molarities of substrate and amine.

Photolysis of Pentafluorobenzene in Acetonitrile in the Absence of Triethylamine. A 25.0-mL solution in acetonitrile of pentafluorobenzene (1.04 g, 0.245 M) and dodecane (250 μL, 1.10 mmol) was prepared. Exactly 10.0 mL of this solution was pipetted out into a quartz tube with a side arm (25.0 mL) fitted with a nitrogen inlet adapter (19/14) with a long stem whose tip almost touched the bottom of the tube. The side arm was connected to an absorption unit containing a known excess of standard NaOH solution. The quartz tube was placed inside the merry-go-round reactor chamber and irradiated with the eight 254-nm lamps surrounding the tube for a period of 30 min, while nitrogen gas was passed through the reaction mixture in a slow stream (1 to 2 bubbles per s for 1 h). Cold air was passed through the chamber during the photolysis in order to maintain the temperature constant at 40 °C. Hydrogen fluoride gas that was evolved during the reaction was swept out by nitrogen into the absorption unit and was converted to NaF. The inlet tube dipping in the NaOH solution was rinsed with distilled water and removed. The aqueous solution containing NaF and excess alkali was titrated with standard potassium hydrogen phthalate solution to the phenolphthalein end point. In a second run, the aqueous solution containing NaF was subjected to quantitative determination of F^- by a modified Volhard's method.[12] Analysis of the product mixture and the sodium hydroxide trap solution revealed the following product components: 1,2,3,5-tetrafluorobenzene (**2**) (0.11%), 1,2,4,5-tetrafluorobenzene (**3**) (1.09%), 1,2,3,4-tetrafluorobenzene (**4**) (0.09%), octafluorobiphenyl (A 0.26, B 0.85, C 0.91, D 0.08%), and HF [3.15% (Volhard's method), 3.01% (titration)].

Quantum Yield Dependence of Pentafluorobenzene Photolysis upon Pentafluorobenzene Concentration. Solutions of pentafluorobenzene in acetonitrile (10.0 mL each) were prepared containing the following concentrations of pentafluorobenzene (internal standard dodecane in parentheses): 0.0256 M (0.643 mM), 0.0504 M (0.756 mM), 0.127 M (0.678), 0.251 M (0.584 mM), 0.376 M (0.688 mM), and 0.500 M (0.622 mM). Duplicate 3.0-mL samples of each solution were degassed in quartz tubes and irradiated at 254 nm for 30 min. Similarly, two 3.0-mL samples of a solution containing cyclopentanone (2.01 M) and dodecane (1.27 mmol) in acetonitrile were degassed and irradiated simultaneously with the pentafluorobenzene samples. At the end of the photolysis period, the pentafluorobenzene samples were extracted six times with pentane (2.0 mL) and the pentane extract was concentrated carefully and then analyzed by gas chromatography.

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Registry No. **1**, 363-72-4; **1** (radical anion), 59331-57-6; **2**, 2367-82-0; **2** (radical anion), 105814-20-8; **4**, 551-62-2; **4** (radical anion), 105814-21-9; $(H_3CCH_2)_3N$, 121-44-8.

(12) Vogel, A. I. *A Text Book of Quantitative Inorganic Analysis*, 3rd ed.; Wiley: New York, 1966; p 269.

Use of Biological Systems for the Preparation of Chiral Molecules. 3. An Application in Pheromone Synthesis: Preparation of Sulcatol Enantiomers

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Both enantiomers of sulcatol, an aggregation pheromone, are prepared by microbial asymmetric reduction of prochiral 6-methylhept-5-en-2-one. Bacteria, yeast, fungi, and commercial enzymes which give high optical purities are used. Enzymatic resolution of (±)-sulcatol by pig pancratic lipase is carried out in anhydrous ether. The use of biological systems in organic chemistry to prepare chiral alcohols is discussed in terms of availability, cost, specific equipment, and chemical and optical yields.

The use of biological systems (enzymes or microorganisms) to prepare chiral alcohols is widespread and very efficient. The alcohol is obtained by reduction of the corresponding ketone.[1] It can be difficult to obtain both enantiomers since most microorganisms obey Prelog's rule and so give the same enantiomer, with *S* configuration.

However, since Sih[2] showed that several alcohol dehydrogenases could be implicated in the reduction of β-keto esters by *Saccharomyces cerevisiae* several authors have succeeded in controlling the stereochemistry of microbiological reductions. Reversibly modifying the substrate can allow the other enantiomer to be obtained.[3]

Besides, given the immense variety of reducing microorganisms, one or more species or strains can be expected to occur that yield alcohols with stereochemistry opposite to that predicted by the Prelog's rule.

In addition, whenever racemic mixtures of alcohols can be readily obtained, separation of the enantiomers can be envisaged. Here again, biological systems are especially useful because of their operational simplicity. Lipases are well-suited to the resolution of chiral alcohols[4] and a very simple procedure was recently developed by A. M. Klibanov.[5]

In the course of work on the stereospecific synthesis of pheromones, we set out to prepare the two enantiomers of sulcatol by means of various biological systems and procedures.

Sulcatol (6-methylhept-5-en-2-ol) is the aggregation pheromone of an ambrosia beetle, an economically important pest in the coniferous forests of the North Pacific

(1) Jones, J. B.; Beck, J. F. "Applications of Biochemical Systems in Organic Chemistry" *Techniques of Chemistry*; Jones, J. B., Sih, C. J., Perlman, D., Eds.; Wiley: New York, 1976; Vol X, p 107. Sih, C. J.; Chen, C. S. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 570.

(2) Shieh, W. R.; Gopalan, A. S.; Sih, C. J. *J. Am. Chem. Soc.* **1985**, *107*, 2993.

(3) Mori, K.; Mori, H.; Sugai, T. *Tetrahedron* **1985**, *41*, 919. Nakamura, K.; Ushio, K.; Oka, S.; Ohno, A. *Tetrahedron Lett.* **1984**, *25*, 3979. Fuganti, C.; Grasselli, P. *Tetrahedron Lett.* **1985**, *26*, 102.

(4) Cambou, B.; Klibanov, A. M. *J. Am. Chem. Soc.* **1984**, *106*, 2687. Ladner, W. E.; Whitesides, G. M. *J. Am. Chem. Soc.* **1984**, *106*, 7250. Hamaguchi, C. S.; Asada, M.; Hasegawa, J.; Watanabe, K. *Agric. Biol. Chem.* **1984**, *48*, 2331. Langrand, G.; Baratti, J.; Buono, G.; Triantaphylides, C. *Tetrahedron Lett.* **1986**, *27*, 29.

(5) Kirchener, G.; Scollar, M. P.; Klibanov, A. M., *J. Am. Chem. Soc.* **1985**, *107*, 7072.

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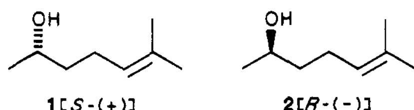
Table I. Reaction Time, Yield, and Optical Purity of Sulcatol Obtained by Reduction of 6-Methylhept-5-en-2-one with Various Biological Systems

biological system	reacn time, h	convern, yield, %	ee,[a] %	$[\alpha]^{25}_J$
bakers' yeast	120	80	94 (*S*)	+14.3°
C. tyrobutyricum (crotonic acid)	48	100	88 (*S*)	*b*
ADH *T. brockii*	144	58	>99 (*S*)	+14.8°
T. brockii (growing)	24	100	>99 (*S*)	*b*
A. niger	24	80	96 (*R*)	-14.3°
C. candidum	17	70	92 (*R*)	*b*
C. tyrobutyricum (glucose)	48	76	80 (*R*)	-11°
resolution with lipase	48	30	88 (*R*)	
			66 (*S*)	*b*
resolution with lipase	96	65	>99 (*S*)	+15.6°
			80 (*R*)	*b*

[a] See Experimental Section for method used. [b] Analytical run, optical rotation not measured.

Coast. The chirality of the molecule is involved in its biological activity: one species, *Gnathotricus sulcatus*, responds to a mixture of 65% of isomer (*S*)-(+)-1 and 35% of isomer (*R*)-(−)-2 and does not respond to either one of these isomers alone.[6] Another species, *Gnathotricus retusus*, is sensitive only to the *S* enantiomer, and its response seems to be inhibited by the *R* enantiomer.[7]



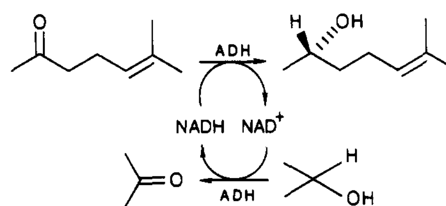
We first looked for microorganisms or enzymes able to reduce 6-methylhept-5-en-2-one to either the *R* or the *S* alcohol with a high optical yield, and we then investigated methods for resolving biologically racemic sulcatol obtained by chemical reduction of the commercial ketone.

Bakers' yeast (*Saccharomyces cerevisiae*), the anaerobic bacterium *Clostridium tyrobutyricum*, the thermophilic anaerobic bacterium *Thermoanaerobium brockii*, and a purified alcohol dehydrogenase were all found to give the *S* enantiomer from 6-methylhept-5-en-2-one. The anaerobic bacterium *C. tyrobutyricum* and two fungi *Geotrichum candidum* and *Aspergillus niger* all gave the *R* enantiomer from the same ketone. Finally, we succeeded in resolving racemic sulcatol by lipase-catalyzed transesterification.

I. Preparation of the *S*-(+) Enantiomer. Three methods for the synthesis of the *S* isomer have been reported: two from naturally occurring substances, L-fucose[8] and (*S*)-(−)-lactate,[9] and the third from (*S*)-(+)-ethyl 3-hydroxybutyrate, obtained by reduction of ethyl 3-oxobutyrate with *S. cerevisiae*.[10] This last pathway requires six steps to obtain sulcatol.

The most direct route to (*S*)-sulcatol might at first sight be thought to be by use of bakers' yeast which is known to give *S* alcohols from the corresponding ketone. However, Mori[10] reported that this reduction did not take place. In our hands, bakers' yeast (*S. cerevisiae*) after cofermentation with sucrose and 6-methylhept-5-en-2-one yielded 80% sulcatol and 20% starting ketone after six

Scheme I



days (Table I). Optical rotation of the product (+14.3°) and determination of its optical purity (see Experimental Section) showed that the reaction is highly stereospecific (97% *S* enantiomer; enantiomeric excess 94%).

This same enantiomer was also prepared by reduction of the starting ketone with the anaerobic bacterium *C. tyrobutyricum*. This bacterium has been extensively studied by H. Simon and co-workers[11] who showed in particular that the type of the enzymes expressed varied according to the composition of the culture medium. We grew *C. tyrobutyricum* with crotonic acid as the sole carbon source. The reduction reaction was carried out with isolated cells under hydrogen. The hydrogenase present in the bacterium regenerates the cofactor. Under these conditions, 6-methylhept-5-en-2-one was reduced to sulcatol in 48 h (Table I). The sulcatol obtained displayed an 88% enantiomeric excess of the *S* isomer.

Finally, we also obtained the (*S*)-(+)-sulcatol using the thermophilic anaerobic bacterium *T. brockii* in two forms: with the commercial alcohol dehydrogenase from this microorganism and with the growing microorganism.

The commercial alcohol dehydrogenase extracted from *T. brockii* was found to catalyse the reduction of 6-methylhept-5-en-2-one. We first immobilized the enzyme according to ref 12, with a yield of 45%. This enzyme requires NADPH as cofactor. Numerous systems have been described for the regeneration of this cofactor, and the coupling of the glucose dehydrogenase and the alcohol dehydrogenase of *T. brockii* has been proposed for the synthesis of chiral alcohols.[13] However, in the case of sulcatol, the regeneration can be achieved by oxidation of isopropyl alcohol by the alcohol dehydrogenase itself (Scheme I).

The activity measured for 6-methylhept-5-en-2-one was 0.6 unit per mg protein, i.e., 10% of the activity for acetone, measured under the same conditions.

We carried out the reaction with 50 mmol and obtained 58% (*S*)-(+)-sulcatol of high optical purity after 6 days reaction time (enantiomeric excess 99%) (Table I).

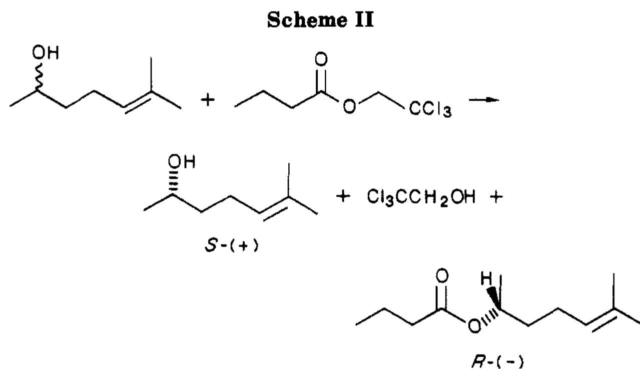
We then reduced 6-methylhept-5-en-2-one with growing *T. brockii* cells. After 24 h the ketone had been totally reduced, giving (*S*)-(+)-sulcatol of high optical purity (enantiomeric excess close to 100%).

The results obtained with *T. brockii* are consistent with those of Keinan[14] obtained in work on the reduction of various ketones with thermostable enzymes.

II. Preparation of the *R*-(−) Enantiomer. This isomer has been prepared chemically from 2-deoxy-D-ribose.[8] Literature data on the reduction of β-keto esters with *G. candidum*[15] and with various strains of *G. candidum* and *A. niger*[16] suggested that these microorganisms

(6) Byrne, K. J.; Swigar, A. A.; Silverstein, R. M.; Borden, J. H.; Stokkink, E. *J. Insect. Physiol.* **1974**, *20*, 1895.
(7) Borden, J. H.; Chong, L.; McLean, J. A.; Slessa, K. N.; Mori, K. *Science (Washington, D.C)* **1976**, *192*, 894. Borden, J. H.; Haudley, J. R.; McLean, J. A.; Silverstein, R. M.; Chong, L.; Slessa, K. N.; Johnsson, B. D.; Schuler, H. R. *J. Chem. Ecol.* **1980**, *6*, 445.
(8) Schuler, H. R.; Slessa, K. N. *Can. J. Chem.* **1977**, *55*, 3280.
(9) Johnson, B. D.; Slessa, K. N. *Can. J. Chem.* **1979**, *57*, 233.
(10) Mori, K. *Tetrahedron* **1981**, *37*, 1341.
(11) Bader, J.; Simon, H., *Arch. Microbiol.* **1980**, *127*, 279.
(12) Pollak, A.; Blumenfeld, H.; Wax, M.; Baughn, R. L.; Whitesides, G. M. *J. Am. Chem. Soc.* **1980**, *102*, 6324.
(13) Wong, C. H.; Drueckhammer, D. G.; Sweers, H. M. *J. Am. Chem. Soc.* **1985**, *107*, 4028.
(14) Keinan, E.; Hafeli, E. K.; Seth, K. K.; Lamed, R. *J. Am. Chem. Soc.* **1986**, *108*, 162.
(15) Wipf, B.; Kupfer, E.; Bertazzi, R.; Leuenberger, G. W. *Helv. Chim. Acta* **1983**, *66*, 485.

Scheme II



might enable us to obtain (*R*)-(−)-sulcatol from 6-methylhept-5-en-2-one. Experiments with *G. candidum* were carried out with growing and resting cells. In both cases extensive metabolization of the material occurred. The stereochemistry of the alcohol obtained varied widely according to the reaction time and the concentration of starting material.

The best results were obtained with 5 g of wet cells suspended in 50 mL of 5% glucose solution for 50 μL of 6-methylhept-5-en-2-one. After 17 h, 76% of sulcatol containing 96% of the *R* enantiomer was obtained (enantiomeric excess 92%, Table I).

Low metabolization and highly reproducible reduction occurred with resting cells of *A. niger* in water, under the same conditions. After 24 h 80% of (*R*)-(−)-sulcatol with an enantiomeric excess of 96% was obtained.

We also obtained (*R*)-sulcatol with the anaerobic bacterium *C. tyrobutyricum*, grown with glucose as sole source of carbon according to Simon[17] the reduction being carried out with isolated cells under hydrogen. After 48 h reaction 80% conversion to sulcatol with an 80% enantiomeric excess of the *R* isomer was achieved.

III. Resolution of Racemic Sulcatol. Racemic sulcatol has already been resolved by the classical method of fractional crystallization of the brucine salt of its phthalic hemiester.[18] The method is lengthy, and large quantities of pure enantiomers are difficult to obtain in this way.

We developed the enzymatic resolution of (±)-sulcatol on the basis of the method described by Klibanov.[5] We used pig pancreatic lipase to catalyze the transesterification of trichloroethanol butyrate and (*R*)-sulcatol in anhydrous ether (See Scheme II).

The (*R*)-sulcatol butyrate is easily separated from unreacted (*S*)-sulcatol by column chromatography. Hydrolysis of the butyrate then yields (*R*)-sulcatol.

The extent of transesterification influences the optical purity of the products. Thus after 4 days and 65% transesterification the enantiomeric excess of the residual (*S*)-sulcatol was more than 99%, and that of the (*R*)-sulcatol obtained from the butyrate was 80%. However, after 2 days and only 30% transesterification, the enantiomeric excess of the residual (*S*)-sulcatol was only 66% while that of the (*R*)-sulcatol obtained by hydrolysis of the ester was 88% (Table I).

Discussion

The results reported here show that biological methods offer simple and rapid means for obtaining sulcatol enantiomers. They also constitute data which can be used to help choose an appropriate method for stereospecifically reducing other carbonyl compounds.

***S* Enantiomer.** The procedure which requires the least specific facilities is the reduction with bakers' yeast, which yields the *S* enantiomer under suitable conditions with an optical purity better than that obtained by chemical synthesis.[9] However, the stereospecificity is not total, as it is generally observed when several alcohol dehydrogenases are present.[1]

The immobilized enzyme gives the best results in terms of both chemical and optical purity. Regeneration of the cofactor is readily achieved here since it requires neither a second enzyme nor a second substrate of low availability. We first set out this procedure to obtain the *R* enantiomer, since the alcohol dehydrogenase from *T. brockii* was known to reduce butanone to an alcohol with stereochemistry opposite to that predicted by Prelog's rule.[19] Just recently though, the specificity of this enzyme has been reinvestigated for a large number of ketones including 6-methylhept-5-en-2-one. The *R* alcohol was obtained only from short chain ketones (C4 and C5) with a moderate enantiomeric excess.[14]

The only disadvantage of the commercial enzyme from *T. brockii* is its high cost, which is likely to make its use on a preparative scale uneconomic.

This drawback can be avoided by using the growing microorganism, which also gives a product of high optical purity. Thus this thermophilic bacterium evidently does not possess several competing alcohol dehydrogenases.

A quite different result was obtained with *C. tyrobutyricum*. We might assume that this microorganism possesses two alcohol dehydrogenases of opposite specificity. Either can predominate according to the culture conditions. The repressive effect of glucose on the biosynthesis of many enzymes is well-known and was studied by Simon in this anaerobic bacterium.[17] The presence of two or more alcohol dehydrogenases has moreover been observed in other microorganisms like in yeasts[2] and in *Aspergillus nidulans*.[20]

***R* Enantiomer.** The best results for the preparation of the (*R*)-(−)-sulcatol were obtained with the resting mycelium of *A. niger*. Chemical yield and optical purity were both high and the reaction was reproducible.

The results obtained with the other fungus, *G. candidum* revealed the presence of several alcohol dehydrogenases, as enantiomeric excesses varied among runs depending on the age of the culture. With 24 h cultures the sulcatol formed is near racemic with a slight excess of the *S* enantiomer. When older, 2–3 days, cultures gave (*R*)-(−)-sulcatol. Apparently, the enzyme giving the *S* enantiomer, which is produced massively in the early stages of growth, is less stable than that giving the *R* enantiomer. Moreover the enzyme giving the *R* enantiomer is apparently inhibited by large amounts of starting material (enantiomeric excess 92% *R* when ketone/wet cells = 1% and 50% *R* when ketone/wet cells = 10%).

Further work is required to optimize stereospecific reducing conditions with this fungus close to yeasts.

Resolution of Racemic Sulcatol. The purest (*S*)-(+)-sulcatol ($[\alpha]^{25}{}_{J}$ 15.6°) was obtained by resolution with lipase, but the method is not totally stereospecific: at 30% transesterification the sulcatol isolated had an enantiomeric excess of only 88% (94% *R* and 6% *S*).

This technique is simple and cheap and can be readily put into operation by chemists unfamiliar with biocon-

(16) Bernardi, R.; Cardillo, R.; Ghiringhelli, D. *J. Chem. Soc., Chem. Commun.* **1984**, 460.
(17) Bader, J.; Gunther, H.; Rambeck, B.; Simon, H. *Hoppe-Seyler's Z. Physiol. Chem.* **1978**, *359*, 19.
(18) Plummer, E. L.; Stewart, T. E.; Byrne, K.; Pearce, G. T.; Silverstein, R. M. *J. Chem. Ecol.* **1976**, *2*, 307.
(19) Zeikus, J. G.; Lamed, R. U.S. Patent 4352885, 1982.
(20) Creaser, E. H.; Porter, R. L.; Britt, K. A.; Pateman, J. A.; Doy, C. H. *Biochem. J.* **1985**, *225*, 449.

Table II. Guide to Choosing the Most Appropriate Method for Any Particular Conversion, Based on Results Obtained for Sulcatol Enantiomers

biological system	enantiomeric excess	chemical yield	specific equipment	cost	operability
		S-(+) Enantiomer			
bakers' yeast	good	fair	no	low	easy
C. tyrobutyricum	fair		yes	medium	very difficult
ADH *T. brockii*	very good	good	no	high	quite difficult
growing *T. brockii*	very good		yes	low	difficult
resolution with lipase	very good	fair	no	low	easy
		R-(−) Enantiomer			
A. niger	good	good	yes	low	difficult
G. candidum	fair	poor	yes	low	difficult
C. tyrobutyricum	fair	fair	yes	low	very difficult
resolution with lipase	fair	fair	no	low	easy

version practice, in particular in small-scale preparation. In large-scale preparation, however, this kinetic resolution would require chemical reduction of ketone first and then separation of the ester product from the unreacted alcohol and ester substrate and would end up in a maximum yield of 50% for an isomer. These operations, particularly that of chromatographic separation of mixtures of neutral compounds could be expensive. Asymmetric reduction, however, does not have these drawbacks, and we have demonstrated that both enantiomers can be obtained with high chemical and optical yields.

Table II sets out various criteria, based on the results obtained, designed to act as a guide to choosing the most appropriate method for any particular conversion. These criteria, though obtained for a specific conversion, i.e., preparation of sulcatol enantiomers, are likely to be valid for the preparation of a great number of other chiral alcohols from corresponding ketones and illustrate the usefulness of biological systems in obtaining optically active molecules.

Experimental Section

6-Methylhept-5-en-2-one was a commercial product (Fluka, purum grade).

Gas-phase chromatography was performed on a Shimadzu C9 Mini-3 instrument fitted with a flame ionization detector and capillary columns. Analytical runs were carried out with a 25 m × 0.32 mm Chrompack column filled with 20% Carbowax and helium as the carrier gas at 1.2 kg/cm^2. The oven temperature was 90 °C. Measurements of enantiomeric excess were made as described below by using a 26 m × 0.22 mm Chrompack column filled with Chirasil-L-Valin and the same carrier gas. Oven temperature was 115 °C.

The bioconversion methods specific to each biological system are given below. In all cases, the crude extracts were analyzed on the Carbowax column: retention time of 6-methylhept-5-en-2-one, 5 min; retention time of sulcatol, 9 min.

Proportions were determined with a Shimadzu CR 3A integrator. The sulcatol was purified by chromatography on silica gel column eluted with pentane/ether (90/10 v/v): liquid, bp 73–75 °C (20 mm). Its identity was confirmed by comparison of its ^{1}H NMR spectrum with that of an authentic sample.

NMR spectra were obtained on a Jeol CX60 apparatus in $CDCl_3$ with Me_4Si as internal standard.

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

Enantiomeric Excess. Enantiomeric excesses were determined by chromatography of derivatized sulcatol on a chiral capillary column. The crude extract or purified sulcatol (1–2 μL) was dissolved in 2 mL of dry CH_2Cl_2 and placed in 100 μL of isopropyl isocyanate in a stoppered tube. After heating for 1 h in a water bath at 95–100 °C the solvent was evaporated along with excess reagent in a stream of nitrogen and the residue taken up in 2 mL of dry CH_2Cl_2.

The solution was analyzed on a Chirasil-L-Valin capillary column. The retention times of the two sulcatol enantiomers were as follows: *R* enantiomer, 18.2 min; *S* enantiomer, 18.7 min.

After each run the proportion of each enantiomer was determined and the enantiomeric excess calculated.

1. Bakers' Yeast (*Saccharomyces cerevisiae*). Bakers' yeast (200 g, Hirondelle brand) was suspended in 4 L of 30 g/L aqueous sucrose. The mixture was kept at 35 °C and shaken throughout the reaction. After 30 min, 4 g of 6-methylhept-5-en-2-one was added. Sucrose (50 g) was added every 24 h. After 5 days the mixture was filtered and the filtrate saturated with $(NH_4)_2SO_4$ and extracted with ether. A mixture of 80% sulcatol and 20% ketone was obtained. Yield after purification: 2.2 g (70%); $[\alpha]^{25}{}_J$ +14.3° (*c* 0.03). Chromatography on chiral column: 97% *S* and 3% *R*.

2. Commercial Alcohol Dehydrogenase from *Thermoanaerobium brockii*. Commercial alcohol dehydrogenase from *T. brockii* (Sigma) was immobilized according to ref 12 by using 10 mg of enzyme/g of activated polymer (PAN 800) in the presence of 1 mM NADP and 10 mM isopropyl alcohol. Yield: 45%.

To 600 mL of a 0.05 M triethanolamine buffer solution (pH 7.5) containing 70 mg of NADP (0.15 mM), 36 g of isopropyl alcohol (1 M), and 3.8 g of 6-methylhept-5-en-2-one (0.05 M) was added 70 mL of gel containing immobilized alcohol dehydrogenase (total activity 22.5 U for reduction of acetone). The mixture was heated to 50 °C under a stream of nitrogen with shaking. After 144 h the gel was removed by centrifugation and the mixture continuously extracted with ether.

The residue contained 58% sulcatol and 42% ketone. After purification 2 g of sulcatol was recovered (yield 90%): $[\alpha]^{25}{}_J$ +14.8° (*c* 0.04 EtOH). Chromatography on chiral column: *S* >99%.

3. *Thermoanaerobium brockii* (ATCC 33075). The microorganism was grown at 70 °C on the medium described by Zeikus et al.[21]

The reaction was carried out in two 250-mL flasks containing 200 mL of medium under nitrogen and fitted with airtight stoppers. Immediately after the flasks were seeded, 100 μL of 6-methylhept-5-en-2-one was added to each. After 24 h the mycelium was removed by centrifuging and the aqueous phase continuously extracted with ether. The residue contained only sulcatol.

The optical purity was determined directly on the crude extract. The optical rotation was not measured. Chromatography on chiral column: *S* >99%.

4. *Clostridium tyrobytyricum* (DSM 1460). This bacterium was grown on a strictly anaerobic medium under nitrogen. The culture medium, containing 6 g/L of crotonic acid as sole carbon source has been described by Bader et al.[11] Growth was monitored by measuring the optical density (OD) at 578 nm. When the OD reached its maximum (1.4–1.5) the culture was filtered in a vertical centrifuge under nitrogen. The cells were washed in a phosphate buffer pH 7 and recovered under nitrogen (1.3–1.4 g/L).

The bioconversions were performed under the following conditions: In a 250-mL conical flask fitted with an airtight screw top and inlet and outlet tubes with taps were placed 52 mL of 0.1 M phosphate buffer (pH 7), 13.5 mg of methyl viologen, 1.3 mg of tetracycline hydrochloride, 8 g of bacteria (fresh weight), and 0.5 g of 6-methylhept-5-en-2-one. All this was done under nitrogen. The nitrogen was replaced by hydrogen. The suspension

(21) Zeikus, J. G.; Hegge, P. W.; Anderson, M. A. *Arch. Microbiol.* **1979**, *122*, 41.

turned blue, and an excess pressure of about 0.5 bar was applied. The suspension was stirred magnetically at 35 °C for 48 h. The bacteria were removed by centrifuging, and the liquid was continuously extracted with ether for 24 h.

The residue contained only sulcatol, complete conversion having taken place. Optical purity was determined on the crude extract. The optical rotation was not measured. Chromatography on chiral column: 94% *S* and 6% *R*.

5. *Aspergillus niger* (ATCC 9142). The following culture medium was used. For 1 L of water: glucose, 20 g; yeast extract, 5 g; soya meal, 5 g; NaCl, 5 g; K_2HPO_4, 5 g; pH adjusted to 6.5.

The culture was set up in 500-mL conical flasks containing 100 mL of medium stirred at 200 rpm at 27 °C. After 24 h the contents of the flasks were filtered and the mycelium washed repeatedly with 8% NaCl solution. The bioconversion was carried out under the following conditions: in a conical flask containing 50 mL of water were placed 5 g of wet mycelium and 50 μL of 6-methylhept-5-en-2-one. The mixture was stirred at 200 rpm at 27 °C.

After a 24-h reaction the mixture was filtered and the filtrate extracted continuously with ether overnight.

The crude extract contained 80% sulcatol and 20% unreacted ketone.

From 10 flasks 2.5 g of purified sulcatol was recovered (yield 80%): $[\alpha]^{25}{}_J$ -14.3° (*c* 0.05, EtOH). Chromatography on chiral column: 98% *R* and 2% S.

6. *Geotrichum candidum* (CBS 233-76). This microorganism is generally classified among the fungi even though it may occur under various forms. The strain used is a yeast-like strain giving large isolated cells.

The following culture medium was used: glucose, 50 g; yeast extract, 10 g; bactopeptone, 10 g; H_2O, 1000 mL. The culture was grown in a 2-L fermenter containing 1 L of medium, well-aerated and maintained at 27 °C. After 48 h growth the mixture was filtered, and the cells were washed repeatedly with 8% NaCl solution.

The bioconversion conditions were identical with those used with *A. niger* except that the water was replaced by 5% glucose solution to prevent high metabolization.

After a 17-h reaction the mixture was filtered and the filtrate extracted continuously with ether overnight. The residue consisted of 70% sulcatol and 30% unreacted ketone. The yield, determined with the aid of an internal standard was 50%. The optical rotation was not measured. The optical purity was determined on the crude extract. Chromatography on chiral column: 96% *R* and 4% *S*.

7. *Clostridium tyrobutyricum* (DSM 1460). To obtain the *R*-(-) enantiomer, the bacterium was grown on a medium containing glucose (10 g/L) as sole carbon source as described by J. Bader et al.[17] Growth was monitored as previously and filtration carried out under anaerobic conditions when the optical density was maximal (2.0 to 2.1).

Recovery of *C. tyrobutyricum* cells and the bioconversion of 6-methylhept-5-en-2-one were carried out under the conditions described previously for the microorganism grown on crotonic acid. The residue consisted of 76% sulcatol and 24% unreacted ketone. After purification, 0.26 g of sulcatol was recovered (yield 70%): $[\alpha]^{25}{}_J$ -11° (*c* 0.015, EtOH). Chromatography on chiral column: 90% *R* and 10% *S*.

8. Resolution of Racemic Sulcatol with Pig Pancreatic Lipase. The resolution of racemic sulcatol was performed as follows: A mixture of 1.3 g (10 mM) of racemic 6-methylhept-5-en-2-ol obtained by $LiAlH_4$ reduction of the corresponding ketone, 2.4 g of trichloroethyl butyrate (11 mM), 2 g of pig pancreatic lipase (Sigma, activity 35 U/mg protein), and 10 mL of anhydrous ether was placed in a vessel sealed with a septum cap and stirred at 27 °C. The degree of completion of the reaction was monitored by gas-phase chromatography. Once the required extent of transesterification had been reached, the lipase was filtered and the ether evaporated off. The residue was chromatographed on a silica gel column with dichloromethane as eluant, giving butyrates and unreacted sulcatol. The butyrates were hydrolyzed with 1 N potassium hydroxyde in alcohol. The alcohol was then evaporated, water added, and the mixture extracted with ether, giving the sulcatol which had reacted.

(a) Reaction time, 48 h: transesterification, 30%; unreacted sulcatol, chromatography on chiral column, 83% *S* and 17% *R*; sulcatol from butyrate, chromatography on chiral column, 94% *R* and 6% *S*.

(b) Reaction time, 96 h: transesterification, 65%; unreacted sulcatol, $[\alpha]^{25}{}_J$ +15.6° (*c* 0.015, EtOH), chromatography on chiral column, *S* >99%; sulcatol from butyrate, chromatography on chiral column, 90% *R* and 10% *S*.

Aryl-Assisted Halogen Exchange and Rearrangements of 2-Bromo-1-chloro-3-arylpropanes and 1-Bromo-3-chloro-2-arylpropanes. Evidence for a Competitive Bromine-Assisted Pathway in the Case of 1,2-Dibromo-3-arylpropanes

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Ion pairing, ring opening, and the rates of formation of aryl-bridged chlorinated intermediates are investigated through reactions of mixed 1,2-dihalo-3-arylpropanes and 1,3-dihalo-2-arylpropanes (aryl = C_6H_4X with X = H and X = *p*-Me) and are compared with previous results for X = *p*-OMe; this allows an estimation of the free energy difference between isomeric aryl-bridged (3-bromopropylene)benzenium ions and bromine-bridged 3-arylpropylenebromonium ions.

Bromonium ions and benzenium ions are well-known intermediates: the former were originally proposed to account for the anti stereochemistry of the addition of bromine to olefins;[1] the latter play an important part in the solvolysis of primary and secondary β-arylalkyl tosylates.[2]

Here we examine an original situation where aryl and bromine groups are present simultaneously and can compete to delocalize a positive charge. This situation is ob-

(1) (a) Roberts, L.; Kimball, J. E. *J. Am. Chem. Soc.* **1937**, *59*, 947. (b) Olah, G. A. *Halonium Ions*; Wiley: New York, 1975; pp 97-124. (c) Freeman, F. *Chem. Rev.* **1975**, *75*, 439. (d) Dubois, J.-E.; Chretien, J. R. *J. Am. Chem. Soc.* **1978**, *100*, 3506. (e) Bienvenüe-Goetz, E.; Dubois, J.-E. *Tetrahedron* **1978**, *34*, 2021. (f) Angelini, G.; Speranza, M. *J. Am. Chem. Soc.* **1981**, *103*, 3792.

(2) (a) Cram, D. J. *J. Am. Chem. Soc.* **1952**, *74*, 2129. (b) Olah, G. A.; Schleyer, P. v. R. *Carbonium Ions*; Wiley: New York, 1972; pp 1347-1383. (c) Schadt, F. L.; Lancelot, C. J.; Schleyer, P. v. R. *J. Am. Chem. Soc.* **1978**, *100*, 228.

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