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Enzymatic Baeyer-Villiger Type Oxidations of Ketones Catalyzed by Cyclohexanone Oxygenase

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Cyclohexanone oxygenase (EC 1.14.13.-) produced by *Acinetobacter* NCIB 9871 is a flavin-containing NADPH-dependent monooxygenase that utilizes dioxygen to convert cyclic ketones into lactones. A variety of ketones were examined in order to determine the substrate specificity, regioselectivity, and enantioselectivity of cyclohexanone oxygenase. Lactones were synthesized using immobilized enzymes. The nicotinamide cofactor required by cyclohexanone oxygenase was regenerated *in situ* with glucose 6-phosphate and glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides*.

INTRODUCTION

Microorganisms are capable of effecting a wide range of oxidative transformations of organic compounds (*1*). Among these transformations, the biological equivalent of the Baeyer-Villiger reaction[2] (*2*)—the insertion of an oxygen atom into organic ketones with the production of lactones—has been implicated in microbial degradations of alkanes, steroids, and cyclic ketones (*3–15*). The objective of this work was to explore the utility of one enzyme—cyclohexanone monooxygenase (EC 1.14.13.-)—capable of catalyzing this tranformation for utility in organic synthesis.

The microorganisms that are capable of removing side chains from steroids are also generally able to carry out an additional Baeyer-Villiger oxidation on the C-17 ketones to give lactones. The microbial double Baeyer-Villiger oxidation of progesterone by *Penicillium chrysogenum*, for example, gives testololactone in 70% yield (*9*). The microbiological Baeyer-Villiger reactions of steroids are not limited to C-17 side chains and D-rings. Eburicoic acid undergoes cleavage of the A-ring by *Glomerella fusarioides* and yields 4-hydroxy-3,4-*seco*-eburica-8,24(28)-diene-3,21-dioic acid in 10% yield (*10*).

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[2] A Baeyer-Villiger rearrangement is defined as an oxygen insertion reaction resulting from the treatment of a ketone with a peracid or other peroxy compound.

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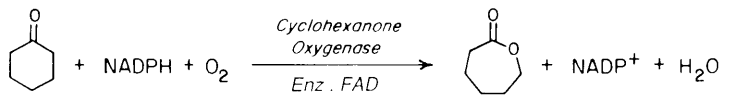
Analogous microbiological Baeyer-Villiger oxidations of simple cyclic ketones to the corresponding lactones have also been reported (*11–15*). The oxidation of 2-heptylcyclopentanone or of 2-pentylcyclopentanone by *Pseudomonas oleovorans* gives the corresponding lactones in low yield, with some suggestion that stereochemical selectivity may be involved (*11*). Microbial oxidation of fenchone with *Corynebacterium* species afford a mixture of 1,2-fencholide and 2,3-fencholide in 42% yield (*12*). In addition, cell-free enzyme preparations from pseudomonad species catalyze the lactonization of camphor and 2,5-diketo camphane (*14, 15*).

Key enzymes in these microbiological oxidations are the dioxygenases and monooxygenases that catalyze the introduction of an oxygen functionality and initiate the carbon–carbon bond cleavage step. Although the most extensively investigated monooxygenases are the aromatic hydroxylases, the lactone-forming enzymes are now well established as important catalysts in the degradation of a range of organic compounds. These enzymes are flavin dependent and carry out a net four-electron reduction of dioxygen: one atom of oxygen is incorporated into the organic substrate; the second appears as water.

Cyclohexanone oxygenase produced by *Acinetobacter* NCIB 9871, belongs to the class of bacterial flavoprotein monooxygenases that catalyze the conversion of cyclic ketones into the corresponding lactones (*16*). Specifically, cyclohexanone oxygenase catalyzes the oxygen insertion and ring expansion of cyclohexanone to form ε-caprolactone,

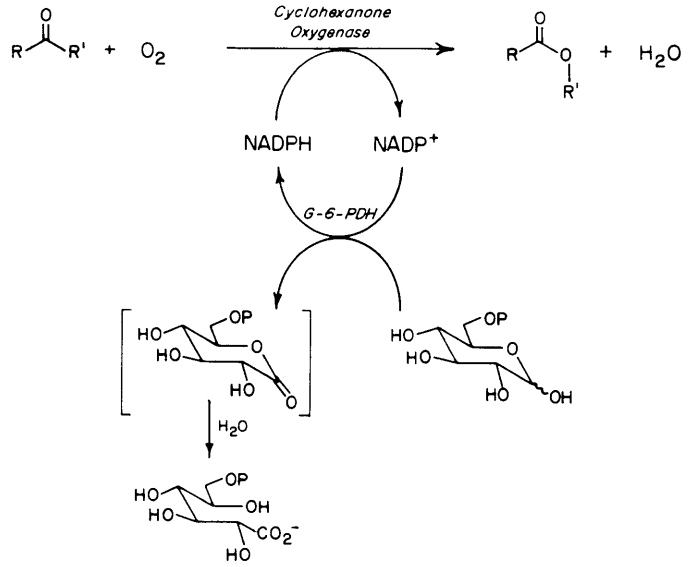


(1)

Mechanistic studies on cyclohexanone oxygenase support the formation of a 4a-hydroperoxyflavin intermediate (*17–19*). This reactive peroxide acts as a nucleophile toward cyclohexanone to form a mixed peroxide, which then decomposes by a Baeyer-Villiger rearrangement to 4a-hydroxyflavin and ε-caprolactone (*17*).

We were interested in the potential synthetic utility of cyclohexanone oxygenase for several reasons. First, cyclohexanone oxygenase displays a broad substrate specificity for cyclic ketones (*20*). Second, work by Schwab (*21*) demonstrates that the lactonization of 2-methylcyclohexanone catalyzed by cyclohexanone oxygenase exhibits regioselectivity comparable with that found for the chemical Baeyer-Villiger oxidation; moreover, there is evidence that the enzyme-catalyzed reaction proceeds with some enantioselectivity (*21*).

Thus, we examined a variety of readily available ketones in order to determine the substrate specificity, regioselectivity, and enantioselectivity of cyclohexanone oxygenase immobilized in a polyacrylamide gel and to ascertain whether this method offered advantages over conventional Baeyer-Villiger procedures. The reduced nicotinamide cofactor NADPH required by cyclohexanone oxygenase was regenerated *in situ* with glucose 6-phosphate and glucose-6-phosphate dehydrogenase from *Leuconostoc mesenteroides* (Scheme I).



SCHEME I. General scheme for the enzymatic synthesis of lactones catalyzed by cyclohexanone oxygenase with regeneration of NADPH using glucose 6-phosphate and glucose-6-phosphate dehydrogenase.

RESULTS AND DISCUSSION

Determination of Substrate Specificity of Cyclohexanone Oxygenase and Measurement of Relative Rates

Table 1 lists the relative activity of cyclohexanone oxygenase toward several ketones. These relative rates were measured by two methods: by following the rate of consumption of NADPH spectrophotometrically and by following the rate of oxygen uptake with an oxygen electrode. These measurements should be interpreted cautiously since all compounds were employed at the same nominal concentration with no regard to differences in solubility or value of K_m. These assays were intentionally run under conditions that should saturate the enzyme and therefore approximate V_{max}.

Cyclohexanone oxygenase was found to catalyze the oxidation of NADPH at the same rate as the reduction of O_2, in the presence of the various ketones examined. Addition of catalase to the assay system had no effect on the rate of oxygen uptake. Substrates and substrate analogs often act as effectors with NADPH-linked monooxygenases (*22*). Effectors combine with the oxidized enzyme (Enz · FAD) and accelerate the rate of flavin reduction by NADPH. In the presence of dioxygen, this process would result in an increased rate of consumption of dioxygen, with some of the dioxygen being reduced to hydrogen peroxide. That is, oxygen insertion into the ketone moiety would be uncoupled from oxida-

TABLE 1

Substrate Specificity of Cyclohexanone Oxygenase and Measurement of Relative Rates[a]

Compound	Relative rate (%)
Cyclohexanone	100
2-Norbornanone	138
D-Fenchone	30
L-Fenchone	60
2-Adamantanone	0
(+)-Dihydrocarvone	32
syn-7-Benzyloxymethyl-2-norbonen-5-one	110
2-Acetylcyclohexanone	0
2-Cyclohexene-1-one	0
(+)-Camphor	46
Progesterone	8
1,4-Cyclohexanedione	90
1,3-Cyclohexanedione	0
4-Phenylcyclohexanone	0
2-Phenylcyclohexanone	19
4-*tert*-Butylcyclohexanone	14

[a] Relative rates were measured spectrophotometrically at 340 nm and by dioxygen uptake at 25°C. Measurements were performed in 1 ml of glycine–NaOH buffer (80 mM, pH 8.0) containing 25 μmol of the indicated ketone (initially dissolved in 50 μl of MeOH), NADPH (0.16 mM), cyclohexanone oxygenase (0.6 U), and an equilibrium concentration of atmospheric diooxygen (0.24 mM). See Experimental for details.

tion of nicotinamide. In an assay system measuring oxygen uptake, the formation of hydrogen peroxide would appear as activity. Since catalase failed to alter the rate of dioxygen consumption, we therefore conclude that those ketones that significantly stimulate oxygen uptake are true substrates for cyclohexanone oxygenase. This observation is also consistent with the conclusion of Ryerson *et al.* (*17*): direct reaction between H_2O_2 and substrate is an unlikely mechanism for the enzyme-catalyzed conversion of ketones to lactones.

The following conclusions concerning the structure–reactivity relationship can be drawn from the results presented in Table 1. First, cyclohexanone oxygenase displays a broad substrate specificity toward *alicyclic* ketones. Second, the following general types of ketones are *not* substrates: α,β-unsaturated ketones and 1,3-diketones (acyclic and alicyclic). These observations are consistent with the findings of Donoghue *et al.* (*16*). Walsh and Chen (*20*) have also demonstrated that the enzyme shows even broader specificity, working on some acyclic ketones and aryl ketones.

The racemic ketone *syn*-7-benzyloxymethyl-2-norbornen-5-one (Corey's ke-

tone), an intermediate in the synthesis of prostaglandins (*23*), was a substrate with a relative rate of 110% (relative to cyclohexanone). A total consumption assay run with a limiting amount of the racemic ketone did not exhibit biphasic kinetic behavior, as would have been expected were the K_m values for each enantiomer significantly different. Product analysis to conclusively determine the enantioselectivity, if any, of the enzyme was not, however, carried out.

Two steroids were investigated as substrates for cyclohexanone oxygenase: androstanolone, which was a substrate with a relative rate of ca. 4%, and progesterone, which was not a substrate.

Enzymatic Syntheses of Lactones

Lactones were synthesized on ca. 30–80 mmol scale from 2-norbornanone **(1),** L-fenchone **(2),** D-fenchone **(3),** (+)-camphor **(4),** and (+)-dihydrocarvone **(5)** (Table 2); the reaction sequence is outlined in Scheme I. The procedure utilizes cyclohexanone oxygenase and a regeneration system for NADPH that is based on glucose 6-phosphate (G-6-P),[3] and glucose-6-phosphate dehydrogenase (G-6-PDH) from *L. mesenteroides*. In a typical synthesis, the reaction mixture contained ketone (0.1 mol), G-6-P · Na_2 (0.12 mol), $NADP^+$ (1 mmol), $MgCl_2$ (6 mmol), EDTA (1 mmol), and the enzymes immobilized in PAN: cyclohexanone oxygenase and G-6-PDH. Although the pH optimum for cyclohexanone oxygenase is 9.0 (*16*), the syntheses of lactones were carried out at pH 8.0 in order to minimize the extent of epimerization of the ketone and the lactone. At this value of pH the enzyme has ca. 70% of its maximum activity.

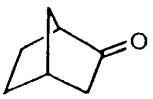
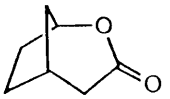
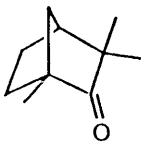
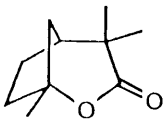
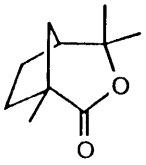
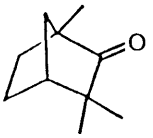
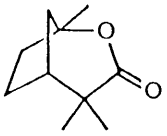
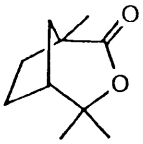
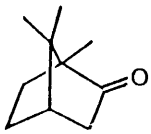
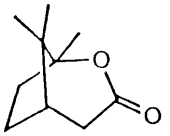
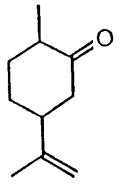
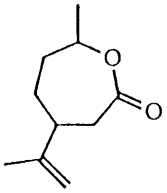
The reactions were allowed to run for 5–10 days: GLC analysis showed complete conversion of ketone to product. After removal of the polyacrylamide gels containing the enzymes, the product was isolated by extraction.

The enzyme-catalyzed oxidation of 2-norbornanone (0.1 mol) afforded, after 5 days, 2-oxabicyclo[3.2.1]octan-3-one **(6)** in 81% yield. ^{1}H NMR and GC/MS data of the enzymatic product were indistinguishable from authentic lactone obtained by oxidation of 2-norbornanone with *m*-chloroperbenzoic acid (*24*). Enzymatic oxidation of L-fenchone (0.1 mol) afforded a mixture of 1,2- and 2,3-fencholides in 76% yield. The ratio of 1,2- and 2,3-lactones was 88 : 12. Enzymatic oxidation of D-fenchone (0.1 mol) afforded the mixture of fencholides in essentially the same proportions, in 79% yield: ^{1}H NMR spectra of the product mixtures obtained were indistinguishable. The spectra exhibited the two signals at δ 1.19 and δ 1.24 due to the *gem*-dimethyl and a single signal at δ 1.47 due to the shielded angular methyl group of the 1,2-fencholide. Two weak signals at δ 1.35 and δ 1.38 were also present corresponding to the *gem*-dimethyl group of the minor component, 2,3-fencholide. The conversion of (+)-camphor to its corresponding lactone proceeded in 89% yield. 6-Isopropenyl-3-methyl-2-oxaheptanone **(10)** was obtained in 75% yield from (+)-dihydrocarvone. These products were identified by comparison with authentic materials obtained by oxidation of the ketones with 40% peracetic acid (*25*).

[3] Abbreviations used: G-6-P, glucose 6-phosphate; G-6-PDH, glucose-6-phosphate dehydrogenase; PAN, poly(acrylamide-*N*-acryloxysuccinimide).

TABLE 2

Summary of the Large-Scale Enzymatic Syntheses of Lactones

Reactant (mmol)	Products	Reaction time (days)	Yield	
			mmol	%
 (100)		5	81	81
2-Norbornanone **(1)**	2-Oxabicyclo[3.2.1]octan-3-one **(6)**			
 (100)	 and 	8	76	76
L-Fenchone **(2)**	1,2-Fencholide **(7)** 2,3-Fencholide **(8)**			
 (100)	 and 	10	79	79
D-Fenchone **(3)**	1,2-Fencholide **(7)** 2,3-Fencholide **(8)**			
 (50)		10	45	89
(+)-Camphor **(4)**	2-Oxabicyclo[3.2.1]1,7,7-trimethyloctan-3-one **(9)**			
 (40)		10	30	75
(+)-Dihydrocarvone **(5)**	6-Isopropenyl-3-methyl-2-oxacycloheptanone **(10)**			

Regeneration of NADPH

The procedure for regeneration of the reduced nicotinamide is based on the dehydrogenation of G-6-P catalyzed by G-6-PDH from *L. mesenteroides* (*26*). This method has several advantages. First, G-6-PDH is commercially available, inexpensive, and easy to manipulate and immobilize. Second, because G-6-PDH from *L. mesenteroides* has no essential thiol groups, this enzyme is relatively stable under aerobic conditions and can be used for regeneration of reduced

nicotinamide cofactors in dioxygen-containing environments; dioxygen is a substrate of cyclohexanone oxygenase. Third, the equilibrium constants for reduced nicotinamide formation are high, because the reaction is rendered essentially irreversible by hydrolysis of the initially formed 6-phosphogluconolactone to 6-phosphogluconate. The disadvantage of this system is that both G-6-P and 6-PG are general acid catalysts for the hydration of the reduced nicotinamide cofactors (*27*).[4]

CONCLUSIONS

This work describes studies concerning the substrate specificity and regioselectivity of cyclohexanone oxygenase; this work was carried out in order to determine whether this enzymatic oxidation of ketones offers advantages over conventional Baeyer-Villiger procedures.

We successfully synthesized several lactones on a 30–80 mmol scale, in high yields, using immobilized enzymes and *in situ* cofactor regeneration. These syntheses should be easily amenable to further scale-up.

Although the data obtained from the substrate specificity experiments show that cyclohexanone oxygenase accepts a broad range of alicyclic ketones as substrates, it does not accept α,β-unsaturated or 1,3-diketones.

The results obtained in the large-scale oxidations of 2-norbornanone, (+)-camphor, and (+)-dihydrocarvone parallel those of the chemical Baeyer-Villiger oxidation: the migratory aptitude of the alkyl groups appears to follow the sequence of *tert*-alkyl > *sec*-alkyl > *prim*-alkyl. It is of interest that the enzymatic oxidation of fenchone gives both possible lactones. The proportions of the 1,2- and 2,3-fencholides obtained (88 : 12) contrast with the chemical oxidation of fenchone with peracetic acid: the 2,3-fencholide is the major product in a 40 : 60 mixture. Although the similarity in the spatial arrangements of the methyl groups at the tertiary C-1 and C-3 positions would suggest a low degree of regioselectivity, a 1,2-attack is favored.

Regarding enantioselectivity, it is clear from the results obtained in the large scale oxidations of racemic 2-norbornanone and D- and L-fenchone that cyclohexanone oxygenase does not exhibit a useful degree of enantioselectivity: both enantiomers are turned over by the enzyme. However, recently, Taschner *et al.* (*28*) have found that cyclohexanone oxygenase is capable of enantioselective synthesis of lactones from mesomeric cyclohexanones.

With respect to regioselectivity, cyclohexanone oxygenase does not seem to offer major advantages over conventional Baeyer-Villiger reaction using peracids. Although, when confronted with a substrate containing a ketone and a sulfide functional group in the same molecule, e.g., 3-thia- and 4-thiacyclohexanones, the enzyme catalyzes the Baeyer-Villiger oxygenative ring expansion exclusively (*29*). This is in contrast to the greater ease of sulfur oxidation in nonenzymatic

[4] The phosphate-catalyzed decomposition of NADPH by G-6-P could probably be avoided by substitution of glucose 6-sulfate.

oxygen-transfer chemistry and is an example of functional group differentiation by an enzyme that is different from conventional oxidation reagents.

We conclude that this enzyme has the combination of availability, specific activity, and stability required to make it a candidate for practical organic synthesis on scales up to 0.1–1 mol. Its enantioselectivity remains to be established carefully. Its structural specificity will be different than that of peracids and may be useful in special circumstances.

EXPERIMENTAL

General

Spectrophotometric measurements were performed at 25°C using a Perkin–Elmer Model 552 spectrophotometer equipped with a constant temperature cell. ^{1}H NMR spectra were recorded at 250 MHz on a Bruker Model WH-250 instrument, using $CDCl_3$ as solvent (internal lock) (kindly performed by Marifaith Hackett). Chemical shifts were measured relative to internal tetramethylsilane. Infrared spectra were recorded on a Perkin–Elmer Model 598 instrument. GC/MS data were obtained on a Hewlett–Packard Model 5990A instrument at an ionizing voltage of 70 eV, using a UC-W98 column (6 ft × 0.125 in.) (kindly performed by Robert DiCosimo). GLC analyses were performed on a Perkin–Elmer Model 3920B instrument equipped with a flame ionization detector, using 10% Carbowax (10 ft × 0.125 in.) and 10% UC-W98 (6 ft × 0.125 in.) columns. Measurements for oxygen concentration were carried out using a Yellow Springs oxygen monitor (Yellow Springs Instrument Co., Yellow Springs, OH).

Materials

Cyclohexanone oxygenase was produced from a culture of *Acinetobacter* NCIB 9871 maintained from a culture donated by Dr. P. W. Trudgill, University College of Wales (Aberystwyrth, UK). Glucose-6-phosphate dehydrogenase from *L. mesenteroides* and $NADP^+$ were purchased from Sigma. Glucose 6-phosphate was prepared enzymatically according to published procedure (*30*). L-Fenchone and D-fenchone were obtained from K and K Laboratories, Inc. (Plainview, NY). All other organic chemicals were obtained from Aldrich. The organic compounds were used without further purification.

Assay Methods[5]

G-6-P, G-6-PDH, and $NADP^+$ were assayed according to the procedure of Bergmeyer (*31*).

Assay of cyclohexanone oxygenase (*32*). The determination of cyclohexanone oxygenase activity is based on the decrease in optical density at 340 nm which results from the conversion of NADPH to NADP. The assay mixture (final vol-

[5] Assays were carried out at 25°C. One unit of enzymatic activity is defined as that amount of enzyme which catalyzes the formation of 1 μmol of product per minute at 25°C.

ume, 3 ml) contained glycine–NaOH buffer (80 mM, pH 8.0), NADPH (0.16 mM), cyclohexanone (48.5 μM), cyclohexanone oxygenase (0.01–0.1 μM; concentration based on flavin, ε_{440} = 11.3 $m\text{M}^{-1}$ cm^{-1}), and oxygen at ambient concentrations (0.24 mM). For the determination of enzymatic activity of immobilized cyclohexanone oxygenase, aliquots (50–100 μl) of the enzyme-containing polyacrylamide gel were used. Units of activity were calculated according to the equation

$$\text{Units} \cdot \text{ml}^{-1} = \frac{A_{340} \cdot \text{Vol}_{\text{assay}}}{6.22}.$$

Growth of Acinetobacter NCIB 9871 Cells and Isolation and Purification of Cyclohexanone Oxygenase

A large-scale (25-liter) fermentation of *Acinetobacter* NCIB 9871 and the isolation and purification of cyclohexanone oxygenase from 80–100 g of cells, obtained from the large-scale fermentation, were carried out following procedures described elsewhere (*17, 32*).

Since the completion of this work an improved protocol for growth of the bacteria and purification of the cyclohexanone oxygenase has been developed. The bacterial growth can be performed on a laboratory bench top with essentially no specialized equipment (*33*).

Immobilization of Cyclohexanone Oxygenase

PAN 1000 (2 g) (*34*) was dissolved in 0.3 M Hepes buffer (8 ml, pH 7.6) containing NADPH (0.2 mM) and cyclohexanone (70 μM). The enzyme solution (15 mg of cyclohexanone oxygenase in 1.5 ml of 21 mM potassium phosphate buffer, pH 7.1, containing 1.2 mM dithiothreitol and 20% glycerol) was added, followed by 1.5 M triethylenetetraamine (0.5 ml). The resulting gel was ground and washed as described elsewhere (*34*). The immobilization yield was 30%.

Determination of Substrate Specificity and Measurements of Relative Rates (Table 1)

(*i*) *Spectrophotometric method.* Cyclohexanone oxygenase activity was measured using the standard assay procedure described above. The rate of decrease in ε_{340} was measured, at 25°C, following the addition of 25 μmol of ketone dissolved in 50 μl of MeOH to 1 ml of a solution containing glycine–NaOH buffer (80 mM, pH 8.0), NADPH (0.16 mM), soluble cyclohexanone oxygenase (0.6 U), and atmospheric oxygen (0.24 mM).

(*ii*) *Oxygen-uptake method.* Assays were carried out in a stirred oxygen-electrode cell at 25°C. The decrease in oxygen concentration was measured by the addition of 10 μl of cyclohexanone oxygenase (0.6 U) to 1 ml of a solution containing glycine–NaOH buffer (80 mM, pH 8.0), NADPH (0.16 mM), and 25 μmol of ketone previously dissolved in 50 μl of MeOH. Catalase (ca. 100 U) was added after 3 min and the reaction allowed to continue.

Subsequent to the completion of this work, an improved protocol for kinetic

assays was developed (*33*). Assays are now conducted at 15°C because of a gradual loss of enzyme activity at higher temperatures.

Enzymatic Syntheses of Lactones

2-Oxabicyclo[3.2.1]octan-3-one **(6).** To a 1-liter solution containing G-6-P · Na_2 (34.6 g, 0.12 mol), $NADP^+$ (0.85 g, 1 mmol), $MgCl_2$ (1.2 g, 6 mmol), EDTA (330 mg, 1 mmol), and 2-norbornanone (11.4 g, 0.1 mol) was added PAN-immobilized G-6-PDH (100 U) and cyclohexanone oxygenase (50 U). The reaction was conducted at pH 8.0 by the addition of 2 N NaOH using a pH controller, and the course of the reaction was followed by GLC for norbornanone derivative and by enzymatic assays for NADP(H) and G-6-P. After 3 days the total concentration of $NADP^+$ and NADPH was 55% of the original value. Additional $NADP^+$ (0.85 g, 1 mmol) was introduced into the system and the reaction continued. After an additional 2 days GLC analysis showed complete conversion of 2-norbornanone to product. The polyacrylamide gels containing the enzymes were separated by centrifugation and the supernatant was continuously extracted with dichloromethane for 1 h. The CH_2Cl_2 extract was dried ($MgSO_4$) and concentrated to afford an oil which crystallized upon cooling (11.4 g). Gas chromatography on 10% Carbowax or 10% UC-W98 gave only a single peak. The product was further purified by molecular distillation which afforded white crystals (10.2 g, 81 mmol, 81% yield); mp: 58–60°C (lit. (*35*) mp 56–57°C). The ^1H NMR spectrum of the product obtained was identical to that of authentic 2-oxabicyclo[3.2.1]-octan-3-one obtained by oxidation of 2-norbornanone with *m*-chloroperbenzoic acid (*24*). GC/MS data obtained for the enzymatic product were similar to those of the synthetic product. The residual activities of the recovered immobilized enzymes were cyclohexanone oxygenase, 77%; G-6-PDH, 80%.

Cyclohexanone oxygenase-catalyzed oxidation of L-fenchone **(2).** The same protocol described for 2-norbornanone was followed for the enzymatic oxidation of L-fenchone (0.1 mol, 15.6 g). After 8 days of reaction GLC analysis showed complete conversion of L-fenchone to product. The reaction mixture was worked up to yield off-white crystals (12.7 g, 76 mmol, 76% yield), mp: 72–76°C (lit. (*35*) mp: 61–63°C). Gas chromatography showed a single broad peak. Analysis of the ^1H NMR spectrum indicated that both the 1,2-fencholide **(7)** and the 2,3-fencholide **(8)** had formed, in a ratio of 88 : 12. The ^1H NMR exhibited the two signals at δ 1.19 and δ 1.24 of the *gem*-dimethyl and the signal at δ 1.47 of the shielded angular methyl for the 1,2-fencholide, and the two signals at δ 1.35 and δ 1.38 corresponding to the *gem*-dimethyl of the 2,3-fencholide.

Cyclohexanone oxygenase-catalyzed oxidation of D-fenchone **(3).** The same protocol described for 2-norbornanone was followed for the oxidation of D-fenchone (0.1 mol, 15.8 g). After 10 days of reaction GLC analysis showed complete conversion of D-fenchone to product. The reaction mixture was worked up as described previously to afford a mixture of 1,2- and 2,3-fencholides (13.2 g, 79 mmol, 79% yield) which was similar by GC and ^1H NMR to the product formed in the enzymatic oxidation of L-fenchone. The ^1H NMR spectrum indicated that the ratio of lactones formed was ca. 88 : 12 with the 1,2-fencholide as the major component.

2-Oxabicyclo[3.2.1]1,7,7-trimethyl octan-3-one **(9).** The enzymatic oxidation of (+)-camphor (50 mmol, 7.6 g) was carried out following the procedure described for 2-norbornanone. The mixture obtained after 10 days of reaction was worked up to afford 2-oxabicyclo[3.2.1]1,7,7-trimethyl octan-3-one (7.5 g, 44.5 mmol, 89% yield). The product was identified by comparison, by GC and NMR, with the synthetic compound obtained from the oxidation of (+)-camphor with 40% peracetic acid (*25*).

6-Isopropenyl-3-methyl-2-oxa-heptanone **(10).** (+)-Dihydrocarvone (40 mmol, 6.2 g) was converted to the corresponding lactone following the protocol described. After 10 days, the reaction was complete and 6-methyl-3-isopropenyl-6-hexanolactone was isolated in 75% yield (50 g, 30 mmol). The product was identified by comparison with synthetic material obtained from the chemical oxidation of (+)-dihydrocarvone with 40% peracetic acid (*25*).

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