A Novel Ketone Monooxygenase from Pseudomonas cepacia

PURIFICATION AND PROPERTIES*

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A ketone monooxygenase was purified from cells of Pseudomonas cepacia grown on 2-tridecanone as sole carbon source. Enzyme stability is maintained by the addition of ethanol, EDTA, and dithiothreitol. Stoichiometric studies show that for 1 mol of undecyl acetate formed, 1 mol of O₂ is consumed and 1 mol of NADPH is oxidized. The monooxygenase, purified to homogeneity, has a molecular weight of approximately 123,000 and consists of two equal subunits with molecular weights of 55,000. The enzyme contains FAD and exhibits absorption maxima at 375 and 488 nm. Enzyme activity is inhibited by thiol-active reagents and the inhibition by the cations, cadmium, copper, zinc, and mercury, is reversed by dithiothreitol, indicating the presence of essential sulfhydryl groups. Substrate specificity tests show that acetate esters are formed from methyl ketones from C-7 through C-14. The monooxygenase is also active on isomers of 2-tridecanone forming esters from 3- through 7-tridecanone. With 6-tridecanone, two esters are formed, heptyl hexanoate and pentyl octanoate, indicating that oxygen is inserted on either side of the carbonyl group. In addition, the enzyme catalyzes the lactonization of the cyclic ketone, cyclopentanone, with the formation of 5-valerolactone.

Methyl ketones are ubiquitous in nature and can be found in such diverse sources as plants, dairy products, and the odorous secretions of insects (1). The apparent lack of accumulation of these compounds would indicate that an efficient recycling process is occurring in the biosphere. Forney et al. (2) were first to report on the degradation of a methyl ketone other than acetone. They isolated an organism, identified as Pseudomonas cepacia, capable of utilizing 2-tridecanone as sole carbon source. Analysis of metabolic intermediates and end products after growth of the organism on 2-tridecanone revealed that the methyl ketone was converted to the ester undecyl acetate followed by hydrolysis to 1-undecanol and acetate (3). The 1-undecanol is oxidized to undecanoic acid and eventually metabolized by β-oxidation. Acetate could be further utilized via the tricarboxylic and glyoxylate cycles.

The enzymatic conversion of 2-tridecanone to undecyl acetate is equivalent to the chemical Baeyer-Villiger oxidation of carbonyl compounds by peracids (4). Efforts were focused at understanding the biological mechanism of this conversion. In O₂ studies with cell-free extracts of P. cepacia revealed that the source of oxygen in undecyl acetate was molecular oxygen (5), thus indicating the functioning of an unusual monooxygenase. The pathway of 2-tridecanone oxidation, showing the incorporation of ¹⁸O₂, is summarized:

This report describes the purification and properties of the monooxygenase that catalyzes the unique conversion of 2-tridecanone to the ester undecyl acetate with emphasis on the remarkable range of substrates utilized by this enzyme.

**Experimental Procedures**

**Materials** — 2-Undecanone (98%, for enzymatic assay) and 2-decanone were obtained from Chemical Samples Co. 2-Tridecanone (for growth of Pseudomonas cepacia), 2-decanone, decyl acetate, nonyl acetate, and octyl acetate were acquired from K and K Laboratories. 2-Undecanone, 2-nonanone, 2-octanone, 2-heptanone, cyclohexanone, cyclopentanone, dodecyl acetate, heptyl acetate, 2-phenyl acetate, ethyl acetate, n-heptanol, n-hexanol, n-pentanol, and N-ethylmaleimide were purchased from Eastman Organic Chemicals Co. 3-Valerolactone was from Aldrich Chemical Co.; 2-tetradecanone from Analabs; n-decanal and n-nonanal from Matheson Co.; and 2-butanol and n-octanol from Fisher Scientific Co. Undecyl acetate was synthesized as described previously (6). Dithiothreitol, NADPH, FAD, glutathione, beef liver catalase, p-chloromercuribenzoate, and o-phenanthroline were from Sigma Chemical Co.

**Bacterial Strain and Cultural Conditions** — A strain of P. cepacia, 4G9 (2) was grown on a basal salts medium (7) supplemented with 0.4% 2-tridecanone (v/v) and 0.1% Difco yeast extract. Cultures

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were grown from a 10% inoculum in 10 liters of media in Micro Fern fermentors (New Brunswick Scientific Co.) for 24 h at 30°C. Perfor- mers were operated at 350 rpm stirrer speed and an air flow of 10 liters/min.

Enzyme Assay—The consumption of oxygen from the conversion of 2-tridecanone to undeacyl acetate was measured at 30°C with a Clark-type polarographic electrode (Yellow Springs Instruments). Reaction mixtures contained 50 μmol of 2-tridecanone, 2.5 μmol of NADPH, 140 μmol of potassium phosphate, pH 7.8, and enzyme in a 3-ml final volume. Reactions were started by the rapid addition of the ketone in 5% ethanol. One unit of enzyme catalyzes the consumption of 1 μmol of oxygen.

Protein Determinations—Protein concentrations were estimated by the biuret method (8) using crystallized bovine serum albumin (Sigma Chemical Co.) as a standard. The method of Warburg and Christian (9) was used to estimate low concentrations of protein.

Purification of 2-Tridecanone Monoxygenase—All purification steps were performed at 0–4°C. Cells from 40 liters of growth medium were harvested by centrifugation and washed twice with 0.05 M potassium phosphate buffer, pH 7.5. A 25% (w/v) cell suspension was prepared in 0.01 M potassium phosphate buffer, pH 7.5, containing 1 mM dithiothreitol, 1 mM EDTA, and 5% ethanol. Cells were disrupted by sonication (at maximum power) in intermittent 1-min exposures for a total of 12 min with a Branson Sonifier model W-185-C. An ethylene glycol bath at −20°C was used to maintain a low temperature during sonication. Unbroken cells and cell debris were removed by centrifugation at 20,000 × g for 20 min. Additional centrifugation of the supernatant was performed at 125,000 × g for 1 h to remove membranous and other sedimentable material. A solution of streptomycin sulfate, 100 mg/ml, was added dropwise to the cell-free extract to a final concentration of 1 mg/ml of protein. After sitting for an additional 30 min, the precipitate was removed by centrifugation at 20,000 × g for 30 min. The supernatant fluid was collected and dialyzed 24 h against two changes of 10 liters each of 0.05 M potassium phosphate at pH 7.5, containing 1 mM dithiothreitol, 5% ethanol and 1 mM EDTA (Buffer A). Solid ammonium sulfate was added slowly with gentle stirring to the streptomycin sulfate supernatant fraction to 45% saturation. After 1 h, the precipitated protein was collected by centrifugation, redissolved in Buffer A and dialyzed overnight against 5 liters of the same buffer. This dialyzed ammonium sulfate fraction was applied to a DEAE-cellulose column (2.5 × 65 cm) equilibrated with Buffer A and operated at a flow rate of 100 ml/h.

The column was washed with 380 ml of the equilibrium buffer followed by 600 ml of 0.075 M KCl in Buffer A and developed with a 2-liter gradient linear in KCl from 0.075 M to 0.5 M in Buffer A. Oxygenase activity eluted at approximately 0.2 M KCl. Fractions containing enzyme activity were pooled, concentrated, and an Amicon ultrafiltration cell using a PM-10 membrane, and dialyzed overnight against 5 liters of Buffer A. The DEAE-cellulose fraction was applied to a column of Sepharose 4B (1 × 20 cm) prepared by the methods of Davis (10) and polymerized at 4°C. The column was washed with 50 ml of Buffer A at a flow rate of 30 ml/h followed by 90 ml of 0.4 M KCl in Buffer A. Oxygenase was eluted with 0.6 M KCl in the same buffer and enzyme fractions were pooled, concentrated, and dialyzed against 5 liters of Buffer A.

The final purification step was by preparative polyacrylamide gel electrophoresis using a Poly-Prep 200 (Buchler Instrument Co.). A 9-cm resolving gel (160 ml), containing 7.5% acrylamide, pH 8.9, was prepared by the methods of Davis (10) and polymerized at 4°C. Spacing gel containing 2.5% acrylamide was prepared at 20% the amount of the resolving gel and photopolymerized at 4°C. The lower chamber containing 4 ml of buffer containing per liter 80 μM His (pH 8.4) HCl which was adjusted to pH 8.1 with Tris. Upper chamber buffer was 0.026 M Tris and 0.10 M glycine, pH 8.3. Dithiothreitol at a final concentration of 1 mM was added to all buffers to stabilize the enzyme during electrophoresis. The apparatus was adjusted to a 1-mm3 elution chamber volume, and the temperature of the upper, lower, and lower buffer flow rates were all 1 ml/min. Ethylene glycol coolant was maintained at 0°C with a recirculating bath. Oxygenase from the AH-Sepharose 4B column was mixed with sucrose to 30% and bromphenol blue was added as a tracking dye. This mixture was overlayed onto the spacer gel and a constant current of 50 mA was applied. The 2-μg-protein band in the resolving gel, the current was increased to 65 mA.

Analytical Polyacrylamide Gel Electrophoresis—The methods of Davis (10) were followed for analytical polyacrylamide gel electrophoresis. Gels containing 7.5% acrylamide, pH 8.9, were electrophoresed at 2-6°C at a constant current of 2 mA/gel. Electrophoresis in the presence of sodium dodecyl sulfate was performed according to the procedures of Weber and Osborn (11). All gels were stained for protein with Cooomassie brilliant blue (11).

Sedimentation Equilibriums and Molecular Weight Measurements—The meniscus depletion or high speed sedimentation equilibrium methods as outlined by Chervenka (12) was employed. Measurements were made at 22,000 rpm in a Beckman model E analytical ultracentrifuge equipped with Rayleigh interference optics. Centrifugation was performed with an An-H rotor at 10.8°C and a protein concentration of 0.6 mg/ml.

Molecular weight was also determined by gel filtration in a Sephadex G-200 column (2 × 54.5 cm) equilibrated with Buffer A. Instructions and protein standards were provided by a Pharmacia Calibration Kit.

Subunit molecular weight was estimated by the sodium dodecyl sulfate-gel electrophoresis methods of Weber and Osborn (11). Gas-Liquid Chromatography and Mass Spectrumry—The end products in the substrate specificity experiments were qualitatively determined by gas-liquid chromatography. Assay mixtures contained 50 μmol of substrate, 5 μmol of NADPH, 135 μmol of potassium phosphate at pH 7.8, and enzyme in a total volume of 3 ml. Substrates and products were extracted in pentane and identified by gas-liquid chromatography with a Becker gas chromatograph model 417 equipped with flame ionization detector using a stainless steel column (14 feet × ½ inch) packed with 10% FFAP (Varian-Aerograph) coated on Chromosorb Q. Identification of the ester products in reactions with methyl ketones as substrates was achieved by comparison of retention times of products and authentic ester standards. Temperatures of the column for the various substrates were as follows: 2-tridecanone and isomers, 2-tetradecane, 2-dodecanone, 2-undecanone, 190°C; 2-decanone, 2-nonanone, 160°C; 2-undecanone, 2-heptanone, 140°C; 2-butane, 80°C. Esters from the oxygenase-catalyzed oxidation of isomers of tridecanone were identified indirectly. Reaction products were assayed by gas-liquid chromatography, hydrolyzed in 4% potassium hydroxide in methanol, and then rechromatographed. The primary alcohols, formed from hydrolysis of the esters, were identified by comparison of retention times of the alcohol and authentic standards. The formation of 5-valerolactone from the oxygenase catalyzed oxidation of cyclopetanone was verified by gas chromatography-mass spectrometry with a Finnegan model 3200 GC-MS equipped with a 10% SP-1000 column and operated at a programmed temperature of 90–220°C and an ionizing voltage of 70 eV.

Other Procedures—Absorption spectra of purified oxygenase were recorded on a Hitachi-Perkin-Elmer double-beam spectrophotometer. Flavin was extracted from purified oxygenase by 5% cold trichloroacetic acid treatment (13). The flavin concentration was determined by paper chromatography in two solvent systems. The first solvent system was composed of 1-butanol-acetic acid-H2O (12:3:5), and the second solvent system contained 5% Na2HPO4, and the second solvent system contained 5% Na2HPO4 in distilled water. Samples of authentic FMN, FAH, and riboflavin were chromatographed with the oxygenase flavin.

RESULTS

Oxygenase Stability and pH Optimum—A major problem encountered during fractionation of the monoxygenase was its instability. As a crude cell-free extract this problem was minimal. However, upon purification, the activity appeared to become increasingly labile. Attempts to stabilize the activity by addition of compounds are summarized in Table I. Samples used in this experiment were ammonium sulfate fractions of a crude extract and were not as sensitive to inactivation as more purified preparations. However, within 11 days, 95% of oxygenase activity was lost during incubation at 0°C in phosphate buffer. Ethanol, dithiothreitol, and EDTA, individually and in combination, stimulated and maintained high levels of oxygenase activity. For these reasons, buffers used during purification of the enzyme contained these compounds. Sensitivity of the enzyme to oxygen was indicated from the observation that higher activity was maintained when the oxygenase was stored under anaerobic conditions. Purified oxygenase, even in Buffer A, was relatively unstable.
Properties of 2-Tridecanone Monoxygenase

Table I

Effect of compounds on the stability of oxygenase activity

Oxygenase fraction was obtained from cell-free extract following streptomycin treatment and precipitation of activity with 40% saturation ammonium sulfate. Assays were performed with 0.75 mg of protein samples by methods described under “Experimental Procedures.” Incubations were at 0°C in 0.05 M phosphate, pH 7.5, plus the additive.

<table>
<thead>
<tr>
<th>Additive</th>
<th>Days incubation at 0°C</th>
<th>% original activity of untreated oxygenase</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1 2 3 5 7 11</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>100 65 46 28 5</td>
<td></td>
</tr>
<tr>
<td>10% Ethanol</td>
<td>73 79 61 40 N.D.</td>
<td></td>
</tr>
<tr>
<td>5% Ethanol</td>
<td>113 154 154 100 59</td>
<td></td>
</tr>
<tr>
<td>10% Acetone</td>
<td>59 16 10 10 N.D.</td>
<td></td>
</tr>
<tr>
<td>5% Acetone</td>
<td>97 87 68 22 31</td>
<td></td>
</tr>
<tr>
<td>10% Glycerol</td>
<td>98 77 68 40 N.D.</td>
<td></td>
</tr>
<tr>
<td>5% Glycerol</td>
<td>100 83 70 29 33</td>
<td></td>
</tr>
<tr>
<td>1 mM EDTA</td>
<td>154 159 149 151 110</td>
<td></td>
</tr>
<tr>
<td>1 mM Morepentoehanol</td>
<td>95 65 33 18 20</td>
<td></td>
</tr>
<tr>
<td>1 mM Glutathione</td>
<td>102 53 13 15 5</td>
<td></td>
</tr>
<tr>
<td>1 mM Dithiothreitol</td>
<td>164 185 115 100 95</td>
<td></td>
</tr>
<tr>
<td>5% Ethanol + 1 mM dithiothreitol</td>
<td>199 164 133 149 111</td>
<td></td>
</tr>
<tr>
<td>5% Ethanol + 1 mM dithiothreitol + 1 mM EDTA</td>
<td>195 215 215 202 190</td>
<td></td>
</tr>
<tr>
<td>1 mM 2-Butanone</td>
<td>99 72 59 33 28</td>
<td></td>
</tr>
<tr>
<td>Anaerobic (N₂ gas)</td>
<td>95 79 80 77 64</td>
<td></td>
</tr>
</tbody>
</table>

* N.D., not determined.

Table II

Purification of 2-tridecanone oxygenase

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume (ml)</th>
<th>Total protein (mg)</th>
<th>Total activity (units)</th>
<th>Specific activity (units/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract</td>
<td>730</td>
<td>14,600</td>
<td>1,131</td>
<td>0.078</td>
</tr>
<tr>
<td>Streptomycin sulfate supernatant (NH₄)₂SO₄ (45% saturation)</td>
<td>790 90</td>
<td>10,900 1,220</td>
<td>0.112</td>
<td></td>
</tr>
<tr>
<td>DEAE-cellulose</td>
<td>55 253</td>
<td>225 0.889</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AH-Sepharose 4B</td>
<td>278 82</td>
<td>174 2.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preparative polyacrylamide gel electrophoresis</td>
<td>82 46</td>
<td>67 1.45</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Sepharose 4B covalently bonded to 1,6-diaminohexane.

and activity would unavoidably be lost in 1 to 2 weeks when stored at 0–20°C. In phosphate buffer, the oxygenase exhibited a broad range of activity above pH 6.5. Optimum activity was observed at pH 7.8 to 8.0 and a gradual decline in activity above pH 8.0.

Enzyme Purification—A summary of steps used in purification of the oxygenase is presented in Table II. Analytical disc gel electrophoresis of purified oxygenase revealed two very closely migrating protein bands. It was concluded that the doublet did not represent contamination because the bands could not be separated by additional preparative electrophoresis, nor did analytical gel electrophoresis in another buffer system (pH 7.5) result in separation of the bands. Also, sodium dodecyl sulfate-polyacrylamide gel electrophoresis and sedimentation equilibrium data are consistent with a homogenous protein. The small increase in fold purification from crude extracts, although due in part to inactivation, indicates that the oxygenase represents a large portion of cellular protein as would be expected for an oxygenase catalyzing the first step in catabolic pathway.

Stoichiometry—The stoichiometry of the oxygenase-catalyzed conversion of 2-tridecanone to undecyl acetate is shown in Table III. The substrates and products are presented in the equation at the top of the table. From these results, it can be concluded that for every mole of undecyl acetate formed, there was 1 mol of NADPH oxidized and 1 mol of oxygen consumed. It was noted that 2-tridecanone oxygenase has an absolute requirement for NADPH. NADH, by itself or with NADP, will not substitute in the reaction.

Molecular Weight and Subunit Composition—From sedimentation equilibrium data, a molecular weight of 123,000 was calculated when 0.74 was assumed as the partial specific volume of the protein. With a calibrated Sephadex G-200 column, a molecular weight of 108,600 was calculated.

Subunit molecular weight, as determined by SDS-polyacrylamide gel electrophoresis, was 55,000 indicating that the oxygenase was composed of two equal subunits.

Flavin Cofactor—Oxygenase activity was always found in yellow fractions suggestive of flavoprotein. The participation...
Properties of 2-Tridecanone Monoxygenase

The specificity of 2-tridecanone monoxygenase for isomers of tridecanone is summarized in Table V. Activity was observed with all isomers, and there was no apparent decrease in oxygen uptake in the presence of catalase as would be expected with substrate analogs. The esters from oxidation of 5-, 4-, and 5-tridecanone were formed by insertion of an oxygen atom on the side of the carbonyl group with the longest alkyl group. However, with 6-tridecanone the detection of two esters indicated that the oxygen atom was inserted on either side of the carbonyl group. 1′-Tridecanone is a symmetrical molecule and insertion of oxygen on either side of the carbonyl group would result in the same ester. It is interesting to note that there was greater activity with 5-, 6-, and 7-tridecanone than with 2-tridecanone, the substrate used for growth of Pseudomonas cepacia.

Investigations on the range of substrates for the oxygenase extended to cyclic ketones. Purified oxygenase with a specific activity of 0.92 μmol of O₂/min/mg of protein with 2-tridecanone was assayed with the substrates cyclopentanone and cyclohexanone. With cyclopentanone, oxygenase specific activity was 0.50 and 0.48, respectively, in the absence and presence of catalase. With cyclohexanone specific activity was only 0.07 without catalase and 0.06 with catalase present. The surprisingly high activity with cyclopentanone was further examined in gas-liquid chromatography assays to determine if the lactonization of this cyclic ketone to 5-valerolactone was catalyzed by the oxygenase. Chromatograms of the reaction products revealed a peak that eluted at the same retention time as authentic 5-valerolactone. The mass spectra of authentic 5-valerolactone and the product formed from the substrate cyclopentanone were identical thus proving that the oxygenase catalyzes a lactonization reaction.

The substrate specificity studies should be interpreted cautiously since all substrates were employed at the same concentration with no regard to solubility and the Kₘ value for the substrate tested. With the soluble substrates, 2-butanone and cyclopentanone, decreases in velocity with decreasing substrate concentration were observed, typical of Michaelis-Menten kinetics. However, with low concentrations of the insoluble substrates, reaction rates were the same as at high concentrations, and when the reaction had proceeded to substrate depletion, the activity came to an abrupt halt. Therefore, Kₘ values for 2-tridecanone and other insoluble ketones could not be calculated. Despite a large excess of substrate, it was questioned if maximum velocity, V_max, was attained, since it was observed that the addition of 10 mg/ml of bovine serum albumin to reaction mixtures would result in an increase in activity of purified oxygenase to 170% of the original activity. It is probable that the albumin was serving as an exogenous source of the flavin cofactor.
to "solubilize" the ketone or make it more available to the oxygenase, analogous to the reported function of serum albumins binding fatty acids and lipids for transport (15). By this reasoning, the observed higher (or lower) activities with other ketones may just be a function of the availability of the substrate to the oxygenase. Attempts to solubilize 2-tridecanone, analogous to the reported function of serum albumin "solubilize" the ketone or make it more available to the ketones may just be a function of the availability of the reasoning, the observed higher (or lower) activities with other mins binding fatty acids and lipids for transport (15). By this tially reversed by the addition of dithiothreitol. These find- with these observations, oxygenase activity was assayed in the presence of various cations. Zinc, cadmium, copper, and essential sulfhydryl groups on the oxygenase. In conjunction logically important since there was a 50-fold difference in K, values for NADPH (2.5 mM versus 0.047 mM) in the absence and presence, respectively, of saturating concentrations of cyclopentanone.

Effects of Inhibitors and Metal Cations—With the metal-complexing agents, azide, cyanide, EDTA, and o-phenanthroline, there was no inhibition of activity at concentrations of 0.01 M. The addition of the thiol-active reagents p-chloromercuribenzoate (1.6 × 10⁻¹ M) and N-ethylmaleimide (1.6 × 10⁻⁴ M) resulted in considerable inhibition of activity, indicative of essential sulfhydryl groups on the oxygenase. In conjunction with these observations, oxygenase activity was assayed in the presence of various cations. Zinc, cadmium, copper, and mercury at 10⁻⁴ M caused inhibition which could be substantially reversed by the addition of dithiothreitol. These find ings, as expected, indicated that the inhibitory action of these metals was due to mercaptide formation with essential sulfhydryl groups.

DISCUSSION

The formation of esters and lactones from linear and cyclic ketones by 2-tridecanone oxygenase is an unusual reaction but not without precedent. Analogous oxygen insertion reactions have been observed in the camphor-oxidizing pathway of Pseudomonas putida (16), the fungal conversion of progesterone to testosterone (17), and the oxidation of the aromatic methyl ketone, acetophenone, to phenyl acetate by an Arthrobacter species (18). Lactonization reactions have been demonstrated in the formation of ε-caprolactone from cyclohexanone by Nocardia globerula (19) and the conversion by cyclopentanone to 5-valerolactone by Pseudomonas N.C.I.B. 9872 (20). The oxygenases that catalyze the formation of these lactones have been purified and shown to be NADPH-specific flavoprotein monooxygenases designated EC 1.14.13.

In many respects, 2-tridecanone oxygenase closely resembles cyclopentanone oxygenase (22, 24). Both enzymes are from species of Pseudomonas; they catalyze the lactonization of cyclopentanone, have absolute requirements for NADPH, and have similar absorption spectra and subunit molecular weight.

2-Tridecanone monooxygenase can be classified as a mixed function oxidase (25), reduced pyridine nucleotide-linked monooxygenase (26), or external monooxygenase (27), depending on the nomenclature preference. The properties of 2-tridecanone monooxygenase indicate that it can be assigned to the groups of flavoprotein monooxygenases designated EC 1.14.13. The properties of many of these enzymes have been reviewed (14, 26). The majority of these oxygenases catalyze the hydroxylation of aromatic rings. 2-Tridecanone oxygenase is distinctively in that its substrate range encompasses aliphatic compounds and is not restricted to just cyclic or aromatic compounds. Greater activity with 5-, 6-, and 7-tridecanone than with 2-tridecanone, the ketone used to grow the organism, illustrates that the name ketone oxygenase might be a more descriptive term. This enzymatic versatility undoubtedly has a great physiological importance in the organism's ability to cope with a range of available nutrients. In a taxonomic study of the genus Pseudomonas, it was observed that strains of P. cepacia, formerly P. multivorans (28), were capable of utilizing 95 to 108 out of 146 organic compounds as sole carbon and energy sources (29). This unparalleled catalytic feat can be put into perspective if one reasons that the organism's nutritional adaptability is related to versatile enzymes such as the ketone oxygenase.

The conversion of ketones to esters or lactones by the oxygenase is the biological equivalent of the chemical Baeyer-Villiger reaction. Although the mechanism of the enzyme has yet to be determined, a theory by Keay and Hamilton (30) based on the oxenoid mechanism (31) offers a possible model for flavoenzyme catalyzed ester or lactone formation from ketones.

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L N Britton and A J Markovetz


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