Stereochemistry of the Microbiological Oxygenation of N-Acylcyclohexylamines[1]

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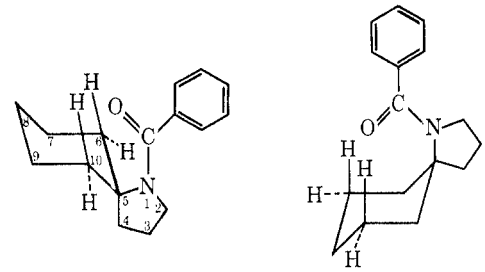
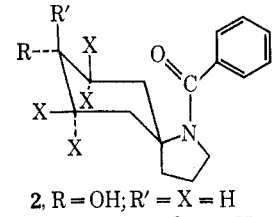
The structures of a series of products obtained from the oxygenation of various N-acylcyclohexylamines by the microorganism *Sporotrichum sulfurescens* have been determined by chemical and physical methods. Thus 1-benzoyl-1-azaspiro[4.5]decan-8-ol (**2**) has been obtained from oxygenation of 1-benzoyl-1-azaspiro[4.5]-decane; N-benzoyl-*trans*-2-methyl-4-oxocyclohexylamine (**7**) and N-benzoyl-*trans*-2-methyl-*trans*-4-hydroxycyclohexylamine (**8**) have been obtained from N-benzoyl-*trans*-2-methylcyclohexylamine; N-benzoyl-*cis*-2-methyl-*trans*-4-hydroxycyclohexylamine (**9**) from N-benzoyl-*cis*-2-methylcyclohexylamine; and (1*S*,2*S*,3*S*,5*R*,-6*R*)-N-(8-hydroxy-3-pinanyl)benzamide (**18**) and its epimer (**19**) from (+)-N-benzoylisopinocampheylamine and its (−) epimer, respectively. The preferred conformation of the 1-benzoyl-1-azaspiro[4.5]decanes has the nitrogen at position 1 in an equatorial configuration and the C-4 carbon in an axial configuration with respect to the cyclohexane ring. The configurations of the hydroxyl substituents found on cyclohexane rings above and in other examples, reported previously, have been determined to be equatorial in all cases, except for compound **9**. These results are consistent with the observation, made previously, that the microbial introduction of a hydroxyl group into cyclic substrates occurs preferentially from a direction opposite, or *trans*, to that of the amide substituent. Finally, equatorial C-4 configurations are assigned to the products of bioconversion of N-cyclohexyl-*p*-chlorobenzamide, N-cyclohexyl-*m*-chlorobenzamide, and N-cyclohexyl-N,N′-dibenzoyl-1,3-diaminopropane by analogy to the above results.

The 1,3- and 1,4-diequatorial relationships of hydroxyl group to amide group in several products of microbial oxygenation reactions has been noted previously.[2] A similar orientation of functional groups in opposite, or *trans*, directions was also seen in several bicyclic systems, which had been oxygenated by the mold *Sporotrichum sulfurescens*. These observations led to the suggestion that the introduction of the C—O bond by the microorganism will preferentially be in a direction opposite, or *trans*, to that of the C—N—C=O functional group.[2] The hydroxylation of several nonrigid N-acylcyclohexylamines has also been reported, but the stereochemistry of the products was not discussed.[3] We now wish to describe the oxygenation of several additional alkylcyclohexylamine derivatives by *S. sulfurescens* and to discuss the stereochemistry of the products.

Oxygenation of 1-benzoyl-1-azaspiro[4.5]decane (**1**) with *S. sulfurescens* gave a single monohydroxylated product (**2**) in 54% yield. Three aspects of the structure of **2** which must be determined are (1) the preferred conformation (**1a** or **1b**) of the spiro ring system, (2) the position of the hydroxyl group, and (3) the configuration of the hydroxyl group. Answers to the first two questions are found in the nmr spectra of alcohol **2**, of the ketone (**3**) obtained from oxidation of **2**, and of the tetradeuterio ketone (**4**) obtained from deuterium exchange with **3**. The nmr spectra of these three compounds are characterized by signals for four protons at 160–210 cps. Two of these protons must result from the downfield shift of the C-2 protons by neighboring nitrogen.[4] Examination of a Dreiding model of **1** suggests that the remaining two protons are the axial hydrogens at either C-6 and C-10 (conformation **1a**) or at C-7 and C-9 (conformation **1b**), which are deshielded by the amide carbonyl group and thereby shifted downfield. All four of the downfield protons under discussion remain in the spectra of **2**, **3**, and **4**, but become more sharply defined as the structure is increasingly modified. Thus an ill-defined multiplet at 172 cps in the spectrum of **1** becomes a six-line pattern (180 cps) in **2**, a four-line pattern (191 cps) in **3**, and a doublet (195 cps) in **4**. These results are consistent with assignment of conformation **1a** to these compounds. With regard to the position of the oxygen function, only three positions (C-3, C-7, and C-8) can accommodate a ketone group and still allow introduction of four adjacent deuterium atoms. The nmr data for the tetradeuterio ketone **4** are inconsistent with placement of the oxygen at C-3 or C-7, and therefore the oxygen function must be found at C-8 in these molecules. The assignment of configuration to the alcohol group in **2**, which has been determined to be equatorial with respect to conformation **1a**, is discussed in a later section of this report.



1a **1b**

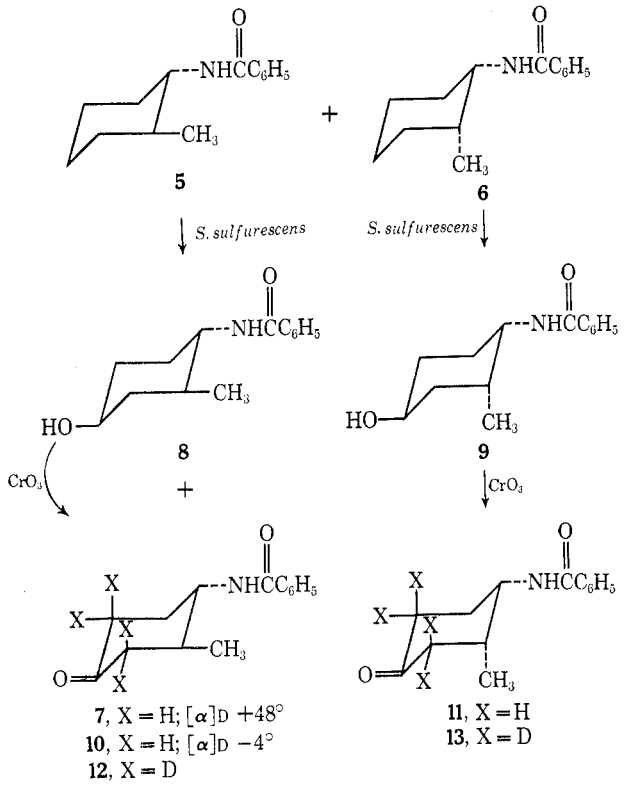


2, R = OH; R′ = X = H
3, R = R′ = =O; X = H
4, R = R′ = =O; X = D

The oxygenation of a mixture (*ca.* 1:1) of the *trans* and *cis* isomers (**5** and **6**, respectively) of N-benzoyl-2-methylcyclohexylamine was found to give a crystalline mixture of products in a yield greater than 50%. In addition, some starting material could be recovered and was found to be greatly enriched in the *trans* isomer. The mixture of products was partially separated into three components by chromatography. The

(1) Stereochemistry of Microbiological Hydroxylation. IV.
(2) R. A. Johnson, M. E. Herr, H. C. Murray, and G. S. Fonken, *J. Org. Chem.*, **33**, 3217 (1968).
(3) G. S. Fonken, M. E. Herr, H. C. Murray and L. M. Reineke, *ibid.*, **33**, 3182 (1968).
(4) *Cf.* R. A. Johnson, *ibid.*, **33**, 3627 (1968).

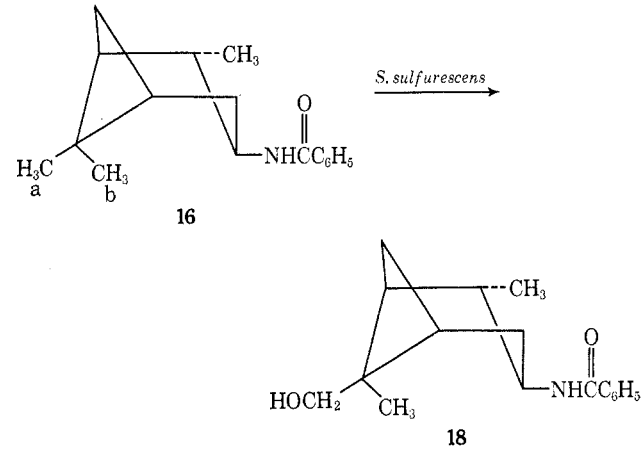
smallest and least polar component was identified as a ketone (**7**, $[\alpha]_D$ +48°) by its infrared spectrum. The two more polar components, **8**, mp 211–213°, and **9**, mp 183–185°, were both monohydroxy derivatives, as determined by infrared and elemental analyses. Although the analytical samples of the two alcohols showed no optical activity, the ketones **10** and **11**, derived by chemical oxidation from the crude samples of **8** and **9**, respectively, had low degrees of optical activity, suggesting that preparation of the analytical samples had resulted in the preferential crystallization of the racemates. Ketones **7** and **10** had nearly identical solid-phase infrared spectra and their nmr spectra were identical. The alcohol **8** was found to be spectrally



identical with the alcohol obtained from bioconversion of pure 1-benzoyl-*trans*-2-methylcyclohexylamine (**14**), as described in the following paragraph. These comparisons establish that compounds **7**, **8**, and **10** are derived from the *trans* isomer of the substrate and that they have been oxygenated at the same position. The positions of the ketone carbonyls in both **10** and **11** were determined to be at C-4 by examination of the nmr spectra of the tetradeuterio derivative of each (**12** and **13** from **10** and **11**, respectively). Only carbonyls at C-4 or C-5 of either ketone can undergo exchange of four adjacent protons. If the ketone were at C-5 in these compounds, the splitting of the C-1 proton would be greatly reduced in the deuterio derivative in comparison with the undeuterated compound. Since such a reduction in coupling is not observed for either ketone, we conclude that the oxygen function in both series of compounds is at the 4 position. It may be further concluded that ketone **11** and alcohol **9** are of the *cis* series, since ketone **10** and alcohol **8** have already been related to the *trans* series of compounds. The configuration of the hydroxyl group in both alcohols is discussed below.

A pure sample of *trans*-2-methylcyclohexylamine was prepared by the procedure of Rathke, Inoue, Varma, and Brown[5] and was converted into the benzamide (**14**) for use as substrate. A single hydroxylated product (**15**, $[\alpha]_D$ −12°) was obtained from bioconversion with *S. sulfurescens*. The position of the hydroxyl group in this product is at C-4, as shown by relationship to the alcohol **8** described above.

Two samples of the substrate N-benzoylisopinocampheylamine[5] were prepared, one (**16**) of which was enriched in the (+) isomer and the other (**17**) enriched in the (−) isomer. Oxygenation of either gave a product having the same structure but of opposite optical rotation. A considerably higher yield (62%) was obtained from **16** than from **17** (28%) under identical conditions. Although the starting materials, (−)- and (+)-α-pinene, used for the preparation of the substrate were not optically pure and it is unlikely that the products are optically pure, the fact that the absolute configuration of the α-pinenes has been determined[6] led us to assign absolute configurations to the predominant products of each bioconversion. These assignments are given in the Experimental Section and are shown below for compounds **16** and **18**. The position at which the



hydroxyl group had been introduced into these products was reduced to one of the geminal methyl groups by inspection of the nmr spectra. Of the two singlet methyl signals found at 73 and 63 cps in the substrate, one has been lost in the spectra of the products and has been replaced by a doublet (which collapses to a singlet upon addition of D_2O) at 213 cps. The remaining singlet methyl signal in the spectra of the products is found at 67 cps, and it is probable that this represents the signal found at 63 cps in the substrate spectrum. Several groups of investigators[7] have assigned the high-field signal in the spectra of pinane derivatives to the methyl group labeled b in formula **16** and the low-field signal to methyl group a. The above evidence suggests that methyl group a has been oxygenated in the present study. Such a result also is consistent with

(5) M. W. Rathke, N. Inoue, K. R. Varma, and H. C. Brown, *J. Amer. Chem. Soc.*, **88**, 2870 (1966).

(6) *Cf.* J. A. Mills and W. Klyne, "Progress in Stereochemistry," W. Klyne, Ed., Academic Press Inc., New York, N. Y., 1954, p 177.

(7) (a) R. L. Erskine and S. A. Knight, *Chem. Ind.* (London), 1160 (1960); (b) B. A. Arbuzov, Z. G. Isaeva, and Y. Y. Samitov, *Dokl. Akad. Nauk SSSR*, **137**, 296, 589 (1961); (c) "NMR Spectra Catalog," Vol. I, Varian Associates, 1962, Spectra No. 272, 274; (d) F. A. Bovey, "NMR Data Tables for Organic Compounds," Interscience Publishers, Inc., New York, N. Y., 1967, pp 286, 288; (e) J. M. Coxon, E. Dansted, M. P. Hartshorn, and K. E. Richards, *Tetrahedron Lett.*, 1149 (1969).

our expectation that, of the two geminal methyl groups, the one which is oriented away from the amide functional group would be the more likely to be oxygenated by *S. sulfurescens*.

We wished to determine the configuration of the hydroxyl groups of several of the above products, and also of several of those reported earlier,[3] to determine if our previously outlined generalization of a *trans* relationship between hydroxyl and amide group orientations is valid. To carry this out, we relied on two types of experiments to determine the hydroxyl configurations. First the ketones derived from the various alcohols were reduced with sodium borohydride. It is known[8] that sterically unhindered ketones will be reduced preferentially to the equatorial alcohols by this reagent. Secondly, the half band width of the carbinol proton was measured, since it is known[9] that an axial proton will have higher coupling constants (*ca.* 20-cps half band width) than will an equatorial proton (*ca.* 8 cps). To take advantage of the second method it was necessary to prepare the trichloromethylurethan derivatives of the alcohols in order to separate the signal of the carbinol proton from that of the proton on the amide-bearing carbon. The half band widths of the latter protons will also vary according to configuration. Reduction of ketones **3** and **10**, benzyl-4-oxocyclohexylcarbamate (**20**),[3] N-cyclohexyl-N-(4-oxo)cyclohexylacetamide (**21**),[3] and N-(4-oxo)-cyclohexylbenzamide (**22**)[3] with sodium borohydride gave, in every case, an alcohol which was identical with the bioconversion alcohol as the sole isolable product. Reduction of **11**, however, gave a noncrystalline product, which was assumed to be a mixture of two alcohols obtained from the reduction of the two possible conformers of the N-benzoyl-*cis*-2-methylcyclohexylamino ketone. The half band widths of the carbinol protons and of the –NCH protons of several bioconversion products are listed in Table I. They are consistent

TABLE I

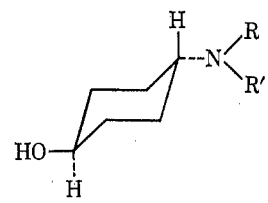
HALF BAND WIDTHS IN THE NMR SPECTRA OF BIOCONVERSION PRODUCTS AS THE TRICHLOROACETYLURETHAN DERIVATIVES

	Carbinol proton		CHN– proton	
Compd	Signal, cps	Half band width, cps	Signal, cps	Half band width, cps
1	296	19	...	...
8	284	21	217	24
9	307	12	261	16
23[a]	287	22	...	...
24[b]	282	20	208	21
30	280	19	...	...

[a] **23** is N-cyclohexyl-N-(4-hydroxy)cyclohexylacetamide, reported in ref 3. [b] **24** is benzyl-4-hydroxycyclohexylcarbamate, reported in ref 3.

with an axial configuration in every case except that of **9**, and therefore confirm that the hydroxyl groups introduced into these molecules by *S. sulfurescens* have equatorial configurations. In the case of compound **9**, the narrower signals suggest that the preferred conformation of the compound has an equatorial methyl group and axial hydroxyl and benzamido substituents. Hydroxylation of **6** to give **9** probably occurs in that conformation of **6** which has the methyl group axial and the amide group equatorial.

Finally, on the basis of the apparent preference for hydroxylation at the equatorial 4 position of N-acylcyclohexylamines, we wish to assign such structures to the products **28**, **29**, and **30** obtained from oxygenation



28, R = H; R′ = CO-*p*-ClC$_6$H$_4$
29, R = H; R′ = CO-*m*-ClC$_6$H$_4$
30, R = COC$_6$H$_5$; R′ = CH$_2$CH$_2$CH$_2$NHCOC$_6$H$_5$

of N-cyclohexyl-*p*-chlorobenzamide (**25**), N-cyclohexyl-*m*-chlorobenzamide (**26**), and N-cyclohexyl-N,N′-dibenzoyl-1,3-diaminopropane (**27**), respectively, with *S. sulfurescens*.

Experimental Section[10]

Biotransformation Process.—The culture used in these experiments was *Sporotrichum sulfurescens* V. Beyma (ATCC 7159). The biotransformation procedure has been described previously,[11] the only variation being that the dispersing agent Ultrawet DS-30 (2.5 ml/l.) was added to the fermentations of substrates **5**, **6**, **14**, **16**, **17**, **24**, **25**, and **26**. Addition of the Ultrawet DS-30 increases the yield of products obtained in these cases, and it is especially noteworthy that without the dispersing agent no products are obtained from substrates **24** and **25**.

Isolation of Products from the Microbiological Oxygenations.—A general procedure for the isolation of bioconversion products has been outlined previously[12] and followed here except as noted.

1-Benzoyl-1-azaspiro[4.5]decane (1).—The substrate **1** was prepared in the usual way from the amine and benzoyl chloride in pyridine. The analytical sample was recrystallized three times from Skellysolve B: mp 86–88°; $\nu_{C=O}$ in Nujol 1600 cm^{-1}; nmr (CDCl$_3$, 37°) 440 (s, C$_6$H$_5$), 200 (t, J = 6 cps, NCH$_2$–), and 172 Hz (broad signal integrating for two protons).

Anal. Calcd for C$_{16}$H$_{21}$NO: C, 78.97; H, 8.70; N, 5.76. Found: C, 78.73; H, 8.86; N, 5.66.

Bioconversion of 1-benzoyl-1-azaspiro[4.5]decane (**1**, 5.0 g, 0.0206 mol) gave 4.2 g of crude product in the middle 25% (v/v) acetone–Skellysolve B eluate fractions. Recrystallization gave 1.57 g of colorless crystals, mp 145–147°. A second crop (1.31 g, total 2.88 g, 0.0111 mol, 54%) of crystals, mp 142–143°, was obtained. Recrystallization gave **1-benzoyl-1-azaspiro[4.5]-decan-8-ol** (**2**) as colorless crystals: mp 143–145°; ν_{OH} 3450, $\nu_{C=O}$ 1600, and $\nu_{C=C}$ 1460 cm^{-1} in Nujol; nmr (CDCl$_3$, 37°) 441 (s, C$_6$H$_5$), 224 (m, –OCH–), 201 (t, J = 6 cps, NCH$_2$–), and 180 HZ (six-line pattern, $J_?$ = 14, 4 cps, axial H at C-6 and C-10).

Anal. Calcd for C$_{16}$H$_{21}$NO$_2$: C, 74.10; H, 8.16; N, 5.40. Found: C, 74.55; H, 8.35; N, 5.36.

1-Benzoyl-1-azaspiro[4.5]decan-8-one (3).—Oxidation of **2** (0.50 g, 0.00193 mol) with Jones reagent gave 0.45 g (0.00175 mol, 90%) of crude ketone. Two recrystallizations from acetone–Skellysolve B gave **3** as colorless crystals: mp 119–121°; $\nu_{C=O}$

(8) D. H. R. Barton, *J. Chem. Soc.*, 1027 (1953).

(9) R. U. Lemieux, R. K. Kullnig, H. J. Bernstein, and W. G. Schneider, *J. Amer. Chem. Soc.*, **80**, 6098 (1958).

(10) Melting points were determined on a Fisher-Johns hot stage and are corrected. Magnesium sulfate was used as the drying agent. Infrared spectra were recorded with either a Perkin-Elmer Infracord or Model 421 spectrophotometer. The nmr spectra were recorded at 60 Mcps with a Varian Model A-60A spectrometer, using tetramethylsilane as an internal standard. Trichloroacetylurethan derivatives of alcohols for determination of nmr spectra were prepared by the addition of a slight excess of trichloroacetylisocyanate to the solution of alcohol in the nmr sample tube. Mass spectra were determined on an Atlas CH4 instrument.

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

(12) R. A. Johnson, H. C. Murray, L. M. Reineke, and G. S. Fonken, *ibid.*, **34**, 2279 (1969).

1700 and 1600 cm^{-1}; in Nujol; nmr ($CDCl_3$, 37°) 442 (s, C_6H_5), 206 (t, J = 6.5 cps, NCH_2–), 191 (four-line pattern, J_{gem} = 12 cps, $J_?$ = 6 cps, axial H at C-6 and C-10), and 90–160 cps (10 protons).

Anal. Calcd for $C_{16}H_{19}NO_2$: C, 74.68; H, 7.44; N, 5.44. Found: C, 74.41; H, 7.35; N, 5.32.

1-Benzoyl-1-azaspiro[4.5]decan-8-one-$d_7d_7d_9d_9$ (4).—Sodium (0.024 g) was added to a solution of 1-benzoyl-1-azaspiro[4.5]-decan-8-one (**3**, 0.100 g) in methyl alcohol-*d* (15 ml). The resulting solution was kept at room temperature for 24 hr. Acetic acid-*d*, prepared from acetic anhydride (30 drops) and D_2O (27 drops), was added to the solution. Additional D_2O (2 ml) was added and the solution was concentrated under reduced pressure to remove methanol. Water and saturated sodium chloride solution was added to the aqueous residue and the mixture was extracted with methylene chloride (three 20-ml portions). The organic layer was dried and concentrated under reduced pressure, giving an oil which soon crystallized. Recrystallization from acetone–Skellysolve B gave colorless needles (0.076 g), mp 122–125°. A second recrystallization gave an analytical sample of **4**: mp 123–125°; $\nu_{C=O}$ 1710 and 1615 cm^{-1} in Nujol; nmr 443 (s, C_6H_5–), 207 (t, J = 6.5 cps, NCH_2–), 195 (d, J_{gem} = 12 cps, axial H at C-6 and C-10), and 90–140 cps (6 protons); mass spectrum m/e 261.

Anal. Calcd for $C_{16}H_{15}D_4NO_2$: C, 73.53; H, 8.87; N, 5.36. Found: C, 73.57; H, 8.88; N, 5.54.

Reduction of 1-Benzoyl-1 -azaspiro[4.5]decan-8-one (3) with Sodium Borohydride.—Reduction of **3** (1.00 g, 3.89 mmol) was carried out as described for reduction of **20**. Recrystallization from acetone–Skellysolve B gave 0.679 g (2.62 mmol, 67%) of **1-benzoyl-1-azaspiro[4.5]decan-8-ol (2)**, mp 141–143°; the ir spectrum of this product in Nujol was identical with the spectrum of the alcohol (**2**) obtained from bioconversion.

Bioconversion of the mixture of N-benzoyl-*trans*- (5) and -*cis*-2-methylcyclohexylamine (6, 25.0 g, 0.115 mol, *ca.* 50% of each component) gave 14.3 g of crude products following chromatography. A first crop (0.580 g) of **N-benzoyl-*trans*-2-methyl-4-oxocyclohexylamine** (**7**), mp 191–194°, was obtained. An additional 0.67 g of solid was obtained from the filtrate. Two recrystallizations from acetone–Skellysolve B gave **7** as long, fine, colorless needles: mp 206–208°; $[\alpha]_D$ +48° (*c* 0.547); ν_{NH} 3280, $\nu_{C=O}$ 1710 and 1640 cm^{-1} in Nujol. The nmr spectrum in $CDCl_3$ was identical with the spectrum of ketone **10**.

Anal. Calcd for $C_{14}H_{17}NO_2$: C, 72.70; H, 7.41; N, 6.06. Found: C, 72.63; H, 7.16; N, 6.26.

N-Benzoyl-*cis*-2-methyl-*trans*-4-hydroxycyclohexylamine (**9**) was obtained as colorless crystals, 3.421 g, mp 180–183°. Two recrystallizations gave **9** as colorless, fine needles: mp 183–185°; $[\alpha]_D$ 0°; $\nu_{OH,NH}$ 3420 and 3310, $\nu_{C=O}$ 1630, $\nu_{amide\ II}$ 1530 cm^{-1} in Nujol; nmr (DMF-d_7, 37°) 255 (m, NCH–), 232 (m, –OCH–), and 56 cps (d, J = 7 cps, $-CH_3$),

Anal. Calcd for $C_{14}H_{19}NO_2$: C, 72.07; H, 8.21; N, 6.00. Found: C, 72.18; H, 8.37; N, 5.87.

N-Benzoyl-*trans*-2-methyl-*trans*-4-hydroxycyclohexylamine (**8**) was obtained as colorless crystals, 1.220 g, mp 193–203°. Two recrystallizations gave **8**: mp 211–213°; $[\alpha]_D$ −1°; $\nu_{OH,NH}$ 3290, $\nu_{C=O}$ 1630, $\nu_{amide\ II}$ 1545 cm^{-1} in Nujol; nmr (DMF-*d*, 37°) 218 (m, –NCH–), 214 (m, –OCH–), and 57 cps (d, J = 6 cps, $-CH_3$).

Anal. Calcd for $C_{14}H_{19}NO_2$: C, 72.07; H, 8.21; N, 6.00. Found: C, 72.19; H, 8.73; N, 5.81.

An oxygenation of the mixture of isomers (2.0 g), which did not proceed to completion, gave recovery of starting material, mp 134–140°, which was greatly enriched in the *trans* isomer as determined by comparison of its infrared spectrum (Nujol) with those of the starting mixture, mp 116–119°, and of the pure *trans* isomer, described below. The alcohols, **9** (0.375 g, mp 181–182°) and **8** (0.200 g, mp 203–210°), were also isolated from this bioconversion.

N-Benzoyl-*trans*-2-methylcyclohexylamine (**14**).—The method of Rathke, *et al.*,[5] was used to prepare pure *trans*-2-methylcyclohexylamine from 1-methylcyclohexene. The amine was converted into the amide by the Schotten–Baumann method. The crude amide was a colorless, crystalline solid, mp 146–149° (lit.[5] mp 151.5–151.8°).

Bioconversion of N-benzoyl-*trans*-2-methylcyclohexylamine (**14**) (2.0 g, 0.22 mmol) gave 0.447 g (1.92 mmol, 21%) of crystals, mp 212–217°. Two recrystallizations gave **N-benzoyl-*trans*-2-methyl-*trans*-4-hydroxycyclohexylamine** (**15**) as colorless, fine needles: mp 213–215°; $[\alpha]_D$ −12° (*c* 0.676). The ir spectrum in Nujol and the nmr spectrum in DMF-d_7 were identical with those of alcohol **8** described above.

Anal. Calcd for $C_{14}H_{19}NO_2$: C, 72.07; H, 8.21; N, 6.00. Found: C, 71.97; H, 8.69; N, 5.90.

N-Benzoyl-*trans*-2-methyl-4-oxocyclohexylamine (**10**).—Oxidation of the crude alcohol **8**, mp 193–203° (0.780 g, 3.34 mmol), with Jones reagent gave 0.588 g (2.54 mmol, 76%) of colorless crystals, mp 185–187°. Two recrystallizations from acetone–Skellysolve B gave the analytical sample of **10** as colorless needles: mp 190–192°; $[\alpha]_D$ −4° (*c* 0.638); ir in Nujol very similar to the spectrum of the ketone **7** isolated from the bioconversion; ν_{NH} 3300, $\nu_{C=O}$ 1710 and 1635 cm^{-1} in Nujol; nmr ($CDCl_3$, 37°) 245 (m, NCH–) and 62 cps (d, J = 5.5 cps, $-CH_3$).

Anal. Calcd for $C_{14}H_{17}NO_2$: C, 72.70; H, 7.41; N, 6.06. Found: C, 72.48; H, 7.57; N, 6.16.

N-Benzoyl-*trans*-2-methyl-4-oxocyclohexylamine-$d_3d_3d_5d_5$ (12).—Ketone **10** (0.093 g) was deuterated by the procedure used to prepare **4**. Recrystallization of the product from acetone–Skellysolve B gave 0.066 g of colorless crystals, nmr ($CDCl_3$, 37°) 245 (m, NCH–) and 62 Hz (d, J = 6 cps, $-CH_3$). Recrystallization of the recovered sample gave colorless crystals of **12**: mp 190–193°; ν_{NH} 3300, ν_{ND} 2460 and 2390, ν_{CD} 2210 and 2110, $\nu_{C=O}$ 1705 and 1625, $\nu_{C=C}$ 1600, 1580, and 1490, $\nu_{amide\ II}$ 1540 cm^{-1} in Nujol; mass spectrum m/e 235 and 236 (M^+).

N-Benzoyl-*cis*-2-methyl-4-oxocyclohexylamine (**11**).—Oxidation of the crude alcohol **9**, mp 180–183° (1.00 g, 4.29 mmol), with Jones reagent gave 0.780 g (3.38 mmol, 78%) of colorless crystals, mp 166–168°. Recrystallization from acetone–Skellysolve B gave an analytical sample of **11**: mp 169–171°; $[\alpha]_D$ −6° (*c* 0.318); ν_{NH} 3300, $\nu_{C=O}$ 1710 and 1635, $\nu_{C=C}$ 1600, 1575, and 1490, $\nu_{amide\ II}$ 1530 cm^{-1} in Nujol; nmr ($CDCl_3$, 37°) 270 (m, NCH–) and 58 cps (d, J = 6.5 cps, $-CH_3$).

Anal. Calcd for $C_{14}H_{17}NO_2$: C, 72.70; H, 7.41; N, 6.06. Found: C, 72.74; H, 7.39; N, 5.98.

N-Benzoyl-*cis*-2-methyl-4-oxocyclohexylamine-$d_3d_3d_5d_5$ (13).—A sample of ketone **11** (0.098 g) was deuterated by the procedure used to prepare **4**. Crystallization of the product from acetone–Skellysolve B gave 0.080 g of colorless needles, nmr ($CDCl_3$, 37°) 269 (m, NCH–), 144 (m, CH_3CH–), 120 (d, J = 7 cps, $-CH_2$–), and 57 cps (d, J = 6.5 cps, $-CH_3$). Recrystallization of the nmr sample gave colorless needles of **13**: mp 167–169°; ν_{NH} 3340, ν_{ND} 2480, 2410, and 2380, ν_{CD} 2210, $\nu_{C=O}$ 1710 and 1630, $\nu_{C=C}$ 1600, 1575, and 1490, $\nu_{amide\ II}$ 1530 cm^{-1} in Nujol; mass spectrum m/e 235 and 236 (M^+).

Reduction of N-Benzoyl-*trans*-2-methyl-4-oxocyclohexylamine (**10**) **with Sodium Borohydride.**—Reduction of **10** (0.341 g, 1.47 mmol) with sodium borohydride was carried out as described for the reduction of **20**. Recrystallization from acetone–Skellysolve B gave 0.124 g (0.532 mmol, 36%) of N-benzoyl-*trans*-2-methyl-*trans*-4-hydroxycyclohexylamine (**8**), mp 203–205°; the ir spectrum in Nujol was identical with that of the alcohol (**8**) obtained from bioconversion above.

Reduction of 11 with sodium borohydride, as described above, gave a viscous gum as the reaction product.

(+)-N-Benzoylisopinocampheylamine (**16**).—(+)-Isopinocampheylamine was prepared from (−)-α-pinene (Aldrich Chemical Co., $[\alpha]_D$ −48°, 102 g) by the procedure of Rathke, Inoue, Varma, and Brown[5] and was isolated as the hydrochloride (63 g). The free base was obtained and the benzamide was prepared under Schotten–Baumann conditions with the exception that the reactants were initially mixed and shaken in the presence of ice–water. The crude benzamide was recrystallized from methanol–water, giving 46.18 g of **16**, mp 117–121°, $[\alpha]_D$ +25° (*c* 0.7860). Two recrystallizations from methanol–water gave an analytical sample: mp 124–126°; ν_{NH} 3300, $\nu_{C=O}$ 1620 cm^{-1} in Nujol; nmr (DMSO, 37°) 267 (quintuplet, J = 8 cps, –NCH–), 73 (s, CH_3), 63.5 (d, J = 7 cps, CH_3), and 63 cps (s, CH_3).

Anal. Calcd for $C_{17}H_{23}NO$: C, 79.33; H, 9.01; N, 5.44. Found: C, 79.32; H, 9.06; N, 5.39.

(−)-N-Benzoylisopinocampheylamine (**17**).—The procedure followed above for the preparation of **16** was repeated using (+)-α-pinene (Aldrich Chemical Co., $[\alpha]_D$ +35°). From 45 g of hydrochloride, 39.02 g of benzamide was obtained following recrystallization from methanol–water, mp 123–126°, $[\alpha]_D$ −25° (*c* 1.046). Recrystallization from methanol–water gave the analytical sample of **17**: mp 124–127°; ν_{NH} 3300, $\nu_{C=O}$ 1620, $\nu_{amide\ II}$ 1535 cm^{-1} in Nujol.

Anal. Calcd for $C_{17}H_{23}NO$: C, 79.33; H, 9.01; N, 5.44. Found: C, 79.07; H, 8.71; N, 5.42.

Bioconversion of (+)-N-benzoylisopinocampheylamine (16) (25.0 g, 0.0973 mol) gave, following chromatography, two crops, 14.487 g and 1.905 g (total 16.392 g, 0.0600 mol, 62%) of **(1*S*,2*S*,3*S*,5*R*,6*R*)-N-(8-hydroxy-3-pinanyl)benzamide (18)**, mp 187–192°. Two recrystallizations from acetone–Skellysolve B gave **18** as colorless, chunky crystals: mp 186–188°; $[\alpha]_D$ +30° (*c* 0.857, EtOH); $\nu_{NH,OH}$ 3500, 3360, 3310, and 3260, $\nu_{C=O}$ 1600 cm^{-1} in Nujol; nmr (DMSO, 37°) 271 (t, J = 5 cps, OH, removed by D_2O), 267 (quintuplet, J = 8 cps, NCH–), 213 (d, J = 5 cps, $-CH_2O-$, collapsed to s by D_2O), 67 (s, CH_3), and 64.5 cps (d, J = 7 cps, CH_3).

Anal. Calcd for $C_{17}H_{23}NO_2$: C, 74.69; H, 8.48; N, 5.12. Found: C, 74.81; H, 8.56; N, 5.28.

Bioconversion of (−)-N-benzoylisopinocampheylamine (17) (25.0 g, 0.0973 mol) gave, following chromatography and recrystallization, a first crop of 7.055 g and a second crop of 0.453 g (total 7.508 g, 0.0275 mol, 28%) of the product **19**, mp 185–191°. Two recrystallizations from acetone–Skellysolve B, the second preceded by decolorization, gave (1*R*,2*R*,3*R*,5*S*,6*S*)-N-(8-hydroxy-3-pinanyl)benzamide (**19**) as colorless crystals, mp 187–190°, $[\alpha]_D$ −26° (*c* 0.798, EtOH). The ir spectrum in Nujol was identical with that of **18**.

Anal. Calcd for $C_{17}H_{23}NO_2$: C, 74.69; H, 8.48; N, 5.12. Found: C, 74.60; H, 8.47; N, 5.42.

Reduction of Benzyl-4-oxocyclohexylcarbamate (20) with Sodium Borohydride.—A solution of sodium borohydride (0.5 g) and **20** (1.00 g, 4.05 mmol) in absolute ethanol (20 ml) was left at room temperature for 20 hr. Sulfuric acid (1 *M*, 5 ml) was added to the solution, which then was made alkaline by the addition of aqueous sodium hydroxide (1 *M*, 10 ml). Water (15 ml) was added and the solution was extracted with methylene chloride (five 15-ml portions). The extract was dried and concentrated under reduced pressure, giving a crystalline product. Recrystallization from acetone–Skellysolve B gave **benzyl-*trans*-4-hydroxycyclohexylcarbamate (24)** as colorless crystals (0.551 g, 2.22 mmol, 55%), mp 161–163° (lit.[3] mp 161°), for bioconversion product benzyl-4-hydroxycyclohexylcarbamate. The ir spectra of the reduction product and the bioconversion product were identical.

Reduction of N-Cyclohexyl-N-(4-oxo)cyclohexylacetamide (21) with Sodium Borohydride.—Reduction of **21** (1.00 g, 4.22 mmol) with sodium borohydride was carried out as described for the reduction of **20**. Recrystallization from acetone–Skellysolve B gave 0.757 g (3.17 mmol, 75%) of **N-cyclohexyl-N-(*trans*-4-hydroxy)cyclohexylacetamide (23)**, mp 171–173°. Recrystallization gave colorless crystals, mp 173–175° (lit.[3] mp 177–178°), for bioconversion product N-cyclohexyl-N-(4-hydroxy)cyclohexylacetamide. The ir spectra of the two alcohols in Nujol were identical.

Reduction of N-(4-Oxo)cyclohexylbenzamide (22) with Sodium Borohydride.—Reduction of **22** (0.019 g, 0.0875 mmol) with sodium borohydride was carried out in the manner described for the reduction of **20**. Recrystallization from acetone–Skellysolve B gave 0.010 g (0.0457 mol, 52%) of colorless N-(4-hydroxy)cyclohexylbenzamide, mp 211–213° (lit.[3] mp 212.5–213.5°); ir spectra (Nujol) were identical.

Bioconversion of N-Cyclohexyl-*p*-chlorobenzamide (25).—The extracts from bioconversion of **25** (2.0 g, 8.42 mmol) were yellowish, crystalline solids. They were dissolved in acetone, decolorized, and crystallized from ether as colorless crystals (0.357 g), mp 264–270°. A second crop of crystals (0.275 g, total 0.632 g, 2.49 mmol, 30%) was collected. Recrystallization from acetone gave N-(*trans*-4-hydroxy)cyclohexyl-*p*-chlorobenzamide (**28**) as colorless needles: mp 275–277°; $\nu_{OH,NH}$ 3360 and 3290, $\nu_{C=O}$ 1630, $\nu_{C=C\ amide\ II}$ 1595, 1570, 1545, and 1490, $\nu_{C_6H_4}$ 845 cm^{-1} in Nujol.

Anal. Calcd for $C_{13}H_{16}ClNO_2$: C, 61.53; H, 6.36; N, 5.52. Found: C, 61.87; H, 6.63; N, 5.45.

Bioconversion of N-cyclohexyl-*m*-chlorobenzamide (26, 2.0 g, 8.42 mmol) gave 0.821 g (3.24 mmol, 38%) of crystals, mp 198–202°, following chromatography and recrystallization. Two recrystallizations from acetone–Skellysolve B gave N-(*trans*-4-hydroxy)cyclohexyl-*m*-chlorobenzamide (**29**) as colorless crystals: mp 201–203°; $\nu_{OH,NH}$ 3340, 3300, and 3250, $\nu_{C=O}$ 1630, $\nu_{C=C}$ 1600 and 1565, $\nu_{amide\ II}$ 1545 cm^{-1} in Nujol.

Anal. Calcd for $C_{13}H_{16}ClNO_2$: C, 61.53; H, 6.36; N, 5.52. Found: C, 61.85; H, 6.51; N, 5.32.

Bioconversion of N-cyclohexyl-N,N′-dibenzoyl-1,3-diaminopropane (27, 2.0 g, 5.50 mmol) gave, following chromatography and recrystallization of the crude product, 1.083 g (2.86 mmol, 52%) of crystals, mp 173–176°. Two recrystallizations from acetone gave colorless crystals of N-(4-hydroxy)cyclohexyl-N,N′-dibenzoyl-1,3-diaminopropane (**30**): mp 174–175°; $\nu_{OH,NH}$ 3280, $\nu_{C=O}$ 1645 and 1615 cm^{-1} in Nujol; nmr ($CDCl_3$, 37°) 208 (m, 6 protons, NCH_2 $-CHNCH_2-$, $-CHO$) and 71 cps (t, J = 7 cps, $-CH_2CH_2CH_2-$).

Anal. Calcd for $C_{28}H_{28}N_2O_3$: C, 72.60; H, 7.42; N, 7.36. Found: C, 72.29; H, 7.22; N, 7.54.

Registry No.—**1**, 23062-70-6; **2**, 23062-09-1; **3**, 23062-71-7; **4**, 23062-72-8; **7**, 23062-10-4; **8**, 23062-11-5; **9**, 23062-12-6; **10**, 23062-13-7; **11**, 23062-15-9; **12**, 23062-16-0; **13**, 23062-14-8; **15**, 23062-17-1; **16**, 23062-18-2; **17**, 23062-19-3; **18**, 23062-20-6; **19**, 23102-75-2; **28**, 23062-21-7; **29**, 23062-22-8; **30**, 23062-23-9.

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