Selective Oxygenation of Adamantanes and Other Substrates by *Beauveria sulfurescens*

Roy A. Johnson,* Milton E. Herr,[1a] Herbert C. Murray,[1b] Constance G. Chidester, and Fusen Han

Upjohn Laboratories, The Upjohn Company, Kalamazoo, Michigan 49001

Received July 14, 1992

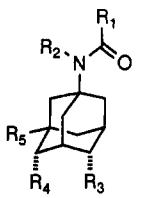
Oxygenation of *N*-(2-adamantyl)benzamide (**7**) with the fungus *Beauveria sulfurescens* (ATCC 7159) gave 4β-benzamidoadamantan-1-ol (**8**), whose structure was determined by X-ray crystallography. Oxygenation of *N*-methyl-*N*-(4-methyleneadamant-1-yl)benzamide (**9**) gave two products, the β-epoxide **10** and the 5-hydroxy β-epoxide **11**, the latter structure determined by X-ray crystallography. Oxygenation of 4-methylene-1-(*p*-toluenesulfonyl)piperidine (**12**) gave 4-hydroxy-4-(hydroxymethyl)-1-(*p*-toluenesulfonyl)piperidine (**14**) and of 4-piperidinoacetophenone (**13**) gave 4-(4-hydroxypiperidino)acetophenone (**15**).

Introduction

In previous reports from these laboratories, we have described the capability of microorganisms to oxygenate organic molecules with selectivities usually unattained with other oxidizing systems.[2] Among the many microorganisms capable of such oxygenations, we focused much attention on the fungus *Beauveria sulfurescens* (ATCC 7159)[3] because this culture is effective in the oxygenation of a wide variety of molecules and we are interested in determining if any structural–stereochemical patterns can be found in the oxygenation products produced by a specific organism. Information of the latter type is needed if we wish to attain a degree of predictability for the sites at which new substrates will be oxygenated by a particular microorganism. Our earlier observations relative to these points with *B. sulfurescens* have been summarized in a review.[2] Recently, Furstoss and co-workers have described the oxygenation of a series of cyclic and polycyclic amides by *B. sulfurescens*[4] and have also considered the questions of selectivity and predictability in oxygenations by this microorganism.[5] Very recently, Roberts and co-workers have examined the hydroxylation and epoxidation of several benzamidoazaspiranes with this microorganism.[6] We now wish to report the results from oxygenation of several additional compounds with *B. sulfurescens*. These results further demonstrate the effectiveness of this organism as a selective oxidizing agent and add information to the understanding of the oxygenation process. While these fermentations were done in the same time period as those of our earlier reports, structural assignments in several cases have been completed only recently as the result of X-ray crystallographic studies.

Results and Discussion

Among the many substrates effectively oxygenated by *B. sulfurescens*, results with the adamantane nucleus are illustrative of the selectivity of the oxygenation process. Oxygenation of *N*-acetyl-1-aminoadamantane (**1**) gave the monohydroxylated products **2** and **3** in 45% and 11% yields, respectively, while oxygenation of *N*-benzoyl-*N*-methyl-1-aminoadamantane (**4**) gave monohydroxylated **5** (15%) and dihydroxylated **6** (52%) as products.[7] The monohydroxy product **5** was shown to be an intermediate in the formation of **6**. The regiochemical selectivity in the oxygenation of these substrates is striking in that all the products except **3** have the hydroxyl groups in a trans configuration relative to the amido substituent. This propensity for trans-hydroxylation presumably reflects in some way the molecular architecture of the enzyme catalytic site and was further explored using adamantanes having a methyl group in either a cis or trans configuration at one of the sites of oxygenation.[8]



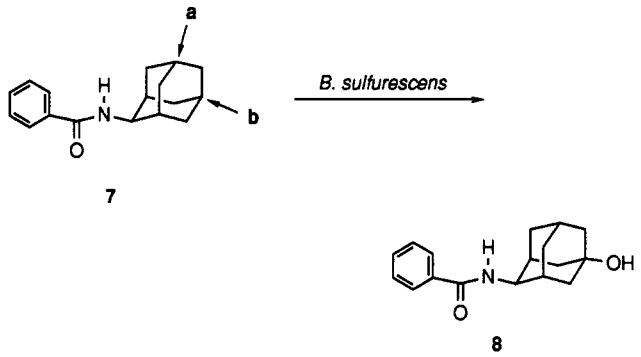
1, $R_1 = CH_3$; $R_2 = R_3 = R_4 = R_5 = H$
2, $R_1 = CH_3$; $R_3 = OH$; $R_2 = R_4 = R_5 = H$
3, $R_1 = CH_3$; $R_5 = OH$; $R_2 = R_3 = R_4 = H$
4, $R_1 = C_6H_5$; $R_2 = CH_3$; $R_3 = R_4 = R_5 = H$
5, $R_1 = C_6H_5$; $R_2 = CH_3$; $R_3 = OH$; $R_4 = R_5 = H$
6, $R_1 = C_6H_5$; $R_2 = CH_3$; $R_3 = R_4 = OH$; $R_5 = H$

Now we have tested this aspect of selectivity with the substrate *N*-benzoyl-2-aminoadamantane (**7**). The potential sites of oxygenation indicated by a and b in structure **7** are oriented cis and trans, respectively, relative to the benzamide group. If there is predictive value in the previous observations of trans hydroxylations, then we expected that hydroxylation of **7** should occur at site b in preference to site a. Fermentation of **7** with *B. sulfurescens* gave a single product (**8**) in 78% yield, one of the highest yields we have observed in an exploratory, nonoptimized fermentation. Elemental and spectral analyses indicated that fermentation had introduced one hydroxyl group into **8**. This hydroxyl group was shown to be on a tertiary carbon when it failed to undergo oxidation by Jones reagent. Determination of the structure of **8** by X-ray crystallography (see Experimental Section) shows that, consistent with expectation, the hydroxyl group has been introduced at site b.

The oxygenation of a variety of olefins by a number of different microorganisms has been reported over the

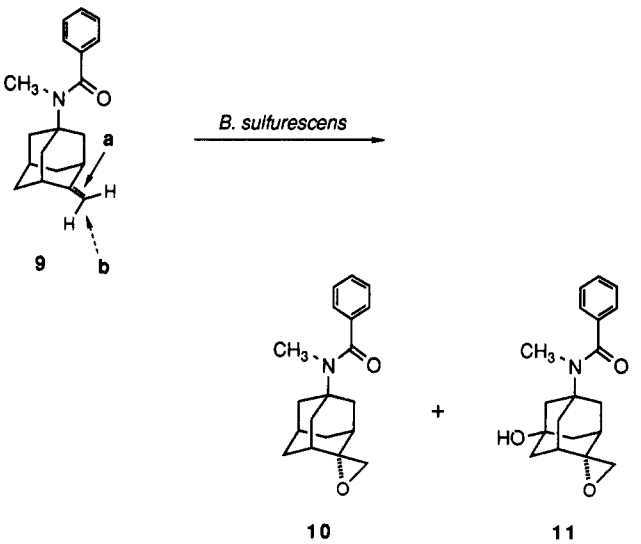
(1) (a) Retired July 31, 1973. (b) Retired January 31, 1974.
(2) For a summary and references to many of these results, see: Johnson, R. A. In *Oxidation in Organic Chemistry*; Trahanovsky, W. S., Ed.; Academic Press: New York, 1978; Part C, p 131.
(3) The classification of this microorganism was changed from *Sporotrichum sulfurescens to Beauveria sulfurescens*: Taylor, J. J. *Mycologia* **1970**, *62*, 797. The culture currently is listed in the catalog of the American Type Culture Collection as *Beauveria bassiana* (ATCC 7159).
(4) Vigne, B.; Archelas, A.; Furstoss, R. *Tetrahedron* **1991**, *47*, 1447 and references cited therein.
(5) Cf., Furstoss, R.; Archelas, A.; Fourneron, J. D.; Vigne, B. In *Enzymes as Catalysts in Organic Synthesis*; Schneider, M. P., Ed.: NATO ASI Ser.; D. Reidel: Norwell, MA, 1986; Vol. 178, p 361.
(6) Carruthers, W.; Prail, J. D.; Roberts, S. M.; Willetts, A. J. *J. Chem. Soc., Perkin Trans. 1* **1990**, 2854.
(7) Herr, M. E.; Johnson, R. A.; Murray, H. C.; Reineke, L. M.; Fonken, G. S. *J. Org. Chem.* **1968**, *33*, 3201.
(8) Herr, M. E.; Johnson, R. A.; Krueger, W. C.; Murray, H. C.; Pschigoda, L. M. *J. Org. Chem.* **1970**, *35*, 3607.

0022-3263/92/1957-7209$03.00/0



7 8

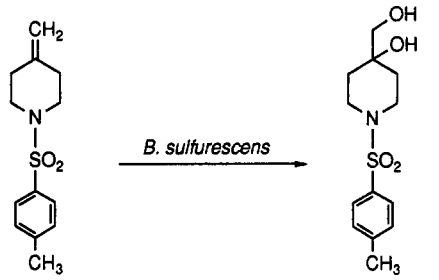
years.[2,9] The oxygenation process usually results in isolation of the epoxide or of a diol, the latter presumably arising from the hydrolytic opening of an intermediate epoxide. At the time the following fermentation was done, oxygenation of olefins by *B. sulfurescens* had not been reported; however, Roberts and co-workers have recently described epoxidation of an endocyclic double bond in an azaspirane by this microorganism.[6] Since the benzamidoadamantanes are good substrates for hydroxylation with *B. sulfurescens*, we sought to introduce a double bond into an adamantane at a site known to undergo hydroxylation. A compound (**9**) satisfying this criterion had been prepared in the course of another series of experiments[8] and was available for use as a substrate as well.



9 10 11

Fermentation of 2 g of **9** in 10 L yielded two new crystalline compounds, a major product (**11**) in 26% yield and a minor product (**10**) in 5% yield. In a larger 125-L fermentation of 27 g of **9**, only the major product (**11**) was isolated and now in 50% yield. Spectral and analytical data for the major product indicated that two atoms of oxygen were added to substrate **9** to form **11** and that the double bond of **9** had undergone a transformation, most likely to an epoxide. Assuming epoxide formation, the question of face selectivity in the enzymatic delivery of oxygen to the olefin becomes of interest. If the same enzyme catalyzes both hydroxylation and epoxidation, then the factors leading to a preference for trans selectivity in hydroxylation may also influence the epoxidation stereochemistry and should give delivery of oxygen from direction b in preference to a as shown for structure **9**. Although spectral data indicated that **11** was a hydroxy–epoxide product, the configurations of these groups were unknown until the recent completion of an X-ray crystallographic structure determination. The structure determined in this way (see Experimental Section) is as shown in the drawing for **11** and shows that enzymatic delivery of oxygen to the olefin has occurred at the "b" face of the olefin, a direction which is opposite, or trans, from the benzamido substituent.

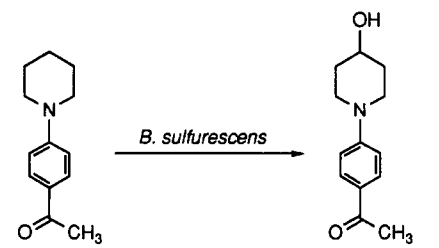
Finally, we report the results of oxygenation of two piperidine derivatives, 4-methylene-1-(*p*-toluenesulfonyl)piperidine (**12**)[10] and 4-piperidinoacetophenone



12 14

(**13**). Substrate **12** like **9** has an olefinic group at a position of the piperidine ring which from previous results[11] is known to be susceptible to enzymic oxygenation. Oxygenation of **12** with *B. sulfurescens* produced a new crystalline compound **14**, for which analytical data clearly showed the addition of two oxygen atoms in the form of hydroxyl groups. The ^{1}H NMR spectrum of the compound contained a signal at δ 3.42, which is appropriate for the hydroxymethyl protons, and further lacked any signal that could be assigned to a tertiary C-4 proton on the piperidine ring. This spectrum is consistent with the assignment of the hydroxyl groups to the positions shown for structure **14**. We suggest that the diol **14** has been derived from an epoxide intermediate. This suggestion is based on the fact that GC analysis of the crude fermentation extract revealed a small peak having the same retention time as that of an authentic sample of an epoxide prepared by epoxidation of **12** with *m*-chloroperbenzoic acid. We did not, however, isolate this epoxide from the fermentation extract since it apparently was present only in small quantity.

The piperidine **13** was examined as a potential substrate because the structure departs slightly from that of an amide derivative. Compound **13** is structurally similar



13 15

to a vinylogous amide and presumably has a considerably

(9) Cf. (a) Bloom, B. M.; Shull, G. M. *J. Am. Chem. Soc* **1955**, *77*, 5767. (b) White, R. F.; Birnbaum, J.; Meyer, R. T.; ten Broeke, J.; Chemerda, J. M.; Demain, A. L. *Appl. Microbiol.* **1971**, *22*, 55. (c) May, S. W.; Schwartz, R. D. *J. Am. Chem. Soc.* **1974**, *96*, 4031. (d) Abraham, W. A.; Hoffmann, H. M. R.; Kieslich, K.; Reng, G.; Stumpf, B. In *Enzymes in Organic Synthesis*; Pitman: London, **1985**; p 146. (e) Takahashi, O.; Umezawa, J.; Furuhashi, K.; Takagi, M. *Tetrahedron Lett.* **1989**, *30*, 1583. (f) Geary, P. J.; Pryce, R. J.; Roberts, S. M.; Ryback, G.; Winders, J. A. *J. Chem. Soc., Chem. Commun.* **1990**, 204. (g) Colbert, J. E.; Katopodis, A. G.; May, S. W. *J. Am. Chem. Soc.* **1990**, *112*, 3993. (h) Zhang, X. M.; Archelas, A.; Furstoss, R. *J. Org. Chem.* **1991**, *56*, 3814.

(10) Brennersin, P.; Grob, C. A.; Jackson, R. A.; Ohta, M. *Helv. Chim. Acta* **1965**, *48*, 146.

(11) Johnson, R. A.; Herr, M. E.; Murray, H. C.; Fonken, G. S. *J. Org. Chem.* **1968**, *33*, 3187.

less basic nitrogen in comparison to that of piperidine. The compound 13 was oxygenated by *B. sulfurescens*, giving a monohydroxylated product 15 in 20% yield. The structure of the product was deduced from the NMR spectrum, and the melting point of the compound is consistent with that reported in the literature for a synthetic compound of the same structure.[12]

The regioselective functionalization of these molecules satisfies the primary objective of these experiments. In addition, we have discussed the patterns of oxygenation in these new products with respect to previous results described for this microorganism. We always attempt to examine new results in the context of a model proposed some time ago by Fonken to account for the selectivity of microbial oxidations.[13] One key element of this model is that there is an interaction between a nucleophilic atom or functional group of the substrate and an electrophilic locus within the enzyme active site which provides orientation to the substrate as it undergoes oxygenation. In recent years the X-ray crystallographic elucidation of the structure of the camphor hydroxylating enzyme from *Pseudomonas putida* has provided a fascinating picture of the active site of this enzyme.[14] This work by Poulos and co-workers has included a structure of the enzyme with its active site occupied by a camphor molecule clearly showing the proximity of the camphor carbonyl oxygen atom to the hydroxyl group of tyrosine-96 of the enzyme.[14a,c] An interaction via hydrogen bonding between carbonyl oxygen and the Tyr-96 hydroxyl group has been suggested[14a] to play an important role in orienting the camphor so that only 5-*exo*-hydroxylation occurs. The similarity of this experimental observation to the hypothesis formulated by Fonken is striking even when considering that the observation is of a bacterium while the hypothesis was put forward for a fungus. We look forward to the time when the active site for the oxygenating enzyme of *B. sulfurescens* is elucidated to the same degree of molecular detail as is available for *P. putida*. If an electrophilic locus similar to Tyr-96 of *P. putida* is found in *B. sulfurescens*, interaction with the electron-rich amide carbonyl oxygen of substrates such as 7, 9, and 12 may play a role in the regioselective oxygenation of these molecules.

Experimental Section

Oxygenation of *N*-(2-Adamantyl)benzamide (7). *trans*-*N*-Benzoyl-4-aminoadamantan-1-ol (8). Using previously described procedures and conditions,[11] a 125-L fermentation of *N*-(2-adamantyl)benzamide (7, 25.0 g, 0.098 mol) with *B. sulfurescens* (ATCC 7159)[3] was allowed to proceed for 72 h. The mycelia were separated from the beer by filtration through Celite, and the beer was extracted with CH_2Cl_2. After concentration of the CH_2Cl_2 extract, the residual material was dissolved in acetone, decolorized with activated charcoal (10 g), and allowed to crystallize giving a first crop (19.65 g) of colorless crystals, mp 208–209 °C. A second crop of 1.404 g (total 21.00 g, 0.077 mol, 78%) of crystals was collected. Recrystallization of a sample of the first crop from acetone–Skellysolve B gave colorless crystals of 8: mp 208–209 °C; IR (mull) 3400, 1645, 1610, 1585, 1545, and 1130 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.77 (m, 2 H, aromatic H), 7.49 (m, 3 H, aromatic H), 4.23 (m, 1 H, C-4 proton), 2.26, 2.20, 1.98, 1.94, 1.83, 1.77, 1.64, 1.61, 1.56, 1.50 (all these peaks are in a complex series of signals for the adamantane moiety). Anal. Calcd for $C_{17}H_{21}NO_2$: C, 75.24; H, 7.80; N, 5.16. Found: C, 75.23; H, 8.13; N, 5.26.

Oxygenation of *N*-Methyl-4-methylene-*N*-(1-adamantyl)benzamide (*N*-Methyl-4-methylene-*N*-tricyclo[3.3.1.1^{3,7}]dec-1-ylbenzamide) (9). 7′-(*N*-Benzoyl-*N*-methylamino)spiro[oxirane-2,2′-tricyclo[3.3.1.1^{3,7}]decane] (10) and 7′-(*N*-Benzoyl-*N*-methylamino)-5′-hydroxyspiro[oxirane-2,2′-tricyclo[3.3.1.1^{3,7}]decane] (11). The concentrated CH_2Cl_2 extracts from a 10-L fermentation of 9 (2.0 g, 0.0071 mol) with *B. sulfurescens* (ATCC 7159) were chromatographed over Florisil (200 g) using gradient elution with increasing proportions of acetone in Skellysolve B and collecting fractions of 75 mL volume. Fractions 11–13 were pooled and contained 0.12 g (0.000 40 mol, 5%) of a less polar crystalline product. Recrystallization of this material from CH_2Cl_2–SSB gave colorless crystals of 10: mp 116–117 °C; ^1H NMR ($CDCl_3$, δ) 7.39 (m, 5 H, aromatic H), 2.88 (s, 3 H, NCH_3), 2.73 (s, 2 H, oxiranyl $-CH_2-$). Anal. Calcd for $C_{19}H_{23}NO_2$: C, 76.73; H, 7.80; N, 4.71. Found: C, 76.30; H, 7.77; N, 4.70.

Fractions 29–35 were pooled and contained 0.58 g (0.0018 mol, 26%) of a more polar crystalline product. Recrystallization of this material from CH_2Cl_2–ether gave colorless crystals (0.30 g) of 11: mp 137–138 °C; ^1H NMR ($CDCl_3$, δ) 7.40 (m, 5 H, aromatic H), 2.90 (s, 3 H, NCH_3), 2.78 (s, 2 H, oxiranyl $-CH_2-$). Anal. Calcd for $C_{19}H_{23}NO_3$: C, 72.82; H, 7.40; N, 4.47. Found: C, 72.33; H, 7.48; N, 4.37.

From a 125-L fermentation of 9 (27.0 g, 0.0960 mol) was isolated following chromatography and crystallization from CH_2Cl_2–ether 15.18 g (0.0485 mol, 50%) of 11, mp 135–137 °C.

Oxygenation of 4-Methylene-1-(*p*-toluenesulfonyl)-piperidine (12). 4-Hydroxy-4-(hydroxymethyl)-1-(*p*-toluenesulfonyl)piperidine (14). The CH_2Cl_2 extracts from a 10-L fermentation of 12 (2.0 g, 0.079 mol) with *B. sulfurescens* were filtered and chromatographed over Florisil (200 g) using gradient elution (4 L, 100% SSB to 50% acetone–SSB) and collecting 90-mL fractions. Fractions 31–37 were pooled and gave a crystalline solid (0.50 g). Recrystallization from acetone–pentane gave 0.46 g (0.0161 mol, 20%) of 14 as colorless crystals: mp 118–120 °C; ^1H NMR ($CDCl_3$, δ) 7.64 (d, 2 H, aromatic H), 7.32 (d, 2 H, aromatic H), 3.57 (d, 2 H, equatorial C-2 and C-6 protons), 3.42 (s, 2 H, $-CH_2O-$), 2.66 (t of d, 2 H, axial C-2 and C-6 protons), 2.44 (s, 3 H, $-CH_3$), 1.66 (m, 4 H, $-CH_2-$). Anal. Calcd for $C_{13}H_{19}NO_4S$: C, 54.71; H, 6.71; N, 4.91; S, 11.24. Found: C, 54.92; H, 6.31; N, 4.91; S, 11.04.

Oxygenation of 4-Piperidinoacetophenone (13). 4-(4′-Hydroxypiperidino)acetophenone (15). The CH_2Cl_2 extracts from oxygenation of 13 (25.0 g, 0.123 mol) with *B. sulfurescens* in a 72-h fermentation were chromatographed over Florisil (10.5- x 50-cm column) packed with SSB. The column was eluted with 10% acetone–SSB, and fractions of 2-L volume were collected. Fractions 15–19 were combined in acetone, decolorized with activated charcoal, filtered, and crystallized from acetone–SSB, giving 5.546 g (0.0253 mol, 20%) of crystalline product, mp 122–125 °C. Three recrystallizations from acetone–SSB gave 15 as colorless crystals: mp 127–129 °C (lit.[12] mp 127–129 °C); IR (mull) 3400, 1645, 1600, 1290, 1225, 1200, 1110, 1075, and 830 cm^{-1}; ^1H NMR ($CDCl_3$, δ) 7.86 (d, 2 H, J = 9.0 Hz, aromatic H), 6.87 (d, 2 H, J = 9.0 Hz, aromatic H), 3.94 (7 lines, 1 H, –CHO–), 3.75 (d of t, 2 H, equatorial –NC*H*H–), 3.12 (seven lines, 2 H, axial –NC*H*H–), 2.52 (s, 3 H, $-CH_3$), 2.00 (m, 2 H, equatorial –C*H*H–), 1.64 (nine lines, 2 H, axial –C*H*H–); ^{13}C NMR ($CDCl_3$) δ 196.3, 153.5, 130.2 (2 C), 126.7, 113.1 (2 C), 67.1, 44.9 (2 C), 33.3 (2 C), 25.8. Anal. Calcd for $C_{13}H_{17}NO_2$: C, 71.20; H, 7.82; N, 6.39. Found: C, 71.17; H, 7.92; N, 6.69.

Crystal data for 8: $C_{17}H_{21}NO_2$, formula wt = 271.4; monoclinic; space group $P2_1/c$; Z = 4; a = 10.007 (1) Å, b = 13.137 (1) Å, c = 12.008 (3) Å, β = 114.37 (2)°, V = 1438.0 (3) Å^3; calculated density = 1.25 g cm^{-3}, absorption coefficient μ = 0.57 mm^{-1}. Intensity data were collected on a clear prism 0.3 × 0.6 × 0.6 mm mounted on a glass fiber on a Siemens $P\bar{1}$ diffractometer controlled by a Harris computer.

Crystal data for 11: $C_{19}H_{23}NO_3$; monoclinic; space group $C2/c$; Z = 8; cell parameters a = 26.339 (3) Å, b = 6.485 (1) Å, c = 21.071 (3) Å, β = 113.26 (1)°. Molecular weight = 313.4; calculated density = 1.25 g cm^{-3}, absorption coefficient μ = 0.57 mm^{-1}. Intensity data were collected on a clear, needle-shaped crystal (0.4 × 0.05 × 0.05 mm) mounted on a glass fiber on a Siemens

(12) Bader, H.; Hansen, A. R.; McCarty, F. J. *J. Org. Chem.* **1966**, *31*, 2321.

(13) Fonken, G. S.; Herr, M. E.; Murray, H. C.; Reineke, L. M. *J. Am. Chem. Soc.* **1967**, *89*, 672.

(14) (a) Poulos, T. L.; Finzel, B. C.; Gunsalus, I. C.; Wagner, G. C.; Kraut, J. *J. Biol. Chem.* **1985**, *260*, 16122. (b) Poulos, T. L.; Finzel, B. C.; Howard, A. J. *Biochemistry* **1986**, *25*, 5314. (c) Poulos, T. L.; Finzel, B. C.; Howard, A. J. *J. Mol. Biol.* **1987**, *195*, 687.

$P2_1$ X-ray diffractometer controlled by a Harris computer.

For both data collections, graphite-monochromatized Cu Kα radiation was used, (λ(Cu Kα) = 1.5418 Å), with $2\theta_{max} = 138°$. Intensity data were measured at low temperature ($T = 148$ (2)° K), using 4°/min (for 8) and 2°/min (for 11) $\theta/2\theta$ step scans with scan widths > 3.4°. Of 2530 unique reflections measured for 8, 2359 had intensities $>3\sigma$. 3081 unique reflections were measured for 11; 2213 intensities were $>3\sigma$. For each data collection, 10 reflections periodically monitored showed no trend toward deterioration, $\sigma^2(I)$ was approximated by $\sigma^2(I)$ from counting statistics + $(dI)^2$, where the coefficient d of I was calculated from the variations in intensities of the monitored reflections and was 0.02 for 8 and 0.03 for 11. Cell parameters were determined by least-squares fit of $K\alpha_1$ 2θ values ($\lambda K\alpha_1 = 1.5402$) for 25 high 2θ reflections.[15] An Lp correction appropriate for a monochromator with 50% perfect character was applied.

Structure Determination of 8. The structure was solved by direct methods, using MULTAN80.[16] Hydrogens were all found in a difference map. Least-squares refinement included coordinates for all atoms and anisotropic thermal parameters for non-hydrogen atoms. The function minimized in the refinement was $\Sigma w(F_o^2 - F_c^{*2})^2$, where weights w were $1/\sigma^2(F_o^2)$, and where F_c^* was as defined by Larson.[17] In the final refinement cycle, all shifts were $<0.2\sigma$. The final agreement index R was 0.049 for 2528 reflections, and the standard deviation of fit was 5.0.

Structure Determination of 11. The trial solution, all 23 non-hydrogen atoms, was obtained using MULTAN80.[16] Subsequent Fourier syntheses verified the structure. Hydrogens were generated using standard planar or tetrahedral geometry. Least-squares refinement included all coordinates, and anisotropic thermal parameters for non-hydrogen atoms and isotropic thermal parameters for 22 of 23 hydrogen atoms, except the one attached to the hydroxyl. The function minimized in the refinement was $\Sigma w(F_o^2 - F_c^2)^2$, where weights w were $1/\sigma^2(F_o^2)$. In the final refinement cycle, all shifts were $<0.50\sigma$ for non-hydrogen atoms, $<0.14\sigma$ for hydrogen atoms. The final agreement index R was 0.060, and the standard deviation of fit was 1.8.

For both structure determinations, atomic form factors were from Doyle and Turner,[18] and, for hydrogen, from Stewart, Davidson, and Simpson.[19] The CRYM system of computer programs was used.[20] The atomic coordinates are deposited at the Cambridge Crystallographic Data Centre.[21]

(15) Duchamp, D. J. *ACS Symp. Ser.* **1977**, No. 46, 98.
(16) Main, P.; Fiske, S. J.; Hull, S. E.; Lessinger, L.; Germain, G.; Declercq, J. P.; Woolfson, M. M. *MULTAN80. A System of Computer Programs for the Automatic Solution of Crystal Structures from X-ray Diffraction Data*; Universities of York, England, and Louvain, Belgium, 1980.
(17) Larson, A. C. *Acta Crystallogr.* **1967**, *23*, 664.
(18) Doyle, P. A.; Turner, P. S. *Acta Crystallogr.* **1968**, *A24*, 390.
(19) Stewart, R. F.; Davidson, E. R.; Simpson, W. T. *J. Chem. Phys.* **1965**, *42*, 3175.
(20) Duchamp, D. J. *CRYM*, a system of crystallographic programs; The Upjohn Company, Kalamazoo, MI, 1984.
(21) The coordinates can be obtained on request from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

Oxygenation of *N*-Cycloheptylbenzamides with *Beauveria sulfurescens*

Roy A. Johnson,* Milton E. Herr,[1a] Herbert C. Murray,[1b] William C. Krueger, Loraine M. Pschigoda, and David J. Duchamp

Upjohn Laboratories, The Upjohn Company, Kalamazoo, Michigan 49001

Received July 14, 1992

The oxygenation of several *N*-cycloheptylbenzamides by fermentation with the fungus *Beauveria sulfurescens* (ATCC 7159) has been studied. Structures have been determined by chemical and physical methods. Enantioselectivity was observed in the oxygenation process, and the absolute configurations of optically active compounds have been determined by circular dichroism spectroscopy, X-ray crystallography, and chemical correlation. Oxygenation of *N*-cycloheptylbenzamide (1) gave *N*-[(1*S*)-4-oxocycloheptyl]benzamide (2) and *N*-[(1*S*,4*S*)-4-hydroxycycloheptyl]benzamide (5). A reversal of enantioselectivity in the oxygenation process was observed with the substrate *N*-cycloheptyl-*N*-methylbenzamide (3), the primary product being *N*-methyl-*N*-[(1*R*)-4-oxocycloheptyl]benzamide (4). Chemical conversion of ketone 2 into *N*-methyl-*N*-[(1*S*)-4-oxocycloheptyl]benzamide (14) confirmed the enantiomeric relationship of the oxygenation products 2 and 4. Enantiomeric excesses of the oxygenation products were not determined since crystallization was used extensively in the purification of crude products.

Introduction

In their study of the oxygenation of alicyclic amides with the fungus *Beauveria sulfurescens*, Fonken and co-workers found that oxygenation of *N*-cycloheptylbenzamide 1 was stereoselective, giving *N*-(4-oxocycloheptyl)benzamide (2) with a rotation of +65°.[2] In contrast, oxygenation of *N*-cycloheptyl-*p*-toluenesulfonamide gave *N*-(4-oxocycloheptyl)-*p*-toluenesulfonamide which was optically inactive.[2] Additional experiments exploring various aspects of stereoselectivity in the oxygenation of cycloheptylamides were subsequently performed in our laboratories, but the intervention of other projects precluded completion of these experiments in reportable form. Now, the use of improved spectroscopic methods has allowed us to make several structural correlations and determinations necessary to complete this work. We report (a) additional details of the oxygenation of 1 as well as of several other closely related substrates; (b) that oxygenation of *N*-cycloheptyl-*N*-methylbenzamide (3) also is stereoselective but occurs primarily at the enantiomeric C-4 methylene group, giving a ketone (4) having an absolute configuration at C-1 opposite that of 2; and (c) the assignment of the absolute configurations of 2, 4, and other related structures.

(1) (a) Retired July 31, 1973. (b) Retired January 31, 1974.
(2) Fonken, G. S.; Herr, M. E.; Murray, H. C.; Reineke, L. M. *J. Org. Chem.* **1968**, *33*, 3182.

Results and Discussion

***N*-Cycloheptylbenzamides.** We repeated the fermentation of *N*-cycloheptylbenzamide (1) with *B. sulfurescens* and modified the isolation procedure by omitting the chromic acid oxidation used by Fonken and co-workers to convert all hydroxylic products to ketones before pu-

0022-3263/92/1957-7212$03.00/0