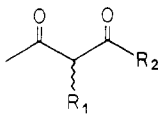
(1) Bolte, J.; Gourcy, J. G.; Veschambre, H. *Tetrahedron Lett.* **1986**, *27*, 565.
(2) Ohta, H.; Ozaki, K.; Tsuchihashi, G. *Agric. Biol. Chem.* **1986**, *50*, 2499.
(3) (a) Schmuff, N. R.; Philips, J. K.; Burkholder, W. E.; Fales, H. M.; Chen, C. W.; Roller, P. P.; Ma, M. *Tetrahedron Lett.* **1984**, *25*, 1533. (b) Sakai, T.; Nakagawa, Y.; Takahashi, J.; Iwabuchi, K.; Ishii, K. *Chem. Lett.* **1984**, 263.
(4) Gerlach, H.; Wetter, H. *Helv. Chim. Acta* **1974**, *57*, 2306.
(5) (a) Gerlach, H.; Kunzler, P. *Helv. Chim. Acta* **1977**, *60*, 638. (b) Ohta, H.; Ozaki, K.; Tsuchihashi, G. *Chem. Lett.* **1987**, 2225.
(6) Belan, A.; Bolte, J.; Fauve, A.; Gourcy, J. G.; Veschambre, H. *J. Org. Chem.* **1986**, *52*, 256.

0022-3263/88/1953-5215$01.50/0

Table I. Microbiological Reduction of 2,4-Diones

	2,4-dione			reactn	convrsn,		abs
	R_1	R_2	microorganism	time, h	%	ee, %	confgn
1	H	CH_3	*G. candidum*	10	100	74	*S*(+)
			A. niger	15	75	95	*R*(−)
			bakers' yeast[a]	144	90	99	*S*(+)
2	H	C_2H_5	*G. candidum*	10	100	99	*R*(−)
			A. niger	24	100	99	*R*(−)
			bakers' yeast[a]	72	100	99	*S*(+)
3	H	$(CH_2)_3CH{=}CH_2$	*G. candidum*	9	100	99	*R*(−)
			A. niger	24	75	95	*R*(−)
			bakers' yeast[a]	72	100	99	*S*(+)
4	H	C_6H_5	*G. candidum*	9	100	90	*R*(−)
			A. niger	24	100	99	*R*(−)
			bakers' yeast	72	90[b]	98	*S*(+)
5	CH_3	CH_3	*G. candidum*	9	75 (56/44)[c]	95 syn	*S*(+)
						98 anti	*S*(+)
			A. niger	24	100 (90/10)[c]	98 syn	*S*(+)
			bakers' yeast[a]	144	30 (80/20)[c]	92 syn	*S*(+)

[a] Already published in ref 1. [b] Mixture of 2-hydroxy and 4-hydroxyketones (85:15). [c] Syn/anti ratios.



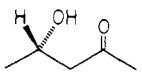
R_1 = H, Me; R_2 = Me, Et, Pent-1-enyl, Ph.

Figure 1.

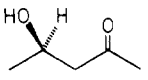
The products were analyzed by gas chromatography (GC) and the enantiomeric excesses were determined by GC on a chiral capillary column as described elsewhere.[1] The absolute configurations were assigned by comparisons with literature data and are discussed for each product.

The results in Table I show that in all of the cases studied, high conversions (72–100%) were realized. Only 2-hydroxy-4-keto compounds were obtained and under the experimental conditions used no diol was observed. The structures of the ketols obtained were confirmed unambiguously by their ^1H NMR spectra.

The first compound studied, pentane-2,4-dione (**1**), was rapidly reduced (10 to 15 h) by both microorganisms, whereas with bakers' yeast the reaction time was much longer (144 h). Ketol **1a** was obtained with a rather low enantiomeric excess with *G. candidum* (74% ee). Its positive optical rotation corresponds to that of the ketol obtained with bakers' yeast, and the absolute configuration has been assigned by Otha et al.[2] as *S*. The ketol obtained with *A. niger* showed a negative rotation and is assumed to have an *R* configuration (**1b**). In this case, the enantiomeric excess was high (95% ee).

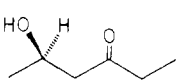


1a(+)

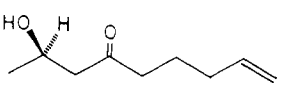


1b(−)

Reduction of hexane-2,4-dione (**2**) and 8-nonene-2,4-dione (**3**) occurred much more rapidly with *G. candidum* than with *A. niger*, but in both cases the ketols were obtained with a very high optical enantiomeric excess and a negative optical rotation. For the same compounds showing a positive rotation, Otha et al. assigned the *S* absolute configuration by comparison with (*S*)-(+)-ethyl 3-hydroxybutanoate.[2] Thus, we assigned the *R* configuration to ketols **2a** and **3a**.

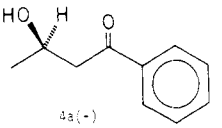


2a(−)



3a(−)

We then studied the reduction of 1-phenyl-1,3-butanedione (**4**) by *G. candidum* and *A. niger*. In both cases only one product, ketol **4a**, was formed. The identical compound with an hydroxy group α to the methyl group and showing a positive optical rotation has been assigned the *S* configuration by Otha et al.[2] and Chenevert.[7] The levorotatory ketol **4a** obtained by the reduction with the two fungi is assumed to have the *R* configuration. The highest enantiomeric excess was obtained with *A. niger* (99% ee).



4a(−)

In our previous work on the microbiological reduction of 2,4-diones by bakers' yeast leading to ketols of *S* configuration,[1] we did not study the reduction of dione **4**. For comparison with the results of the present work on *R* ketols, we carried out the reduction of **4** with *S. cerevisiae* (commercial bakers' yeast). In this case, ketol **4b** was the predominant product (90% of the mixture) but was accompanied by a significant amount of the isomeric ketol **4c**.

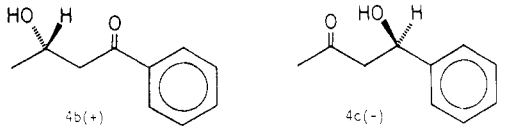
Some difficulty was encountered in obtaining pure **4b** and **4c**, which required several successive column chromatographic separations followed by preparative thin layer chromatographic separations. The two isomers are very difficult to separate and to characterize chromatographically (TLC R_f values are almost identical and GC retention times are very close). However, their ^1H NMR spectra exhibit different chemical shifts for the methyl groups. Ketol **4b** was obtained optically pure (98% ee) and showed a positive rotation. This result agrees with those obtained by Ohta et al.[2] and Chenevert[7] for the *S* ketol. Thus, we have assigned the *S* configuration to isomer **4b**. However, the optical rotation found for **4b** (+66°) is higher than the values reported by these authors (respectively, +39° and +55°).

To determine the enantiomeric excesses of all ketols described in this study, we analyzed the corresponding diols, chemically obtained, by GC on a capillary column as already described.[1] This procedure enabled us to assign the *S* configuration of ketol **4c**, which gave predominantly the same *SS* diol as isomer **4b**, with a high enantiomeric

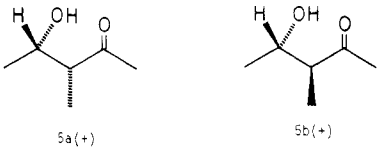
(7) Chenevert, R.; Thiboutot, S. *Can. J. Chem.* **1986**, *64*, 1599.

excess (96% ee). This assignment agrees with the literature.[8] In addition, the negative sign of the optical rotation of **4c** is consistent with literature data for similar optically active alcohols bearing α phenyls group and the *S* configuration.[9]

The difference in optical rotation found between **4b** and the ketol obtained by Ohta and by Chenevert may be attributed to the presence of some ketol **4c** in the latter. The negative optical rotation of **4c** would have lowered the value measured for **4b**.



We completed the study of the 2,4-diones with the branched diketone, 3-methylpentane-2,4-dione (**5**). The reduction of **5** with *G. candidum* occurred rapidly and gave a mixture of syn and anti isomers (**5a** and **5b**) as shown by NMR analysis in accordance with Santelli.[10] These two ketols were separated by column chromatography and were shown to have positive optical rotations. Since both gave the same *SS* diol upon reduction, they are assigned the 4*S* configuration at the carbon bearing the hydroxyl group.



Since amounts of syn and anti diastereoisomers of 4*S* configuration were obtained, one hypothesis would be that there is a high enantiofacial selectivity in the reduction of the enantiotopic carbonyl groups (4*S* hydroxy compounds) but no diastereofacial selectivity since 3*R* and 3*S* methyl ketols are obtained.

Reduction by *A. niger* also gave a ketol of 4*S* configuration and the reaction was not only highly stereospecific (99% ee) but also very diastereoselective (90% syn diastereoisomer). We have previously shown that analogous syn and anti ketols of *S* configuration are obtained by reduction with bakers' yeast.[1] However, a syn ketol of configuration 4*R* can be obtained, with low enantiomeric excess (30%), by reduction of **5** with an anaerobic bacteria *Clostridium tyrobutyricum*.[11]

Overall, these results and those of our previous work with bakers' yeast show that an appropriate choice of the microorganism allows one to obtain the 2-hydroxy ketol of either *S* or *R* configuration from the corresponding 2,4-dione.

With the shortest chain diones, the two fungi tested led to *S* ketols, while longer chain substrates gave *R* ketol. A similar finding of structure-dependent stereospecificity was reported by Keinan et al.[12] for the reduction of monoketones with *Thermoanaerobium brockii*. These authors obtained the *R* alcohol from short-chain ketones (C_4, C_5) and the *S* alcohol from long-chain ketones. Presumably, these microorganisms have several alcohol dehydrogenases and the one with the active site best matching the shape of the ketone reacts preferentially.

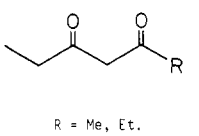


Figure 2.

Table II. Microbiological Reduction of 3,5-Diones

	3,5-dione R	microorganism	reactn time, h	convn, % (3-OH/5-OH)	ee, %	abs confgn
6	C_2H_5	*S. cerevisiae*[a]	72	100	30	*R*(−)
		G. candidum	9	100	70	*R*(−)
		A. niger	24	100	95	*R*(−)
7	C_3H_7	*S. cerevisiae*	72	100 (33/67)	98	*R*(−)
		G. candidum	8	100 (65/35)	99	*R*(−)
		A. niger	24	100 (100)	99	*R*(−)

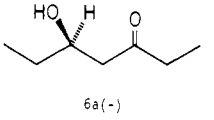
[a] Already described in previous work.[1]

2. Reduction of 3,5-Diones. While investigating the microbiological reduction of diones by bakers' yeast,[1] we noticed a structure-dependent change in the stereochemistry of the reaction with heptane-3,5-dione. Whereas 2,4-diones gave a single ketol of *S* configuration, the 5-hydroxyheptan-3-one obtained by reduction with bakers' yeast was predominantly of configuration 5*R*, although with a low enantiomeric excess (30%).

The reduction of two diones, R = ethyl or propyl in Figure 2, was investigated with the same two fungi and compared with the reduction with bakers' yeast.

These reactions took place under the same experimental conditions as those described for the 2,4-diones. As shown by NMR analysis, reduction of either of the carbonyl groups in the unsymmetrical dione **7** may occur. The results are given as 3-OH/5-OH ratios in Table II.

Heptane-3,5-dione (**6**) was rapidly reduced by *G. candidum* to the corresponding ketol **6a**. Although the enantiomeric excess of this ketol was higher than that for the ketol previously obtained with bakers' yeast,[1] it was still rather poor (70% ee). The best results for this compound were obtained with *A. niger*, which in 24 h gave 5-hydroxyheptan-3-one (**6a**) with a high yield and a high enantiomeric excess (95% ee). In all cases, a levorotatory optical rotation was observed.



To study the regioselectivity of the reaction, we carried out the reduction of octane-3,5-dione (**7**) with bakers' yeast, *G. candidum*, and *A. niger*.

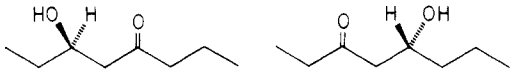
A mixture of ketols **7a** and **7b** (33:67) was obtained with bakers' yeast (reaction time 3 days). The two isomers were purified by column chromatography.

Their ^1H NMR spectra at 300 MHz, though different, did not enable us to determine their structures unequivocally. Therefore two-dimensional COSY-type NMR spectra were run. From the successive proton coupling data obtained, it was determined that 3-hydroxyoctan-5-one (**7a**) exhibited the shortest GC retention time and 5-hydroxyoctan-3-one (**7b**) was the major product. The enantiomeric excesses of these two ketols were determined by reduction to the related diols and GC analysis on a chiral stationary phase. Both isomers showed a high enantiomeric excess (98% ee) and both had levorotatory optical rotations.

(8) Schneider, F.; Simon, R. *Synthesis* **1986**, 582.
(9) (a) Kenyon, J.; Partridge, M. *J. Chem. Soc.* **1936**, 128. (b) Downer, E.; Kenyon, J. *J. Chem. Soc.* **1939**, 1956. (c) Cram, D. J.; MacCarty, J. E. *J. Am. Chem. Soc.* **1957**, *79*, 2866.
(10) Santelli, M.; Viala, J. *Tetrahedron* **1978**, *34*, 2327.
(11) Belan, A.; Fauve, A.; Veschambre, H., unpublished results.
(12) Keinan, E.; Kafeli, E. K.; Seth, K. K.; Lamed, R. *J. Am. Chem. Soc.* **1986**, *108*, 162.

The reaction with *G. candidum* was completed after 8 h and also gave a mixture of ketols. However, in contrast to the results with bakers' yeast, this fungus gave predominantly 3-hydroxyoctan-5-one (**7a**) (Table II). Both ketols were purified, and their structures were confirmed by comparison of their ^1H NMR spectra at 300 MHz with those of the ketols obtained by using *S. cerevisiae*. The optical purity of the two ketols obtained with *G. candidum* was also high (99% ee). Both gave a negative optical rotation.

After 24 h of reaction, with *A. niger*, only 3-hydroxyoctan-5-one (**7a**) was formed, showing a negative optical rotation and a high enantiomeric excess (98% ee).



7a(-) 7b(-)

The three major ketols obtained in the microbiological reduction of 3,5-diones all showed negative optical rotations. The derived 3,5-diols consistently exhibit shorter retention times in chiral-phase GC analysis, corresponding to the *R,R* isomers. Furthermore, ketol **7a** of *R* configuration has been prepared by an aldol-type condensation of chiral *p*-tolylsulfinyl methyl ketone.[8] The *R* configuration is assigned to ketols **6a** and **7b** by analogy to **7a**.

The microbiological reduction of the 3,5-diones studied provides only ketols of *R* configuration. The regioselectivity of the reaction depends on the microorganism used. Bakers' yeast and *G. candidum* give different ketols as the main product, and so the desired product can be obtained by selecting the appropriate microorganism. The highly regioselective reduction by *A. niger* affords 3-hydroxyoctan-5-one (**7a**) as the only product.

Conclusion

Taken together, the results of this and previous[1] work show that microbiological reduction of β-diketones yields enantiomeric ketols in most cases.

Either of the enantiomers of the corresponding 2-hydroxy 4-ketones can be obtained from 2,4-diketones by suitable choice of the microorganism. We have not succeeded in obtaining enantiomeric ketols from 3,5-diones. In this case, the choice of the microorganism leads to either the 3-hydroxy or the 5-hydroxy isomer.

We are presently investigating the reduction of various β-diketones with other microorganisms in an effort to obtain all possible stereoisomers for each compound.

Experimental Section

General Methods. Analytical gas chromatography was performed on a capillary column filled with 20% Carbowax (20 m × 0.32 mm). The carrier gas was helium at 1.2 kg/cm^2. Conversions in percent were determined by integration of the GC chromatogram. Whenever possible retention times of the products were compared to those of racemic samples obtained by chemical methods. Enantiomeric excesses were determined by chromatography using a 26 m × 0.22 mm capillary column packed with chirasil-L-valin as described.[1]

Column chromatography separations were carried out on Merck silica gel (70–230 mesh) with pentane/ether as the eluent (90/10 v/v). Purification of all compounds was carried out by bulb-to-bulb distillation. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ solution. Optical rotation values were determined at 25 °C for the mercury J line (λ = 578 nm). In each case GC chromatograms, NMR spectra, and optical rotation values were compared to literature data as referenced. The purity of the purified ketols was determined to be >95% by GC and NMR.

General Procedure for Microbiological Reductions. Culture conditions for *Geotrichum candidum* (CBS 233-76) and *Aspergillus niger* (ATCC 9142) have already been described.[6]

***G. candidum*.** After 48 h of growth, the culture (1 L) was filtered and mycelium (80 g) was washed repeatedly with NaCl solution (8 g/L). Wet mycelium was suspended in 500-mL conical flasks (5 g) containing 50 mL of 5% glucose solution and 50 μL of dione. The flasks were shaken at 200 rpm at 27 °C. After reaction, the mixture was filtered, the filtrate was extracted continuously with ether overnight, and the extract was analyzed by GC.

***A. niger*.** The conditions were identical with those used with *G. candidum* except that glucose solution was replaced by distilled water.

Bakers' Yeast (*S. cerevisiae*). Bakers' yeast (Hirondelle brand 50 g) was suspended in 1 L of 30 g/L aqueous sucrose. The mixture was kept at 35 °C and shaken throughout the reaction. After 30 min, 1 g of dione was added. Sucrose (12 g) was added every 24 h. After 3 days the mixture was centrifuged and the supernatant extracted continuously with ether overnight.

Reduction of Pentane-2,4-dione (1). This was a commercial product (Fluka).

***G. candidum*.** After 10 h of reaction, the dione had all reacted. After workup, 4-hydroxypentan-2-one (**1a**) was obtained with a yield of 55% determined by using an internal standard (3-methylcyclohex-2-en-1-one). The yield of **1a** was 0.3 g (35%) after bulb-to-bulb distillation.

(4*S*)-(+)-Hydroxypentan-2-one (1a): GC t_R 9 min (oven temperature 95 °C); ^1H NMR (60 MHz) δ 1.13 (d, J = 6 Hz, 3 H), 2.10 (s, 3 H), 2.55 (d, J = 6 Hz, 2 H), 3.80 (s, 1 H, exchangeable with D_2O), 3.90 to 4.50 (m, 1 H); $[\alpha]^{25}_J$ +40° (*c* 0.04, $CHCl_3$); GC analysis on chiral column 87% *S* and 13% *R* (74% ee *S*); NMR and optical rotation are in accordance with the literature.[2,13]

***A. niger*.** After 15 h of reaction, the residue consisted of 75% ketol and 25% unreacted dione. After workup ketol **1b** was obtained with a yield of 50% determined by using the same internal standard and 30% after bulb-tobulb distillation.

(4*R*)-(−)-4-Hydroxypentan-2-one (1b). NMR spectrum and retention time are identical with those obtained for the product formed with *G. candidum*: $[\alpha]^{25}_J$ −60° (*c* 0.02, $CHCl_3$); lit.[2] for optical antipode $[\alpha]$ +64°; GC analysis on chiral column 95% ee *R*.

Reduction of Hexane-2,4-dione (2). This was prepared from acetone and ethyl propionate according to Adams et al.[14]

***G. candidum*.** The reaction was complete in 10 h. After workup, ketol **2a** was obtained with a yield of 60% determined with an internal standard (3-methylcyclohex-2-en-1-one).

(2*R*)-(−)-2-Hydroxyhexan-4-one (2a): isolated yield 0.4 g, (50%); GC t_R 12.7 min (oven temperature 105 °C); ^1H NMR (60 MHz) δ 1.05 (t, 3 H, J = 7 Hz), 1.20 (d, 3 H, J = 6 Hz), 2.20 to 2.60 (m, 2 H), 2.60 (d, 2 H, J = 6 Hz), 4.00 to 4.60 (m, 1 H), 4.10 (s, exchangeable with D_2O); $[\alpha]^{25}_J$ −74° (*c* 0.03, $CHCl_3$); lit.[1,2] for optical antipode $[\alpha]$ +73°, +61°; GC analysis on chiral column >99% ee *R*.

***A. niger*.** The reaction was complete after 24 h. The enantiomeric excess of 2-hydroxyhexan-4-one (**2a**) was determined directly from the crude extract. The optical rotation was not measured. GC analysis on chiral column: >99% ee *R*.

Reduction of 8-Nonene-2,4-dione (3). This was prepared from pentane-2,4-dione and 1-bromo-4-butene according to Gerlach et al.[5]

***G. candidum*.** The reaction was complete after 9 h. The only product was 2-hydroxy-8-nonen-4-one (**3a**). From 1 g of dione, 0.7 g of ketol was recovered after workup and isolated yield was 65% after bulb-to-bulb distillation.

(2*R*)-(−)-2-Hydroxy-8-nonen-4-one (3a): GC t_R 7.8 min (oven temperature 140 °C); ^1H NMR (60 MHz) δ 1.20 (d, 3 H, J = 7 Hz), 1.40 to 2.60 (m, 8 H), 3.15 (s, 1 H, exchangeable with D_2O), 4.10 to 4.50 (m, 1 H), 4.80 to 5.30 (m, 2 H), 5.50 to 6.10 (m, 1 H); $[\alpha]^{25}_J$ −55° (*c* 0.05, $CHCl_3$); lit.[1] $[\alpha]$ +58° for optical antipode; GC analysis on chiral column >99% ee *R*.

***A. niger*.** After 24 h of reaction, 75% of the diketone had reacted. The only product was the ketol **3a**. The optical rotation was not measured. GC analysis on chiral column: 95% ee *R*.

Reduction of 1-Phenylbutane-1,3-dione (4). The starting diketone is a commercial product (Aldrich).

(13) Tanabe, T. *Bull. Soc. Chim. Jpn.* **1973**, *46*, 2233.
(14) Adams, J. T.; Hauser, C. R. *J. Am. Chem. Soc.* **1944**, *66*, 1220.

G. candidum. The reaction was complete after 9 h. The only product was 2-ketol **4a**. It was purified by column chromatography.

(3*R*)-(−)-3-Hydroxy-1-phenylbutan-1-one (4a): isolated yield 70%; ^{1}H NMR (60 MHz) δ 1.25 (d, J = 6 Hz, 3 H), 3.00 to 3.20 (m, 2 H), 3.50 (s, 1 H, exchangeable with D_2O), 4.10 to 4.70 (m, 1 H), 7.30 to 8.20 (m, 5 H); $[\alpha]^{25}_J$ −58° (c 0.05, $CHCl_3$); lit.[15] [α] −41.1°; GC analysis on chiral column 90% ee *R*.

A. niger. Reaction was complete in 24 h. After purification the ketol **4a** was obtained with an isolated yield of 65%.

4a: NMR spectrum identical with that previously obtained; $[\alpha]^{25}_J$ −67° (c 0.02, $CHCl_3$); GC analysis on chiral column 99% ee *R*.

Bakers' Yeast (*S. cerevisiae*). After 72 h of reaction, the residue contained essentially a mixture of ketols **4b** and **4c** (conversion rate 90%). 3-Hydroxy-1-phenylbutan-1-one (**4b**) was purified by chromatography on a silica gel column. The end fractions from this column yielded the other ketol (**4c**) purified by further column chromatography on silica gel, followed by preparative thin-layer chromatographic separations. The overall yield was 80%. The 4-ketol **4c** accounted for 10% to 15% of the ketol mixture.

(3*S*)-(+)-3-Hydroxy-1-phenylbutan-1-one (4b): ^{1}H NMR (60 MHz) identical with that of **4a**; $[\alpha]^{25}_J$ +66° (c 0.04, $CHCl_3$); lit.[2,7] [α] +39°, +55°; GC analysis on chiral column 98% ee *S*.

(4*S*)-(−)-4-Hydroxy-4-phenylbutan-2-one (4c): ^{1}H NMR (60 MHz) δ 2.10 (s, 3 H), 2.70 to 2.90 (m, 2 H), 3.40 (s, 1 H, exchangeable with D_2O), 5.00 to 5.30 (m, 1 H), 7.00 (s, 5 H); $[\alpha]^{25}_J$ −43.5° (c 0.015, $CHCl_3$); lit.[8] [α] −55°; GC analysis on chiral column 96% ee *S*.

Reduction of 3-Methylpentane-2,4-dione (5). This was prepared by methylation of pentane-2,4-dione by the method of Johnson et al.[16]

G. candidum. After 9 h of reaction, the residue obtained consisted of 25% dione and 75% of syn and anti ketols **5a** and **5b** in a ratio of 56/44. The yield determined with an internal standard, 3-methylcyclohex-2-en-1-one, was 50%. The two ketols were separated by chromatography on a silica gel column. Racemic ketols were already described.[10]

(3*R*,4*S*)-(+)-3-Methyl-4-hydroxypentan-2-one (syn, 5a): GC t_R 12.4 min (oven temperature 95 °C); ^{1}H NMR (300 MHz) δ 1.08 (d, J = 6 Hz, 3 H), 1.10 (d, J = 6 Hz, 3 H), 2.20 (s, 3 H), 2.57 (m, 1 H), 2.85 (s, 1 H, exchangeable with D_2O), 4.15 (m, 1 H); ^{13}C NMR (75.47 MHz) δ 213.6 (C-4), 67.3 (C-2), 52.3 (C-3), 29.3 (C-5), 20.1 (C-1), 10.1 (Me-3); $[\alpha]^{25}_J$ +37° (c 0.06, $CHCl_3$); GC analysis on chiral column 95% ee *S*.

(3*S*,4*S*)-(+)-3-Methyl-4-hydroxypentan-2-one (anti, 5b): GC t_R 12.9 min (oven temperature 95 °C); ^{1}H NMR (300 MHz) δ 1.10 (d, J = 6 Hz, 3 H), 1.23 (d, J = 6 Hz, 3 H), 2.24 (s, 3 H), 2.60 (m, 1 H), 2.80 (s, 1 H, exchangeable with D_2O), 3.93 (m, 1 H); ^{13}C NMR (75.47 MHz) δ 213.8 (C-4), 69.4 (C-2), 56.1 (C-3), 29.6 (C-5), 20.8 (C-1), 10.1 (Me-3); $[\alpha]^{25}_J$ +4° (c 0.04, $CHCl_3$); GC analysis on chiral column 90% ee *S*.

A. niger. Reaction was complete after 24 h. The residue contained essentially the ketol **5a** (syn). **5a** (syn): retention time and NMR spectra identical with those of the *syn* isomer obtained with *G. candidum*; [a]$^{25}_J$ +41° (c 0.05, $CHCl_3$); GC analysis on chiral column 98% ee *S*.

Reduction of Heptane-3,5-dione (6). This was prepared from butan-2-one and ethyl propionate according to Adams et al.[14]

G. candidum. The reaction was complete after 9 h. After workup, 5-hydroxyheptan-3-one (**6a**) was obtained. The yield determined by using the same internal standard was 60%. After bulb-to-bulb distillation the isolated yield was 55%. Racemic ketol is described in ref 17.

(5*R*)-(−)-5-Hydroxyheptan-3-one (6a): GC t_R 11.9 min (oven temperature 105 °C); ^{1}H NMR (60 MHz) δ 1.00 (t, J = 6 Hz, 6 H), 1.10–1.60 (m, 2 H), 2.20 to 2.70 (m, 4 H), 3.25 (s, 1 H, exchangeable with D_2O), 3.60 to 4.20 (m, 1 H); $[\alpha]^{25}_J$ −38° (c 0.03, $CHCl_3$); GC analysis on chiral column 85% *R* and 15% *S* (70% ee *R*).

A. niger. The reaction was complete in 24 h and yielded only 5-hydroxyheptan-3-one (**6a**). The yield determined with an internal standard was 65%.

6a: $[\alpha]^{25}_J$ −51° (c 0.03, $CHCl_3$); GC analysis on chiral column 95% ee *R*.

Reduction of Octane-3,5-dione (7). This was prepared from butan-2-one and ethyl butyrate according to Adams et al.[14]

Bakers' Yeast (*S. cerevisiae*). After 3 days of reaction the mixture obtained contained 28% octane-3,5-dione and 72% of a mixture of the two ketols **7a** and **7b** in a ratio of 33/67. The yield determined with the internal standard was 80%. The two ketols were separated on a silica gel column: isolated yield 70%.

(3*R*)-(−)-3-Hydroxyoctan-5-one (7): GC t_R 9.5 min (oven temperature 120 °C); ^{1}H NMR (300 MHz) δ 0.92 (t, J = 6 Hz, 3 H), 0.94 (t, J = 6 Hz, 3 H), 1.37 to 1.70 (m, 4 H), 2.30 to 2.65 (m, 4 H); 3.35 (s, 1 H exchangeable with D_2O); 3.70 to 4.02 (m, 1 H); $[\alpha]^{25}_J$ −56.5° (c 0.03, $CHCl_3$); lit.[8] [α] −47.1°. We noticed that the product obtained by these authors is compound **7a** instead of compound **6a** as noted in their Table 2. GC analysis on chiral column: 98% ee *R*.

(5*R*)-(−)-5-Hydroxyoctan-3-one (7b): GC t_R 10.2 min (oven temperature 120 °C); ^{1}H NMR (300 MHz) δ 0.90 (t, J = 6 Hz, 3 H), 1.04 (t, J = 6 Hz, 3 H), 1.28 to 1.55 (m, 4 H), 1.39 to 2.63 (m, 4 H), 2.91 (s, 1 H, exchangeable with D_2O), 3.98 to 4.10 (m, 1 H); $[\alpha]^{25}_J$ −55° (c 0.06, $CHCl_3$); GC analysis on chiral column 98% ee *R*.

G. candidum. Reaction was complete in 8 h and yielded a mixture of ketols **7a** and **7b** in a ratio of 65/35. The yield determined by using the internal standard was 80%. The two ketols were separated by column chromatography: isolated yield 70%. Retention times and NMR spectra at 300 MHz were identical with those of isomers obtained with *S. cerevisiae*.

7a: $[\alpha]^{25}_J$ −57° (c 0.05, $CHCl_3$); GC analysis on chiral column 99% ee *R*.

7b: $[\alpha]^{25}_J$ −56° (c 0.02, $CHCl_3$); GC analysis on chiral column 99% ee *R*.

A. niger. Reaction was complete in 24 h; a single ketol was present in the residue, **7a**.

7a: t_R and NMR spectrum identical with those of ketol **7a** obtained previously; isolated yield 70%; $[\alpha]^{25}_J$ −57° (c 0.03, $CHCl_3$); GC analysis on chiral column 99% ee *R*.

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(15) Narasaka, K.; Miwa, T.; Hayashi, H.; Ohta, M. *Chem. Lett.* **1984**, 1399.

(16) Johnson, A. W.; Markham, E.; Price, P. *Org. Synth.* **1962**, *42*, 75; **1965**, *45*, 68.

(17) le Goaller, R.; Pierre, J. L. *Can. J. Chem.* **1978**, *56*, 481.