$P2_1$ 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*

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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.

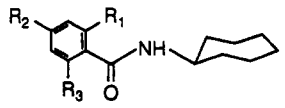
(1) (a) Retired July 31, 1973. (b) Retired January 31, 1974.
(2) Fonken, G. S.; Herr, M. E.; Murray, H. C.; Reineke, L. M. *J. Org. Chem.* **1968**, *33*, 3182.

Results and Discussion

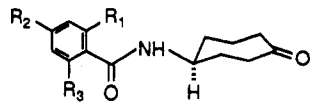
***N*-Cycloheptylbenzamides.** We repeated the fermentation of *N*-cycloheptylbenzamide (1) with *B. sulfurescens* and modified the isolation procedure by omitting the chromic acid oxidation used by Fonken and co-workers to convert all hydroxylic products to ketones before pu-

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

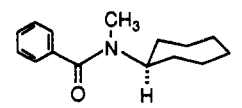
Chart I



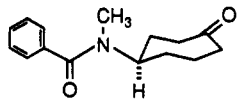
1, $R_1 = R_2 = R_3 = H$
6, $R_1 = CH_3$, $R_2 = R_3 = H$
10, $R_1 = R_2 = R_3 = CH_3$



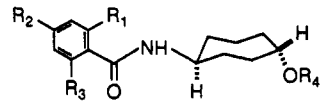
2, $R_1 = R_2 = R_3 = H$
7, $R_1 = CH_3$, $R_2 = R_3 = H$
11, $R_1 = R_2 = R_3 = CH_3$



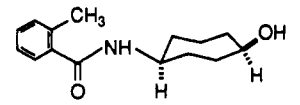
3



4



5, $R_1 = R_2 = R_3 = R_4 = H$
8, $R_1 = CH_3$, $R_2 = R_3 = R_4 = H$
15, $R_1 = R_2 = R_3 = H$, $R_4 = COCH_2Br$



9

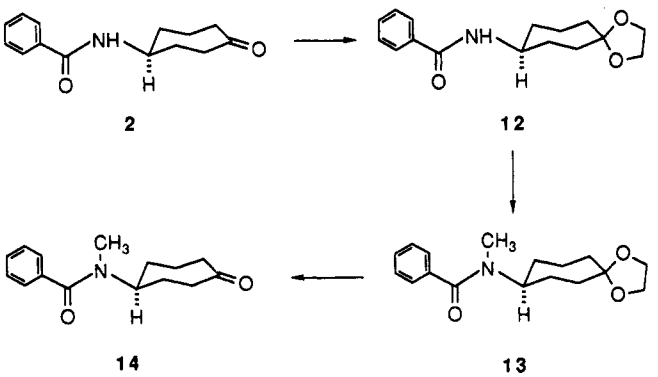
rification. In this way we obtained ketone **2** (14%), alcohol **5** (13%), and recovered substrate (18%) from fermentation of 25 g of **1**. From a second fermentation of the same size which proceeded to complete utilization of substrate, we obtained ketone **2** (38%) and alcohol **5** (15%). After three recrystallizations of each, the ketone **2** exhibited an optical rotation of +73° and the alcohol **5** a rotation of +25°. The ketone had spectral properties identical to those described for the product obtained previously from this fermentation.[2] To correlate the position of the hydroxyl group in **5** with that of the carbonyl group in **2**, a sample of alcohol **5** was oxidized to the ketone **2**. The ketone obtained in this way had $[\alpha]_D$ +72° after one crystallization, and its other spectral properties were identical to those of the ketone obtained directly from the fermentation. These results allow us to conclude that alcohol **5** and ketone **2** are derived from the preferential oxygenation of the same enantiogenic carbon in substrate **1**, but since we did not measure rotations of the unrecrystallized products we cannot determine the degree of enantiomeric selection in the oxygenation process.

Results similar to the above were observed in the oxygenation of *N*-cycloheptyl-2-methylbenzamide (**6**) with the exception that a second alcohol was obtained as a minor product. From this fermentation, we obtained ketone **7** (42%, $[\alpha]_D$ +38°), alcohol **8** (22%, $[\alpha]_D$ +15°), and the minor alcohol **9** (3%, 0°), the optical rotations in each case being measured after three recrystallizations. Both alcohols **8** and **9** were oxidized with Jones reagent (using first crystallization samples) and gave ketone **7** with $[\alpha]_D$ +65° and +73°, respectively. From comparison of the ^1H NMR spectra of alcohols **8** and **9** with the spectrum of alcohol **5**, it is clear that **8** and **5** have the same relative configurations at C-4, and therefore, the configuration of the minor alcohol **9** at C-4 must be epimeric with that of **8**.

From oxygenation of *N*-cycloheptyl-2,4,6-trimethylbenzamide (**10**), only ketone **11** was isolated in low yield (6%, $[\alpha]_D$ +47°).

***N*-Cycloheptyl-*N*-methylbenzamide.** This compound (**3**) was initially viewed as a minor structural variant of benzamide **1** and was examined as a substrate because of availability and a desire to extend the structural scope for this class of compounds. Preliminary examination of the fermentation extract suggested a close parallel to the results obtained with benzamides **1** and **6**. The extract contained three new products which tentatively were ascribed to a ketonic product and two hydroxylic products. Attempts to purify the two alcohols were unsuccessful, so the bulk of material was oxidized to ketone. Samples of ketone (total yield, 43%) isolated either directly from the fermentation or following chromic acid oxidation exhibited optical rotations of −28° to −44° after the initial crystallizations. The rotation of the ketone (using a sample with an initial $[\alpha]_D$ of −35°) was increased to −82° after five recrystallizations. The fact that the sign of rotation of this NCH_3 ketone (**4**) was negative while that of the NH ketone **2** was positive caught our attention. The chiroptical properties of these two ketones were further examined by circular dichroism (CD) spectroscopy, and as discussed in the following section, the CD spectra of the two ketones displayed Cotton effects of opposite signs.

Scheme I



2 **12** **14** **13**

The seemingly minor difference of NH vs NCH_3 amide in these two substrates appeared to have caused a reversal in enantioselectivity of oxygenation by *B. sulfurescens*. To confirm that this was the case, we carried out a chemical correlation of the two sets of compounds (see Scheme I). A sample of the NH amido–ketone **2**, $[\alpha]_D$ +65°, was first coverted to the ketal **12**, then methylated to give **13**, and finally deketalized to produce an NCH_3 amido–ketone (**14**) in an overall yield of 67%. The crystalline ketone **14** so obtained had an optical rotation of +84° which in comparison to the ketone **4** obtained from fermentation of **3** is of similar magnitude but is opposite in sign, confirming that the absolute configurations at C-1 in **14** and **4** are opposite.

The difference in enantioselectivity of *B. sulfurescens* for the two substrates was unexpected. In considering an explanation, we note that the conformational changes caused by the simple replacement of NH by NCH_3 in benzamides can be large. The phenyl rings in benzanilide, for example, are found in a trans configuration whereas in *N*-methylbenzanilide these groups are in a cis configu-

Table I. Chiroptical Data

compound	$[\alpha]_D$	CD,[a] nm (θ)
2	+73°	288 (6960)
4	-82°	288 (-6890)
7	+73°	288 (6550)
14	+84°	288 (7700)
(*R*)-(-)-4-Me-cycloheptanone[b]	-137°	281 (-10000)
14[c]	+95°	288 (7809)

[a] CD data in methanol. [b] Reference 5. [c] *N*-Methyl-*N*-(4-oxocycloheptyl)benzamide from physoperuvine, ref 6.

ration.[3] We see evidence in the ^1H NMR spectra of 1 and 3 for conformational differences of this type in these two amides. Only one signal at δ 4.12 is seen for the C-1 cycloheptyl proton in the spectrum of 1, suggesting a homogeneous magnetic environment and implying that rotation around the C-1 carbon–nitrogen bond is relatively unrestricted. The amide substituents of 1 are expected to be in a trans relationship to one another. In the spectrum of 3, two signals at δ 3.66 and 4.68 and in a ratio of 3:2 are seen for the C-1 cycloheptyl proton. Additionally, two signals are seen for the NCH_3 group in 3 at δ 2.96 and 2.79, also in the ratio of 3:2. Observation of pairs of signals, as seen for 3, usually is interpreted to be the result of two conformational populations of approximately equal energy levels so that a signal is seen for each conformation.[4] In the case of 3, the two most likely conformations are the cis-trans forms of the amide. The conformation in which phenyl and cycloheptyl groups are trans will be similar in shape to that of amide 1 while the conformation in which phenyl and methyl are trans will have a very different shape. One explanation for the difference in enantioselectivity of oxygenation is that the molecular shape of this latter conformation of 3 has a better fit in the catalytic site of the enzyme and as such presents the enantiomeric methylene group for oxygenation.

Absolute Configurations. The absolute configurations of the compounds described in this report have been assigned from the CD spectra of the ketones and confirmed by an X-ray crystallographic study of a suitable derivative of alcohol 5. The CD spectra of ketones 2, 4, 7, and 14 were measured, and the results are summarized in Table I. Ketones 2 and 7 obtained by fermentation both have positive n $\rightarrow \pi^*$ Cotton effects in their CD spectra while the NCH_3 ketone 4 from fermentation has a negative Cotton effect. Ketone 14, obtained by chemical modification of ketone 2, shows a positive Cotton effect. These results are consistent with the correlation described in the preceding section showing that enantiomeric ketones are obtained depending on whether the substrate is the NH or the NCH_3 amide. For the assignment of absolute configuration, these CD spectra may be compared with the CD spectrum of the model compound, (-)-(4*R*)-methylcycloheptanone, whose absolute configuration is known and which has a negative n $\rightarrow \pi^*$ Cotton effect.[5] We believe it reasonable to assume that the 4-benzamidocycloheptanones described in this report and 4-methylcycloheptanone have similar conformational populations, permitting assignment of the absolute configuration by comparison of their CD spectra. Accordingly, ketones 2, 7, and 14 are assigned the 1*S* configuration and ketone 4 is assigned the 1*R* configuration. A similar conclusion was drawn in assigning an absolute configuration to the *N*-(4-oxocycloheptyl)-*N*-methylbenzamide derived from the natural product physoperuvine.[6]

Determination of the relative configurations of amide and hydroxyl substituents in 5 as well as confirmation of the assignment of absolute configuration was obtained from an X-ray structure determination of a crystalline derivative of 5. The bromoacetate 15 of 5 was prepared, and crystals of this compound suitable for an X-ray crystallographic study were obtained. The structure obtained upon solution of the crystal data is represented by the drawing of 15 and confirms the 1*S* configuration. The relative configuration of the C-4 bromoacetoxy substituent is trans to the C-1 benzamido group and consequently may be assigned the 4*S* absolute configuration. From these results and the chemical interconversions presented in the preceding discussion, assignment of absolute configurations can be extended directly to compounds 2, 4, 5, and 14. Comparison of the high-field ^1H NMR spectrum of 5 with the spectra of 8 and 9 (see the Experimental Section) clearly show that 8 must have a trans relationship between amide and hydroxyl group, and therefore, 9 must have a cis relationship between these groups. The absolute configurations of 8 and 9 follow from their structural relationship to ketone 7, whose absolute configuration was determined by the CD spectroscopy summarized in Table I.

Experimental Section

Oxygenation of *N*-Cycloheptylbenzamide (1). *N*-[(1*S*,4*S*)-*trans*-4-Hydroxycycloheptyl]benzamide (5) and *N*-[(1*S*)-4-Oxocycloheptyl]benzamide (2). Part A. The concentrated CH_2Cl_2 extracts of the filtered beer from oxygenation of 1 (25.0 g, 0.115 mol) with *B. sulfurescens*[7] (ATCC 7159) were chromatographed over Florisil (10.5 × 50 cm) packed with Skellysolve B (SSB). Elution (2-L fractions) was with 10% acetone–SSB (9 fractions), 25% acetone–SSB (7 fractions), and 50% acetone–SSB (6 fractions). Starting material (1, 4.6 g) was recovered from fractions 5 and 6 (see part B for a second fermentation of 1 which consumed all of the substrate and gave a higher yield of products). Fractions 11–14 were combined in acetone, decolorized with activated charcoal, and crystallized from acetone–SSB to give a first crop (2.304 g), mp 138–144 °C, and a second crop (1.405 g, total 3.709 g, 0.0160 mol, 14%), mp 138–144 °C, of 2. Two additional recrystallizations of the first crop from acetone–SSB gave an analytical sample of 2: mp 146–148 °C; $[\alpha]_D$ +73° (*c* 0.453, $CHCl_3$); IR (mull) 3320, 1705, 1690, 1630, 1600, 1575, 1530 cm^{-1}; ^1H NMR ($CDCl_3$, δ) 7.75 (m, 2 H, aromatic), 7.51 (m, 1 H, aromatic), 7.41 (m, 2 H, aromatic), 6.38 (m, 1 H, NH), 4.16 (m, 1 H, NHC*H*), 2.40–2.69 (m, 4 H, CH_2COCH_2), 1.87–2.00 (m, 1 H), 1.61–1.85 (m, 2 H), 1.46–1.59 (m, 1 H); ^{13}C NMR ($CDCl_3$) 213.74, 166.58, 134.50, 131.56, 128.60, 126.86, 51.67, 43.49, 39.72, 36.25, 30.44, 20.92. Anal. Calcd for $C_{14}H_{17}NO_2$: C, 72.70; H, 7.41; N, 6.06. Found: C, 72.72; H, 7.36; N, 5.85.

Fractions 16–18 were combined in acetone, decolorized, and crystallized from acetone–SSB to give a first crop (2.902 g), mp 142–157 °C, and a second crop (0.618 g, total 3.520 g, 0.0151 mol, 13%), mp 114–118 °C, of 5. Two additional recrystallizations of the first crop gave an analytical sample of 5: mp 159–161°; $[\alpha]_D$ +25° (*c* 0.3178, CH_3OH); IR (mull) 3420, 3370, 3320, 1630, 1600, 1580, 1540, 1490 cm^{-1}; ^1H NMR ($CDCl_3$, δ) 7.73 (m, 2 H, aromatic),

(3) Yamaguchi, K.; Matsumura, G.; Kagechika, H.; Azumaya, I.; Ito, Y.; Itai, A.; Shudo, K. *J. Am. Chem. Soc.* **1991**, *113*, 5474.

(4) Cf.: (a) Ōki, M. *Applications of Dynamic NMR Spectroscopy to Organic Chemistry*; VCH Publishers, Inc.: Deerfield Beach, FL, 1985; pp 41–105. (b) Karuso, P.; Kessler, H.; Mierke, D. F. *J. Am. Chem. Soc.* **1990**, *112*, 9434.

(5) Djerassi, C.; Burrows, B. F.; Overberger, C. G.; Takekoshi, T.; Gutsche, C. D.; Chang, C. T. *J. Am. Chem. Soc.* **1963**, *85*, 949. Optical rotary dispersion data in this article were converted to CD data using relationships described in Crabbé, P. *Optical Rotary Dispersion and Circular Dichroism in Organic Chemistry*; Holden-Day: San Francisco, 1965; pp 13–19.

(6) Ray, A. B.; Oshima, Y.; Hikino, H.; Kabuto, C. *Heterocycles* **1982**, *19*, 1233.

(7) Previously known as *Sporotrichum sulfurescens*. For reclassification, see: Taylor, J. J. *Mycologia* **1970**, *62*, 797. This microorganism is currently listed in the catalog of the American Type Culture Collection as *Beauveria bassiana* (ATCC 7159).

7.49 (m, 1 H, aromatic), 7.42 (m, 2 H, aromatic), 4.17 (m, 1 H, NCH), 3.89 (m, 1 H, CHOH), 1.85–2.14 (m, 4 H), 1.38–1.78 (m, 6 H); ^{13}C NMR 166.30, 134.69, 131.17, 128.37, 126.62, 72.01, 50.52, 37.05, 34.81, 33.33, 29.64, 18.86. Anal. Calcd for $C_{14}H_{19}NO_2$: C, 72.07; H, 8.21; N, 6.00. Found: C, 72.22; H, 8.39; N, 5.80.

Part B. From a second 125-L fermentation of **1** (25.0 g, 0.115 mol) with *B. sulfurescens* there were obtained after chromatography and crystallization of appropriate fractions a first crop (4.505 g), mp 135–140 °C, and a second crop (1.872 g, total 6.377 g, 0.0276 mol, 24%) of ketone **2**. From later fractions there also were obtained as a crystalline 1:1 mixture a first crop (5.875 g), mp 121–123 °C, second crop (0.637 g), and third crop (1.241 g, total 7.753 g, 0.0334 mol, 29%) of ketone **2** and alcohol **5**. Recrystallization from acetone–SSB gave the 1:1 mixture of ketone and alcohol with unchanged mp, 121–123 °C.

***N*-[(1*S*)-4-Oxocycloheptyl]benzamide (2) from Oxidation of Alcohol 5.** A sample of **5** from first-crop crystals described in part A of the preceding experiment (0.610 g, 0.00262 mol) in an acetone solution (30 mL) was oxidized with Jones' reagent. The crystalline crude reaction product was recrystallized from acetone–SSB to give a first crop (0.448 g), mp 148–150 °C, and a second crop (0.065 g, total 0.513 mol, 84%), mp 145–147 °C, of **2**. The first crop was used for measurement of optical rotation, which was $[\alpha]_D$ +72° (*c* 1.061, $CHCl_3$).

***N*-Cycloheptyl-2-methylbenzamide (6).** A mixture of cycloheptylamine (56.6 g, 0.50 mol), 2-methylbenzoyl chloride (77.3 g, 0.50 mol), 50% aqueous NaOH (60 mL), and ice water was shaken vigorously in a large separatory funnel. The crude solid product was collected by filtration, air-dried, and crystallized from CH_3OH–H_2O to give a first crop (70.0 g) of **6**, mp 121–123 °C. Three additional recrystallizations from CH_3OH–H_2O gave an analytical sample of **6**: mp 125–127 °C; ^{1}H NMR ($CDCl_3$, δ) 7.27–7.33 (m, 2 H, aromatic), 7.16–7.21 (m, 2 H, aromatic), 4.14 (m, 1 H, NHC*H*), 2.43 (s, 3 H, CH_3), 2.00–2.08 (m, 2 H), 1.45–1.73 (m, 10 H). Anal. Calcd for $C_{15}H_{21}NO$: C, 77.88; H, 9.15; N, 6.05. Found: C, 77.65; H, 9.50; N, 6.26.

Oxygenation of *N*-Cycloheptyl-2-methylbenzamide (6). *N*-[(1*S*)-4-Oxocycloheptyl]-2-methylbenzamide (7), *N*-[(1*S*,4*S*)-4-Hydroxycycloheptyl]-2-methylbenzamide (8), and *N*-[(1*S*,4*R*)-4-Hydroxycycloheptyl]-2-methylbenzamide (9). The extracts from oxygenation of **6** (25.0 g, 0.108 mol) with *B. sulfurescens* were chromatographed on a column of Florisil (10.5 × 50 cm) which had been dry packed with SSB. Elution (2-L fractions) was with 10% acetone–SSB (7 fractions), 25% acetone–SSB (7 fractions), and with 50% acetone–SSB (5 fractions). TLC (20% acetone–$CHCl_3$) of the fractions showed fractions 10–13 to be mainly a single product, fraction 14 to be a mixture of two more polar products, and fractions 15–18 to be mainly the most polar of the latter products. Fractions 10–13 were combined in acetone, decolorized, and crystallized from acetone–SSB to give a first crop (7.910 g), mp 128–130 °C, and a second crop (3.370 g, total 11.280 g, 0.0460 mol, 42%), mp 121–124 °C, of crystals. Two recrystallizations from acetone–SSB gave **7** as shiny, colorless needles: mp 131–133 °C; $[\alpha]_D$ +38° (*c* 0.9912, $CHCl_3$); IR (mull) 3260, 3060, 1700, 1630, 1600, 1580, 1550, 1490 cm^{-1}; ^{1}H NMR ($CDCl_3$, δ) 7.26–7.32 (m, 2 H, aromatic), 7.15–7.21 (m, 2 H, aromatic), 4.10 (m, 1 H, NHC*H*), 2.35–2.68 (m, 4 H), 2.39 (s, 3 H, CH_3), 2.07–2.25 (m, 2 H), 1.82–1.97 (m, 1 H), 1.56–1.81 (m, 2 H), 1.39–1.53 (m, 1 H); ^{13}C NMR 213.68, 169.16, 136.41, 135.79, 130.94, 129.85, 126.58, 125.71, 51.46, 43.44, 39.68, 36.18, 30.36, 20.86, 19.67. Anal. Calcd for $C_{15}H_{19}NO_2$: C, 73.44; H, 7.81; N, 5.71. Found: C, 73.69; H, 7.96; N, 5.72.

Fraction 14 (crude weight, 2 g) was rechromatographed over silica gel packed in SSB and eluted with increasing proportions of acetone in SSB. Fractions of 335-mL volume were collected. Fractions 15–21 were combined in acetone and crystallized from acetone–SSB to give a first crop (0.580 g), mp 127–129 °C, and second crop (0.276 g, total 0.856 g, 0.00347 mol, 3%), mp 124–126 °C, of crystals. Two recrystallizations from acetone–SSB gave **9** as colorless needles: mp 126–129 °C; $[\alpha]_D$ 0°; IR (mull) 3420, 3240, 3060, 1640, 1620, 1595, 1550 cm^{-1}; ^{1}H NMR ($CDCl_3$, δ) 7.26–7.37 (m, 2 H, aromatic), 7.16–7.24 (m, 2 H, aromatic), 4.20 (m, 1 H, NHC*H*), 4.01 (m, 1 H, CHOH), 2.44 (s, 3 H, CH_3), 1.93–2.12 (m, 2 H), 1.58–1.92 (m, 4 H), 1.35–1.57 (m, 4 H). Anal. Calcd for $C_{15}H_{21}NO_2$: C, 72.84; H, 8.56; N, 5.66. Found: C, 73.07; H, 8.96; N, 6.12.

Fractions 15–18 of the first column were combined in acetone, decolorized, and crystallized from acetone–SSB to give 6.013 g (0.0243 mol, 22.5%) of the most polar product, mp 128–132 °C. Two recrystallizations from acetone–SSB gave **8** as long, colorless needles: mp 145–147 °C; $[\alpha]_D$ +15° (*c* 0.9634, $CHCl_3$); IR (mull) 3420, 3360, 3280, 1630, 1600, 1535 cm^{-1}; ^{1}H NMR ($CDCl_3$, δ) 7.26–7.33 (m, 2 H, aromatic), 7.13–7.22 (m, 2 H, aromatic), 4.12 (m, 1 H, NHC*H*), 3.83 (m, 1 H, CHOH), 2.40 (s, 3 H, CH_3), 1.78–2.11 (m, 5 H), 1.30–1.74 (m, 5 H). Anal. Calcd for $C_{15}H_{21}NO_2$: C, 72.84; H, 8.56; H, 5.66. Found: C, 72.87; H, 8.79; N, 5.60.

Oxidations of 8 and 9 to 7. A sample of alcohol **9** (first crop crystals, 0.333 g, 1.35 mmol) was oxidized with Jones' reagent. The crystalline crude product was recrystallized from acetone–SSB, giving 0.234 g (0.955 mmol, 70%) of **7** as crystals, mp 125–128 °C; $[\alpha]_D$ +65° (*c* 1.0370, $CHCl_3$). Likewise, a sample of alcohol **8** (first crop crystals, 0.752 g, 3.04 mmol) was oxidized with Jones' reagent, and after crystallization from acetone–SSB gave 0.652 g (2.66 mmole, 87%) of ketone **7**. Two recrystallizations from acetone–SSB gave colorless needles of **7**: mp 136–137 °C; $[\alpha]_D$ +73° (*c* 0.9038, $CHCl_3$); infrared spectra (mull) of the oxidation products are identical with the infrared spectrum of the ketone **7** isolated in the preceding experiment.

***N*-Cycloheptyl-2,4,6-trimethylbenzamide (10).** A mixture containing cycloheptylamine (slight excess), 2,4,6-trimethylbenzoyl chloride, 50% aqueous NaOH, and ice water was shaken vigorously in a large separatory funnel. The solid crude product was extracted with ether from the reaction mixture, and the ether solution was washed with dilute aqueous HCl to remove excess cycloheptylamine. The ether solution was washed with water, dried ($MgSO_4$), filtered, and concentrated. The residue was crystallized from CH_3OH–H_2O, giving colorless crystals, mp 164–168 °C. Two additional recrystallizations from CH_3OH–H_2O gave **10** as colorless needles: mp 165–166 °C; ^{1}H NMR ($CDCl_3$) δ 6.83 (s, 2 H, aromatic protons), 4.20 (m, 1 H, C-1 proton), 2.28 (m, 9 H, CH_3), 2.07 (m, 2 H), 1.7–1.4 (m, 10 H). Anal. Calcd for $C_{17}H_{25}NO$: C, 78.71; H, 9.72; N, 5.40. Found: C, 78.68; H, 9.61; N, 5.76.

Oxygenation of *N*-Cycloheptyl-2,4,6-trimethylbenzamide (10). *N*-[(1*S*)-4-Oxocycloheptyl]-2,4,6-trimethylbenzamide (11). The concentrated CH_2Cl_2 extracts of the filtered beer from the 125-L fermentation of **10** (25.0 g, 0.0965 mol) with *B. sulfurescens* were chromatographed over Florisil (10.5- × 50-cm column) packed with SSB. Elution (2-L fractions) was with 10% acetone–SSB (10 fractions), 20% acetone–SSB (8 fractions) and 30% acetone–SSB (6 fractions) and gave a single product in fractions 12–16 and a mixture of products in later fractions. Fractions 12–16 were pooled, dissolved in acetone, decolorized, and crystallized from acetone–SSB, giving crystals (1.723 g, 0.0063 mol, 6%), mp 156–158 °C, of **11**. Two recrystallizations from acetone–SSB gave shiny colorless needles of **11**: mp 158–159 °C; $[\alpha]_D$ +47° (*c* 0.885, $CHCl_3$); IR (mull) 3220, 3030, 1700, 1625, 1545, 1325 cm^{-1}; ^{1}H NMR ($CDCl_3$, δ) 6.82 (s, 2 H, aromatic), 4.19 (m, 1 H, NHC*H*), 2.35–2.69 (m, 4 H), 2.26 (s, 3 H, CH_3), 2.25 (s, 6 H, CH_3), 2.12–2.30 (m, 2 H), 1.73–1.97 (m, 2 H), 1.55–1.72 (m, 1 H), 1.37–1.50 (m, 1 H). Anal. Calcd for $C_{17}H_{23}NO_2$: C, 74.69; H, 8.48; N, 5.12. Found: C, 73.79; H, 8.67; N, 5.74.

Oxygenation of *N*-Cycloheptyl-*N*-methylbenzamide (3). *N*-Methyl-*N*-[(1*R*)-4-oxocycloheptyl]benzamide (4). A. 10-L Fermentation. The CH_2Cl_2 extract residue from fermentation of **3** (2.0 g, 0.00865 mol) with *B. sulfurescens* (10 L) was dissolved in acetone and oxidized with Jones' reagent. Following workup, the crude oxidation product was chromatographed over Florisil (200 g) by gradient elution with 6 L of solvent, SSB to 45% acetone in SSB. Fractions of 70-mL volume were collected, and the desired product was eluted in fractions 33–40. These fractions were pooled to give 0.73 g of product. Recrystallization from acetone–SSB gave a first crop (0.183 g), mp 119–121 °C, and a second crop (0.110 g), mp 120–123 °C, of ketone **4**: $[\alpha]_D$ −31° (*c* 0.9270, $CHCl_3$) on second crop crystals; IR (mull) 1700, 1620, 1600, 1580, 790, 710 cm^{-1}; ^{1}H NMR ($CDCl_3$, δ) 7.40 (br s, 5 H, aromatic), 4.66 (m, 0.65 H, N(Me)C*H*), 3.63 (m, 0.35 H, N(Me)C*H*), 2.98 (s, 1.2 H, NCH_3), 2.82 (s, 1.8 H, NCH_3). Anal. Calcd for $C_{15}H_{19}NO_2$: C, 73.44; H, 7.81; N, 5.71. Found: C, 73.43; H, 8.01; N, 5.49.

B. 125-L Fermentation. The CH_2Cl_2 extracts from fermentation of **3** (25.0 g, 0.108 mol) with *B. sulfurescens* (125 L) were divided into two equal portions. One part was chromato-

graphed directly and the other part was first oxidized with Jones' reagent and then chromatographed. Chromatography of the first, unoxidized part was done over Florisil (1 kg) using gradient elution from 100% SSB to 40% acetone in SSB and collecting fractions of 200-mL volume. Fractions 17–20 contained starting material **3** (1.0 g), fractions 32–42 contained ketone **4** (5.5 g), fractions 43–46 contained a mixture of ketone and alcohol (0.52 g), and fractions 47–52 (1.97 g), fractions 53–57 (3.48 g), and fractions 58–67 (0.40 g) contained a mixture of two alcohols in varying proportions. Fractions 32–42 were dissolved in acetone, decolorized, and crystallized from acetone–SSB to give 3.90 g of **4**, mp 121–124 °C, $[\alpha]_D$ −28° (*c* 0.7386, $CHCl_3$), with IR and NMR spectral properties identical to those of **4** isolated above in part A.

Fractions 47–52 were dissolved in acetone, decolorized, and crystallized from acetone–SSB, giving 0.780 g (0.00316 mol, 3%) of a crystalline alcohol: mp 82–90 °C; ^1H NMR ($CDCl_3$, δ) 7.38 (s, 5 H, aromatic), 4.64 (m, 0.48 H, N(Me)C*H*), 4.02 (m, 0.48 H, C*H*OH), 3.74 (m, 0.52 H, C*H*OH), 3.62 (m, 0.48 H, N(Me)C*H*), 2.97 (s, 1.56 H, NCH_3), 2.80 (s, 1.44 H, NCH_3). The filtrate was combined with fractions 53–67, and the combined material was oxidized with Jones' reagent to give, after crystallization from acetone–SSB, 1.29 g of **4**: mp 125–127 °C; $[\alpha]_D$ −44° (*c* 0.6986, $CHCl_3$).

The second part of the fermentation extract was oxidized with Jones' reagent in acetone. After workup, the crude product was chromatographed over Florisil (1 kg) by gradient elution, 100% SSB to 25% acetone in SSB, with fractions of 200-mL volume. Fractions 13–19 contained starting material **3** (0.98 g) and fractions 39–56 contained ketone **4** (8.75 g). Recrystallization of fractions 39–56 from acetone–SSB gave 6.20 g (total recrystallized ketone from fermentation and from oxidation of alcohols 11.39 g, 0.0465 mol, 43%) of **4**: mp 122–125 °C; $[\alpha]_D$ −35° (*c* 0.8980, $CHCl_3$); other spectral (IR and NMR) properties are identical to those of **4** described above. One gram of the 6.20-g sample was recrystallized five times from acetone–SSB to give colorless crystals of **4**: mp 133–135 °C; $[\alpha]_D$ −82° (*c* 0.5240, $CHCl_3$).

Preparation of *N*-Methyl-*N*-[(1*S*)-4-oxocycloheptyl]-benzamide (14) from *N*-[(1*S*)-4-Oxocycloheptyl]benzamide (2). A. Ketalization of 2. A mixture of **2** ($[\alpha]_D$ +65°, 2.31 g, 0.010 mol), benzene (75 mL), *p*-toluenesulfonic acid (0.175 g), and ethylene glycol (10 mL) was heated to reflux for 25 h in an apparatus that allowed the condensate to return to the reaction flask by passing through a water scavenging trap of calcium carbide granules. The cooled mixture was treated with pyridine (5 drops), diluted with benzene, washed with 5% $NaHCO_3$ solution, washed with H_2O, and dried over Na_2SO_4. The solution was filtered and the solvent removed under reduced pressure, leaving a residue which was lixiviated with ether to give ketal **12** (2.40 g): mp 134–142 °C; a single spot on TLC; IR (mull) 3050, no ketone carbonyl band, 1640, 1610, 1580 cm^{-1}.

B. Methylation of 12. A mixture of NaH (0.70 g of a 59% suspension in mineral oil) and dry xylene (10 mL) was stirred under N_2 and was treated with a hot solution of ketal **12** in xylene (40 mL). The resulting mixture was stirred and heated at reflux temperature for 19 h, cooled, treated with MeI (3.0 mL, 6.8 g), and then heated to reflux for 2.5 h under an acetone–dry ice condenser. The mixture was cooled, additional MeI (3.0 mL) was added, and heating was resumed for 3 h more. The cooled mixture was filtered, and the solid was washed with benzene. The combined filtrates were concentrated under reduced pressure, and the residue was lixiviated with ether to give **13** (2.30 g): mp 82–86 °C; IR (mull) no NH band, 1620, 800, 735 cm^{-1}.

C. Hydrolysis of Ketal 13. A solution of **13** (2.20 g) in ethanol (30 mL) and 12% aqueous HCl (6 mL) was stirred 5 min and then concentrated to dryness under reduced pressure. The solid residue was crystallized from acetone–SSB to give **14** (1.65 g, 0.0673 mol, 67% from **2**): mp 135–138 °C; $[\alpha]_D$ +84° (*c* 0.9796, $CHCl_3$). Anal. Calcd for $C_{15}H_{19}NO_2$: C, 73.44; H, 7.81; N, 5.71. Found: C, 73.66; H, 7.89; N, 5.71.

***N*-[(1*S*,4*S*)-4-Hydroxycycloheptyl]benzamide Bromoacetate (15).** A mixture of **5** (0.165 g, 0.708 mmol) in toluene (15 mL) containing pyridine (0.4 mL) was stirred, and bromoacetyl bromide (0.2 mL) was added. The resulting mixture was stirred at rt for 4 h, H_2O (25 mL) and ether (15 mL) were added, and this mixture was stirred overnight. The mixture was transferred to a separatory funnel with additional H_2O (25 mL) and ether (24 mL). The two layers were separated, and the organic layer was washed with 2 N aqueous HCl, saturated $NaHCO_3$, and H_2O and then was dried (Na_2SO_4), filtered, and concentrated to give a white solid (0.176 g). Crystallization of this solid from EtOAc–hexane gave 0.094 g of colorless crystals of **15**, mp 125–126 °C. A second crystallization of 0.071 g of this sample from EtOAc–hexane gave colorless crystals (0.040 g) of **15**: mp 129–130 °C; $[\alpha]_D$ +15° (*c* 2.97, EtOH); ^1H NMR ($CDCl_3$, δ) 7.74 (dt, 2 H, aromatic), 7.39–7.53 (m, 3 H, aromatic), 5.03 (m, 1 H, OC*H*CO), 4.18 (m, 1 H, NHC*H*), 3.82 (s, 2 H, COCH_2), 1.60–2.20 (m, 8 H), 1.40–1.55 (m, 2 H); ^{13}C NMR 166.68, 166.50, 134.73, 131.44, 128.58, 126.80, 76.40, 50.70, 43.61, 35.02, 33.23, 29.80, 29.33, 26.34, 19.24. Anal. Calcd for $C_{16}H_{20}BrNO_3$: C, 54.25; H, 5.69; N, 3.95. Found: C, 54.28; H, 5.80; N, 3.86.

X-ray Structure Determination of 15. $C_{16}H_{20}NO_3Br$, formula wt = 354.2; monoclinic; space group $P2_1$; $Z = 2$; $a = 9.773$ (1) Å, $b = 5.447$ (1) Å, $c = 15.790$ (2) Å, $\beta = 109.90$ (1)°, $V = 790.4$ (1) Å^3; calculated density = 1.49 g cm^{-3}, absorption coefficient $\mu = 3.39$ mm^{-1}. Intensity data were collected on a clear needle, 0.06 × 0.11 × 0.25 mm, mounted on a glass fiber on a Siemens *P*1 diffractometer.

Graphite monochromatized Cu Kα radiation was used, (λ(Cu Kα) = 1.5418 Å), with $2\theta_{max} = 145°$. Intensity data were measured at room temperature, using $\theta/2\theta$ scans with scan widths ≥3.2° and a variable scan rate (4–24 deg/min, depending on the intensity of the reflection being measured). The total time spent counting background, half at each end of the scan, was equal to the time spent scanning. Of the 1735 unique reflections measured, 1185 had intensities > 3σ. Ten reflections periodically monitored showed no trend towards deterioration; $\sigma^2(I)$ was approximated by $\sigma^2(I)$ from counting statistics + $(0.005I)^2$, where the coefficient of I was calculated from the variations in intensities of the monitored reflections. Cell parameters were determined by least squares fit of Kα_1 2θ values (λ(Kα_1) = 1.5402 Å) for 25 high 2θ reflections.[8] A polarization correction appropriate for a monochromator with 50% perfect character was applied, and the data were corrected for absorption.[9]

The structure was solved by direct methods, using DIREC.[10] Least squares refinement included coordinates for all atoms and anisotropic thermal parameters for non-hydrogen atoms. The function minimized in the refinement was $\sum w(F_o^2 - F_c^2)^2$, where weights w were $1/\sigma^2(F_o^2)$. In the final refinement anomalous dispersion factors[11] were included; in the final cycle all shifts were ≤0.3σ. The opposite enantiomer was also refined to convergence including anomalous dispersion factors. Starting from the final refinement of each enantiomer, a computer search was made for the 50 reflections most affected by anomalous dispersion, and the agreement index R was calculated for just these 50 reflections. R was 0.084 for the enantiomer we report and 0.110 for the other enantiomer, confirming that the enantiomer indicated by circular dichroism is the correct one. The final agreement index R was 0.053 for all 1735 reflections, and 0.036 for the 1185 reflections having $F_o^2 \geq 3\sigma$. The standard deviation of fit was 1.7. Atomic form factors were from *International Tables for X-ray Crystallography*,[12] and, for hydrogen, from Stewart, Davidson, and Simpson.[13] The CRYM system of computer programs was used.[10] The atomic coordinates are deposited at the Cambridge Crystallographic Data Centre.[14]

(8) Duchamp, D. J. *ACS Symp. Ser.* **1977**, No. 46, 98–121.
(9) Busing, W. R.; Levy, H. A. *Acta Crystallogr.* **1957**, *10*, 180.
(10) Duchamp, D. J. 1984, DIREC, a direct methods program for solving crystal structures, and *CRYM*, a system of crystallographic programs. The Upjohn Company, Kalamazoo, MI.
(11) Cromer, D. T. *Acta Crystallogr.* **1965**, *18*, 17.
(12) *International Tables for X-ray Crystallography*; Kynoch Press: Birmingham, 1962; Vol. III, pp 202–205.
(13) Stewart, R. F.; Davidson, E. R.; Simpson, W. T. *J. Chem. Phys.* **1965**, *42*, 3175–3187.
(14) The coordinates can be obtained on request from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.