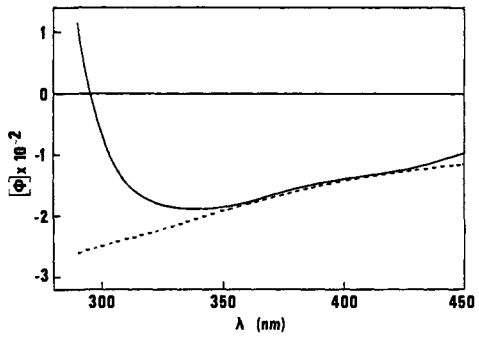

Figure 7. ORD curves of (2*R*)-*N*-benzoyl-2-amino-4-methylpentane (**1**) in methanol (- - -) and dioxane (—).

maxima from 272 and 278 nm in the same solvents (Table I).

The strong CD band of **17** at 227 nm (methanol) can be attributed to the n → π* transition of the amide group. As in the case of the *o*-methyl derivative **16**, the band occurs at shorter wavelengths than in the case of the *N*-monosubstituted benzamides, about 235 nm *vs.* 255 nm in dioxane. The origin of the positive asymmetry in the long wavelength wing of this band in nonpolar media (Figure 5) is unclear. Another CD band associated with the shoulder observed in absorption (Figure 5) may be located near 250 nm, or the asymmetry may be due to distortion produced by a strong negative band below 220 nm.

In conclusion, we suggest that the gross features and solvent effects in the CD spectra of benzamides **1**–**21** can be best explained by assuming that the n → π* transition of the benzamido chromophore is optically active and, except for **11**–**13**, is located between the B_{1u} and B_{2u} transitions. The CD solvent changes of benzamides **1**–**8** do not suggest that tautomerism or mesomerism effects are operative as have past ORD studies conducted on similar molecules.[6] These ORD studies were carried out between 600 and 300 nm, and the sign reversal of the plain curve for some molecules was attributed to a change in electronic structure of the amide portion of the molecule with solvent. We also notice such a "sign reversal" in the ORD of **1** in the wavelength range 450–290 nm (Figure 7). The curve is negative in methanol and, although the rotations near 400 nm begin negative in dioxane, the final result in dioxane is a plain positive curve. The ORD behavior of **1** is easily understood from the CD of **1**. The sign of the plain curve depends upon which of the three CD bands dominates in the ORD between 450 and 290 nm. According to our interpretation, the positive n → π* band determines the sign in dioxane because of a shift to longer wavelength, while the negative B_{1u} or B_{2u} bands dominate the ORD in methanol. In the case of *N*-benzoyl-2-aminobutane (**5**), the ORD sign reversal was not observed (**6b**). According to the CD of **5** (Table I), this is because the n → π* transition is weak and does not significantly distort the long wavelength ORD tail of the B_{1u} transition in any solvent. It is conceivable that the peculiar ORD solvent effects of other *N*-benzoyl derivatives of amines observed in the past could be explained in the same way.

Acknowledgments. We wish to acknowledge helpful discussions concerning the nmr spectra of some molecules with Dr. George Slomp and Dr. B. V. Cheney.

The Microbiological Oxygenation of Acyclic *N*-Alkylbenzamides[1]

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Abstract: In searching for new classes of substrates that are oxygenated by *Sporotrichum sulfurescens*, acyclic *N*-alkylbenzamides of four to seven carbon chain length have been found to give a variety of oxygenated products. In general, the longer chain length substrates are oxygenated primarily at the subterminal carbon farthest from the benzamido group. Substrates of shorter chain length are oxygenated at either the terminal or the subterminal carbon atom. The chiral center at the benzamido carbon exerts an influence on the stereochemical course of the reaction in several cases, suggesting an "active" role for the benzamido group in the oxygenation process.

Microbial oxygenations of acyclic compounds have received considerable attention, largely in the hands of microbiologists whose primary interest has been in the metabolic fate of hydrocarbon molecules.[2] Once the hydrophobic barrier of the hydrocarbon chains has been successfully breached by an initial oxidative attack, the oxygenated products generally are much more susceptible to further metabolism as an energy source for the microorganism, thereby precluding any substantial accumulation of useful chemical intermediates. Only when this further metabolism has been effectively blocked have significant quantities of the initially formed products been obtained. As an example, fermentation of hydrocarbons with *Torulopsis apicola*[3] or *T. gropengiesseri*[4] was found to give insoluble glycolipids, into which the oxygenated intermediates were incorporated. Hydrolysis of the glycolipids released

(1) Stereochemistry of Microbiological Hydroxylation, Part VI.

(2) *Cf.* E. J. McKenna and R. E. Kallio, *Annu. Rev. Microbiol.*, **19**, 183 (1965); A. C. van der Linden and G. J. E. Thijsee, *Advan. Enzymol.*, **27**, 469 (1965).

(3) A. P. Tulloch, J. F. T. Spencer, and P. A. J. Gorin, *Can. J. Chem.*, 40, 1326 (1962).

(4) D. F. Jones and R. Howe, *J. Chem. Soc. C*, 2801 (1968).

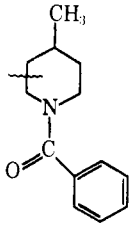
Table I. Acyclic Benzamides Used for Substrates

Compound	Mp, °C	Lit. mp, °C	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N
$(CH_3)_2CHCH_2CH_2NHCOC_6H_5$ (**2**)	55–57	57–58[a]						
$(CH_3)_2CHCH_2CH(CH_3)NHCOC_6H_5$ (**4**)	92–94		76.05	9.33	6.82	76.38	9.16	7.27
$(CH_3)_2CHCH_2CH_2CH(CH_3)NHCOC_6H_5$ (**7**)	78–80		76.66	9.65	6.39	76.54	9.75	6.26
$(CH_3)_2CHCH_2CH_2CH_2CH(CH_3)NHCOC_6H_5$ (**9**)	78–81		77.20	9.94	6.00	77.00	9.87	6.12
$(CH_3)_3CCH_2CH(CH_3)NHCOC_6H_5$ (**11**)	124–126		76.66	9.65	6.39	76.57	9.98	6.41
$(CH_3)_3CCH_2C(CH_3)_2NHCOC_6H_5$ (**15**)	67–69	70–71[b]						
$CH_3CH_2CH(CH_3)CH_2CH(CH_3)NHCOC_6H_5$ (**17**)	37–41		76.66	9.65	6.39	76.56	9.37	6.11
$(CH_3CH_2)_2CHNHCOC_6H_5$ (**20**)	85–88		75.35	8.96	7.32	75.15	9.02	7.27
$CH_3CH_2CH_2CH(CH_3)NHCOC_6H_5$ (**22**)	76–78		75.35	8.96	7.32	75.18	9.16	7.40
$CH_3CH_2CH_2CH_2CH(CH_3)NHCOC_6H_5$ (**27**)	81–83	82–83[c]						
$CH_3CH_2CH_2CH_2CH(CH_2CH_3)NHCOC_6H_5$ (**30**)	93–95	92–93[c]						
$CH_3CH_2CH_2CH_2CH_2CH(CH_3)NHCOC_6H_5$ (**33**)	68–70	71–72[c]						

[a] H. L. Wheeler, *Amer. Chem. J.*, **23**, 135 (1900). [b] J. J. Ritter and P. P. Minieri, *J. Amer. Chem. Soc.*, **70**, 4045 (1948). [c] D. C. Iffland and T-F. Yen, *ibid.*, **76**, 4180 (1954).

these intermediates. Similarly, in the oxygenation of some alkylaromatics, metabolism of the intermediates *via* the β-oxidation pathway was slowed by the aromatic ring on the hydrocarbon chain[5] or was inhibited by cooxidation processes.[6]

In recent studies of microbial oxygenations, our main interests have been in the preparation of novel chemical intermediates and in the nature of the oxygenation process.[7] While studying the oxygenation of the bicyclic molecule, 1-benzoyl-*trans*-decahydroquinoline,[8] and the monocyclic 1-benzoylalkylpiperidines, such as **1**,[9] by *Sporotrichum sulfurescens*, we became curious as to whether the cyclic system of the latter could be further fragmented (as illustrated) and still serve as substrate for oxygenation by this microorganism. To examine



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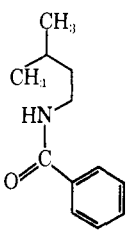
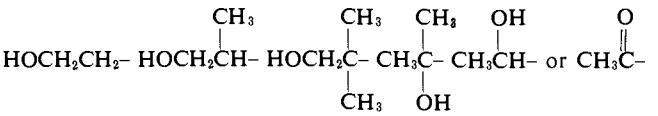


2

this possibility, we have prepared several branched chain benzamides, such as *N*-(3-methylbutyl)benzamide (**2**) and have examined their bioconversion by *S. sulfurescens*. Results of preliminary experiments were encouraging and led us to prepare the series of substrates listed in Table I. Bioconversions of these substrates by a standard procedure[9] have given yields of products in the range of 5–70%. Yields in most cases are based on the actual amounts of products isolated. The elucidation of the structures and stereochemistry of these products provides the subject of this report.

It has been possible to assign structures to the products almost entirely on the basis of their infrared and nmr spectra. The oxidation state of the products, either alcohol or ketone, was easily determined from the infrared spectra. The position of oxygenation was also readily determined since all of the products fall into one of the classes

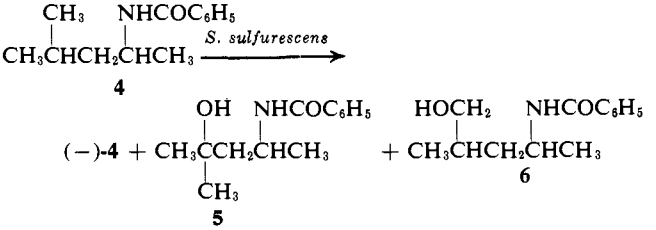


The signals for each of these combinations of protons are distinct in the nmr spectra of these compounds.[10] When combined with the known structure of the substrate and the characteristic signals for the protons in the vicinity of the nitrogen substituents, this information is sufficient for assignment of structures to the bioconversion products. The spectral data have been confined to the Experimental Section and the discussion is presented in terms of the structures based on these data.

Results and Discussion

Branched Chain Benzamides. A single hydroxylated product, *N*-(3-hydroxy-3-methylbutyl)benzamide (**3**), was obtained in low yield (13%) from bioconversion of our initially chosen substrate, *N*-(3-methylbutyl)-benzamide (**2**). Recovery of considerable starting material (28%) from this bioconversion indicated that the rate of oxygenation was very slow.

The substrate *N*-(1,3-dimethylbutyl)benzamide (**4**), a homolog of **2**, is perhaps the most interesting substrate of the present study and consequently, its bioconversion has been examined in greatest detail. Two major products were isolated from bioconversion of racemic **4**. These were the tertiary alcohol **5** (22%) and the primary alcohol **6** (29%), both of which were



initially isolated as viscous oils and were purified by distillation under vacuum. The oily products **5** and **6**

(5) (a) D. M. Webley, R. B. Duff, and V. C. Farmer, *Nature (London)*, **178**, 1467 (1956); (b) J. B. Davis and R. L. Raymond, *Appl. Microbiol.*, **9**, 383 (1961).

(6) (a) J. D. Douros, Jr., and J. W. Frankenfeld, *ibid.*, **16**, 320 (1968); (b) R. L. Raymond, V. W. Jamison, and J. O. Hudson, *ibid.*, **15**, 857 (1967).

(7) R. A. Johnson, M. E. Herr, H. C. Murray, and G. S. Fonken, *J. Org. Chem.*, **33**, 3217 (1968).

(8) R. A. Johnson, H. C. Murray, L. M. Reineke, and G. S. Fonken, *ibid.*, **33**, 3207 (1968).

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

(10) Examples may be found in "NMR Spectra Catalog," Vol. 1 and 2, Varian Associates, Palo Alto, Calif., 1962, 1963.

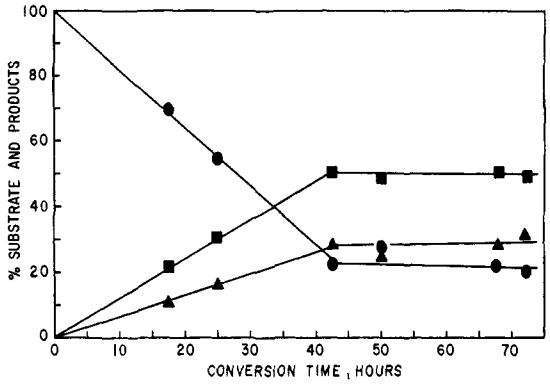
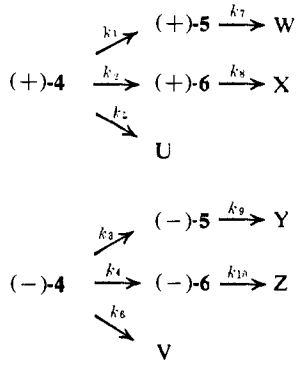


Figure 1. Relative quantities of substrate and product *vs.* conversion time in extracts from the bioconversion of (*RS*)-*N*-(1,3-dimethylbutyl)benzamide (●). Products are *N*-(1,3-dimethyl-4-hydroxybutyl)benzamide (■) and *N*-(1,3-dimethyl-3-hydroxybutyl)-benzamide (▲).

were optically active and had specific rotations of −9 and +18°, respectively. Subsequently, these oils partially crystallized, giving crystalline solids having either less or no rotation at the sodium D line, indicating that neither oily product was optically pure. In addition to these two major products, some starting material (7%) was recovered from the bioconversion and, surprisingly, was found to be optically acitive ([α]D −21°). These results strongly suggested that (a) each enantiomer of the substrate was being hydroxylated preferentially, although not exclusively, at a different position,[11] *i.e.*, $k_1 \neq k_2$ and $k_3 \neq k_4$ (Scheme I), and (b) the (+)-enantiomer was being hydroxylated at a faster rate than was the (−)-enantiomer, *i.e.*, $k_1 + k_2 > k_3 + k_4$. It must be remembered that additional, undetected pathways leading to hypothetical degradation products U–Z could also lead to the observed results. However, a great degree of coincidence would be required of these latter pathways in order to be consistent with the observed results.

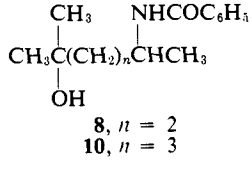
Scheme I



A semiquantitative experiment was carried out in which the change in the ratio of products to substrate with time was determined. This was accomplished by withdrawing samples from the bioconversion reaction at intervals and analyzing extracts of the samples by vapor phase chromatography (vpc). The relative amounts of products and substrate, expressed as a percentage of the total extract, are plotted against the time intervals shown in Figure 1. The fact that the total amount of material (products and substrate) recovered in each extraction was nearly constant (see Table II, Experimental Section) gives significance to the remarkable correlation found between the total amount of products appearing and the amount of substrate disappearing (or remaining) in this experiment. Also notable is the cessation of oxygenation after about 40 hr with no further change in the composition or quantities of substrate and product up to 75 hr. If degradation of the products by pathways leading to U, V, etc., were occurring, the quantities of one or both remaining in the fermentation would be expected to decrease with time after conversion of the substrate had stopped.[12]

In order to determine if the (+)-enantiomer of the substrate was being oxygenated predominantly on the primary methyl carbon and the (−)-enantiomer on the tertiary methine carbon, we resolved 2-amino-4-methylpentane by known methods[13] and prepared the optically active benzamides from the resolved amines. Optically pure[13a] (−)-amine gave (−)-benzamide, having a specific rotation of −22°. Bioconversion of this enantiomer of **4** gave (−)-**5** as the major product (41%) and (−)-**6** as the minor product (14%). Some starting material was also recovered. Bioconversion of the (+)-enantiomer of **4** gave the opposite result, the major product now being (+)-**6** (36%) and the minor product being (+)-**5** (12.5%). The absolute configuration of (+)-2-amino-4-methylpentane has been related to L-leucine[14] and therefore has the (*S*) absolute configuration at C-2. Likewise, the (+)-enantiomers of **4**, **5**, and **6** therefore have (*S*) configurations at the amido carbon while the (−)-enantiomers have the (*R*) configuration. It may be noted here that the (1*R*)-(−)-enantiomer of **4**, which apparently is oxygenated at a slower rate than the (+)-enantiomer, is related in absolute configuration to the unnatural isomers (*R*) of the amino acids.

Bioconversion of the next two higher homologs (**7** and **9**) of branched alkylbenzamide **4** resulted in good yields of single products (**8**, 59%; and **10**, 50%, respectively) in each case. Both products were identified as tertiary alcohols by their nmr spectra and both were optically inactive at the sodium D line. However, in the case of **7**, a small amount (4%) of substrate was recovered and was found to be optically active ([α]D −15°). No substrate was recovered from the bioconversion of **9**. A chemical preparation of **10** has been reported.[15]



8, $n = 2$
10, $n = 3$

The bioconversions of more highly branched homologs of **4** were also examined. Bioconversion of *N*-(1,3,3-trimethylbutyl)benzamide (**11**) gave *N*-(4-hydroxy-1,3,3-trimethylbutyl)benzamide (**12**) as the only

(11) Hydroxylation of enantiomers at different positions by *S. sulfurescens* has been noted in the cases of 1-benzoyl-*trans*-decahydroquinoline[8] and 1-benzoyl-2-methylpiperidine.[9]

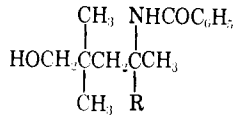
(12) The rate of microbial oxygenation reactions may be a function of a number of factors, *e.g.*, (a) length of the microbial life cycle, (b) pH of the medium, (c) available nutrients, such as sugar, (d) inhibition by products, etc. Changes in any of these could explain the stopping of oxygenation short of completion.

(13) (a) B. Halpern and J. W. Westley, *Chem. Commun.*, 34 (1966); (b) R. H. Holm, A. Chakravorty, and G. O. Dudek, *J. Amer. Chem. Soc.*, 86, 379 (1964).

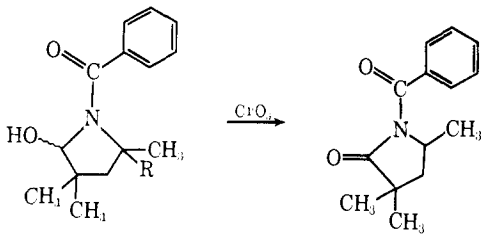
(14) P. Karrer and P. Dinkel, *Helv. Chim. Acta*, 36, 122 (1953).

(15) T. Seiyaku, *Kenkyu Nempo*, 4, 21 (1950); *Chem. Abstr.*, 54, 8627 (1960).

major product which could be isolated consistently from this reaction. However, on one occasion an additional product was isolated from this fermentation and was assigned the structure of 1-benzoyl-3,3,5-trimethyl-2-pyrrolidinol (**13**) on the basis of the infrared and nmr



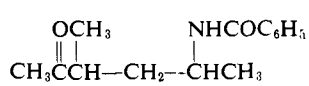
12, R = H
16, R = CH_3



13, R = H **14**

spectra of it and of the compound (**14**) derived from it by chromic acid oxidation.[16] Pyrrolidinol **13** was also rapidly oxidized in the injection port of a vapor chromatograph. Bioconversion of *N*-(1,1,3,3-tetramethylbutyl)benzamide (**15**) similarly resulted in isolation of a single product, which was the primary alcohol **16**.

The final branched chain benzamide used as a substrate in this study was *N*-(1,3-dimethylpentyl)benzamide (**17**). Bioconversion of **17** gave two products, one a ketone (**18**) in low yield (4%) and the second an alcohol (**19**) in modest yield (21%). The ketone, shown



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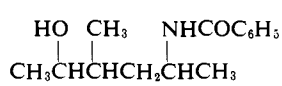
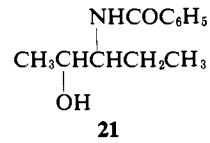


19

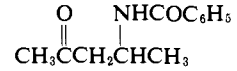
by nmr to be a methyl ketone, was crystalline and optically active ([α]D +59°) whereas the alcohol failed to crystallize and was optically inactive. Structure **19** could be deduced from the nmr spectrum of the alcohol and was confirmed by oxidation of the alcohol to ketone **18**. This oxidation was carried out on a sample which contained a small amount of bioconversion ketone, which likely explains the low order of optical activity (+8°) observed for the product. Since alcohol **19** contains three asymmetric carbons, the product could be one or several of eight possible stereoisomers (four *d,l* pairs), of which it may be expected that members of different *d,l* pairs could be separated by various chromatographic means. It seemed very possible that the noncrystalline nature of alcohol **19** may be the result of a mixture of some of these isomers. However, the hydroxylic product could not be separated into more than one component by several tlc systems, as well as by paper chromatography, suggesting that **19** is being obtained as a mixture of one *d,l* pair.

Straight Chain Benzamides. Acyclic benzamides having no branching of the alkyl chain also were oxygenated by *S. sulfurescens*. *N*-(1-Ethylpropyl)benzamide (**20**) was hydroxylated in low yield, giving alcohol **21** (10%, [α]D −31°) as the only product. Starting material also was recovered from this fermentation.

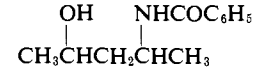


21

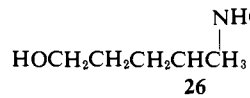
Oxygenation of *N*-(1-methylbutyl)benzamide (**22**) gave a mixture of products from which one ketone and three alcohols were isolated. A small amount of substrate was also recovered and was optically active ([α]D − 15°). The ketone (**23**) crystallized in two crops; the first was a racemate whereas the second was



23



24, mp 57–61°; **25**, mp 91–93°

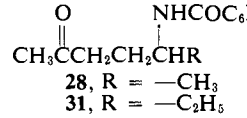


26

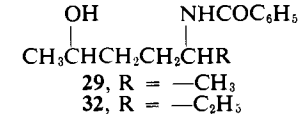
optically active ([α]D +20°). Two of the alcohols, separable by chromatographic techniques, were identified as the 3-hydroxy compounds, **24** ([α]D −11°) and **25** ([α]D −14°), by their nmr spectra. These two products must be threo and erythro isomeric forms. The third alcohol was the primary hydroxy compound **26** ([α]D +23°). The absolute configuration of alcohol **26** has been determined and is 1*S*.[17]

It is interesting to compare these results with those of the branched substrate **4** of the same five-carbon chain length. It seems probable that the (+)-enantiomer of substrate **22** is preferentially oxygenated at the terminal carbon and at the 3 position to give either the erythro or threo isomer, which perhaps is further dehydrogenated to ketone **23**, whereas the (−)-isomer is preferentially oxygenated at the 3 position to give both the erythro and threo isomers, the result being the mixture of products described above. Erythro and threo isomers were isolated only from oxygenation of substrate **22** in the present study, although *d,l* mixtures of a single chiral alcohol were obtained in several other cases.

Bioconversion of *N*-(1-methylpentyl)benzamide (**27**), the higher homolog of **22**, gave *N*-(1-methyl-4-oxopentyl)benzamide (**28**, 9–11%, [α]D +6 to +17°) and *N*-(4-hydroxy-1-methylpentyl)benzamide (**29**). Again, recovered starting material was optically active ([α]D −19°). As in the case of alcohol **19**, above, alcohol **29** may exist as several isomers. However, thin-layer and paper chromatography again were unable to show more than one spot for the alcohol. Oxidation of **29** with chromic acid gave ketone **28** (86%, [α]D 0°). Similarly, bioconversion of *N*-(1-ethylpentyl)benzamide (**30**) gave a ketonic product (**31**, 15%) and an amor-



28, R = $-CH_3$
31, R = $-C_2H_5$



29, R = $-CH_3$
32, R = $-C_2H_5$

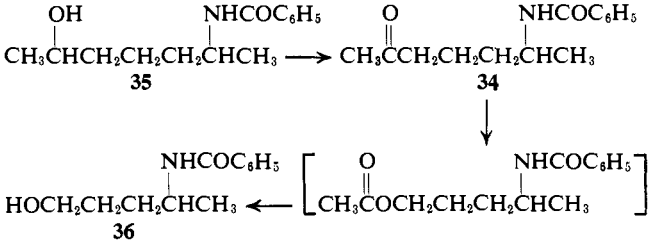
phous, glassy hydroxylic product (**32**, 54%). Oxidation of alcohol **32** with chromic acid gave ketone **31**.

It is likely that the isolation of ketones from several of the above bioconversions represents the action of alcohol dehydrogenase enzyme(s) on the secondary alcohols formed as a result of the initial hydroxylation

(16) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).

(17) See W. C. Krueger, R. A. Johnson, and L. M. Pschigoda, *J. Amer. Chem. Soc.*, **93**, 4865 (1971).

reaction. Formation of the ketones by simple air oxidation seems unlikely since a stereoselectivity in the step involved between alcohol and ketone is apparent in some cases (*e.g.*, **19** → **18**). A further step in the metabolism of compounds such as these is suggested by the results obtained from oxygenation of *N*-(1-methylhexyl)benzamide (**33**). In addition to a ketonic product (**34**, 6%, $[\alpha]D$ −13°) and a secondary hydroxylic product (**35**, 5% $[\alpha]D$ −6°), results which are similar to the two preceding examples, there was obtained a primary alcohol (**36**, 2% $[\alpha]D$ +21°), which was shorter by two carbons than the substrate molecule. It seems possible that **36** was formed from ketone **34** *via* an undetected intermediate acetate. Reactions such as this are analogous to the chemical Baeyer–Villiger reaction



and were first documented for the microbial cleavage of the progesterone side chain.[18] Very recently, this sequence of reactions has been proposed for a similar hydrocarbon degradation by the bacterium *Pseudomonas multivorans*, from which an acetate was isolated.[19] The same sign of optical rotation observed for **36** and the identical compound (**26**), obtained from oxygenation of **22**, is probably coincidental.

With the hope of obtaining some insight into the oxygenation process, the patterns of stereochemistry and substitution in these products have been further examined. Thus, it was interesting to find that optically active substrate could be recovered from these reactions in at least four different cases (from substrates **4**, **7**, **22**, and **27**). More significantly, a study of the circular dichroism (CD) curves of these four compounds has shown that all have the same absolute configuration (1*R*) at the asymmetric carbon.[17] It appears, therefore, that the rates of oxygenation of the individual enantiomers are directly related to the absolute configuration at the benzamido carbon, the 1*S*-enantiomer reacting in each case at a faster rate than the 1*R*-enantiomer. Additionally, oxygenation of the five-carbon chain substrates, **4** and **22**, occurred at a different position in each enantiomer, the 1*R*-enantiomers being oxygenated primarily at the C-3 carbon while the 1*S*-enantiomers were oxygenated at the terminal (C-4) carbon atom. These results clearly show that stereochemistry at C-1 influences the oxygenation reactions. This influence may be exerted at two different sites if one arbitrarily separates the entire enzyme–substrate complex into (a) the site at which oxygenation occurs, and (b) the hypothetical site of interaction between enzyme and an electron-rich center in the substrate molecule (the amide group in the present substrates).[20] The proximity of the C-1 chiral center to the benzamide and the direct relationship between absolute configuration and the rates of oxygenation suggest that the stereochemical influence is exerted at site (b). If correct, this conclusion provides evidence for an "active" role[21] on the part of the electron-rich center during oxygenation of substrates such as these by *S. sulfurescens*.

The positions of oxygenation found in these bioconversion products are of further interest. Oxygenation occurs mainly at the carbon next to the terminal methyl group farthest from the amide substituent except in several of the substrates of shorter chain length. In the latter cases, oxygenation of the terminal carbon is also observed. There appears, on the basis of these observations, to be an inherent preference for oxygenation at the β-carbon in these substrates which is, in part, counteracted by a preference for oxygenation at a carbon not closer than some minimum distance (perhaps the ~5.5 Å of a previous proposal by Fonken, *et al.*[20]) in the substrates of shorter chain length. Whether the preference for oxygenation at carbon next to methyl is due to a fundamental preference for oxygenation at a more highly substituted carbon (as is typical of free radical and carbonium-ion type reactions) or for some as yet unexplained preference for a β-carbon cannot be determined from the present results.

Experimental Section[23]

Biotransformation Process. The culture used in these experiments was *Sporotrichum sulfurescens* v. Beyma (ATCC 7159). The biotransformation procedure has been described previously,[9] the only variation being that the dispersing agent Ultrawet DS-30 (2.5 ml/l) was added to the fermentations with the substrates.

Bioconversion of *N*-(3-Methylbutyl)benzamide (2). The methylene chloride extract from the bioconversion of **2** (2.0 g, 0.0105 mol) with *S. sulfurescens* was chromatographed on a Florisil column (3.8 × 35 cm), which was dry packed with Skellysolve B. Elution was with 335-ml fractions [five each of 5%, 10%, 20%, and 30% (v/v) acetone–Skellysolve B]. Fractions 12 and 13 were combined in acetone, decolorized, and crystallized from acetone–Skellyslove B, giving 0.233 g (1.12 mmol, 11%) of crystals, mp 104–106°. Two recrystallizations from acetone–Skellysolve B gave *N*-(3-hydroxy-3-methylbutyl)benzamide (**3**) as shiny, colorless flakes: mp 106–107°; $\nu_{OH,NH}$ 3260, $\nu_{C=O/C=C/amide\ II}$ 1640 sh, 1630, 1620, 1605, 1575, 1550, 1495 cm^{-1} in Nujol; $\delta_{CDCl_3}^{37°}$ 3.60 (four-line signal, J = 6 Hz, 2 H, $-CH_2N<$), 1.77 (triplet, J = 6 Hz, 2 H, $>CCH_2-$), 1.27 (singlet, 6 H, $-CH_3$).

Anal. Calcd for $C_{12}H_{17}NO_2$: C, 69.54; H, 8.27; N, 6.76. Found: C, 69.44; H, 8.44; N, 6.89.

A larger scale bioconversion of **2** (25.0 g, 0.131 mol) gave recovery of starting material (7.16 g), having an infrared spectrum identical with substrate, and **3** (3.495 g, 0.0169 mol, 13% or 18% based on recovered starting material), mp 103–107°.

Bioconversion of *N*-(1,3-Dimethylbutyl)benzamide (4). The residue from the concentrated methylene chloride extract from the bioconversion (125 l.) of **4** (25.0 g, 0.122 mol) with *S. sulfurescens* was redissolved in a minimum of methylene chloride and the solution was placed on a Florisil chromatography column (10.5 × 50 cm), which was dry packed with Skellysolve B. Elution was with 2-l. fractions, beginning with one of Skellysolve B followed by three

(18) G. S. Fonken, H. C. Murray, and L. M. Reineke, *J. Amer. Chem. Soc.*, 82, 5507 (1960).

(19) F. W. Forney and A. J. Markovetz, *J. Bacteriol.*, 96, 1055 (1968).

(20) G. S. Fonken, M. E. Herr, H. C. Murray, and L. M. Reineke, *J. Amer. Chem. Soc.*, 89, 672 (1967).

(21) By an "active" role for the electron-rich center, we refer to the type of interaction proposed by Fonken, *et al.*, for the oxygenation of macrocyclic alcohols[20] and also considered by Bridgeman, *et al.*, in the oxygenation of steroid ketones.[22] A "passive" role would be of the second type considered for the steroid ketones case[22] in which the influence of the electron-rich center would be to "block its own site, and might also influence the reactivities of neighboring positions." [22]

(22) J. E. Bridgeman, J. W. Browne, P. C. Cherry, M. G. Combe, J. M. Evans, E. R. H. Jones, A. Kasal, G. D. Meakins, V. Morisawa, and P. D. Woodgate, *Chem. Commun.*, 463 (1969).

(23) Melting points were determined on a Fisher-Johns hot stage and are corrected. Magnesium sulfate was used as a drying agent. Infrared spectra were determined with either a Perkin-Elmer Infracord or Model 421 spectrophotometer. The nmr spectra were determined at 60 Mc with a Varian Model A-60A spectrometer, using tetramethylsilane as an internal standard. Specific rotations were determined at 24.0 ± 0.5°.

of 5% (v/v) acetone in Skellysolve B, eight of 10% acetone in Skellysolve B, and six of 20% acetone in Skellysolve B. Fractions 6 and 7 were combined in acetone, decolorized, and recrystallized from acetone–Skellysolve B. Colorless crystals (1.790 g, 7%) of optically active substrate (**4**) were obtained: mp 105–107°; $[\alpha]D$ −21° (*c*, 0.758, chloroform); ν_{NH} 3390, $\nu_{C=O}$ 1630 cm^{-1} in Nujol and identical with the infrared spectrum of **4** prepared from resolved amine.

Fractions 11–14 (crude weight, 6.751 g) were combined and distilled under reduced pressure, giving 5.461 g (0.0247 mol, 22% based on recovered starting material) of **5** as a light yellow oil: bp 155–157° (0.10 mm); $[\alpha]D$ −9° (*c* 0.420, chloroform); $\nu_{OH,NH}$ 3320, $\nu_{C=O}$ 1640, $\nu_{C=C}$ 1605, 1580, 1490, $\nu_{amide\ II}$ 1545 sh, 1535, $\nu_{C_6H_5}$ 715, 700 cm^{-1} on liquid; $\delta^{37°}_{CDCl_3}$ 4.25 (multiplet, 1 H, –CHN<), 1.80, 1.73, 1.65, 1.63 (four-line signal, 2 H, $-CH_2-$), 1.28 (doublet, J = 6 Hz, 3 H, $-CH_3$), 1.21 (singlet, 6 H, *gem*-CH_3).

Anal. Calcd for $C_{13}H_{19}NO_2$: C, 70.55; H, 8.65; N, 6.33. Found: C, 70.83; H, 8.92; N, 6.36.

The viscous oil partially crystallized and from acetone–Skellysolve B, 2.035 g (0.0092 mol, 8%) of **5** was obtained as colorless crystals, mp 83–85°. Two recrystallizations from acetone–Skellysolve B gave *N*-(**1,3-dimethyl-3-hydroxybutyl**)**benzamide** (**5**) as colorless crystals: mp 84–86°; $[\alpha]D$ 0° (chloroform); $\nu_{OH,NH}$ 3270, $\nu_{C=O}$ 1625, $\nu_{C=C}$ 1605, 1580, 1490 $\nu_{amide\ II}$ 1545, $\nu_{C_6H_5}$ 720 cm^{-1} in Nujol.

Anal. Found: C, 70.71; H, 8.70; N, 6.24.

Fractions 16–19 (crude weight, 8.935 g) were combined and distilled under reduced pressure, giving 7.300 g (0.0328 mol, 29% based on recovered starting material) of *N*-(**1,3-dimethyl-4-hydroxybutyl**)**benzamide** (**6**) as a light yellow oil: bp 172–177° (0.09 mm); $[\alpha]D$ +18° (*c* 0.623, chloroform); $\nu_{OH,NH}$ 3310, $\nu_{C=O}$ 1635, $\nu_{C=C}$ 1605, 1580, 1490, $\nu_{amide\ II}$ 1545, $\nu_{C_6H_5}$ 710, 700 cm^{-1} on the liquid; $\delta^{37°}_{CDCl_3}$ 4.20 (multiplet, 1 H, –CHN<), 3.43 (multiplet, 2 H, $-OCH_2-$), 1.88–1.42 (multiple signals, 3 H, –CH–, $-CH_2-$), 1.20 (doublet, J = 6 Hz, 3 H, $-CH_3$), 0.89 (doublet, J = 6 Hz, 3 H, $-CH_3$).

Anal. Calcd for $C_{13}H_{19}NO_2$: C, 70.55; H, 8.65; N, 6.33. Found: C, 69.75; 69.79; H, 8.67, 8.81; N, 6.42.

The oil partially crystallized, from which a small amount of crystalline **6** was obtained by recrystallization from acetone–Skellysolve B. Two additional recrystallizations gave **6** as colorless crystals: mp 91–92°, $[\alpha]D$ +8° (*c* 0.7916, $CHCl_3$); $\nu_{NH,OH}$ 3300, $\nu_{C=O}$ 1630, $\nu_{amide\ II}$ 1535 cm^{-1} in Nujol.

Anal. Found: C, 70.40; H, 9.03; N, 6.26.

Resolution of 2-Amino-4-methylpentane. A. (2*R*)-2-Amino-4-methylpentane. The procedure of Halpern and Westley[13] was followed. After five recrystallizations from methanol, (2*R*)-2-amino-4-methylpentane tartrate (47.1 g) was obtained from 2-amino-4-methylpentane (101 g) and (+)-tartaric acid (150 g). The free amine was obtained by addition of aqueous sodium hydroxide and extraction with ether. Following drying and concentration, the amine was distilled through a 15-cm Vigreux column at atmospheric pressure. A forerun (4.588 g, bp 90–105°) and main fraction (9.012 g, bp 105–106°), $[\alpha]D$ −11.70° (neat) (lit.[13] −10.78°, −11.2°[24] (neat)), were collected.

B. (2*S*)-2-Amino-4-methylpentane. From the filtrates of the above resolution, amine enriched in the 2*S*-isomer was obtained (~80 g) and was combined with racemic amine (53 g). Resolution was carried out as above with (*l*)-(−)-tartaric acid (200 g). After five recrystallizations from methanol, (2*S*)-2-amino-4-methylpentane tartrate (86.2 g) was obtained. The free amine was obtained and distilled through a 15-cm Vigreux column. Forerun (12.929 g, bp 50–105°) and main fraction (15.001 g, bp 105–106.3°), $[\alpha]D$ +11.73° (neat), were collected.

(1*R*)-*N*-(1,3-Dimethylbutyl)benzamide. A mixture of (2*R*)-2-amino-4-methylpentane (13.4 g), benzoyl chloride (21.0 g), aqueous 50% sodium hydroxide, and ice water were shaken together and then stirred in a beaker. An additional 4 g of benzoyl chloride was added. A solid formed and was collected. Two recrystallizations from methanol–water gave an analytical sample of amide: mp 107–108°; $[\alpha]D$ −22° (*c* 0.950).

Anal. Calcd for $C_{13}H_{19}NO$: C, 76.05; H, 9.33; N, 6.82. Found: C, 76.17; H, 9.09; N, 6.69.

(1*S*)-*N*-(1,3-Dimethylbutyl)benzamide. The main fraction (15.0 g) of the (2*S*)-amine was converted to the benzamide by the same procedure used above. The crude product, 28.50 g, mp 106–108°, had $[\alpha]D$ +21° (*c* 1.051). Two recrystallizations from methanol–water gave colorless needles: mp 107–109°; $[\alpha]D$ +24° (*c* 0.914).

Anal. Calcd for $C_{13}H_{19}NO$: C, 76.05; H, 9.33; N, 6.82. Found: C, 76.02; H, 9.49; N, 7.18.

From the forerun (12.93 g), additional benzamide (19.86 g) was obtained and had, after recrystallization from methanol–water, mp 106–108° and $[\alpha]D$ +21° (*c* 0.932).

Bioconversion of (1*R*)-(−)-*N*-(1,3-Dimethylbutyl)benzamide. A methylene chloride solution of the extracts from bioconversion of (1*R*)-(−)-*N*-(1,3-dimethylbutyl)benzamide (25.0 g, 0.122 mol) was placed on a Florisil column (10.5 × 50 cm) and eluted as described for the bioconversion of **4** above. Starting material (3.1 g) was obtained in the early fractions. The less polar product (12.89 g crude weight) was pooled in acetone, decolorized, and crystallized from cold acetone–Skellysolve B. Colorless crystals (11.011 g, 0.0498 mol, 41%), mp 76–82°, were obtained. Two recrystallizations from acetone–Skellysolve B gave colorless crystals of (1*R*)-(−)-*N*-(1,3-dimethyl-3-hydroxybutyl)benzamide: mp 78–80°; $[\alpha]D$ −28° (*c* 0.982); $\nu_{NH,OH}$ 3420, 3370, 3330, $\nu_{C=O,\ C=C,\ amide\ II}$ 1640, 1625, 1600, 1575, 1540 sh, 1530, 1490, $\nu_{C_6H_5}$ 725, 695 cm^{-1} in Nujol.

Anal. Calcd for $C_{13}H_{19}NO_2$: C, 70.55; H, 8.65; N, 6.33. Found: C, 70.76; H, 8.90; N, 6.38.

The more polar product (3.65 g crude weight, 14%) was a viscous oil which failed to crystallize.

Bioconversion of (1*S*)-(+)-*N*-(1,3-Dimethylbutyl)benzamide. A methylene chloride solution of the extracts from bioconversion of (1*S*)-(+)-*N*-(1,3-dimethylbutyl)benzamide (25.0 g, 0.122 mol) was placed on a Florisil column (10.5 × 50 cm), which was packed with Skellysolve B. Elution was as described for the bioconversion of **4** above. The fractions containing the less polar product (crude weight 4.57 g) were combined in acetone, decolorized, and crystallized from cold acetone–Skellysolve B, giving 3.374 g (0.0153 mol, 12.5%) of crystals, mp 76–78°. Two recrystallizations from acetone–Skellysolve B gave (1*S*)-(+)-*N*-(**1,3-dimethyl-3-hydroxybutyl**)**benzamide** as colorless, rectangular crystals: mp 78–80°, $[\alpha]D$ +22° (*c* 0.850); infrared spectrum in Nujol is identical with that of (1*R*)-(−)-*N*-(1,3-dimethyl-3-hydroxybutyl)benzamide, obtained above.

Anal. Calcd for $C_{13}H_{19}NO_2$: C, 70.55; H, 8.65; N, 6.33. Found: C, 70.58; H, 8.75; N, 6.70.

The more polar fractions (9.8 g crude weight, 36%) were pooled in acetone, decolorized, and crystallized from cold acetone–Skellysolve B, giving colorless crystals, mp 65–67°. Two recrystallizations from cold acetone gave (1*S*)-(+)-*N*-(**1,3-dimethyl-4-hydroxybutyl**)**benzamide** as colorless crystals: mp 68–71°; $[\alpha]D$ +33° (*c* 0.891); $\nu_{NH,OH}$ 3320 sh, 3270, $\nu_{C=O}$ 1635, $\nu_{C=C,amide\ II}$ 1600 sh, 1575, 1550, 1490, ν_{C6H5} 715 cm^{-1} in Nujol.

Anal. Calcd for $C_{13}H_{19}NO_2$: C, 70.55; H, 8.65; N, 6.33. Found: C, 70.82; H, 9.21; N, 6.59.

Determination of the Quantities of Products and Substrate in the Bioconversion of *N*-(1,3-Dimethylbutyl)benzamide (4) as a Function of Time. A 10-l. bioconversion of **4** (2.0 g) was carried out in the usual manner. Samples containing the equivalent of 10 mg of substrate (50 ml) were withdrawn from the fermentation at various time intervals (see Table II), extracted with methylene chloride (4 × 50 ml), and the combined extracts concentrated to dryness. The extracts were then dissolved in 0.4 ml of 1:1 (v/v) methanol–ethylene dichloride and 0.0020 ml (containing the equivalent of 0.050 mg of substrate) injected onto a 3-ft 2% (w/w) Epon 1001 epoxy resin 70–80 mesh GasChrom Z (Applied Science) column, which was being swept with 35–40 cc/min of helium. The chromatograph was programmed for 9°/min temperature rise from 175 to 240° on an F & M Model 609 gas chromatograph.

The substrate **4** and the products **5** and **6** were detected at 6, 10, and 13 min from the time of injection, respectively. The measured responses for detection of these compounds were corrected on the basis of the response of standardized pure control samples. Table II presents the amount of each component obtained as a percentage of the total material detected for each sample.

Bioconversion of *N*-(1,4-Dimethylpentyl)benzamide (7). The residue from the concentrated extract of the bioconversion of **7** (25.0 g, 0.114 mol) with *S. sulfurescens* was dissolved in methylene chloride and the solution was placed on a Florisil chromatography column (10.5 × 50 cm), which was dry-packed with Skellysolve B. Elution was with 2-l. fractions (one of Skellysolve B, four of 5% (v/v) acetone in Skellysolve B, four of 10% acetone–Skellysolve B, and seven of 25% acetone–Skellysolve B). Fractions 7–9 were combined in acetone. Crystallization did not occur from acetone–Skellysolve B, but was successful from methanol–water. Colorless

(24) R. H. Mazur, *J. Org. Chem.*, **35**, 2050 (1970).

Table II. Relative Quantities of Products and Remaining Substrate as a Function of Time in the Bioconversion of *N*-(1,3-Dimethylbutyl)benzamide

	Time, hr					
	18	25	43	50	68	72
% **4**	68.5	54	22	27	22	20
% **5**	10.5	16	28	25	28	31
% **6**	21	30	50	48	50	49
Sample recovery (γ)	38	43	32	44	50	39
Sample injected (γ)	50	50	50	50	50	50

crystals, 0.983 g (4%), of optically active substrate **7** were obtained: mp 99–102°; $[\alpha]_D$ −15° (*c* 0.884, chloroform).

Fractions 12 and 13 were combined in acetone, decolorized, and recrystallized from acetone–Skellysolve B. Two crops (12.00 g and 2.24 g, 0.0606, 53%, 59% based on recovered starting material) of crystals were obtained: mp 105–107°. Two recrystallizations from acetone–Skellysolve B gave ***N*-(1,4-dimethyl-4-hydroxypentyl)benzamide (8)** as colorless crystals: mp 105–107°; $[\alpha]_D$ 0°; $\nu_{NH,OH}$ 3330, 3280, $\nu_{C=O}$ 1635, $\nu_{C=C}$ 1605, 1580, 1495, $\nu_{amide\ II}$ 1550, $\nu_{C_6H_5}$ 700, 680 cm^{-1} in Nujol; $\delta^{37°}_{CDCl_3}$ 4.15 (multiplet, 1 H, –CHN<), 1.55 (multiplet, 4 H, $-CH_2CH_2-$), 1.20 (doublet, J = 6 Hz, 3 H, $-CH_3$), 1.17 (singlet, 6 H, *gem*-CH_3).

Anal. Calcd for $C_{14}H_{21}NO_2$: C, 71.45; H, 9.00; N, 5.95. Found: C, 71.56; H, 9.11; N, 6.08.

Bioconversion of *N*-(1,5-Dimethylhexyl)benzamide (9). The residue from the concentrated extract from the bioconversion of **9** (25.0 g, 0.107 mol) with *S. sulfurescens* was chromatographed on Florisil in the same way as described for the bioconversion of **7**. Fractions 12–15 were combined in acetone, decolorized, and crystallized from acetone–Skellysolve B, giving 13.137 g (0.0528 mol, 50%) of crystals: mp 105–110°. Two recrystallizations from acetone–Skellysolve B gave colorless crystals of ***N*-(1,5-dimethyl-5-hydroxyhexyl)benzamide (10)**: mp 110–112°; $[\alpha]_D$ 0° (chloroform); $\nu_{NH,OH}$ 3370 sh, 3340, $\nu_{C=O}$ 1640, $\nu_{C=C,amide\ II}$ 1620, 1605, 1575, 1545, 1495; $\delta^{37°}_{CDCl_3}$ 4.23 (multiplet, 1 H, –CHN), 1.47 (multiplet, 6 H, $-CH_2CH_2CH_2-$), 1.21 (doublet, J = 6 Hz, 3 H, CH_3), 1.16 (singlet, 6 H, *gem*-CH_3); lit.[15] mp 105–108°.

Anal. Calcd for $C_{15}H_{23}NO_2$: C, 72.25; H, 9.30; N, 5.62. Found: C, 72.40; H, 9.16; N, 5.93.

Bioconversion of *N*-(1,3,3-Trimethylbutyl)benzamide (11). The extracts from bioconversion of **11** (2.0 g, 9.13 mmol) were chromatographed on a Florisil column (3.8 × 35 cm), packed with Skellysolve B. Elution (335-ml fractions) with 5% (v/v) acetone–Skellysolve B removed starting material. Elution with 10% acetone–Skellysolve B gave two different crystalline products. The fractions of the less polar product were combined in acetone, decolorized, and recrystallized from acetone–Skellysolve B, giving 0.167 g (0.717 mmol, 8%) of crystals, mp 117–126°. Two recrystallizations from acetone–Skellysolve B gave **1-benzoyl-3,3,5-trimethyl-2-pyrrolidinol (13)** as colorless crystals: mp 126–129°; ν_{OH} 3350, $\nu_{C=O,C=C}$ 1605, 1575, 1520, 1495 cm^{-1} in Nujol; $\delta^{37°}_{CDCl_3}$ 4.78–3.90 (3 H, OH, –CHN<, –CHO), 1.80 (doublet, J = 8 Hz, 2 H, $-CH_2-$), 1.33 (broad doublet ?, 3 H, $-CH_3$), 1.03 (singlet, 3 H, $-CH_3$), 0.85 (singlet, 3 H, $-CH_3$).

Anal. Calcd for $C_{14}H_{19}NO_2$: C, 72.07; H, 8.21; N, 6.00. Found: C, 71.97; H, 8.00; N, 6.32.

The fractions of the more polar product were combined in acetone, decolorized, and recrystallized from acetone–Skellysolve B, giving 0.091 g (0.387 mmol, 4%) of crystals, mp 91–93°. Two recrystallizations from acetone–Skellysolve B gave ***N*-(4-hydroxy-1,3,3-trimethylbutyl)benzamide (12)** as colorless crystals: mp 91–93°; $[\alpha]_D$ 0°; $\nu_{NH,OH}$ 3360 sh, 3290, $\nu_{C=O}$ 1630, $\nu_{C=C}$ 1600, 1580, $\nu_{amide\ II}$ 1525, $\nu_{C_6H_5}$ 690 cm^{-1} in Nujol; $\delta^{37°}_{CDCl_3}$ 4.18 (multiplet, 1 H, –CHN<), 3.37 (doublet, J = 7 Hz, 2 H, $-OCH_2-$), 1.25 (doublet, J = 6.5 Hz, 3 H, $-CH_3$), 0.94 (singlet, 3 H, $-CH_3$), 0.88 (singlet, 3 H, $-CH_3$).

Anal. Calcd for $C_{14}H_{21}NO_2$: C, 71.45; H, 9.00; N, 5.95. Found: C, 71.57; H, 8.88; N, 6.33.

Bioconversion of **11** in other cases gave only **12** as the major product.

Oxidation of 1-Benzoyl-3,3,5-trimethyl-2-pyrrolidinol (13). A. With Jones Reagent. Excess Jones reagent was added to a solution of **13** (0.055 g) in acetone (3 ml). After typical work-up, a viscous, oily product was obtained, which had $\nu_{OH,NH}$ none, $\nu_{C=O}$ 1740, 1675, $\nu_{C=C}$ 1600, 1580, 1495, $\nu_{C_6H_5}$ 730, 695 cm^{-1} on the oil; $\delta^{37°}_{CDCl_3}$ 4.41 (sextet, $J \simeq 7$ Hz, 1 H, –CHN<), 2.18 (doublet of doublets, J_{gem} = −13 Hz, J = 8 Hz, 1 H, C-4 proton), 1.61 (doublet of doublets, J_{gem} = −13 Hz, J = 8 Hz, 1 H, C-4 proton), 1.41 (doublet, J = 6 Hz, 3 H, $-CH_3$), 1.25 (singlet, 3 H, $-CH_3$), 1.13 (singlet, 3 H, $-CH_3$).

B. During Vapor Phase Chromatography. When **13** was submitted to vpc analysis for the purpose of determining whether it was identical with the corresponding vpc peak on the crude beer assay, it was found to undergo partial oxidation to a compound having the same retention time as the above oxidation product. This oxidation presumably occurred in the injection port of the instrument. The instrument used was an F & M Model 5756 chromatograph with flame detector. The injection port temperature was 250°. The column was a 3.5 ft 3% (w/w) phenylsilicone polymer XE-61 on 100–120 mesh Supelcoport column and was programmed for an 8°/min temperature rise from 150 to 275° while being swept with 35–40 cc of helium/min.

Bioconversion of *N*-(1,1,3,3-Tetramethylbutyl)benzamide (15). Chromatography on Florisil (10.5 × 50 cm) of the extracts from bioconversion of **15** (25.0 g, 0.107 mol) gave a crystalline product from the early 10% (v/v) acetone–Skellysolve B fractions. These fractions were combined in acetone, decolorized, and recrystallized from acetone–Skellysolve B, giving 3.112 g (0.0124 mol, 11%) of product, mp 103–105°. Two recrystallizations from acetone–Skellysolve B gave ***N*-(4-hydroxy-1,1,3,3-tetramethylbutyl)benzamide (16)** as colorless needles: mp 106–108°; $\nu_{NH,OH}$ 3200, 3070, $\nu_{C=O}$ 1630, $\nu_{C=C}$ 1600, $\nu_{amide\ II}$ 1560, $\nu_{C_6H_5}$ 715 cm^{-1} in Nujol; $\delta^{37°}_{CDCl_3}$ 3.44 (singlet, 2 H, $-OCH_2-$), 1.69 (singlet, 2 H, $-CH_2-$), 1.53 (singlet, 6 H, $-CH_3$), 0.98 (singlet, 6 H, $-CH_3$).

Anal. Calcd for $C_{15}H_{23}NO_2$: C, 72.25; H, 9.30; N, 5.62. Found: C, 72.39; H, 9.25; N, 5.80.

Bioconversion of *N*-(1,3-Dimethylpentyl)benzamide (17). A methylene chloride solution of the extracts from bioconversion of **17** (25.0 g, 0.114 mol) was placed on a Florisil column (10.5 × 50 cm), which had been dry packed with Skellysolve B. Elution was with 2-l. fractions of increasing proportions of acetone in Skellysolve B. Elution with 10% (v/v) acetone (fractions 6–13) in Skellysolve B gave oily crystals in fractions 9–13. The oil was removed from the crystals of fraction 13 by washing with acetone and these crystals were combined with fractions 9–12 in acetone, decolorized, and crystallized from acetone–Skellysolve B giving 1.249 g (0.00536 mol, 4.7%) of product, mp 96–107°. Five recrystallizations from acetone–Skellysolve B gave ***N*-(1,3-dimethyl-4-oxopentyl)benzamide (18)** as colorless needles: mp 116–118°; $[\alpha]_D$ +59° (*c* 1.065); ν_{NH} 3310, $\nu_{C=O}$ 1705, 1635, $\nu_{C=C}$ 1605, 1580, 1490, $\nu_{amide\ II}$ 1535 cm^{-1} in Nujol; $\delta^{37°}_{CDCl_3}$ 4.25 (multiplet, 1 H, –CHN), 2.63 (multiplet, 1 H, –C(O)CH–), 2.10 (singlet, 3 H, $-C(O)CH_3$), 1.25 (doublet, J = 6.5 Hz, 3 H, $-CH_3$), 1.17 (doublet, J = 7 Hz, 3 H, $-CH_3$).

Anal. Calcd for $C_{14}H_{19}NO_2$: C, 72.07; H, 8.21; N, 6.00. Found: C, 71.90, 72.55; H, 8.86, 8.24; N, 5.92, 6.14.

Fractions 15–17 (eluted with 25% acetone in Skellysolve B) were combined and rechromatographed on a silica gel column (500 g, 5 × 57 cm), which was packed as a slurry in 50% (v/v) benzene in ethyl acetate. Elution was with 335-ml fractions of the same solvent system. Tlc (silica gel plates developed with ethyl acetate) of the fractions from this column showed fractions 5–9 (2.21 g) to be a mixture of ketone and alcohol and fractions 10–19 to contain only ***N*-(1,3-dimethyl-4-hydroxypentyl)benzamide (19)**, which was a viscous oil (5.79 g, 0.0246 mol, 21.6%): $\nu_{OH,NH}$ 3320, $\nu_{C=O}$ 1635, $\nu_{C=C}$ 1605, 1580, 1490, $\nu_{amide\ II}$ 1540, $\nu_{C_6H_5}$ 710, 700 cm^{-1} on the oil; $\delta^{37°}_{CDCl_3}$ 4.13, 3.58 (broad signals, 2 H, –CHN< and –OCH), 1.21 (doublet, J = 6 Hz, 3 H, $-CH_3$), 1.10 (doublet, J = 6 Hz, 3 H, $-CH_3$), 0.87 (doublet, J = 6 Hz, 3 H, $-CH_3$).

Anal. Calcd for $C_{14}H_{21}NO_2$: C, 71.45; H, 9.00; N, 5.95. Found: C, 70.91; H, 9.11; N, 5.89.

Oxidation of fractions 8, 9, and 20 (1.68 g) from the above silica gel column, with Jones reagent gave, after crystallization from acetone–Skellysolve B, 0.632 g of crystalline ketone **18**, mp 88–92°. Two recrystallizations from acetone–Skellysolve B gave colorless needles: mp 92–94°; $[\alpha]_D$ +8° (*c* 1.091); infrared spectrum in Nujol is identical with that of ketone **18** from bioconversion.

Anal. Calcd for $C_{14}H_{19}NO_2$: C, 72.07; H, 8.21; N, 6.00. Found: C, 72.35; H, 8.34; N, 6.15.

Bioconversion of *N*-(1-Ethylpropyl)benzamide (20). Bioconversion of **20** (2.0 g, 0.0105 mol) gave ***N*-(1-ethyl-2-hydroxypropyl)-benzamide (21)** (0.229 g, 1.11 mmol, 10.5%), mp 109–112°. Two recrystallizations from acetone–Skellysolve B gave **21** as colorless crystals: mp 114–116°; $[\alpha]_D$ −31° (*c* 0.769, $CHCl_3$); $\nu_{OH,NH}$ 3400 sh, 3280, $\nu_{C=O}$ 1630, $\nu_{C=C}$ 1600, 1575, 1490, $\nu_{amide\ II}$ 1540,

$\nu_{C_6H_5}$ 700 cm^{-1} in Nujol; $\delta^{37°}_{CDCl_3}$ 3.92 (multiplet, 3 H, OH, -CHN<, -CHO-), 1.67 (quintet, J = 7 Hz, 2 H, $-CH_2-$), 1.17 (doublet, J = 6 Hz, 3 H, $-CH_3$), 0.93 (triplet, J = 7 Hz, 3 H, $-CH_3$).

Anal. Calcd for $C_{12}H_{17}NO_2$: C, 69.54; H, 8.27; N, 6.76. Found: C, 69.86; H, 8.17; N, 6.86.

Bioconversion of **20** (25.0 g) gave recovered starting material (14.13 g), mp 93–96°, and **21** (1.558 g), mp 103–112°.

Bioconversion of *N*-(1-Methylbutyl)benzamide (22). A. The extracts from bioconversion of **22** (2.0 g, 0.0104 mol) were chromatographed on Florisil (3.8 × 35 cm) packed with Skellysolve B. Eluted first (increasing proportions of acetone in Skellysolve B were used to elute the column) was starting material (0.433 g, 21%): mp 78–88°; $[\alpha]D$ −15° (*c* 0.933, $CHCl_3$). Eluted next was a viscous gum (0.242 g, crude weight), which was identified as ***N*-(3-hydroxy-1-methylbutyl)benzamide (25)** by its nmr spectrum: $\delta^{37°}_{CDCl_3}$ 4.67–3.38 (broad multiplets, 3 H, OH, -CHNH<, -CHO-), 1.28 (doublet, J = 7 Hz, $-CH_3$), 1.14 (doublet, J = 6 Hz, $-CH_3$). Eluted last was 0.639 g (crude weight) of ***N*-(4-hydroxy-1-methylbutyl)benzamide (26).** Crystallization from acetone–Skellysolve B gave 0.137 g of colorless crystals, mp 90–93°. One recrystallization from acetone–Skellysolve B gave **26** as colorless needles: mp 92–93.5°; $[\alpha]D$ +23° (*c* 0.575, $CHCl_3$); infrared spectrum in Nujol is identical with the spectrum of **36**, below.

B. The extracts from bioconversion of **22** (25.0 g, 0.131 mol) were chromatographed on Florisil (10.5 × 50 cm) packed with Skellysolve B. Starting material (1.686 g), mp 93–95°, was eluted first. Elution with 25% (v/v) acetone–Skellysolve B (2-l. fractions) gave several fractions (numbers 13–18) having various mixtures of products. Fractions 13 and 14 were combined and rechromatographed on silica gel (150 g), which was packed with chloroform. Elution was with 100-ml fractions of 5% (v/v) acetone in chloroform. Fractions 7–10 and fractions 12–20 were shown by tlc to be separate components. Fractions 7–10 were combined in acetone, decolorized, and crystallized from acetone–Skellysolve B. The first crop (0.921 g) had mp 98–100°. Two recrystallizations from acetone–Skellysolve gave ***N*-(1-methyl-3-oxobutyl)benzamide (23)** as colorless crystals: mp 98–100°; $[\alpha]D$ 0° ($CHCl_3$); ν_{NH} 3300, $\nu_{C=O}$ 1710, 1630, $\nu_{amide\ II}$ 1545 cm^{-1} in Nujol; $\delta^{37°}_{CDCl}$ 4.51 (six-line pattern, J = 7 Hz, 1 H, >NCH-), 2.77 (doublet, J = 5.5 Hz, 1 H, -COCH-), 2.70 (doublet, J = 6 Hz, 1 H, -COCH-), 2.14 (singlet, 3 H, CH_3CO-), 1.28 (doublet, J = 7 Hz, 3 H, $-CH_3$).

Anal. Calcd for $C_{12}H_{15}NO_2$: C, 70.22; H, 7.37; N, 6.82. Found: C, 70.28; H, 7.47; N, 6.89.

The second crop (0.245 g) had mp 88–89°. Recrystallization from acetone–Skellysolve B gave colorless crystals of **23**: mp 88–90°; $[\alpha]D$ +20° (*c* 0.9796, $CHCl_3$); ν_{NH} 3300, $\nu_{C=O}$ 1710, 1630, $\nu_{amide\ II}$ 1540 cm^{-1} in Nujol.

Anal. Found: C, 70.08; H, 7.43; N, 6.80.

Fractions 12–20 were combined in acetone, decolorized, and crystallized from acetone–Skellysolve B. The product was a gummy, crystalline material. Some less gummy crystals were separated and recrystallized from acetone–Skellysolve B, giving soft crystals of ***N*-(3-hydroxy-1-methylbutyl)benzamide (24)**: mp 57–61°; $[\alpha]D$ −11° (*c* 0.6762, $CHCl_3$); $\nu_{NH,OH}$ 3500, 3400, $\nu_{C=O}$ 1625, $\nu_{amide\ II}$ 1540 cm^{-1} in Nujol; $\delta^{37°}_{CDCl_3}$ 1.28, 1.26 (two doublets, J = 7 Hz, 6 H, $-CH_3$).

Anal. Calcd for $C_{12}H_{17}NO_2$: C, 69.54; H, 8.27; N, 6.76. Found: C, 69.40; H, 8.07; N, 6.70.

Fractions 15 and 16 of the original column were combined and rechromatographed on silica gel (300 g) packed with chloroform. Elution was with 250-ml fractions of 5% acetone in chloroform. Fractions 7 and 8 were combined in acetone, decolorized, and crystallized from acetone–Skellysolve B, giving two crops of ketone **23**, 0.213 g and 0.241 g, mp 97–99°. The infrared spectrum was identical with that of ketone **23** obtained above. Fractions 11–16 were combined in acetone, decolorized, and crystallized from acetone–Skellysolve B, giving 1.096 g of **24**, mp 60–63°. Fractions 17–30 were combined in acetone, decolorized, and crystallized from acetone–Skellysolve B, giving 0.676 g of crystals, mp 88–90°. Two recrystallizations from acetone–Skellysolve B gave ***N*-(3-hydroxy-1-methylbutyl)benzamide (25)** as colorless needles: mp 91–93°; $[\alpha]D$ −14° (*c* 0.9992, $CHCl_3$); $\nu_{NH,OH}$ 3360, 3270, $\nu_{C=O}$ 1640, $\nu_{amide\ II}$ 1550, $\nu_{C_6H_5}$ 700 cm^{-1} in Nujol; $\delta^{37°}_{CDCl_3}$ 4.00 (eight-line pattern, $J \simeq$ 6.5 Hz, 2 H, HOCH-, NHCH-), 1.67 multiplet, 2 H, $-CH_2-$), 1.27 and 1.20 (two doublets, J = 6 Hz, 6 H, $-CH_3$).

Anal. Calcd for $C_{12}H_{17}NO_2$: C, 69.54; H, 8.27; N, 6.76. Found: C, 69.48; H, 8.43; N, 6.77.

Bioconversion of *N*-(1-Methylpentyl)benzamide (27). A. The extracts from bioconversion of **27** (2.0 g, 9.74 mmol) were chromatographed on Florisil (3.8 × 35 cm) packed with Skellysolve B. Eluted first was starting material, which was recrystallized from acetone–water, giving 0.112 g of crystals: mp 95–97°; $[\alpha]D$ −19° (*c* 0.956, $CHCl_3$). Eluted next and crystallized from acetone–Skellysolve B was ***N*-(1-methyl-4-oxopentyl)benzamide (28**, 0.200 g), mp 106–112°. Two recrystallizations from acetone–Skellysolve B gave **28** as colorless, fine needles: mp 121–123°; $[\alpha]D$ +17° (*c* 0.637, $CHCl_3$); ν_{NH} 3400, $\nu_{C=O}$ 1700, 1630, $\nu_{C=C}$ 1600 sh, 1490 sh, $\nu_{amide\ II}$ 1530, $\nu_{C_6H_5}$ 700 cm^{-1} in Nujol; $\delta^{37°}_{CDCl_3}$ 4.13 (multiplet, 1 H, -CHN), 2.54 (triplet, J = 6.5 Hz, 2 H, $-C(O)CH_2-$), 2.08 (singlet, 3 H, $CH_3C(O)-$) 1.82 (quartet, J = 6.5 Hz, 2 H, $-CH_2-$), 1.23 (doublet, J = 6 Hz, 3 H, $-CH_3$).

Anal. Calcd for $C_{13}H_{17}NO_2$: C, 71.20; H, 7.82; N, 6.39. Found: C, 71.13; H, 7.87; N, 6.69.

Eluted finally was a semicrystalline solid (see part B).

B. The extracts from a large-scale bioconversion of **27** (23.0 g 0.112 mol) were chromatographed on silica gel (10.5 × 50 cm). Eluted first was ketone **28**, which was decolorized in acetone and crystallized from acetone–Skellysolve B, giving 2.747 g (0.0125 mol, 11%) of crystals, mp 103–107°. Two recrystallizations from acetone–Skellysolve B gave colorless crystals of **28**: mp 107–112°; $[\alpha]D$ +6° (*c* 1.049, $CHCl_3$); ν_{NH} 3340, 3300, $\nu_{C=O}$ 1710, 1635, $\nu_{C=C}$ 1600, 1580, 1520, 1490, $\nu_{amide\ II}$ 1545, $\nu_{C_6H_5}$ 1490 cm^{-1} in Nujol.

Anal. Calcd for $C_{13}H_{17}NO_2$: C, 71.20; H, 7.82; N, 6.39. Found: C, 71.47; H, 7.74; H, 6.28.

Eluted second was partially crystalline ***N*-(4-hydroxy-1-methylpentyl)benzamide (29)** (18.4 g crude weight). A portion (7.2 g crude weight) was crystallized from methylene chloride–Skellysolve B by cooling in a freezer, giving 3.185 g of white solid, mp 62–72°.

Oxidation of 29. Oxidation of a sample of **29** (mp 62–72°, 0.729 g, 3.30 mmol) with Jones reagent gave, from acetone–Skellysolve B, crystalline ketone **28** (0.620 g, 2.83 mmol, 86%), mp 111–114°. Two recrystallizations from acetone–Skellysolve B gave colorless needles: mp 111–113° (lit.[25] mp 111–114°); $[\alpha]D$ 0° ($CHCl_3$); ν_{NH} 3300, $\nu_{C=O}$ 1710, 1635, $\nu_{C=C}$ 1600, 1580, 1490, $\nu_{amide\ II}$ 1545, $\nu_{C_6H_5}$ 700 cm^{-1} in Nujol.

Anal. Found: C, 71.51; H, 7.79; N, 6.42.

Bioconversion of *N*-(1-Ethylpentyl)benzamide (30). A methylene chloride solution of the extracts from bioconversion of **30** (25.0 g, 0.114 mol) was placed on a Florisil column (10.5 × 50 cm), which was dry packed with Skellysolve B. Fractions of 2 l. were collected. Fraction 5 (10% v/v acetone in Skellysolve B) contained starting material, which was recrystallized from methanol–water, giving colorless crystals (0.213 g): mp 101–103°; $[\alpha]D$ 0°. On the basis of tlc (10% methanol–benzene on silica gel plates) results, fractions 9–11 (20% acetone in Skellysolve B) were combined (6.6 g crude weight) in acetone, decolorized, and recrystallized from acetone–Skellysolve B, giving 4.175 g (0.0179 mol, 15.7%) of colorless crystals, mp 79–88°. Two recrystallizations from acetone–Skellysolve B gave ***N*-(1-ethyl-4-oxopentyl)benzamide (31)** as colorless crystals: mp 86–89°; $[\alpha]D$ 0°; ν_{NH} 3300, $\nu_{C=O}$ 1705, 1635, $\nu_{C=C}$ 1605, 1580, 1490, $\nu_{amide\ II}$ 1535, $\nu_{C_6H_5}$ 700 cm^{-1} in Nujol; $\delta^{37°}_{CDCl}$ 4.05 (multiplet, 1 H, CH-N), 2.56 (triplet, J = 6.5 Hz, 2 H, $-C(O)CH_2-$), 2.09 (singlet, 3 H, $CH_3C(O)-$), 2.03–1.33 (overlapping signals, 4 H, $-CH_2-$), 0.94 (triplet, J = 6.5 Hz, 3 H, $-CH_3$).

Anal. Calcd for $C_{14}H_{19}NO_2$: C, 72.07; H, 8.21; N, 6.00. Found: C, 72.28; H, 8.17; N, 6.05.

Fractions 12–15 (20% acetone in Skellysolve B) were combined in acetone and decolorized, and crystallization from acetone–Skellysolve B was attempted. Crystals failed to form and as the solution was allowed to evaporate to dryness (4 months), a glassy mass of ***N*-(4-hydroxy-1-ethylpentyl)benzamide (32)** remained, 14.683 g (0.0624 mol, 54%).

Oxidation of **32** (1.538 g, 6.55 mmol) with Jones reagent gave, after crystallization from acetone–Skellysolve B, 1.260 g (5.42 mmol, 92%) of crystalline ketone **31**, mp 85–88°. Two crystallizations from acetone–Skellysolve B gave **31** as very fine, colorless needles: mp 87–89°; $[\alpha]D$ 0° ($CHCl_3$); infrared spectrum in Nujol is identical with that of ketone **31** obtained directly from bioconversion.

Anal Found: C, 72.02; H, 8.36; N, 5.81.

Bioconversion of *N*-(1-Methylhexyl)benzamide (33). The residue from the methylene chloride extract of the bioconversion of **33** (25.0 g, 0.114 mol) with *S. sulfurescens* was redissolved in methylene chloride and placed on a Florisil column (10.5 × 50 cm), which was

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dry packed with Skellysolve B. Elution was with 2-l. fractions (one of Skellysolve B, five each of 5% (v/v) and 10% acetone in Skellysolve B, ten of 20% acetone–Skellysolve B, and five of 30% acetone–Skellysolve B). Fractions 14 and 15 were combined in acetone, decolorized, and crystallized from acetone–Skellysolve B. A first crop of 1.617 g (6.93 mmol, 6%) of colorless needles was obtained, mp 103–105°. Recrystallization from acetone–Skellysolve B gave colorless crystals of **N-(1-methyl-5-oxohexyl)benzamide (34)**: mp 111–113°; $[\alpha]D$ −13° (*c* 0.812, $CHCl_3$); ν_{NH} 3290, $\nu_{C=O}$ 1710, 1635, $\nu_{C=C}$ 1580, 1490, $\nu_{amide\ II}$ 1540, $\nu_{C_6H_5}$ 705 cm^{-1} in Nujol; $\delta^{37°}_{CDCl_3}$ 4.18 (multiplet, 1 H, –CHN<), 2.48 (triplet, J = 6 Hz, 2 H, $C(O)CH_2$–), 2.12 (singlet, 3 H, $CH_3C(O)$–), 1.60 (multiplet, 4 H, $-CH_2-$), 1.23 (doublet, J = 6.5 Hz, 3 H, $-CH_3$).

Anal. Calcd for $C_{14}H_{19}NO_2$: C, 72.07; H, 8.21; N, 6.00. Found: C, 72.27; H, 8.44; N, 6.26.

Fractions 18–20 were combined in acetone, decolorized, and crystallized from acetone–Skellysolve B, giving 1.317 g (5.60 mmol, 5%) of colorless crystals. Three recrystallizations from acetone–Skellysolve B gave **N-(5-hydroxy-1-methylhexyl)benzamide (35)** as colorless needles, but failed to sharpen the melting point, which was 107–115°: $[\alpha]D$ −6° (*c* 0.699, $CHCl_3$); $\nu_{NH,OH}$ 3290, $\nu_{C=O}$ 1635, $\nu_{C=C}$ 1605, 1580, 1490, $\nu_{amide\ II}$ 1545, $\nu_{C_6H_5}$ 700 cm^{-1} in Nujol; $\delta^{37°}_{CDCl_3}$ 4.21 (multiplet, 1 H, >CHN<), 3.80 (multiplet, 1 H, >CHO–), 1.49 (6 H, $-CH_2-$), 1.24 (doublet, J = 6 Hz, 3 H, $-CH_3$), 1.17 (doublet, J = 6 Hz, 3 H, $-CH_3$).

Anal. Calcd for $C_{14}H_{21}NO_2$: C, 71.45; H, 9.00; N, 5.95. Found: C, 71.80; H, 9.03; N, 5.98.

Fraction 24 was dissolved in acetone, decolorized, and crystallized from acetone–Skellysolve B. A first crop of 0.607 g of a mixture of two kinds of crystals was obtained. The major component consisted of long, fine needles, mp 88–91°, while the minor component was clear, rectangular crystals, which were less soluble in acetone. The latter were identified by their infrared spectrum as *l*-leucylprolyl anhydride. Two recrystallizations, carried out by dissolving the needles in acetone and carefully separating the solution from the remaining rectangular crystals and then crystallizing from acetone–Skellysolve B, served to completely remove the anhydride and gave **N-(4-hydroxy-1-methylbutyl)benzamide) (36)** as colorless needles: mp 93–95° $[\alpha]D$ +21° (*c* 0.389, $CHCl_3$); $\nu_{NH,OH}$ 3380, 3290, $\nu_{C=O}$ 1635, $\nu_{C=C}$ 1605, 1580, 1490, $\nu_{amide\ II}$ 1540, $\nu_{C_6H_5}$ 700 cm^{-1} in Nujol; $\delta^{37°}_{CDCl_3}$ 4.17 (multiplet, 1 H, >CHN<), 3.60 (multiplet, 2 H, $-CH_2O$), 1.60 (multiplet, 4 H, $-CH_2-$), 1.20 (doublet, J = 6.5 Hz, 3 H, $-CH_3$).

Anal. Calcd for $C_{13}H_{17}NO_2$: C, 69.54; H, 8.27; N, 6.76. Found: C, 69.23; H, 8.18; N, 7.06.

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The Influence of a Methyl Substituent on the Microbiological Oxygenation of Cyclic Compounds[1]

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Abstract: The influence that a methyl substituent has upon the oxygenation of cyclic substrates with *Sporotrichum sulfurescens* has been examined. Previous and present results suggest that oxidative attack at a given position will be enhanced by a methyl substituent, provided the hydrogen to be replaced can approximate a trans orientation in relation to a second enzyme attachment site within the substrate molecule. However, if the methyl group assumes this trans orientation, it may block oxidative attack at that position.

A preference for attack at the more highly substituted subterminal carbon of acyclic *N*-alkylbenzamides was suggested by a study of the oxygenation of these substrates with *Sporotrichum sulfurescens*.[2] This observation raised the question of what effect a methyl substituent might have on the oxygenation of cyclic substrates. Consequently, we have examined those of our previous results pertinent to this question and have carried out several additional experiments with the hope of answering this question.

Previous Results. Several of the many cyclic substrates that are oxygenated by *S. sulfurescens* have had methyl substituents. Both the cis and trans isomers of *N*-benzoyl-2-methylcyclohexylamine underwent oxygenation at the 4 position of the cyclohexane ring.[3] The methyl group in these substrates apparently is too near the amide functional group to exert influence and oxygenation occurs by replacement of the equatorial C-4 hydrogen. The latter position is more nearly at an optimum distance of 5.5 Å from the amide carbonyl oxygen, considered to be a site of attachment to the enzyme system.[4] Similarly, 1-benzoyl-2-methylpiperidine did not undergo oxygenation at the C-2 position.[5] However, 1-benzoyl-3-methylpiperidine was, in part, hydroxylated at the 3 position, but was not hydroxylated at the 5 position (geometrically equivalent to the 3 position with respect to the benzamido functional group), suggesting that a methyl substituent may have an influence on the position at which oxygenation occurs. 1-Benzoyl-4-methylpiperidine (**1**) also was oxygenated partially at the 4 position, the other major product resulting from oxygenation of the methyl group. It was speculated that oxygenation to give the 4-hydroxy compound might be occurring only when the piperidine ring was in the conformation having an axial 4-methyl group.[5]

Present Results. With the above results in mind, the substrate 1-benzoyl-4-methyl-1(4*H*)-hexahydroazepine (**2**) has been prepared. This substrate presents two positions (the 4 and 5 positions), which differ only by

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