Microbiological Oxygenation of Alicyclic Amides

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Received February 7, 1968

Continuing our broad study of the microbiological oxygenation of simple monocyclic systems, a series of N-cycloalkylamides and N,N-dicycloalkylamides was subjected to fermentation by *Sporotrichum sulfurescens*. Consonant with the hypothetical enzyme–substrate model proposed earlier, N-cyclododecylacetamide was monooxygenated at the 5, 6, and 7 positions, while a variety of N-cyclohexylamides and N-cycloheptylamides underwent oxygenation at the 4 position. N-Cyclooctylamides gave two products, thought to be monoxygenated at the 4 and 5 positions. When confronted with two alicyclic rings of different sizes in the same N,N-dicycloalkylacetamide molecule, the organism seemed to prefer the cycloheptyl to the cyclohexyl ring, and showed little tendency to attack a cyclopentyl ring.

The hypothetical enzyme–substrate model proposed[1] for the microbiological oxygenation of monoalicyclic alcohols such as cyclododecanol suggested as an additional line of investigation the microbiological oxygenation of cycloalkylamines. When cyclododecylamine was exposed to *Sporotrichum sulfurescens* ATCC 7159 in a complex nutrient medium a mixture of N-(hydroxycyclododecyl)acetamides was obtained that was oxidized to a mixture of N-(oxocyclododecyl)-acetamides with Jones chromic acid reagent.[2] Appropriate chromatography and crystallization afforded N-(6-oxocyclododecyl)acetamide as the preponderant product, a lesser amount of N-(7-oxocyclododecyl)-acetamide, and a very small amount of N-(5-oxocyclododecyl)acetamide. The same products were obtained when N-cyclododecylacetamide was used as the substrate. Evidently N-acetylation (either microbial or chemical) is a necessary prerequisite to ring hydroxylation, since a variety of other microorganisms failed to acetylate or hydroxylate cyclododecylamine, but readily hydroxylated N-cyclododecylacetamide. Even *S. sulfurescens* failed to bring about hydroxylation of cyclododecylamine when the fermentation was conducted in Czapek–Dox medium, under which conditions N-acetylation did not take place.

Unequivocal structure proofs were carried out for the isomeric N-(5-, 6-, and 7-oxocyclododecyl)acetamides, wherein each compound was reduced with sodium borohydride to the corresponding N-(hydroxycyclododecyl)acetamide, which was acetylated. Treatment of the N-(acetoxycyclododecyl)acetamide with dinitrogen tetraoxide by the method of White[3] gave the N-nitroso product, with subsequent elimination of nitrogen and rearrangement to the diacetate. Hydrolysis and subsequent chromic acid oxidation led to the previously described cyclododecanediones.[1,4]

Attempted microbial hydroxylation of N-cyclohexylacetamide was not successful, perhaps for the same reasons presented in the earlier[1] discussion of experiments with cyclohexanol. When the microorganism was presented with the more lipophilic N-cyclohexylbenzamide, hydroxylation to N-(4-hydroxycyclohexyl)-benzamide took place. The structure of this product was established by oxidizing it to the keto derivative, which was identical with N-(4-oxocyclohexyl)benzamide synthesized from 4-hydroxycyclohexylamine.[5]

Similarly, microbial hydroxylation of benzyl cyclohexylcarbamate[6] gave benzyl 4-hydroxycyclohexylcarbamate, identical with material synthesized by N-acylation of 4-hydroxycyclohexylamine with carbobenzoxy chloride. Microbial hydroxylation of N-cyclohexyl-*p*-toluenesulfonamide gave, after Jones reagent oxidation, N-(4-oxocyclohexyl)-*p*-toluenesulfonamide identical with synthetic material obtained by N-acylation of 4-hydroxycyclohexylamine with *p*-toluenesulfonyl chloride followed by Jones reagent oxidation.

Microbial hydroxylation of N-cycloheptylbenzamide,[7] N-cycloheptyl-*p*-toluenesulfonamide, and benzyl cycloheptylcarbamate gave (in each case after Jones reagent oxidation to convert alcohol–ketone mixtures entirely into ketone) the corresponding 4-oxo compounds. The structures for these compounds were established by synthesis, using diazomethane ring expansion of the corresponding 4-oxocyclohexyl compounds.

Interestingly, although the N-(4-oxocycloheptyl)-benzamides obtained by bioconversion and by synthesis had identical infrared and nmr spectra, the bioconversion product was optically active whereas the synthetic product was a racemate. The same racemate was obtained by reduction of microbially produced N-(4-oxocycloheptyl)-*p*-toluenesulfonamide to 4-hydroxycycloheptylamine, followed by benzoylation, and oxidation. Thus the microorganism apparently attacks stereoselectively at one of the 4-methylene groups when the nitrogen bears the benzoyl group, but not when it bears the *p*-toluenesulfonyl group. In the case of the benzyl cycloheptylcarbamate bioconversion, the optical activity of the product was of such a low order that it is impossible to assess possible stereospecificity with the present data.

Microbial hydroxylation of N-cyclooctyl-*p*-toluenesulfonamide gave two keto amides whose structures have not been established unequivocally. The higher melting compound is tentatively assigned the structure N-(5-oxocyclooctyl)-*p*-toluenesulfonamide on the basis of its anomalous nmr spectrum, which suggests that the material exists as an equilibrium internal redox mixture of keto amide and N-(5-hydroxycyclooctylidene)-*p*-toluenesulfonamide. The transannular redox reaction would be consistent with a 1,5 relationship of the

(1) G. S. Fonken, M. E. Herr, H. C. Murray, and L. M. Reineke, *J. Amer. Chem.*, **89**, 672 (1967).
(2) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).
(3) E. H. White, *J. Amer. Chem. Soc.*, **77**, 6008, 6011 (1955).
(4) E. T. Niles and H. R. Snyder, *J. Org. Chem.*, **26**, 330 (1961).
(5) E. Ferber and H. Bruckner, *Ber.*, **72**, 995 (1939).
(6) A. B. Foster, M. Stacy, and S. V. Vardheim, *Acta Chem. Scand.*, **13**, 281 (1959).
(7) M. Mousseron, R. Jacquier, and H. Christol, *Bull. Soc. Chim. Fr.*, **24**, 346 (1957).

functional groups. The lower melting compound is tentatively assigned the structure N-(4-oxocyclooctyl)-*p*-toluenesulfonamide by elimination of alternatives *via* consideration of the nmr spectrum.

By melting point analogy to the N-cyclooctyl-*p*-toluenesulfonamide compounds, the higher melting keto amide from microbial hydroxylation of N-cyclooctylbenzamide is tentatively considered to be N-(5-oxocyclooctyl)benzamide, and the lower melting compound N-(4-oxocyclooctyl)benzamide.

N,N-Dicycloalkylacetamides should also fulfill the polarity (lipophilicity) and geometry requirements of the enzyme–substrate model. The dicycloalkyl amide system would be expected to be particularly useful for measuring the relative effects of ring conformations and sizes on the tendency to hydroxylate. N,N-Dicyclopentylacetamide, when subjected to *S. sulfurescens*, afforded only a very low yield of impure material that was not characterized but is believed to be a hydroxylated derivative. On the other hand, bioconversion of N,N-dicyclohexylacetamide by *S. sulfurescens* gave a good yield of N-cyclohexyl-N-(4-hydroxycyclohexyl)-acetamide. The structure was established by comparison with an authentic sample, synthesized by condensing 4-hydroxycyclohexylamine with cyclohexanone in a Leuckart reaction[8] to give cyclohexyl-(4-hydroxycyclohexyl)amine, which was acetylated to the O,N-diacetyl derivative and then saponified to the desired compound. N-Cyclohexyl-N-cyclopentylacetamide was hydroxylated in modest yield to N-cyclopentyl-N-(4-hydroxycyclohexyl)acetamide, whose structure was established by synthesis from 4-hydroxycyclohexylamine and cyclopentanone *via* the Leuckart reaction, followed by acylation. N-Cyclohexyl-N-cycloheptylacetamide, prepared *via* a Leuckart reaction using cycloheptanone and cyclohexylamine, gave a hydroxylated product that was most readily isolated as the corresponding ketone. This was shown not to be N-(4-hydroxycyclohexyl)-N-cycloheptylacetamide by synthesizing this compound from 4-hydroxycyclohexylamine, following the route outlined above for the N-cyclohexyl-N-(4-hydroxycyclohexyl)acetamide synthesis. Assuming that the microbially introduced hydroxyl group was probably in the cycloheptyl ring, its position was readily established by oxidizing N-cyclohexyl-N-(4-hydroxycyclohexyl)acetamide to the corresponding ketone and then subjecting the latter to ring expansion with diazomethane. The resulting N-cyclohexyl-N-(4-oxocycloheptyl)acetamide was identical with that obtained by oxidation of the bioconversion product, which may then be formulated as N-cyclohexyl-N-(4-hydroxycycloheptyl)acetamide.

N,N-Dicycloheptylacetamide underwent microbial oxygenation at the 4 position to give N-cycloheptyl-N-(4-oxocycloheptyl)acetamide (after Jones reagent oxidation), whose structure was established by synthesis *via* diazomethane ring expansion of N-cycloheptyl-N-(4-oxocyclohexyl)acetamide.

The organism may prefer the seven-membered over the six-membered ring because of the greater number of 5.5-Å spacings possible from the carbonyl oxygen to the two equivalent methylene groups in the conformationally less rigid seven-membered ring.

(8) As a leading reference, see M. L. Moore, *Org. Reactions*, **5**, 301 (1949).

Experimental Section[9]

Bioconversion of N-Cyclododecylacetamide.—The fermentation procedure with *Sporotrichum sulfurescens* ATCC 7159 and the subsequent beer extraction procedure have been described previously.[1] The crude extract residue from bioconversion of 25 g of N-cyclododecylacetamide contained a mixture of N-(oxocyclododecyl)acetamides and N-(hydroxycyclododecyl)acetamides, with the former preponderant. Chromatography on Florisil gave N-(6-oxocyclododecyl)acetamide and N-(7-oxocyclododecyl)acetamide in the 25% acetone–petroleum ether and 50% acetone–petroleum ether eluates, respectively. The appropriate fractions were combined and rechromatographed, and the eluted materials were recrystallized from acetone to give two crops of N-(6-oxocyclododecyl)acetamide (5.06 g, mp 143–148° and 3.23 g, mp 145–147°) and two crops of N-(7-oxocyclododecyl)acetamide (1.53 g, mp 195–197° and 0.50 g, mp 191–193°). For analysis, a sample of N-(6-oxocyclododecyl)acetamide was rerecrystallized from acetone to mp 150.5–151.5°.

Anal. Calcd for $C_{14}H_{25}NO_2$: C, 70.25; H, 10.53; N, 5.85. Found: C, 70.26; H, 10.49; N, 6.14.

For analysis, a sample of N-(7-oxocyclododecyl)acetamide was recrystallized from acetone to mp 196.5–198°.

Anal. Calcd for $C_{14}H_{25}NO_2$: C, 70.25; H, 10.53; N, 5.85. Found: C, 70.51; H, 10.45; N, 5.81.

Bioconversion of Cyclododecylamine.—Bioconversion of 25 g of cyclododecylamine afforded a mixture of N-(oxocyclododecyl)-acetamides and N-(hydroxycyclododecyl)acetamides. Repeated chromatography as described above, with pooling and recrystallization of appropriate fractions as indicated by paper chromatographic assay, gave three crops of N-(6-oxocyclododecyl)-acetamide (7.92 g, mp 147–148°, 3.20 g, mp 143–145°, and 1.96 g, mp 135–139°), two crops of N-(7-oxocyclododecyl)acetamide (1.43 g, mp 200–201°, and 1.12 g, mp 194.5–198°), as well as about 3.69 g of mixed N-(hydroxycyclododecyl)acetamides. This last was oxidized in acetone with Jones chromic acid reagent to a mixture of N-(oxocyclododecyl)acetamides that was separated by chromatography, to give N-(5-oxocyclododecyl)acetamide in the early 20% acetone–Skellysolve B eluate fractions. Recrystallization from acetone–petroleum ether and finally from ether gave 0.17 g, mp 128–129°.

Anal. Calcd for $C_{14}H_{25}NO_2$: C, 70.25; H, 10.53; N, 5.85. Found: C, 70.60; H, 10.75; N, 6.19.

N-(6-Acetoxycyclododecyl)acetamide.—The reduction of 7.17 g of N-(6-oxocyclododecyl)acetamide in 250 ml of 95% ethanol with a solution of 6.0 g of sodium borohydride in 60 ml of 0.1 *N* sodium hydroxide solution at room temperature for 2 hr gave, after appropriate work-up, 6.80 g of crude solid hydroxy amide, whose infrared spectrum showed the disappearance of carbonyl and introduction of hydroxyl, with amide still intact. Acetylation of 4 g of the crude hydroxy amide with acetic anhydride in pyridine gave 4.65 g of crude product that was recrystallized from ether–petroleum ether to give 3.57 g of N-(6-acetoxycyclododecyl)acetamide, mp 96–100°.

Anal. Calcd for $C_{16}H_{29}NO_3$: C, 67.81; H, 10.31; N, 4.94. Found: C, 67.98; H, 10.48; N, 4.83.

Cyclododecane-1,6-dione from N-(6-Acetoxycyclododecyl)-acetamide.—Liquid dinitrogen tetraoxide (1.0 ml) was transferred to a cold (10°) solution of 10 ml of acetic acid and 10 ml of methylene chloride. Freshly fused sodium acetate (1.97 g) and N-(6-acetoxycyclododecyl)acetamide (1.42 g) were added and the mixture was stirred in an ice water bath for 15 min. It was poured into ice water, stirred, and adjusted to pH 6 with sodium hydroxide solution. The mixture was extracted with methylene chloride, and the extract was washed with 5% sodium bicarbonate solution and dried (sodium sulfate). The filtered solution was made up to 200 ml of methylene chloride. One-half of this solution was taken to dryness under reduced pressure (cold) to give a yellow oil of unstable N-nitroso compound. A vacuum was applied to this material while warming on a steam bath. In

(9) All melting points were determined using a Fisher-Johns block. "Petroleum ether" refers to a product, bp 60–70°, of the Skelly Corp. called Skellysolve B. Florisil is a synthetic magnesium silicate product of the Floridin Co. Gas–liquid partition chromatograms were carried out on a 3-ft, stainless steel column of 2.6% SE-30 silicone oil on 100–200 mesh Gas Chrom Z (W/W). Thin layer chromatograms were run on E. Merck silica gel GF plates (250 μ) with 20% methanol in benzene. Detection was with Dragendorff reagent [E. Roberts and C. C. Delwicke, *J. Biol. Chem.*, **205**, 565 (1953)]. Contrast was enhanced by overspray with 1:1 methanolic sulfuric acid followed by 0.1 *N* iodine–potassium iodide reagent.

a few seconds a violent reaction occurred and nitrogen gas was eliminated, leaving an oily residue that was heated at reflux in petroleum ether for 30 min and the solvent was then distilled off. The residue was heated at reflux for 30 min with 10 ml of methanol and 2 ml of 10% sodium hydroxide solution. The cooled mixture was diluted with water, extracted with ether, washed with water, dried (sodium sulfate), and evaporated. The residue of crude diol was chromatographed on Florisil, using gradient elution with 5–30% acetone–petroleum ether. The diol, found in the 14–18% acetone eluate fractions, was oxidized with Jones reagent. Crystallization of the crude product from acetone–petroleum ether gave cyclododecane-1,6-dione, mp 89–91° (lit.[4] mp 94–95°), whose infrared spectrum and thin layer chromatographic mobility were identical with those of an authentic specimen.

N-(7-Oxocyclododecyl)acetamide and N-(5-Oxocyclododecyl)-acetamide Degradation.—The degradative procedure described above was applied to 1.0 g of N-(7-oxocyclododecyl)acetamide. The crude diol, eluted from Florisil with petroleum ether containing 14–18% acetone, was oxidized with Jones reagent to give cyclododecane-1,7-dione, mp 134–135° (lit.[1] mp 134–136°). The infrared spectrum and paper chromatographic mobility were identical with those of an authentic specimen.

Because of the extreme paucity of N-(5-oxocyclododecyl)acetamide, degradation could be undertaken only on 125 mg of material, which yielded only enough cyclododecane-1,5-dione for characterization by thin layer chromatography and gas–liquid partition chromatography, where it showed mobilities identical with those of an authentic sample.[1]

N-(4-Hydroxycyclohexyl)benzamide.—The extract residue from the bioconversion of 2.0 g of N-cyclohexylbenzamide was taken up in methylene chloride and filtered to give 0.46 g of crude product, and the filtrate was chromatographed on Florisil. The product was eluted slowly by 25% acetone–petroleum ether and readily by acetone. Appropriate fractions were combined with the crude material from the initial filtration and recrystallized from acetone–petroleum ether to give 0.64 g of N-(4-hydroxycyclohexyl)benzamide, mp 213.5–214°. The analytical sample was recrystallized from acetone to mp 212.5–213.5°.

Anal. Calcd for $C_{13}H_{17}NO_2$: C, 71.20; H, 7.82; N, 6.39. Found: C, 70.83; H, 7.91; N, 6.47.

N-(4-Oxocyclohexyl)benzamide. A. From Bioconversion Product.—N-(4-Hydroxycyclohexyl)benzamide (100 mg) was oxidized with Jones reagent to give, after recrystallization from acetone–petroleum ether, 80 mg of N-(4-oxocyclohexyl)benzamide, mp 174–175°.

Anal. Calcd for $C_{13}H_{15}NO_2$: C, 71.86; H, 6.96; N, 6.45. Found: C, 72.00; H, 6.97; N, 6.85.

B. Synthesis.—Diacylation of 1.4 g of 4-hydroxycyclohexylamine with 3 ml of benzoyl chloride in pyridine gave crude N-(4-hydroxycyclohexyl)benzamide benzoate, which was hydrolyzed to the crude free alcohol by heating with 6 *N* methanolic potassium hydroxide on a steam bath. Oxidation with Jones reagent gave the crude keto amide (0.23 g, mp 152–170°), which was chromatographed on Florisil. The product was eluted with 10% acetone–methylene chloride, giving N-(4-oxocyclohexyl)-benzamide, mp 172–173.5°, whose infrared spectrum was identical with that of the bioconversion-derived keto amide. The mixture melting point was undepressed.

Benzyl 4-Hydroxycyclohexylcarbamate. A. Bioconversion.—The extract residue from the bioconversion of 20 g of benzyl cyclohexylcarbamate was dissolved in 250 ml of hot acetone and treated with 20 g of Nuchar C-190N; the mixture was filtered through Celite, and the filter cake was washed with hot acetone. The product was precipitated by concentrating the combined acetone filtrate and wash, and diluting with petroleum ether: yield 5.03 g.; mp 159–161°. The analytical sample, recrystallized from acetone–petroleum ether, had mp 161°.

Anal. Calcd for $C_{14}H_{19}NO_3$: C, 67.44; H, 7.68; N, 5.62. Found: C, 67.38; H, 7.51; N, 5.94.

B. Synthesis.—A mixture of 2.3 g of 4-hydroxycyclohexylamine, 10 ml of tetrahydrofuran, and 5 ml of 2 *N* sodium hydroxide was stirred and chilled in an ice bath while adding, alternately in small portions, 10 ml of 2 *N* sodium hydroxide and 5.0 ml of carbobenzoxy chloride during 25 min. The mixture was diluted with water and stirred; the solid was recovered by filtration and washed with water and a little ether. This was taken up in acetone and filtered to remove insoluble material. The filtrate was concentrated and diluted with petroleum ether to precipitate the product, mp 161°, whose infrared spectrum was the same as that of the bioconversion product. The mixture melting point was undepressed.

Oxidation of benzyl 4-hydroxycyclohexylcarbamate from either preparation with Jones reagent afforded the same **benzyl 4-oxocyclohexylcarbamate**, mp 82–83°.

Anal. Calcd for $C_{14}H_{17}NO_3$: C, 67.99; H, 6.93; N, 5.67. Found: C, 67.74; H, 6.86; N, 5.61.

N-(4-Oxocyclohexyl)-*p*-toluenesulfonamide. A. Bioconversion.—The extract residue from the bioconversion of 2.0 g of N-cyclohexyl-*p*-toluenesulfonamide was chromatographed on Florisil. The product, eluted as an oil with 22–30% acetone–petroleum ether, was oxidized with Jones reagent to give 0.627 g of N-(4-oxocyclohexyl)-*p*-toluenesulfonamide, mp 111–112°. The analytical sample crystallized from ether melted at 116–117°.

Anal. Calcd for $C_{13}H_{17}NO_3S$: C, 58.40; H, 6.41; N, 5.24; S, 12.00. Found: C, 58.53; H, 6.63; N, 5.00; S, 12.06.

B. Synthesis.—A mixture of 5.0 g of 4-hydroxycyclohexylamine, 50 ml of 2 *N* sodium hydroxide, and 8.0 g of *p*-toluenesulfonyl chloride was shaken vigorously for 10 min and allowed to stand for 30 min. A gummy residue extracted from the acidified mixture with methylene chloride was oxidized with Jones reagent and the resulting product was crystallized from methylene chloride–ether to give N-(4-oxocyclohexyl)-*p*-toluenesulfonamide, mp 108–109°, whose infrared spectrum and thin layer chromatographic and gas–liquid chromatographic mobility were identical with that of the bioconversion product.

N-(4-Oxocycloheptyl)benzamide. A. Bioconversion.—The extract residue from the bioconversion of 2.0 g of N-cycloheptylbenzamide was chromatographed on Florisil. About 0.9 g of substrate was found in the 10% acetone–petroleum ether eluate, about 0.6 g of crude N-(4-oxocycloheptyl)benzamide in the early 25% acetone–petroleum ether eluate, and about 0.3 g of N-(4-hydroxycycloheptyl)benzamide in the later 25% acetone–petroleum ether eluate. All product fractions were combined and oxidized with Jones reagent, and the resultant ketoamide was chromatographed on Florisil. Recrystallization of the early 25% acetone–petroleum ether eluate fraction from acetone–petroleum ether gave 0.82 g of N-(4-oxocycloheptyl)benzamide, mp 143–145°. The analytical sample had mp 144–146° and $[\alpha]D$ +65° (*c* 1, $CHCl_3$).

Anal. Calcd for $C_{14}H_{17}NO_2$: C, 72.70; H, 7.41; N, 6.06. Found: C, 72.42; H, 7.61; N, 6.05.

B. Synthesis. 1. From N-(4-Oxocycloheptyl)-*p*-toluenesulfonamide.—To a mixture of 6.0 g of lithium aluminum hydride in 150 ml of anhydrous tetrahydrofuran was added with stirring a solution of 6.0 g of N-(4-oxocycloheptyl)-*p*-toluenesulfonamide in 75 ml of anhydrou stetrahydrofuran. The mixture was heated at reflux for 18 hr, chilled in an ice bath, and carefully treated dropwise with 25 ml of water. Ether (300 ml) was added and, after stirring for 30 min, the solids were removed by filtration and washed well with ether. The combined filtrate and wash solution was tried (sodium sulfate) and the solvent was evaporated. The residue was well stirred with 25 ml of 10% hydrochloric acid and the insoluble residue of N-(4-hydroxycycloheptyl)-*p*-toluenesulfonamide was separated from the aqueous acid phase. The acid solution was made strongly basic by the addition of 10 ml of 50% sodium hydroxide solution. The alkaline solution was chilled and shaken with 2.0 ml of benzoyl chloride for 30 min. The insoluble product was recovered by filtration, washed with water and ether, and dried. This material was taken up in acetone and oxidized with Jones reagent. The resulting product was recrystallized from acetone–hexane: yield 0.356 g; mp 137–138°; mixture melting point with biosynthetic N-(4-oxocycloheptyl)benzamide, 132–135°; $[\alpha]D$ −1° (*c* 1, $CHCl_3$). The infrared spectrum in chloroform and the nmr spectrum in deuteriochloroform were identical with those of the optically active biosynthetic material.

Anal. Calcd for $C_{14}H_{17}NO_2$: C, 72.70; H, 7.41. Found: C, 72.64; H, 7.40.

2. From N-(4-Oxocyclohexyl)benzamide.—A suspension of 0.5 g of N-(4-oxocyclohexyl)benzamide in 10 ml of methanol was chilled in an ice bath and treated with 10 ml of a cold ether solution containing excess diazomethane. The mixture was removed from the ice bath and allowed to stand in an open beaker in the hood until the solvent was evaporated. The residue was again subjected to the same procedure. This product was chromatographed over Florisil. Gradient elution with acetone (0–25%) in petroleum ether, followed by recrystallization from acetone–hexane, gave 260 mg of N-(4-oxocycloheptyl)benzamide, mp 137–139°, from the 21–25% acetone eluate fractions.

Recrystallization (from acetone–hexane) of material eluted from the column by 17–20% acetone gave a small amount of **6-benzamido-1-oxaspiro[2.5]octane**, mp 178–179°. The nmr spectrum in deuteriochloroform showed a sharp signal at 2.70 ppm (δ) for the isolated methylene of the oxaspiro ring.

Anal. Calcd for $C_{14}H_{17}NO_2$: C, 72.70; H, 7.41; N, 6.06. Found: C, 72.64; H, 7.51; N, 6.27.

Benzyl Cycloheptylcarbamate.—A solution of 33.9 g of cycloheptylamine in 150 ml of pyridine was chilled and stirred in an ice–methanol bath while adding 56.3 g of carbobenzoxy chloride. The mixture was stirred for 15 min in the cold bath and 30 min with no temperature control, diluted with 350 ml of water, and allowed to stand for 18 hr in an open beaker. Concentrated hydrochloric acid (100 ml) was added and the mixture was extracted with ether. N,N'-Dicycloheptylurea crystals (6.78 g) were separated at the interface. The ether extract was washed with dilute hydrochloric acid, water, and 5% sodium bicarbonate, and dried (sodium sulfate) and the solvent was removed. The residue was chromatographed over Florisil, using gradient elution with petroleum ether containing increasing proportions of acetone from 0 to 30%. The benzyl cycloheptylcarbamate was eluted with 10–14% acetone–petroleum ether to give 19.0 g, mp 56°.

Anal. Calcd for $C_{15}H_{21}NO_2$: C, 72.84; H, 8.56; N, 5.66. Found: C, 73.43; H, 8.52; N, 5.50.

Benzyl-4-Oxocycloheptylcarbamate. A. Bioconversion.—The residue from the bioconversion of 56.36 g of benzyl cycloheptylcarbamate was oxidized with Jones reagent to give 42.0 g, of dark oil, 35 g of which was chromatographed over Florisil, using gradient elution with petroleum ether containing increasing proportions of acetone from 0 to 30%. The benzyl 4-oxycycloheptylcarbamate was eluted as an oil by 20–30% acetone–petroleum ether: yield 15.1 g; $[\alpha]_D$ +9° (*c* 1, $CHCl_3$). This compound finally crystallized to a low melting solid after prolonged storage in a desiccator.

Anal. Calcd for $C_{15}H_{19}NO_3$: C, 68.94; H, 7.33; N, 5.36. Found: C, 69.16; H, 7.49; N, 5.28.

The semicarbazone, prepared in the usual way, was recrystallized from methylene chloride–ether and then from aqueous methanol: mp 188–191°.

Anal. Calcd for $C_{16}H_{22}N_4O_3$: C, 60.36; H, 6.97; N, 17.60. Found: C, 59.94; H, 7.31; N, 17.52.

B. Synthesis.—Treatment of benzyl 4-oxocyclohexylcarbamate with diazomethane as described above gave an oily product whose infrared spectrum and gas–liquid partition chromatographic mobility were identical with those of the benzyl 4-oxocycloheptylcarbamate from the bioconversion.

Benzyl 4-Hydroxycycloheptylcarbamate.—The reduction of 5.0 g of benzyl 4-oxocycloheptylcarbamate in 50 ml of methanol with a solution of 5.0 g of sodium borohydride in 30 ml of 0.1 *N* sodium hydroxide gave a yellow oil. Chromatography on Florisil, using gradient elution, gave the desired compound in the 15–22% acetone–petroleum ether eluate fractions. Recrystallization from ether–petroleum ether gave 2.98 g of benzyl 4-hydroxycycloheptylcarbamate, mp 63–68°.

Anal. Calcd for $C_{15}H_{21}NO_3$: C, 68.41; H, 8.04; N, 5.32. Found: C, 68.36; H, 8.00; N, 5.28.

N-Cycloheptyl-*p*-toluenesulfonamide.—Shaking a mixture of 25.0 ml of cycloheptylamine, 40.0 g of *p*-toluenesulfonylchloride, and 200 ml of 8% sodium hydroxide solution and crystallizing the isolated crude product from methanol–water gave 84.05 g of product, mp 63–64°.

Anal. Calcd for $C_{14}H_{21}NO_2S$: C, 62.88; H, 7.99; N, 5.24; S, 11.99. Found: C, 62.46; H, 8.04; N, 5.10; S, 12.21.

N-(4-Oxocycloheptyl)-*p*-toluenesulfonamide. A. Bioconversion.—The extract residue from the bioconversion of 25.0 g of N-cycloheptyl-*p*-toluenesulfonamide was oxidized with Jones reagent. The neutral residue obtained by methylene chloride extraction was triturated with 150 ml of ether to give 14.77 g of solid material, which was chromatographed on Florisil. Elution with 20% acetone–petroleum ether, followed by recrystallization from ether, gave 11.58 g of N-(4-oxocycloheptyl)-*p*-toluenesulfonamide, mp 109–111°. The analytical sample, recrystallized from ether, had mp 110–112°.

Anal. Calcd for $C_{14}H_{19}NO_3S$: C, 59.76; H, 6.81; N, 4.98; S, 11.40. Found: C, 59.80; H, 6.94; N, 4.80; S, 11.36.

B. Synthesis.—Treatment of N-(4-oxocyclohexyl)-*p*-toluenesulfonamide with diazomethane as described above gave a residue that was chromatographed over Florisil, using gradient elution. N-(4-Oxocycloheptyl)-*p*-toluenesulfonamide was eluted with 12–15% acetone–petroleum ether and then recrystallized from ether to mp 109–110°. The mixture melting point with the bioconversion product was not depressed, and the infrared spectrum was identical with that of the bioconversion product.

N-Cyclooctylbenzamide.—Benzoylation of 25.5 g of cyclooctylamine by the Schotten–Baumann procedure gave 44.0 g of N-cyclooctylbenzamide, mp 110–111°. A sample recrystallized from acetone–water melted at 112–113°.

Anal. Calcd for $C_{15}H_{21}NO$: C, 77.88; H, 9.15; N, 6.06. Found: C, 78.20; H, 9.26; N, 6.03.

Bioconversion of N-Cyclooctylbenzamide.—The dried extract residue from the bioconversion of 20.0 g of N-cyclooctylbenzamide was chromatographed on Florisil. Elution with 20% acetone–petroleum ether, followed by recrystallization from methylene chloride–ether, gave 3.02 g of (presumably) N-(4-oxocyclooctyl)-benzamide, mp 136–139°.

Anal. Calcd for $C_{15}H_{19}NO_2$: C, 73.43; H, 7.81; N, 5.71. Found: C, 73.11; H, 7.71; N, 5.59.

Elution with 25% acetone–petroleum ether, followed by recrystallization from acetone–petroleum ether, gave 9.20 g of (presumably) N-(5-oxocyclooctyl)benzamide, mp 164–166°.

Anal. Calcd for $C_{15}H_{19}NO_2$: C, 73.43; H, 7.81; N, 5.71. Found: C, 73.51; H, 7.85; N, 5.84.

N-Cyclooctyl-*p*-toluenesulfonamide was prepared from 12.7 g of cyclooctylamine by the Schotten–Baumann procedure and crystallized from aqueous methanol to give 23.6 g of N-cyclooctyl-*p*-toluenesulfonamide, mp 66–67°.

Anal. Calcd for $C_{15}H_{23}NO_2S$: N, 4.98; S, 11.38. Found: N, 4.68; S, 11.49.

Bioconversion of N-Cyclooctyl-*p*-toluenesulfonamide.—The extract residue from the bioconversion of 20.0 g of N-cyclooctyl-*p*-toluenesulfonamide was chromatographed on Florisil. Gradient elution with petroleum ether plus increasing proportions of acetone from 0–30% afforded (presumably) N-(5-oxocyclooctyl)-*p*-toluenesulfonamide in the 10–15% acetone–petroleum ether eluates. Recrystallization from ether gave 2.23 g, mp 163–164°.

Anal. Calcd for $C_{15}H_{21}NSO_3$: C, 60.99; H, 7.17; N, 4.74; S, 10.86. Found: C, 61.04; H, 7.17; N, 5.09; S, 10.84.

The (presumed) N-(4-oxocyclooctyl)-*p*-toluenesulfonamide was eluted with 18–21% acetone–petroleum ether, and was recrystallized from ether to give 6.04 g, mp 107–108°.

Anal. Calcd for $C_{15}H_{21}NSO_3$: C, 60.99; H, 7.17; N, 4.74; S, 10.86. Found: C, 60.93; H, 7.40; N, 4.69; S, 10.73.

General Procedure for Conducting Leuckart Reductive Amination[8] of Ketones.—In general the amine was added to 98+% formic acid with cooling; the ketone was then added directly to the still warm mixture. Boiling pellets were added to control the evolution of generated carbon dioxide and the mixture was heated at reflux for 5 hr. Dilution with water, acidification of the cooled mixture with hydrochloric acid, and extraction with ether removed unreacted ketone. The aqueous acid solution was boiled to remove dissolved ether and then heated at reflux for 1–4 hr to hydrolyze formates of unreacted starting materials or products. In some cases hydrochloride salts separated directly from the cooled mixture; otherwise the mixture was made basic with 50% sodium hydroxide and the product was extracted with ether.

Cyclohexylcyclopentylamine Hydrochloride.—The Leuckart reaction of cyclohexylamine (57 ml), cyclopentanone (67 ml), and formic acid (24 ml) produced 50.38 g of amine hydrochloride, mp 271°. The preparation of the free base has been reported.[10]

Anal. Calcd for $C_{11}H_{22}NCl$: C, 64.84; H, 10.89; Cl, 17.40. Found: C, 64.83; H, 11.03; Cl, 17.40.

N-Cyclohexyl-N-cyclopentylacetamide.—Acetylation of 41 g of cyclohexylcyclopentylamine (generated from the hydrochloride by sodium hydroxide treatment) with acetic anhydride in pyridine gave, after recrystallization from acetone–petroleum ether, 37.5 g of N-cyclohexyl-N-cyclopentylacetamide, mp 53–54°.

Anal. Calcd for $C_{13}H_{23}NO$: C, 74.59; H, 11.07; N, 6.69. Found: C, 74.70; H, 11.18; N, 6.66.

N-Cyclopentyl-N-(4-hydroxycyclohexyl)acetamide. A. Bioconversion.—The extract residue from the bioconversion of 15.0 g of N-cyclohexyl-N-cyclopentylacetamide was chromatographed on Florisil using gradient elution with 0–30% acetone–petroleum ether. The yield of N-cyclopentyl-N-(4-hydroxycyclohexyl)-acetamide, eluted with 15–30% acetone–petroleum ether, and

(10) K. Jewers and J. McKenna, *J. Chem. Soc.*, 2209 (1958).

crystallized from methylene chloride–ether, was 5.99 g, mp 144–146°.

Anal. Calcd for $C_{13}H_{23}NO_2$: C, 69.29; H, 10.29; N, 6.22. Found: C, 69.21; H, 10.18; N, 6.37.

B. Synthesis.—The Leuckart reaction of 11.5 g of 4-hydroxycyclohexylamine, 13.2 ml of cyclopentanone, and 7.5 ml of formic acid afforded 3.05 g of cyclopentyl-(4-hydroxycyclohexyl)amine, mp 160–162°. The analytical sample was recrystallized from acetone to mp 165–167°.

Anal. Calcd for $C_{11}H_{21}NO$: C, 72.08; H, 11.55; N, 7.64. Found: C, 71.95; H, 11.47; N, 7.58.

Acetylation of cyclopentyl-(4-hydroxycyclohexyl)amine with acetic anhydride in pyridine, followed by hydrolysis of the ester with methanolic sodium hydroxide gave N-cyclopentyl-N-(4-hydroxycyclohexyl)acetamide, mp 145–147° (from aqueous methanol), identical with material from the bioconversion.

N-Cyclopentyl-N-(4-oxocyclohexyl)acetamide.—Oxidation of 2 g of N-cyclopentyl-N-(4-hydroxycyclohexyl)acetamide with Jones reagent gave a quantitative yield of crude N-cyclopentyl-N-(4-oxocyclohexyl)acetamide that was recrystallized from aqueous acetone to mp 144–145°.

Anal. Calcd for $C_{13}H_{21}NO_2$: C, 69.92; H, 9.48; N, 6.27. Found: C, 70.22; H, 9.50; N, 6.55.

N-Cyclohexyl-6-(4-hydroxycyclohexyl)acetamide. A. Bioconversion.—The extract residue from the bioconversion of 25 g of N,N-dicyclohexylacetamide was recrystallized twice from acetone to give 7.85 g of N-cyclohexyl-N-(4-hydroxycyclohexyl)acetamide, mp 172–173.5°. A second crop (7.5 g, mp 167–169°), was obtained by chromatography of mother liquor solids on Florisil (elution with 25% acetone–petroleum ether), followed by recrystallization from acetone. The analytical sample (from acetone) had mp 177–178°.

Anal. Calcd for $C_{14}H_{25}NO_2$: C, 70.25; H, 10.53; N, 5.85. Found: C, 70.19; H, 10.27; N, 5.52.

B. Synthesis.—The Leuckart reaction of 23 g of 4-hydroxycyclohexylamine, 10.4 ml of cyclohexanone and 15 ml of formic acid afforded 2.25 g of cyclohexyl-(4-hydroxycyclohexyl)amine, mp 181° from acetone.

Anal. Calcd for $C_{12}H_{23}NO$: C, 73.04; H, 11.75; N, 7.10. Found: C, 73.13; H, 11.54; N, 7.13.

4-(Cyclohexylamino)cyclohexanol (1.0 g) was acetylated with acetic anhydride in pyridine. After hydrolysis of the ester with methanolic sodium hydroxide 0.335 g of N-cyclohexyl-N-(4-hydroxycyclohexyl)acetamide, mp 167–169°, crystallized from the neutralized (acetic acid) and partially evaporated mixture. The infrared spectrum was identical with that of the bioconversion product.

N-Cyclohexyl-N-(4-oxocyclohexyl)acetamide.—Oxidation of 0.20 g of N-cyclohexyl-N-(4-hydroxycyclohexyl)acetamide with Jones reagent and recrystallization of the crude product from acetone–petroleum ether gave 0.13 g of N-cyclohexyl-N-(4-oxocyclohexyl)acetamide, mp 142–146°. The analytical sample, recrystallized from the same solvent, had mp 142–144.5°.

Anal. Calcd for $C_{14}H_{23}NO_2$: C, 70.85; H, 9.77; N, 5.90. Found: C, 71.07; H, 9.76; N, 6.15.

The **oxime**, prepared in ethanol–pyridine at reflux, was recrystallized from aqueous methanol to mp 179–183°.

Anal. Calcd for $C_{14}H_{24}N_2O_2$: C, 66.63; H, 9.59; N, 11.10. Found: C, 66.96; H, 9.60; N, 11.33.

N-Cycloheptylcyclohexylamine Hydrochloride.—The Leuckart reaction was carried out with 37.7 ml of formic acid, 112 ml of cyclohexylamine, and 59.0 ml of cycloheptanone. When the reaction mixture was diluted with 500 ml of water and acidified with 100 ml of concentrated hydrochloric acid, the hydrochloride salt precipitated. After chilling, this was recovered by filtration, washed with a little cold water and with ether, and dried to yield 76 g of N-cycloheptylcyclohexylamine hydrochloride, mp 264°. For analysis a sample was recrystallized from methanol–ether.

Anal. Calcd for $C_{13}H_{26}NCl$: C, 67.35; H, 11.31; Cl, 15.30. Found: C, 66.99; H, 11.08; Cl, 15.41.

N-Cycloheptyl-N-cyclohexylacetamide.—The acetylation of 50 g of N-cycloheptylcyclohexylamine hydrochloride was carried out as described above for N-cyclohexylcyclopentylamine. The product, obtained as an oil, solidified upon standing under reduced pressure for 3 days to give 43.16 g of N-cycloheptyl-N-cyclohexylacetamide, mp 48–49°. The analytical sample, recrystallized from acetone–water had mp 48–49°.

Anal. Calcd for $C_{15}H_{27}NO$: C, 75.89; H, 11.47; N, 5.90. Found: C, 75.92; H, 11.46; N, 5.90.

Bioconversion of N-Cycloheptyl-N-cyclohexylacetamide.—A standard bioconversion of 2 g of N-cycloheptyl-N-cyclohexylacetamide in 10 l. of beer afforded an extract residue that contained N-cyclohexyl-N-(4-hydroxycycloheptyl)acetamide but that was, for convenience, oxidized with Jones reagent to the keto amide by methods described above. Chromatography on Florisil (elution with 16% acetone–petroleum ether) gave an oil that soon crystallized, mp 76–79°. For analysis the N-cyclohexyl-N-(4-oxocycloheptyl)acetamide was recrystallized from petroleu mether to mp 80–82°.

Anal. Calcd for $C_{15}H_{25}NO_2$: C, 71.67; H, 10.03; N, 5.57. Found: C, 71.90; H, 10.04; N, 5.76.

When the bioconversion of N-cycloheptyl-N-cyclohexylacetamide was carried out on a 20-g scale, it afforded 8 g of N-cyclohexyl-N-(4-oxocycloheptyl)acetamide, mp 73–76°.

The **2,4-dinitrophenylhydrazone**, mp 221–223°, was prepared in the standard fashion and recrystallized from ethanol.

Anal. Calcd for $C_{21}H_{29}N_5O_5$: C, 58.45; H, 6.77; N, 16.23. Found: C, 58.25; H, 6.39; N, 16.49.

N-Cycloheptyl-N-(4-oxocyclohexyl)acetamide.—The Leuckart reaction of 23.0 g of 4-hydroxycyclohexylamine, 11.8 ml of cycloheptanone, and 15 ml of formic acid gave 1.34 g of crude product,[11] mp 150° (from acetone).

Anal. Calcd for $C_{13}H_{25}NO$: C, 73.88; H, 11.92; N, 6.63. Found: C, 72.38; H, 12.17; N, 6.85.

Acetylation of this material with acetic anhydride in pyridine, followed by alkaline hydrolysis of the ester as described above and oxidation with Jones reagent, gave N-cycloheptyl-N-(4-oxocyclohexyl)acetamide, mp 121° (from acetone–water).

Anal. Calcd for $C_{15}H_{25}NO_2$: C, 71.67; H, 10.03; N, 5.57. Found: C, 71.48; H, 9.90; N, 5.65.

N-Cyclohexyl-N-(4-oxocycloheptyl)acetamide.—Treatment of N-cyclohexyl-N-(4-oxocyclohexyl)acetamide with diazomethane as described above gave an oil whose thin layer chromatographic and gas chromatographic mobility was the same as that of the N-cyclohexyl-N-(4-oxocycloheptyl)acetamide described above, and which afforded a 2,4-dinitrophenylhydrazone, mp 221–223°, identical with that derived from the bioconversion product.

N,N-Dicycloheptylamine Hydrochloride.—The Leuckart reaction of 64 ml of cycloheptylamine, 89 ml of cycloheptanone, and 18.8 ml of formic acid gave 80.8 g of N,N-dicycloheptylamine hydrochloride, mp 230° (from methanol–ether).

Anal. Calcd for $C_{14}H_{28}NCl$: C, 68.40; H, 11.48; Cl, 14.42. Found: C, 68.53; H, 11.13; Cl, 14.52.

N,N-Dicycloheptylacetamide was prepared by the acetylation procedure described above. From 73.6 g of dicycloheptylamine hydrochloride there was obtained 72.3 g of N,N-dicycloheptylacetamide, mp 61–64°. The analytical sample from acetone melted at 63–64°.

Anal. Calcd for $C_{16}H_{29}NO$: C, 76.44; H, 11.63; N, 5.57. Found: C, 76.19; H, 11.67; N, 5.75.

N-Cycloheptyl-N-(4-oxocycloheptyl)acetamide. A. Bioconversion.—The extract residue from the bioconversion of 2.5 g of N,N-dicycloheptylacetamide was chromatographed on Florisil. Gradient elution with petroleum containing increasing proportions of acetone from 0 to 30% afforded crude N-cycloheptyl-N-(4-hydroxycycloheptyl)acetamide in the 25–30% acetone eluates. Oxidation with Jones reagent gave 0.43 g of N-cycloheptyl-N-(4-oxocycloheptyl)acetamide, mp 99–101°. The analytical sample was recrystallized from acetone–petroleum ether to mp 106–108°.

Anal. Calcd for $C_{16}H_{29}NO_2$: C, 72.41; H, 10.26. Found: C, 72.29; H, 10.49.

B. Synthesis.—Treatment of N-cycloheptyl-N-(4-oxocyclohexyl)acetamide with diazomethane as described above gave N-cycloheptyl-N-(4-oxocycloheptyl)acetamide, mp 94–96° from acetone–petroleum ether. Although thin layer chromatographic and gas–liquid partition chromatographic analysis showed the presence of a small amount of residual N-cycloheptyl-N-(4-oxocyclohexyl)acetamide, the major component was chromatographically and spectrally (infrared) identical with the N-cycloheptyl-N-(4-oxocycloheptyl)acetamide from the bioconversion.

Registry No.—N-(6-Oxocyclododecyl)acetamide, 16801-59-5; N-(7-oxocyclododecyl)acetamide, 16801-60-8; N-(5-oxocyclododecyl)acetamide, 16801-61-9; N-

(11) Cycloheptyl(4-hydroxycyclohexyl)amine is the crude product.

(6-acetoxycyclododecyl)acetamide, 16853-01-3; N-(4-hydroxycyclohexyl)benzamide, 13941-93-0; N-(4-oxocyclohexyl)benzamide, 13942-05-7; benzyl 4-hydroxycyclohexylcarbamate, 16801-62-0; benzyl 4-oxocyclohexylcarbamate, 16801-63-1; N-(4-oxocyclohexyl)-*p*-toluenesulfonamide, 16801-64-2; N-(4-oxocycloheptyl)-benzamide, 14156-24-2; 6-benzamido-1-oxaspiro[2.5]-octane, 16801-78-8; benzyl cycloheptylcarbamate, 16801-66-4; benzyl 4-oxocycloheptylcarbamate, 16801-67-5; semicarbazone of benzyl 4-oxocycloheptylcarbamate, 16801-68-6; benzyl 4-hydroxycycloheptylcarbamate, 16801-69-7; N-cycloheptyl-*p*-toluenesulfonamide, 16801-70-0; N-(4-oxocycloheptyl)-*p*-toluenesulfonamide, 16801-71-1; N-cyclooctylbenzamide, 13364-13-1; N-(4-oxocyclooctyl)benzamide, 16801-73-3; N-(5-oxocyclooctyl)benzamide, 16853-02-4; N-cyclooctyl-*p*-toluenesulfonamide, 16801-74-4; N-(5-oxocyclooctyl)-*p*-toluenesulfonamide, 16801-75-5; N-(4-oxocycloacetyl)-*p*-toluenesulfonamide, 16801-76-6; cyclohexylcyclopentylamine hydrochloride, 16801-77-7; N-cyclohexyl-N-cyclopentylacetamide, 16803-22-8; N-cyclopentyl-N-(4-hydroxycyclohexyl)acetamide, 16803-23-9; cyclopentyl-(4-hydroxycyclohexyl)amine, 16803-24-0; N-cyclopentyl-N-(4-oxocyclohexyl)acetamide, 16803-25-1; N-cyclohexyl-N-(4-hydroxycyclohexyl)acetamide, 16803-26-2; cyclohexyl(4-hydroxycyclohexyl)amine, 16803-27-3; N-cyclohexyl-N-(4-oxocyclohexyl)acetamide, 16803-28-4; oxime of N-cyclohexyl-N-(4-oxocyclohexyl)acetamide, 16803-29-5; N-cycloheptylcyclohexylamine hydrochloride, 16803-30-8; N-cycloheptyl-N-cyclohexylacetamide, 16803-31-9; N-cyclohexyl-N-(4-oxocycloheptyl)acetamide, 16803-32-0; 2,4-dinitrophenylhydrazone of compound preceding, 16803-33-1; cycloheptyl(4-hydroxycyclohexyl)amine, 16803-34-2; N-cycloheptyl-N-(4-oxocyclohexyl)acetamide, 16803-35-3; N,N-dicycloheptylamine hydrochloride, 16803-36-4; N,N-dicycloheptylacetamide, 16803-37-5; N-cycloheptyl-N-(4-oxocycloheptyl)acetamide, 16803-38-6.

The Microbiological Oxygenation of Azacycloalkanes. Structural Determinations and Some Chemical Modifications Leading to Transannular Reactions

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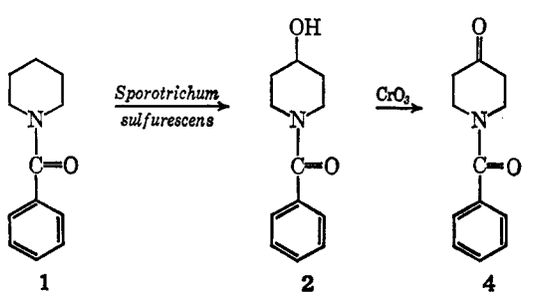
Received February 7, 1968

Microbiological oxygenation with *Sporotrichum sulfurescens* has been shown to occur at C-4 of 1-benzoylpiperidine (**1**), at C-3 and C-4 of 1-benzoylhexamethylenimine (**6**), at C-4 of 1-*p*-toluenesulfonylhexamethylenimine (**7**), and at C-4 and C-5 of 1-benzoylheptamethylenimine (**15**) and 1-benzoyloctamethylenimine (**34**). Further chemical modifications in the hepta- and octamethylenimine series have led to a number of transannular reactions. Included in these reactions is the conversion of 1-benzoylhexahydro-5(2H)-azocinone (**18**) into the iminium salt (**26**) *via* 9-benzoyl-1,4-dioxa-9-azaspiro[4.7]dodecane (**20**), 9-benzyl-1,4-dioxa-9-azaspiro[4.7]dodecane (**21**), and 1,4-dioxa-9-azaspiro[4.7]dodecane (**25**). A similar reaction series converted 1-benzoyloctamethylenimine (**34**) into the iminium salt (**41**).

The microbiological oxygenation of organic molecules recently has been extended to include macrocyclic alcohols[1a] and the acyl derivatives of cyclic and macrocyclic amines.[1b] Included in the study of the oxygenation of macrocyclic alcohols with *Sporotrichum sulfurescens* was a consideration of the molecular geometry of the substrate, which led to the formulation of a hypothetical enzyme–substrate model. Among the features proposed in this model was an optimal spacing of 5.5 Å between an electron-rich center of the substrate and the point of enzymatic oxygenation.[1a] We now describe the microbiological oxygenation of a series of heterocyclic compounds, which includes piperidine and hexa-, hepta-, and octamethylenimine as their benzoyl derivatives as well as the *p*-toluenesulfonyl derivative of hexamethylenimine, with *S. sulfurescens*. In these substrates, the electron-rich group is considered to be the oxygen of the carbonyl or sulfonyl groups. The structures of the products have been determined by chemical means with the aid of spectroscopic techniques. The oxygenation of this series of compounds follows that proposed by the enzyme–substrate model outlined previously[1a] and provides a new method of inserting oxygen functions at positions accessible with difficulty by chemical means. These new compounds therefore became available for further chemical studies which are included in the following discussion.

In general, the biotransformation products were extracted from the filtered beer of the fermentations with methylene chloride. A typical fermentation and work-up is described in detail in the Experimental Section. The concentrated methylene chloride extracts were either chromatographed on Florisil columns or were oxidized with Jones reagent[2] and chromatographed. This latter procedure converts the hydroxylic products into ketonic products and simplifies the purification of the biotransformation products in this heterocyclic series. The yields of oxygenated products are generally in the range of 25–60%.

Piperidine Series.—The structure of the product from the biotransformation of 1-benzoylpiperidine (**1**) with



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(1) (a) G. S. Fonken, M. E. Herr, H. C. Murray, and L. M. Reineke, *J. Amer. Chem. Soc.*, **89**, 672 (1967); (b) G. S. Fonken, M. E. Herr, H. C. Murray, and L. M. Reineke, *J. Org. Chem.*, **33**, 3182 (1968).

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