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-methylhexylbenzamide** (**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 $C_{12}H_{17}NO_2$: C, 69.54; H, 8.27; N, 6.76. Found: C, 69.23; H, 8.18; N, 7.06.

Acknowledgments. We thank J. R. Heald, J. M. Noteboom, I. N. Pratt, M. J. Sutton, S. L. Towne, and H. M. Wiessner for technical assistance and Dr. E. C. Olson and associates for physical and analytical data.

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

Roy A. Johnson,* Milton E. Herr, Herbert C. Murray, and Lester M. Reineke

Contribution from the Research Laboratories, The Upjohn Company, Kalamazoo, Michigan 49001. *Received October 27, 1970*

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.

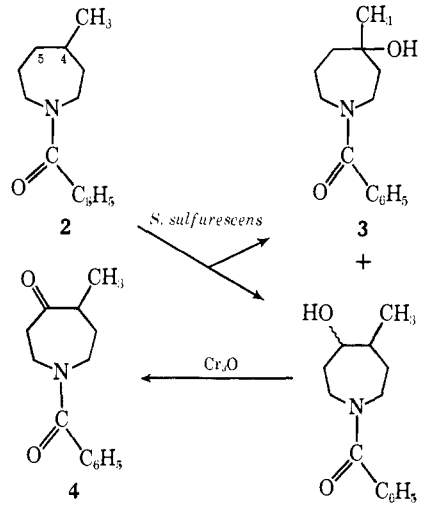
A preference for attack at the more highly substituted subterminal carbon of acyclic *N*-alkylbenzamides was suggested by a study of the oxygenation of these substrates with *Sporotrichum sulfurescens*.[2] This observation raised the question of what effect a methyl substituent might have on the oxygenation 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

(1) Stereochemistry of Microbiological Hydroxylation, Part VII.
(2) R. A. Johnson, H. C. Murray, and L. M. Reineke, *J. Amer. Chem. Soc.*, **92**, 4872 (1971).
(3) R. A. Johnson, M. E. Herr, H. C. Murray, and G. S. Fonken, *J. Org. Chem.*, **35**, 622 (1970).
(4) G. S. Fonken, M. E. Herr, H. C. Murray, and L. M. Reineke, *J. Amer. Chem. Soc.*, **89**, 672 (1967).
(5) R. A. Johnson, H. C. Murray, L. M. Reineke, and G. S. Fonken, *J. Org. Chem.*, **34**, 2279 (1969).

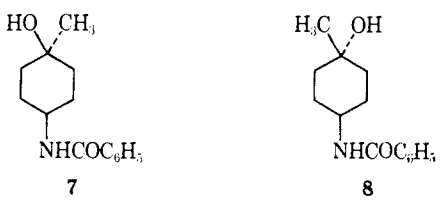
the presence or absence of a methyl substituent, to the oxygenating enzyme. Furthermore, the conformational mobility of this compound is greater than for those having six-membered rings.[6] This factor is of importance in allowing the methyl group to assume various conformational positions with a minimum of energy change, making more real the comparison of the tertiary C-4 and the secondary C-5 carbons as sites for oxygenation. The major product (29%) isolated from oxygenation of **2** with *S. sulfurescenes* was the tertiary alcohol **3**, identified by the presence of a singlet for the methyl signal in the nmr spectrum. The sec-



2 **3** **4**

ondary carbon at C-5 was oxygenated to a lesser extent (11%), the product being isolated by oxidation of the secondary alcohol to a ketone (**4**) to facilitate separation from tertiary alcohol **3**. The unsubstituted analog of substrate **2**, 1-benzoyl-1(4*H*)-hexahydroazepine, is known to be primarily (55%) oxygenated at the C-4 position (equivalent to C-5 in **2**).[7] It therefore appears that in the present case, the methyl substituent does have a directive influence upon the position of oxidative attack.

The cis and trans isomers of *N*-benzoyl-4-methylcyclohexylamine, **5** and **6** (Figure 1), respectively, appeared suitable for further testing the effect of a methyl substituent on the oxygenation of cyclic compounds, since *N*-benzoylcyclohexylamine is hydroxylated at the 4 position, giving *N*-benzoyl-*trans*-4-hydroxycyclohexylamine.[3] Oxygenation of a readily available mixture of **5** and **6** gave two tertiary alcohols (**7**, 30%, and **8**, 0.8%) as the only isolated products. Since previous results



7 **8**

have established that the hydroxyl group is introduced trans with respect to the amide group in molecules such as these,[3,8] it was suspected that the major product (**7**)

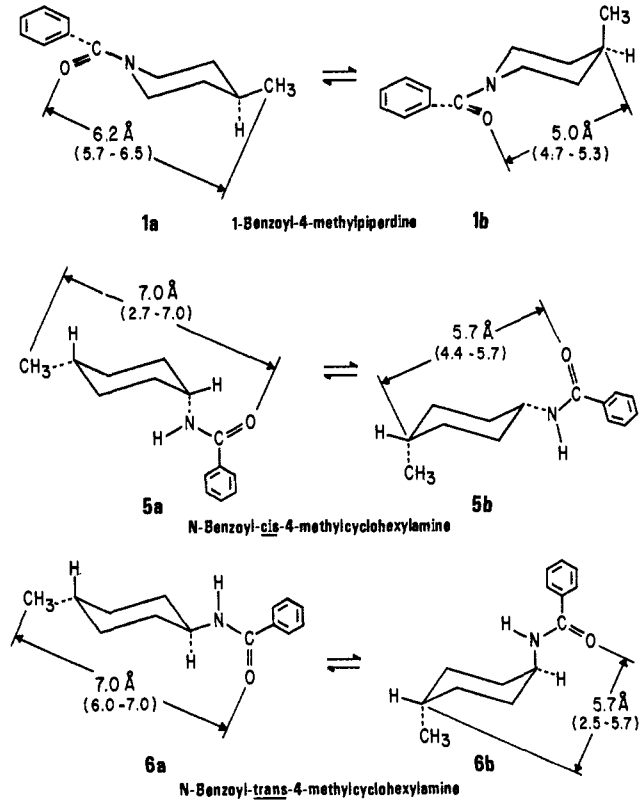


Figure 1. Preferred conformations of some substrate molecules Distances refer to presumed conformation of lowest energy (range of distances are for other conformations possible).

was derived from the cis isomer of the substrate (**5**) because conformational flipping to give an axial 4-methyl group (and an equatorial 4-hydrogen, conformation **5b** Figure 1) is much more favorable in this isomer. This suspicion was shown to be correct when the product obtained from oxygenation of a nearly pure (>95%) sample of the cis isomer was found to be identical with the product **7**. It seems, therefore, that oxygenation occurs readily at the tertiary 4 position when the methyl group can easily assume an axial conformation (cis isomer), but is largely blocked when the methyl group prefers to remain in the equatorial conformation (trans isomer).

These results are further supported by the oxygenation of the cis and trans (with respect to the amide group) 4-methyl derivatives of *N*-benzoyl-*N*-methyl-1-adamantanamine.[9] The rigid adamantane structure of course prevents conformational flipping such as is possible in the *N*-benzoyl-4-methylcyclohexylamines. It also provides positions that, when unsubstituted, are known to be readily oxygenated.[10] The major products from oxygenation of the cis-4-methyl isomer (**9**) were the 4,6-dihydroxy and the 4,7-dihydroxy[11] derivatives.[9] Therefore, in this isomer, it appears that the methyl group is directing oxygenation to the 4 position. Oxygenation of the *trans*-4-methyl isomer (**10**) gave a 6-hydroxy product in low yield.[9] No product of oxy-

(6) *Cf.*, (a) E. L. Eliel, N. L. Allinger, S. J. Angyal, and G. A. Morrison, "Conformational Analysis," Interscience, New York, N. Y., 1965, pp 207–210; (b) M. Hanack, "Conformation Theory," Academic Press, New York, N. Y., 1965, pp 158–162.

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

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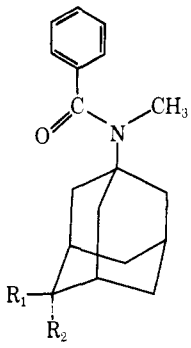
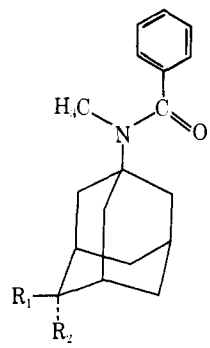
(11) The assignment of the 7 position to one of the hydroxy groups in this derivative is only tentative. The other hydroxyl group is definitely at C-4, however.[9]



9a, $R_1 = CH_3$; $R_2 = H$
10a, $R_1 = H$; $R_2 = CH_3$

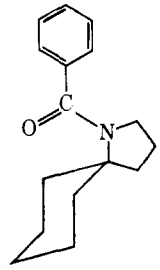


9b, $R_1 = CH_3$; $R_2 = H$
10b, $R_1 = H$; $R_2 = CH_3$

genation at the 4 position could be detected, and here it appears that the methyl group is blocking attack at this position.

Further Discussion. The additional question may be raised as to why the methyl group itself is not oxygenated in some of these substrates, since in the case of 1-benzoyl-4-methylpiperidine partial oxygenation of the methyl group is observed.[5] Consideration of the conformations of these substrates and of the distances between the amide carbonyl oxygen and the potential sites of oxygenation offer a possible explanation.

Several conformational variables are present in these substrates. Interconversion of chair forms permits substituents to assume either axial or equatorial configurations. The amide group itself enjoys considerable conformational freedom, particularly in the case of a primary amide. In Figure 1 are shown the amide conformations presumed to be of lowest energy for three substrates (**1**, **5**, and **6**) having 4-methyl substituents in axial and in equatorial configurations. In adamantane substrates **9** and **10** the conformational freedom of interest is that resulting from rotation around the adamantyl-nitrogen C–N bond. The extremes of this rotation are represented by conformers **9a** and **9b** and should differ only slightly in energy. The conformationally related substrate 1-benzoyl-1-azaspiro[4.5]decane has been shown[3] to prefer conformation **11**, identical with conformation **9a**. Since **11** is



11

hydroxylated at position C-7 in good yield, we conclude that this conformation is acceptable to the oxygenating enzyme. Thus, to examine the distances between amide carbonyl oxygen and oxygenation site, we may consider conformers **1a**, **1b**, **5a**, **5b**, **6a**, **6b**, **9a**, and **10a**.

Examination of those conformers assumed to be oxygenated (**1a**, **1b**, **5a**, **9a**) reveals distances between carbonyl oxygen and oxygenation site that vary from a minimum of 4.5 Å (**9a**) to a maximum of 6.2 Å (**1a**). These distances represent a range centered around 5.5 Å, the distance that has been suggested as optimum between a hypothetical enzyme attachment site and the sites of oxygenation on cyclododecanol by *S. sulfurescens*.[4] In those conformers that are not oxygenated (**5a**, **6a**, **6b**, **10a**), either the intersite distances are larger, varying from 6.0 Å (**10a**) to 7.0 Å (**5a** and **6a**), or the conformations are of considerably higher energy than the minimum possible for the molecule. For example, conformer **6b**, which has the appropriate separation between carbonyl oxygen and potential oxygenation site, is the conformer of **6** of higher energy, having two axial substituents. These substituents increase the energy of conformer **6b** over **6a** by 3.0–3.5 kcal/mol.[12] This energy difference represents an equilibrium concentration of roughly one **6b** to 250 **6a**, a level of **6b** that apparently is too low to result in significant oxygenation. Consequently, it may be suggested that this energy exceeds the energetic limit of the enzyme to hold the substrate molecule in conformation **6b** for the necessary length of time to achieve oxygenation.

Summary. The above discussion suggests that the following factors influence the oxygenation of cyclic molecules by *S. sulfurescens*. (1) Oxidative attack may occur at positions as far as 6.0–6.2 Å distant from the hypothetical enzyme attachment site (*e.g.*, amide carbonyl oxygen). (2) Oxidative attack may occur at positions as near as 4.5 Å from the carbonyl oxygen. Distances less than this have not been adequately challenged. (3) Oxidative attack at a given position will be enhanced by a methyl substituent at that point, within the above spatial limitations and provided that the methyl group is not oriented trans to the amide group. In the latter case, oxygenation may be either blocked, or may occur on the methyl carbon.

Experimental Section[13]

Biotransformation Process. The culture used in these experiments was *Sporotrichum sulfurescens* v. Beyma (ATCC 7159). The biotransformation procedure has been described previously.[7]

1-Benzoyl-4-methylenehexahydro-4*H*-azepine. A solution of *n*-butyllithium in hexane (~1.6 *M*, 37.0 ml) was added to a mixture of methyltriphenylphosphonium bromide (20.9 g, 0.0585 mol) and dry benzene (300 ml) which was under an atmosphere of N_2. A solution of 1-benzoyl-4-oxohexahydro-4*H*-azepine[7] (12.705 g, 0.0585 mol) in benzene was added dropwise to the above mixture. The resulting mixture was heated to 60–70° for 3 hr, then cooled and allowed to evaporate partially. Water (200 ml) was added to the reaction mixture. The benzene layer was separated, dried, and concentrated under reduced pressure. The residual material was chromatographed on a column of silica gel (500 g) which was packed as a slurry with 50% (v/v) cyclohexane–ethyl acetate. The column was eluted with the same system (500-ml fractions). The oily product was found in fractions 3–6. Distillation gave 7.49 g (0.0348 mol, 59%) of **1-benzoyl-4-methylenehexahydro-4*H*-azepine** as a colorless liquid: bp 123–125° (0.10 mm); $\nu_{C=O}$ 1630, $\nu_{C=C}$ 1600, 1575, 1495, $\nu_{C_6H_5}$ 785, 730, 705 cm^{-1} neat; δ_{CDCl_3} 290 (singlet, 2 H, $=CH_2$), 212 (broad multiplet, 4 H, $-CH_2NCH_2-$) cps.

Anal. Calcd for $C_{14}H_{17}NO$: C, 78.10; H, 7.96; N, 6.51. Found: C, 78.03; H, 8.09; N, 6.83.

1-Benzoyl-4-methylhexahydro-4*H*-azepine (2). A solution of 1-benzoyl-4-methylenehexahydro-4*H*-azepine (7.25 g, 0.0337 mol) in methanol (75 ml) was shaken with hydrogen (40 psi) and PtO_2 (1 g) in a Parr hydrogenation apparatus. Uptake of hydrogen had stopped after 5 hr. The catalyst was removed by careful filtration and the filtrate was concentrated under reduced pressure, giving the oily product. Distillation gave 6.000 g (0.0276 mol,

(12) Reference 6a, pp 44–45.

(13) 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.

82%) of colorless **1-benzoyl-4-methylhexahydro-4*H*-azepine (2)**: bp 120–128° (0.08–0.09 mm); $\nu_{C=O}$ 1630, $\nu_{C=C}$ 1600, 1575, 1495, $\nu_{C_6H_5}$ 785, 730, 705 cm^{-1} neat; $\delta^{37°}_{CDCl_3}$ 222, 205 (multiplets, 4 H, $-CH_2NCH_2-$), 128–170 (6 H, $-CH_2-$), 59 (doublet, 3 H, J = 5.5 Hz, $-CH_3$).

Bioconversion of 1-Benzoyl-4-methylhexahydro-4*H*-azepine (2). The extracts from bioconversion of **2** (2.0 g, 9.23 mmol) with *S. sulfurescens* were chromatographed on Florisil (3.8 × 35 cm), which was dry packed with Skellysolve B. Elution with 10% (v/v) and with 25% acetone in Skellysolve gave mixtures of ketonic and hydroxylic products as determined by tlc and ir and nmr spectra. Decolorization and crystallization of several later fractions from acetone–Skellysolve B gave 0.442 g of colorless crystals, mp 91–93°. Two recrystallizations from acetone–Skellysolve B gave shiny, colorless crystals, identified by their nmr spectrum as **1-benzoyl-4-hydroxy-4-methylhexahydro-4*H*-azepine (3)**: mp 94–96°; $[\alpha]D$ −4° (*c* 0.747, $CHCl_3$); ν_{OH} 3370, $\nu_{C=O}$ 1600, $\nu_{C=C}$ 1575, 1530, 1505, $\nu_{C_6H_5}$ 740, 715, 705 cm^{-1} in Nujol; $\delta^{37°}_{CDCl_3}$ 235–190 (multiplet, 4 H, $-CH_2NCH_2-$), 102 (multiplet, 6 H, 3-CH_2-), 72.5 (singlet, 3 H, $-CH_3$) cps.

Anal. Calcd for $C_{13}H_{17}NO_2$ (233.30): C, 72.07; H, 8.21; N, 6.00. Found: C, 71.46, 72.48; H, 8.14, 8.03; N, 6.19.

The remaining chromatography fractions containing products and the filtrates from the above crystallizations were combined in acetone and oxidized with excess Jones reagent.[14] Following work-up, the product mixture (1.02 g) was chromatographed on Florisil (100 g), which was packed with Skellysolve B. The products were eluted with 20% (v/v) acetone in Skellysolve B. Two fractions of ketone, one fraction of mixture (~1:3 ketone to alcohol), and four fractions of alcohol were obtained. Tlc (20% methanol in benzene) showed this separation of products. The ketone (**4**) (crude weight, 0.24 g, 1.03 mmol, 11%) failed to crystallize; $\nu_{C=O}$ 1705, 1605 cm^{-1} on the oil; $\delta^{37°}_{CDCl_3}$ 242 (broad multiplet, 2 H, >CHNCH<), 202 (multiplet, 2 H, >CHNCH<), 160 (multiplet, 3 H, $-CH_2COCH<$), 102 (four-line pattern, 2 H, $-CH_2-$), 66 (doublet, 3 H, J = 6.5 Hz, $-CH_3$) cps. The alcohol-containing fractions (crude weight, 0.37 g) gave 0.182 g of crystalline **3**, mp 93–95°; total yield of **3**, 0.624 g (2.68 mmol, 29%).

Bioconversion of the Mixture, *N*-Benzoyl-*cis*-4-methylcyclohexylamine (5) and *N*-Benzoyl-*trans*-4-methylcyclohexylamine (6). The methylene chloride extracts from bioconversion of this mixture (25.0 g, 0.115 mol, containing about 40% of one component and 60% of the other as determined by vpc analyses on several columns) were chromatographed on a column of Florisil (10.5 × 50 cm) which was dry packed with Skellysolve B. The column was eluted with 2-l. fractions of increasing proportions of acetone in Skellysolve B. Fraction 10 (20% acetone in Skellysolve B, crude weight 0.34 g) was recrystallized from acetone–Skellysolve B, giving 0.227 g (0.975 mmol, 0.8%) of colorless crystals, mp 154–156°. Two recrystallizations from acetone–Skellysolve B gave ***N*-benzoyl-*trans*-4-methyl-4-hydroxycyclohexylamine (8)**: mp 156–158°; $\nu_{OH,NH}$ 3380, 3300, $\nu_{C=O}$ 1635, $\nu_{amide\ II}$ 1540, $\nu_{C_6H_5}$ 700 cm^{-1} in Nujol; δ_{CDCl_3} 385 (1 H, doublet, J = 8 Hz, -NH-), 234 (1 H, broad, -CHN-), 130–190 (8 H, $-CH_2-$), 72 (3 H, singlet, CH_3).

Anal. Calcd for $C_{14}H_{19}NO_2$: C, 72.07; H, 8.21; N, 6.00. Found: C, 71.96; H, 8.24; N, 6.02.

Fractions 13–18 (crude weight, 9.61 g) were combined on the basis of tlc, decolorized, and recrystallized from acetone–Skellysolve B, giving 8.221 g (35.2 mmol, 30%) of colorless crystals, mp 161–163°. Two recrystallizations gave *N*-benzoyl-*cis*-4-methyl-4-hydroxycyclohexylamine (**7**) as colorless crystals: mp 171–173°; $\nu_{OH,NH}$ 3350, 3310, $\nu_{C=O}$ 1640, $\nu_{amide\ II}$ 1545, $\nu_{C_6H_5}$ 695 cm^{-1} in Nujol; δ_{CDCl_3} 373 (1 H, broad, -NH-), 242 (1 H, broad, -CHN-), 76 (3 H, singlet, $-CH_3$).

Anal. Calcd for $C_{14}H_{19}NO_2$: C, 72.07; H, 8.21; N, 6.00. Found: C, 71.86; H, 8.04; N, 5.84.

Bioconversion of *N*-Benzoyl-*cis*-4-methylcyclohexylamine (5). The extracts from bioconversion of **5** (0.300 g, 1.38 mmol, >95% *cis* isomer of mp 125–128°, lit.[15] mp 130–130.5°) in shake flasks were chromatographed on Florisil (30 g). A single product was isolated. Decolorization and crystallization from acetone–Skellysolve B gave 0.022 g (0.0945 mmol, 7%) of ***N*-benzoyl-*cis*-4-methyl-4-hydroxycyclohexylamine (7)** as colorless crystals, mp 172–174°; infrared spectrum in Nujol is identical with the sample of **7** described above.

Acknowledgments. It is a pleasure to thank Dr. G. S. Fonken for many discussions of these results. We thank J. R. Heald, J. M. Noteboom, I. N. Pratt, M. J. Sutton, S. L. Towne, and H. M. Wiessner for technical assistance.

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Nucleophilic Attack by Zinc(II)–Pyridine-2-carbaldoxime Anion on Phosphorylimidazole. A Model for Enzymatic Phosphate Transfer[1]

G. J. Lloyd[2] and Barry S. Cooperman*[3]

Contribution from the Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104. *Received July 18, 1970*

Abstract: Phosphorylimidazole (PIm) monoanion has been found to transfer rapidly its phosphate group to the anionic oxygen of Zn^{2+}–pyridine-2-carbaldoxime (Zn^{2+}–PCA). The results suggest that transfer proceeds *via* a ternary complex. In the absence of Zn^{2+}, no evidence is found for PCA anion attack on either PIm or *N*-methylphosphorylimidazole (*N*-MePIm). The catalytic role of Zn^{2+} is explained on the basis of its ability to lower the electrostatic barrier toward anionic attack on an anionic center and to provide a template for proper alignment of the oxime oxygen and the phosphorus of PIm. The relevance of these studies to the nucleoside diphosphokinase reaction is discussed.

Nucleophilic attack by anionic oxygen at the phosphorus of phosphate monoester dianions is a common enzyme-mediated reaction, the enzymes responsible generally requiring divalent metal ions for activity.[4]

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(2) Thouron Fellow, 1969–1970.

(3) To whom requests for reprints and all inquiries should be addressed.

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