(76), 107 (63), 121 (100), 272 (26), 273 (6). Anal. Calcd for $C_{20}H_{32}$: C, 88.16; H, 11.84. Found: C, 88.41; H, 11.98.

(−)-4(*E*),8(*E*),12(*Z*)-Casbene (45): IR (film) 3010, 2890, 1440, 1375, 1260, 1155, 1125, 1055, 945, 870, 840, 10 cm^{-1}; ^1H NMR (300 MHz) δ 5.05 (m, 2 H), 4.87 (d, J = 10.9 Hz, 1 H), 2.59 (dt, J = t 13.3 Hz, d 4.1 Hz), 1.80–2.35 (m, 9 H), 1.70 (s, 3 H), 1.58 (s, 3 H), 1.53 (s, 3 H), 1.25–1.45 (m, 3 H), 1.02 (s, 3 H), 0.88 (s, 3 H), 0.43 (m, 1 H (dt, J(t) = 9.0 Hz, J(d) = 2.7 Hz); ^{13}C NMR ($CDCl_3$, 22.49 MHz) δ 133.80, 133.22, 124.15, 123.81, 123.08, 40.24, 38.78, 31.27, 29.90, 29.17, 26.49, 25.42, 24.59, 23.17, 20.10, 19.91, 16.64, 16.10, 15.81; $[\alpha]^{22}_D$ −277.2°; mass spectrum calcd for $C_{20}H_{32}$ 272.2504, found 272.2503, m/e (relative intensity) 93 (81), 107 (60), 121 (100), 272 (37), 273 (9).

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Inducibility of an Enone Reductase System in the Fungus *Beauveria sulfurescens*: Application in Enantioselective Organic Synthesis

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Microbiological reduction of α,β-unsaturated carbonyl compounds is studied. Inducibility of the enone reductase system of *Beauveria sulfurescens* is reported. The best inducer is shown to be cyclohex-2-en-1-one. An appropriate procedure using induced resting mycelium is developed to reduce substituted cyclohexenones that are shown to be unable to induce the reducing enzyme. Optically pure *trans*-(2*R*,6*R*)-(−)-2,6-dimethylcyclohexan-1-one and *trans*-(2*R*,6*R*)-(−)-2,6-dimethylcyclohexan-1-ol are obtained from (±)-2,6-dimethylcyclohex-2-en-1-one along with optically pure (6*S*)-(−)-2,6-dimethylcyclohex-2-en-1-one.

In previous papers, we reported that the reduction of α,β-unsaturated carbonyl compounds is widespread among living organisms. Aerobic[1] and anaerobic bacteria,[2] fungi,[3] protozoan,[4] and plant[5] and animal[6] cells were all shown to reduce the carbon–carbon double bond. The stereochemical characteristics of this reaction are identical for all the organisms, while the reduction of the carbonyl group, when occurring, leads to either an *R* or *S* alcohol, depending on the cell involved.

According to those findings, one could predict the absolute configuration of substituted saturated ketones obtained via microbial reduction. Yet, the synthetic applications of the stereochemical rule proposed by our group for the reduction of C–C double bonds by a fungus *Beauveria sulfurescens*[3a] have been shown to be limited: the reaction occurs only on small molecules bearing a small α-substituent and a hydrogen atom in the β-position. Thus, many compounds of synthetic interest could not be reduced by the fungus under the conditions generally used.

In the hope of extending the scope of the reaction, we studied the reducing activity of the fungus under various growing conditions. The results reported here describe an efficient procedure developed to reduce rapidly and enantiospecifically prochiral substrates previously shown to remain untransformed.

Results and Discussion

Before describing our results, it would be appropriate to mention how we intended to explore the biology of *B. sulfurescens*. When working on the reduction of prochiral disubstituted cyclohexenones, we noticed that some of them could not be reduced by the fungus, even after 6–10 days of reaction.[3a] Beside enzymatic hindrances, cell permeation effects were thought to be involved. For these experiments, bioconversions had been performed in situ, the compound to be reduced being added to a 24-hour-old culture and incubated at least 48 h in the growth medium. No reducing activity could be detected in the growth medium free of mycelium leading us to assume that the reducing enzymes might be endocellular.

To support this assumption and to clarify whether or not cell permeation of the substrate was involved, we investigated the reducing activity of the fungus in mycelium homogenates. As a model experiment, we studied the reduction of cyclohex-2-en-1-one (cyclohexenone), shown to be thoroughly reduced, first into saturated ketone and then into saturated alcohol, in previous experiments.[3a]

The harvested mash of a 24-hour-old culture of *B. sulfurescens* was disrupted with an X-Press (freeze-pressing principle), resuspended in a buffer, and centrifuged to yield a supernatant that was immediately incubated with cyclohexenone and various cofactors.

The first results showed that no activity could be evidenced in cell-free extracts whatever the cofactor added. Saturated cyclohexanone could be detected only in experiments where the growth medium had been supplemented with the substrate. Thus, prior to any cofactor requirement, the reducing system must have been induced.

Following these results, we carried out a study on induction. We found it much easier, and cheaper, to work with resting mycelium, instead of homogenates, to study the influence of various inducers on the time course changes of cyclohexenone and to study applications in enantioselective synthesis.

B. sulfurescens was grown in conical flasks containing a glucose/peptone medium. After 24 h on a gyratory shaker, each flask received an aliquot of cyclohexenone. The induction time varied from 20 min to 24 h. After incubation, the mycelium of each flask was removed by filtration. Cyclohexenone reduction was followed by GPC

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Table I. Reduction of Cyclohex-2-en-1-one in the Growth Medium of *B. sulfurescens*: Induction of Enone Reductase

incubn time	20 min	45 min	1 h	2 h	3 h	4 h	5 h	24 h
conversion yield,[a] %	6	10	16	63	93	96	100	100
wet mycelium wt, g/100 mL	5.0	5.1	5.1	5.3	5.4	5.8	6.6	9.0
control without subst wt, g/100 mL	5.5	5.7	5.8	6.0	6.2	6.5	7.0	10.0

[a] Conversion of 50 μL of cyclohexenone added to 24-hour-grown fungus in 500-mL flask containing 100 mL of medium. 100% reduction means that no starting ketone could be detected by GPC of the crude extract. Isolated yields of reduced compound were lower.

Table II. Reduction of Cyclohex-2-en-1-one by Partially Induced Resting Mycelium of *B. sulfurescens*

induction time of resting mycelium[a]	0	20 min	2 h	4 h	6 h	12 h	24 h	48 h
conversion yield,[b] % after 30 min	6	8	40	55	35	26	14	6

[a] 0 induction time is a control assay obtained with noninduced mycelium. [b] Conversion of 50 μL of cyclohexenone in 50 mL of distilled water by 5 g of wet pressed mycelium.

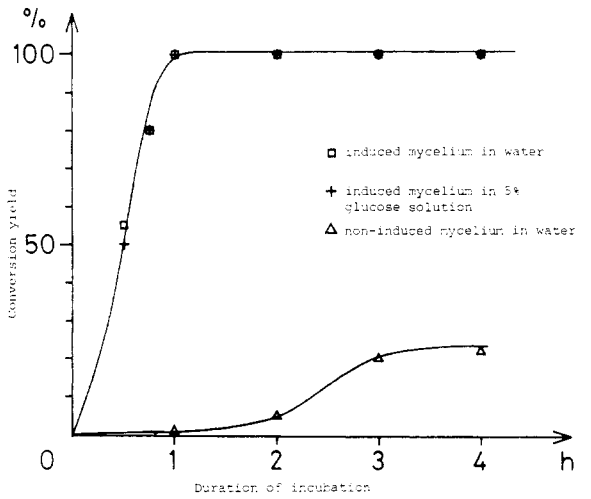


Figure 1. Time course changes of cyclohex-2-en-1-one by resting mycelium of *B. sulfurescens*.

analysis of the extracted filtrates. Conversion yields and mycelium weights are given in Table I.

No reducing activity was observed during the first hour, which might be the minimal time required for the enzyme biosynthesis to start. As already observed on the protozoan *Tetrahymena pyriformis*,[4] the toxicity of cyclohexenone is shown by an inhibition of the fungal growth until the complete reduction of the inducer substrate. After 24 h, the mycelium weight obtained was slightly lower than the weight obtained when no cyclohexenone had been added.

Table II shows the results obtained for cyclohexenone reduction by partially induced resting mycelium. After 24-h growth and various incubation times, mycelium was harvested, washed three times with saline, resuspended in water, and incubated 30 min with only cyclohexenone; bioconversion conditions were identical for all assays (see the Experimental Section).

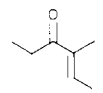
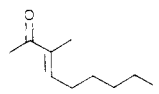
The best results were obtained with resting mycelium previously induced for 4 h. It is noteworthy that the enzyme life span is quite short, as poor results are obtained when induction time is longer than 12 h.

Time course changes of cyclohexenone by induced resting mycelium under different bioconversion conditions were studied (Figure 1). Even though noninduced mycelium has been shown to be unable to reduce cyclohexenone, cyclohexanone was detected after 2 h and the amount increased slowly as if the reducing system were induced by contacting the substrate, although no nutritional source had been added (Figure 1).

No energy sources were needed to perform bioconversions with resting mycelium. Addition of glucose, up to 5%, did not increase the yield of saturated products.

As observed for reductions in growth medium, 10–15% of saturated alcohol was obtained from the saturated ketone, the mycelium being induced or not. Alcohol dehydrogenases involved in this second reduction reaction were not induced by cyclohexenone or by any of the reduction products (Table III). They are constitutive enzymes that might be inhibited by cyclohexenone as the saturated alcohol appears only when all the substrate is reduced.

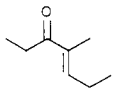
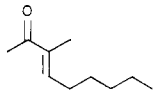
Table III. Reduction of Cyclohex-2-en-1-one by Variously Induced Resting Mycelium of *B. sulfurescens*

	inducer	residual substr, %	satd ketone, %	satd alcohol
	none	94	6	0
a[a]		0	99	tr
		95	5	0
		95	5	0
a		0	99	tr
b		94	6	0
c		75	25	0
b		94	6	0
c		60	40	0
b		80	20	0

[a] Key: (a) reduced by the growing fungus in 48 h;[3a] (b) not reduced by the growing fungus, even after 10 days;[3a] (c) reduced by the growing fungus in 3–10 days.[3a]

Table III displays results obtained for the reduction of cyclohexenone by resting mycelium grown for 24 h, induced 4 h with various α,β-unsaturated carbonyl compounds, washed, and resuspended as described in the Table II experiments. The reaction period was 45 min for all bioconversions performed under identical conditions.

Table IV. Conversion of α,β-Unsaturated Ketones by Cyclohexenone-Induced Resting Mycelium of *B. sulfurescens*[a]

substrate	conversion yield, %	incubn time, h	previous results
	100	6	90%, 48 h[3a]
	100	24	not reduced[3a]
	50	24	50%, 5–10 days[8]
	50	24	not reduced[3a]
	0	48	not reduced[3a]

[a] Reduction of 50 μL of unsaturated ketone in 50 mL of distilled water by 5 g of wet pressed mycelium.

These results clearly indicate that α,β-unsaturated ketones (a) that were thoroughly reduced by the growing mold are good enzyme inducers. Substrates that used to require longer reaction time (c) are weak inducers, while substrates that had never been reduced (b) are not able to induce the biosynthesis of the reducing enzyme.

Commercial cyclohexenone was then used as an inducer each time enone reductases were required. Table IV displays some of our results obtained for the reduction of various α,β-unsaturated ketones by induced resting mycelium.

Reductions by induced resting mycelium remarkably improved. Incubation times are lower and product recovery is easier as only substrate and fungus are present in the reaction medium. Moreover, we are now able to reduce substrates that never underwent the fungal reduction and have been shown not to be enzyme inducers. 3-Methylcyclohexenone is not reduced though, corroborating with our rule that requires a hydrogen atom at the β-position of the substrate.[3a]

A synthetic application of these results had been studied on a substrate previously reported to withstand fungal reduction: (±)-2,6-dimethylcyclohex-2-en-1-one. This compound was obtained from commercial mixture of cis and trans isomers (72:28) of 2,6-dimethylcyclohexan-1-one by bromination–dehydrobromination according to Mironov.[7] Contrary to 2,5-dimethylcyclopent-2-en-1-one,[8] this prochiral ketone could not be reduced by the growing fungus. As shown in Table IV, it could be reduced only by cyclohexenone-induced resting mycelium. It can exist in two enantiomeric forms, **1a** and **1b**. According to our stereochemical rule,[3a] upon reduction by *B. sulfurescens* these two forms could only lead to saturated ketones being *R* in configuration at C-2, **2a** and **2b**, and then to *S* alcohols, being cis with respect to the C-2 substituent.

In fact, as already shown in Table IV, we could not drive the reaction to completion. After 10 h of contact with induced mycelium, products were obtained and their relative proportions not further changed even after 2 days (bacterial contamination might occur for longer incubation times). In Table V are the results obtained by GPC analysis for various incubation times; retention times, different for all compounds, were identical with those of authentic samples.

Table V. Reduction of 2,6-Dimethylcyclohex-2-en-1-one by Cyclohexenone-Induced Resting Mycelium[a]

incubn time, h	resid substr, %	satd ketone, % cis:% trans	saturd alcohol, %
6	53	6:28	13
10	41	10:35	14
24	37	10:38	15
48	36	11:40	23

[a] Conversion of 50 μL of 2,6-dimethycyclohex-2-en-1-one in 50 mL of distilled water by 5 g of wet mycelium.

The results in Table V show that the major saturated compound was the trans isomer of 2,6-dimethylcyclohexan-1-one (**2a**). As previously assumed, it could only arise from **1a**, while the minor product of the biological reduction, *cis*-2,6-dimethylcyclohexan-1-one (**2b**) could only come from **1b** (Scheme I). Thus, as already observed for the reduction of 2,5-dimethylcyclopent-2-en-1-one,[8] the rate constants for the reduction reaction are very different for the two enantiomeric forms of the substrate. The difference is even bigger when alcohol dehydrogenases are involved as only one saturated alcohol is obtained, as shown by GPC analysis (chemical reduction leads to three diastereoisomers having different retention times).

Following these analytical results, a large-scale reaction was performed with fermenter-grown induced resting mycelium (see the Experimental Section). GPC analysis of the crude extract obtained after 10 h of incubation gave the same four products as shown in Table V. After separation and purification by various chromatographic methods, they were analyzed and identified.

Pure *cis*-2,6-dimethylcyclohexan-1-one (**2b**) (11%) was easily identified. Its structure was confirmed by ^{1}H NMR, and no optical activity was detected.

Pure *trans*-2,6-dimethylcyclohexan-1-one (**2a**) (33%) was eluted along with unreacted substrate by column chromatography. They were separated by preparative GPC. This saturated ketone had a high optical activity; $[\alpha]^{25}_J$ −118° [lit.[9] +108.9° for (+) enantiomer]. Its ^{1}H NMR spectrum, recorded in presence of chiral europium, showed it to be optically pure when compared to spectrum obtained for the (±) trans-saturated ketone (see the Experimental Section). This compound was expected to be *trans*-(2*R*,6*R*)-(−)-2,6-dimethylcyclohexan-1-one (**2a**), optically pure.

Pure saturated alcohol (17%) was obtained by column chromatography of the reaction mixture. Since this was already done by our group for cycloheximide synthons,[10] relative and absolute configurations could be ascertained by comparing its GPC retention time and ^{13}C NMR spectral values to those obtained by Grenier-Loustalot et al.[11] for all diastereoisomers.

Retention time and spectral values of the saturated alcohol obtained were almost identical with those obtained by these authors for their (±)-(e,e,a) alcohol[11] where the methyl groups are trans (Table VI). The optical activity was $[\alpha]^{25}_J$ −35°. For 2,6-dimethylcyclohexanol having trans

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Table VI. Comparison of GPC Retention Times and Carbon NMR Data of 2,6-Dimethylcyclohexanols Chemically and Biologically Obtained

steric orientation				chemical shifts, δ							
OH	Me-2	Me-6	ret time, min	C-1	C-2	C-3	C-4	C-5	C-6	Me-2	Me-6
				Compounds Chemically Obtained[a]							
a	e	e	5.02	74.8	38.0	27.7	26.3	27.7	38.0	18.9	18.9
e	e	e	5.64	81.9	40.2	34.8	26.0	34.8	40.2	19.2	19.2
e	e	a	7.34	77.5	33.6	32.1	20.3	31.0	33.8	18.5	13.9
				Compound Biologically Obtained							
			7.42	77.7	33.4	31.6	20.0	30.7	33.7	18.1	13.7

[a] Data from ref 11.

methyl groups, the literature gives the (2*R*,6*R*) configuration for $[\alpha]^{20}_{J}$ −43.3°.[12] Its ^{1}H NMR spectrum, recorded in the presence of a chiral reagent, was compared to the spectrum of (±)-**3a**, chemically obtained, and (−)-**3a** was proven to be optically pure. Thus, the only saturated alcohol obtained by fungal reduction would be (2*R*,6*R*)-(−)-2,6-dimethylcyclohexan-1-ol (**3a**).

These configurations would be in total agreement with our stereochemical rule.[3a] No absolute configuration is given for the C-1 hydroxyl group when methyl groups are trans even though our previous studies showed that *B. sulfurescens* always leads to *S* alcohols. In this case, the most that can be said is that the hydroxyl group is equatorial and cis with respect to one of the methyl groups.

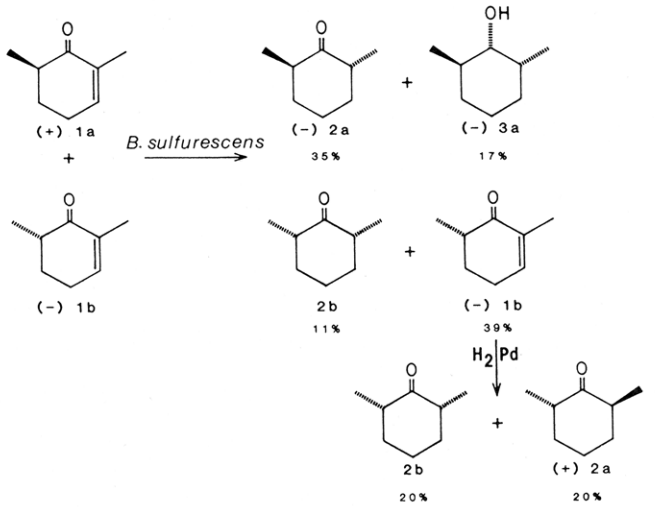
Unreacted 2,6-dimethylcyclohex-2-en-1-one (39%) was separated from the trans-saturated ketone by preparative GPC after column chromatography. Its optical activity was high compared to the literature value: $[\alpha]^{25}_{J}$ −82° (lit.[13] $[\alpha]_{586}$ −46.6°). Its ^{1}H NMR analysis with chiral europium showed only one doublet for C-6 methyl while two doublets were present in the spectrum recorded under the same conditions for the (±) starting ketone.

Obtaining chiral unreacted substrate via biological reaction on prochiral racemate is not surprising as enzymes are chiral reagents. As observed for 2,5-dimethylcyclopent-2-en-1-one,[8] a preferential attack on **1a** was considered, leading us to assume that the remaining chiral substrate would be **1b**. To ascertain the absolute configuration at C-6, we chemically reduced the unreacted ketone obtained. Following our hypothesis, the saturated ketone obtained would be a mixture of cis and trans isomers, the latter being the enantiomer of the trans saturated ketone obtained via fungal reduction.

The crude mixture of isomers obtained from the unreacted ketone showed an excess of dextrorotarory optical activity [$[\alpha]^{25}_{J}$ +20°], i.e., of opposite sign than **2a**. The unreacted ketone obtained proved to be the enantiomer (6*S*)-(−)-2,6-dimethylcyclohex-2-en-1-one (**1b**).

According to those results, Scheme I could be drawn for obtaining various optically active reduced compounds from an α,α'-disubstituted unsaturated ketone.

Scheme I. Proposed Pathway of 2,6-Dimethylcyclohex-2-en-1-one Reduction by *B. sulfurescens*



Conclusion

Our initial target, to find a route overcoming the limitations of our stereochemical rule and thus extending the scope of the reaction, was partially reached by a simple manipulation of the metabolic regulation of the fungus. This induction technique may be applicable to a kinetic resolution (recovery of an optically pure α,β-unsaturated ketone) as well as to a chiral reduction (optically pure reduction products) of other prochiral unsaturated ketones.

Recent research efforts have focused on bioconversions mostly because of the ability of enzymes to discriminate between enantiomers in enantioselective synthesis. Yet, when working with biological material as a reagent, chemists should remember that enzymes are proteins, i.e., their biosynthesis is genetically controlled. The present study shows, among other things, that chemists can learn how to manipulate the genetic control of a living organism for synthetic purposes.

Experimental Section

General Methods. a. Instrumentation. Analytical gas-phase chromatography (GPC) was performed on a Shimadzu C-9 Mini-3 instrument fitted with a flame ionization detector and a capillary column filled with 20% Carbowax (Chrompack, 20 m × 0.32 mm). Helium was the carrier gas at 1.2 kg/cm^2. The oven temperature was 100 °C. Retention times of bioconversion products were compared to those of authentic samples easily obtained by chemical reduction. Conversion yields were determined by a Shimadzu CR 3A integrator connected to the chromatograph.

Preparative gas-phase chromatography was performed with a Varian Aerograph 90-P with a catharometric detector. The 6-m-long aluminum column was filled with 20% Carbowax 20 M on Chromosorb W. The carrier gas was hydrogen at 2 bar.

Column chromatography was realized on Merck silica gel (70–230 mesh) with pentane/ether as the eluent (95:5, v/v).

Purification of each compound was realized by a bulb-to-bulb distillation on a Buchi GKR-50 apparatus.

Proton magnetic resonance (^{1}H NMR) spectra were recorded on a Brucker 300 MSL spectrometer at 300 MHz in $CDCl_3$ solution with $CDCl_3$ as the internal standard or on a Jeol CX 60 instrument at 60 MHz in $CDCl_3$ solution with TMS as the internal standard.

Carbon magnetic resonance (^{13}C NMR) spectra were recorded on a Jeol FX 60 spectrometer at 15.03 MHz for $CDCl_3$ solution. Chemical shifts are given relative to a TMS standard.

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Optical rotation values were determined on a Perkin-Elmer 141 polarimeter at 25 °C for the mercury J line (λ = 578 nm).

No elementary analysis was performed. The products obtained being either described or commercial products, analysis of their retention times, NMR spectra, and optical rotation values were considered as sufficient for identification.

b. Chemicals. Cyclohex-2-en-1-one was a commercial product (Fluka, puriss grade). The α,β-unsaturated ketones used in the induction study were already described in a previous paper.[3a]

(±)-2,6-Dimethylcyclohex-2-en-1-one (1a + 1b). Bromination of 2,6-dimethylcyclohexan-1-one (Aldrich, mixture of cis and trans isomers, 72:28) was carried out according to Mironov's slightly modified method.[7] A 30-g sample of 2,6-dimethylcyclohexan-1-one (33 mL) was placed in a 500-mL round-bottomed flask equipped with a thermometer, a condenser, and a tap funnel and containing 25 mL of $CHCl_3$, 40 mL of H_2O, and 17 g of $MgCO_3$. Br_2 (2–3 drops) was added to the stirred suspension. The temperature was raised to 45 °C for the reaction to start. As soon as the solution became discolored, 20 mL of Br_2 was added dropwise (T = 40–45 °C) and the solution stirred for 30 min at room temperature. The reaction mixture was poured into Et_2O (100 mL) and decanted. The aqueous layer was washed with ether, and the combined Et_2O layers were dried over $MgSO_4$.

Dehydrobromination was as follows: The Et_2O solution was refluxed for 2 h over $MgCO_3$ (37 g), washed three times with NaOH (5 N) solution and three times with water, and dried over $MgSO_4$. After evaporation of the solvent, the crude extract was shown to contain 80% 2,6-dimethylcyclohex-2-en-1-one and 20% 2,6-dimethylphenol, which were separated by column chromatography (pentane/ether, 95:5 as eluent).

After evaporation of the combined fractions, 9 mL of (**1a** + **1b**) was obtained. No bulb-to-bulb distillation had been performed to avoid 2,6-dimethylphenol formation: ^{1}H NMR (300 MHz) δ 1.14 (d, J = 7 Hz, 3 H), 1.63–1.77 (m, 1 H), 1.78 (s, 3 H), 2.0–2.10 (m, 1 H), 2.32–2.46 (m, 3 H), 6.70 (m, 1 H).

Bioconversion Methods. a. Cultivation of the Fungus and Induction. *B. sulfurescens* ATCC 7159 was grown in 500-mL conical flasks containing 100 mL of the following medium: glucose, 30 g/L; peptone, 10 g/L; K_2HPO_4, 1 g/L; $MgSO_4$, 0.5 g/L; KCl, 0.5 g/L; $ZnSO_4$, 0.3 g/L; $FeSO_4 \cdot 7H_2O$, 0.01 g/L in tap water. After 24-h cultivation on a rotary shaker at 27 °C, inducer was added (50 μL/flask) and the mixture incubated for various periods with continuous shaking. After incubation, the filtrate of the cultured broth was extracted continuously with ether overnight. The ether extract was dried and concentrated. The crude extract was analyzed by GPC.

b. Conversion by Induced Resting Mycelium. After incubation with 0.05% inducer, the mycelium was harvested by filtration, washed three times with saline (NaCl, 9 g/L), and sucked for 10 min.

Wet mycelium (5 g) was resuspended in 500-mL conical flasks containing 50 mL of distilled water, supplemented or not with 5% glucose, and 50 μL of cyclohexenone or other α,β-unsaturated ketone. The reactions were carried out at 27 °C with stirring, along with controls with noninduced resting mycelium.

After incubation, the filtrate of the reaction medium was extracted with ether overnight and analyzed by GPC.

c. Large-Scale Reduction with Induced Resting Mycelium. *B. sulfurescens* was grown in a 2-L fermenter (Biolafitte) containing 1 L of growing medium. After 24-h cultivation at 27 °C with an aeration rate of 300 mL/L·min^{-1}, 0.5 mL of cyclohexenone in 1 mL of DMSO was added and induction was done for 4 h.

Wet mycelium (50 g) was obtained and distributed in ten 500-mL flasks each containing 50 μL of 2,6-dimethylcyclohex-2-en-1-one to be reduced in 50 mL of distilled water, as already described for bioconversions with resting mycelium. (Oxygen was required for the enzymatic reduction to occur. Low conversion yields had been obtained for large-scale assays carried out in 500-mL flasks containing more than 5 g of mycelium and 50 μL of substrate in 50 mL of distilled water each.) Repeated assays have been carried out to obtain 1.5 g of crude extract from 3 g of starting ketone. Separation of cis-saturated ketone **2b** and trans-saturated alcohol **3a** from a mixture of trans-saturated ketone **2a** and residual substrate **1b** had been performed by column chromatography with pentane/ether (95:5) as eluent. Trans-saturated ketone and residual substrate were then separated by preparative GPC at 145 °C.

***cis*-2,6-Dimethylcyclohexan-1-one (2b)**: 0.15 g; GPC, retention time 3.95 min; R_f 0.7; bulb-to-bulb distillation, oven temperature 200 °C; $[\alpha]^{25}_J$ 0; ^{1}H NMR (60 MHz) δ 1.10 (d, J = 6 Hz, 6 H), 1.2–2.8 (m, 8 H).

***trans*-(2*R*,6*R*)-(−)-2,6-Dimethylcyclohexan-1-one ((−)-2a)**: 0.5 g; GPC, retention time 4.30 min; R_f 0.6; preparative GPC, retention time 7 min; bulb-to-bulb distillation, oven temperature 200 °C; $[\alpha]^{25}_J$ −118° (c 0.02, CH_3OH) [lit.[9] $[\alpha]^{25}_J$ +108.9° (neat)]; ^{1}H NMR (300 MHz) δ 1.12 (d, J = 7 Hz, 6 H), 1.5–1.64 (m, 2 H), 1.71–1.82 (m, 2 H), 1.87–2.03 (m, 2 H), 2.50–2.66 (m, 2 H).

Optical purity: (±)-**2a** was obtained by column chromatography of the commercial mixture of 2,6-dimethylcyclohexan-2-one, pentane/ether (95:5) as eluent.

^{1}H NMR (300 MHz): (±)-**2a** (4 mg) and tris[3-(trifluoromethyl)hydroxymethylene-*d*-camphorato]europium(III) (22.5 mg) in 0.4 mL of $CDCl_3$, methyls 2 and 6 as two doublets at δ 1.31 and 1.33 (J = 7 Hz); (−)-**2a** (4.6 mg) and tris[3-(trifluoromethyl)hydroxymethylene-*d*-camphorato]europium(III) (11.5 mg) in 0.4 mL of $CDCl_3$, methyls 2 and 6 as one doublet at δ 1.23 (J = 7 Hz).

***trans*-(2*R*,6*R*)-(−)-2,6-Dimethylcyclohexan-1-ol ((−)-3a)**: 0.25 g; GP, retention time 7.4 min; R_f 0.35; bulb-to-bulb distillation, oven temperature 250 °C; $[\alpha]^{25}_J$ −35° (c 0.02, cyclohexane) [lit.[12] $[\alpha]^{20}_J$ −43.3° (cyclohexane)]; ^{1}H NMR (300 MHz) δ 0.95 (d, J = 7 Hz, 3 H), 0.97 (d, J = 6 Hz, 3 H), 1.35–1.53 (m, 5 H), 1.60–1.75 (m, 2 H), 1.90–2.01 (m, 1 H), 3.28–3.35 (m, 1 H); ^{13}C NMR (15.03 MHz) δ 77.7 (C-1), 33.7 (C-6), 33.4 (C-2), 31.6 (C-3), 30.7 (C-5), 20.0 (C-4), 18.1 (Me-2), 13.7 (Me-6).

Optical purity: (±)-**3a** was obtained by preparative GPC of the mixture of diastereoisomers chemically obtained from the ketone ($LiAlH_4$ treatment) [oven temperature 145 °C; retention time 14 min].

^{1}H NMR (300 MHz): (±)-**3a** (10 mg) and tris[3-(trifluoromethyl)hydroxymethylene-*d*-camphorato]europium(III) (22.5 mg) in 0.4 mL of $CDCl_3$, methyls 2 and 6 as four doublets at δ 2.01 and 2.08 (J = 6 Hz), 2.27 and 2.33 (J = 7 Hz), proton 1 as two singlets at δ 5.90 and 5.96; (−)-**3a** (4.5 mg) and tris[3-(trifluoromethyl)hydroxymethylene-*d*-camphorato]europium(III) (15.5 mg) in 0.4 mL of $CDCl_3$, methyls 2 and 6 as two doublets at δ 1.97 (J = 6 Hz) and 2.28 (J = 7 Hz), proton 1 as one singlet at δ 5.89.

(6*S*)-(−)-2,6-Dimethylcyclohex-2-en-1-one (1b): 0.6 g; GPC, retention time 6.45 min; R_f 0.6; preparative GPC, retention time 10 min; bulb-to-bulb distillation, oven temperature 120 °C (13 mm); $[\alpha]^{25}_J$ −82° (c 0.06, CH_3OH) [lit.[13] $[\alpha]_{546}$ −46.6° (c 0.6, CH_3OH)]; ^{1}H NMR (300 MHz) δ 1.14 (d, J = 7 Hz, 3 H), 1.63–1.77 (m, 1 H), 1.78 (s, 3 H), 2.0–2.10 (m, 1 H), 2.32–2.46 (m, 3 H), 6.70 (m, 1 H).

^{1}H NMR (300 MHz): (±)-**1** (4 mg) and tris[3-(trifluoromethyl)hydroxymethylene-*d*-camphorato]europium(III) (7.5 mg) in 0.4 mL of $CDCl_3$, methyl 6 as two doublets at δ 1.32 and 1.34 (J = 7 Hz); (−)-**1b** (4 mg) and tris[3-(trifluoromethyl)hydroxymethylene-*d*-camphorato]europium(III) (8 mg) in 0.4 mL of $CDCl_3$, methyl 6 as one doublet at δ 1.31 (J = 7 Hz).

Reduction of **1b**: (−)-**1b** (0.1 g) in 100 mL of ether was reduced under hydrogen pressure (1 atm) in the presence of palladium/carbon for 4 h. After filtration and evaporation of the solvent, the optical activity of the mixture of isomers was measured; $[\alpha]^{25}_J$ +20° (c 0.04, CH_3OH).

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Registry No. (±)-**1**, 73723-50-9; **1**, 40790-56-5; (+)-**1a**, 109785-13-9; (−)-**1b**, 109785-14-0; (±)-*trans*-**2**, 57340-70-2; (+)-**2a**, 77882-09-8; (−)-**2a**, 109785-15-1; **2b**, 766-42-7; (−)-**3a**, 109785-16-2; EtCOC(Me)=CHMe, 52883-78-0; ACC(Me)=CH$(CH_2)_4$Me, 54615-56-4; 2,6-dimethyl-2-bromocyclohexanone, 55234-03-2; 2-cyclohexen-1-one, 930-68-7; cyclohexanone, 108-94-1; cyclohexanol, 110-82-7; 2-methyl-2-cyclohexen-1-one, 1193-18-6; 3-methyl-2-cyclohexen-1-one, 1193-18-6; 2,5-dimethyl-2-cyclopenten-1-one, 4041-11-6.