(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

ROY A. JOHNSON, MILTON E. HERR, HERBERT C. MURRAY, AND GUNTHER S. FONKEN

Biochemical Research Division, The Upjohn Company, Kalamazoo, Michigan 49001

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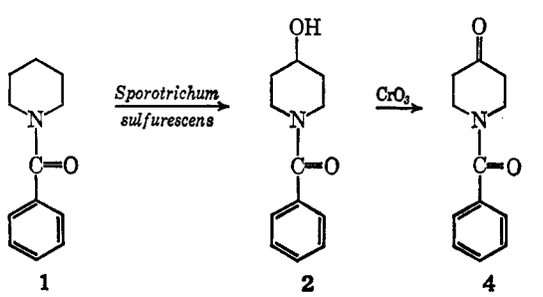
Microbiological oxygenation with *Sporotrichum sulfurescens* has been shown to occur at C-4 of 1-benzoylpiperidine (**1**), at C-3 and C-4 of 1-benzoylhexamethylenimine (**6**), at C-4 of 1-*p*-toluenesulfonylhexamethylenimine (**7**), and at C-4 and C-5 of 1-benzoylheptamethylenimine (**15**) and 1-benzoyloctamethylenimine (**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



1 **2** **4**

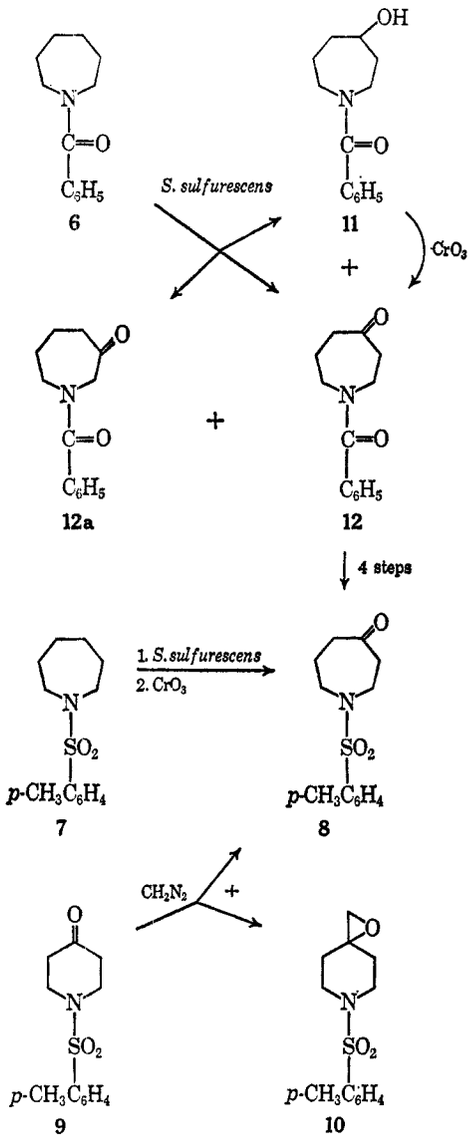
(1) (a) G. S. Fonken, M. E. Herr, H. C. Murray, and L. M. Reineke, *J. Amer. Chem. Soc.*, **89**, 672 (1967); (b) G. S. Fonken, M. E. Herr, H. C. Murray, and L. M. Reineke, *J. Org. Chem.*, **33**, 3182 (1968).

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

Sporotrichum sulfurescens was shown to be 1-benzoyl-4-piperidinol (**2**) by comparison of derivatives. The phenylurethan (**3**) of **2** has the reported melting point 184.5–186.5°.[3] Oxidation of **2** with Jones reagent[2] gave the oily 1-benzoyl-4-piperidone (**4**), whose 2,4-dinitrophenylhydrazone (**5**) has the reported mp 196–198°.[3]

Hexamethylenimine Series.—Both the benzoyl (**6**) and *p*-toluenesulfonyl (**7**) derivatives of hexamethylenimine (see Scheme 1) were used as substrate for bio-

SCHEME I



conversion with *S. sulfurescens*. The nature of the acyl function had little effect on this conversion since in each case a similar mixture of hydroxy and ketonic products was isolated. The presence of the two types of products was shown by partial chromatographic separation followed by inspection of infrared spectra. To facilitate purification and identification of the products, which may include stereoisomers, the product mixture was oxidized,[2] giving entirely ketonic material. Thus a single ketone (**8**), having dimorphic crystalline forms, mp 81 and 90°, was obtained from the bioconversion of **7**. The probable position of the ketone in **8** is at C-4 of the saturated ring. This was proved by synthesis of **8** from 1-*p*-toluenesulfonyl-4-piperidone[4] (**9**) *via* a diazomethane ring expansion.[5] In addition to **8**, the ring expansion gave the oxide (**10**), resulting from insertion of a methylene group into the carbonyl double bond. While such insertions are not uncommon,[5] of interest in this case is the nmr spectrum of **10**. Sharp signals are found at δ 2.62 ppm and 2.43 ppm, corresponding to the isolated methylene and the aromatic methyl protons, respectively, and, in addition, the remaining spectrum in the methylene proton region consists of a series of at least 26 signals, suggesting nonequivalence of the ring methylene protons.

The microbiological oxygenation of 1-benzoylhexamethylenimine (**6**) occurred primarily at the 4 position as it did for 1-*p*-toluenesulfonylhexamethylenimine (**7**). This was proved by interrelating the two series in the following manner. (See Scheme I.) The amide–ketone (**12**), obtained by oxidation of the biotransformation products from **6**, was converted into the amide–ketal, which was reduced to the amine–ketal (**13**) with lithium aluminum hydride. The benzyl group of **13** was removed readily by hydrogenolysis over palladium-on-carbon catalyst, giving the secondary amine (**14**). The *p*-toluenesulfonate was prepared before removal of the ketal with dilute acidic acetone. The ketone obtained in this manner was identical with the ketone (**8**) obtained from the bioconversion of **7** in crystalline form, mp 90°. A second product (**12a**) was isolated in addition to **12** during the work-up[6] of a large-scale, oxidized bioconversion of **6**. This crystalline ketone (**12a**) was shown by the nmr spectrum (see Table I) to be the result of oxygenation at the 3 position.

TABLE I

CHEMICAL SHIFTS OF PROTONS OF KETONIC PRODUCTS IN DEUTERIOCHLOROFORM[a]

Compd	$-CH_2CH_2CH_2-$	$-CH_2CH_2\overset{O}{\overset{\|}{C}}-$	$-CH_2CH_2\overset{\|}{N}-$	$-\overset{O}{\overset{\|}{C}}CH_2\overset{\|}{N}-$
8	1.89 (2)	2.63 (4)	3.43 (4)	
12	1.79 (2)	2.68 (4)	3.72 (4)	
12a	1.77 (4)	2.60 (2)	3.61 (2)	4.16 (2)[b]
12a[c]	1.77 (4)	2.58 (2)	3.64 (2)	4.14 (2)[b]
18	2.19 (4)[d]	2.39 (4)[d]	3.47 (4)[e]	
19	1.95 (4)	2.58 (4)	3.42 (4)[f]	
35	1.82 (6)	2.48 (4)	3.42 (4)	
36	1.52 and 2.03 (6)	2.47 (2) and 2.8 (2)	3.38 (2) and 3.73 (2)	

[a] Chemical shifts were measured as δ values in parts per million (number of protons), multiplets unless indicated otherwise. [b] Singlet. [c] In d_7-DMF at 95°. [d] Broad signals at 2.19 and 2.39 overlap. [e] Triplet (J = 4.5 cps). [f] Triplet (J = 5.5 cps).

The significant signal in this spectrum is the sharp singlet seen at δ 4.14 ppm, resulting from the methylene adjacent to both the ketone carbonyl and the nitrogen.

Heptamethylenimine Series.—The bioconversion of 1-benzoylheptamethylenimine (**15**) (see Scheme II) with *S. sulfurescens* gave an oily mixture of products, which were shown by infrared spectra following chromatography to be hydroxylic and ketonic in nature.

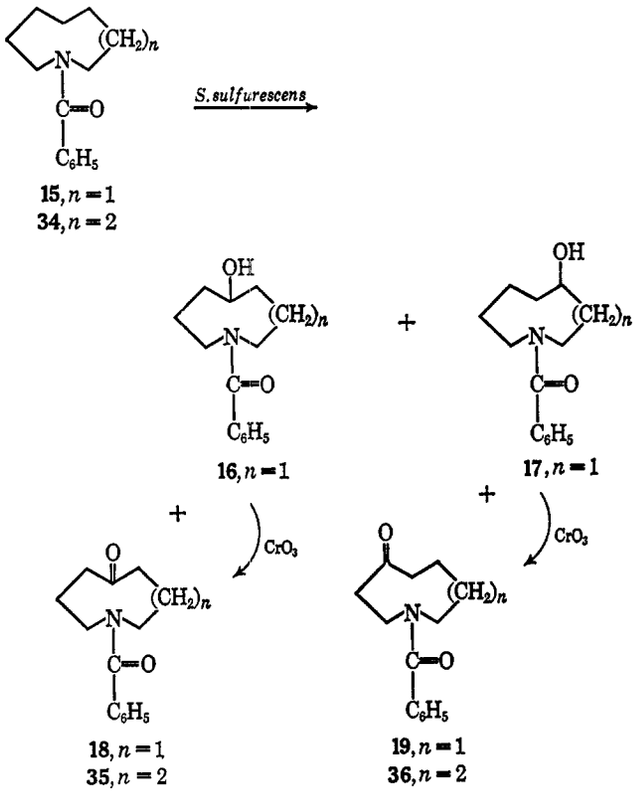
(3) S. M. McElvain and R. E. McMahon, *J. Amer. Chem. Soc.*, **71**, 901 (1949).

(4) F. Arndt and A. Kalischek, *Chem. Ber.*, **63**, 587 (1930).

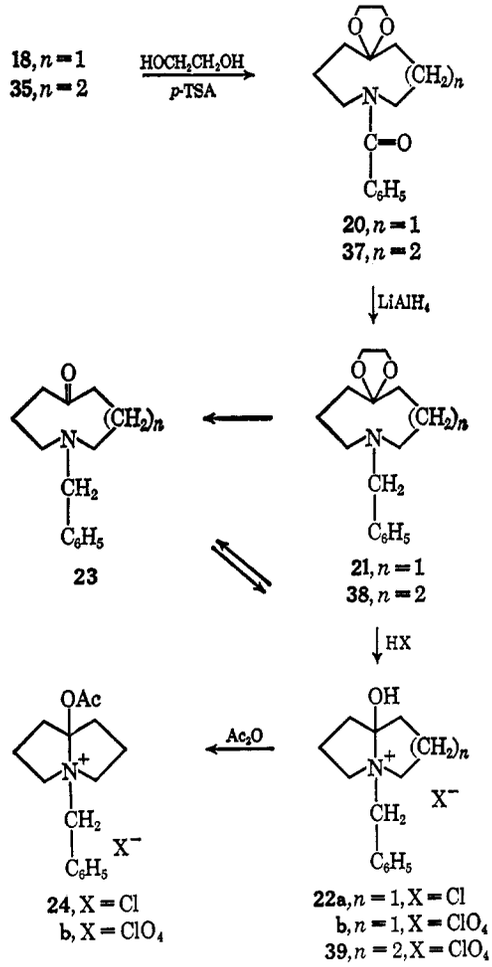
(5) (a) E. Muller and M. Bauer, *Ann. Chem.*, **654**, 92 (1962); (b) E. Mosettig and A. Burger, *J. Amer. Chem. Soc.*, **52**, 3456 (1930).

(6) We thank Dr. Jackson B. Hester, Jr., and Mr. J. R. Greene for these experimental results.

SCHEME II



SCHEME III



Although a crystalline alcohol (**16**) was obtained from this mixture, the work-up was simplified if the entire crude reaction product was oxidized.[2] Using this technique, a more polar major ketonic product (**18**) and a less polar minor ketonic product (**19**) were separated upon chromatography on a Florisil column. The crystalline alcohol (**16**) was oxidized separately to the major ketonic product (**18**); therefore the structure of **16** follows from the evidence presented below in support of the structure of ketone (**18**). In the light of past experience with the microbiological oxygenation of cyclic compounds,[1a] it was felt that oxygenation of **15** had occurred in the 4 and 5 positions of the eight-membered heptamethylenimine ring, with the latter considered the most probable. If this were true, it can be seen that the major ketonic product (**18**) would be a molecule having the needed requirements for undergoing transannular interactions and reactions,[7] provided that the appropriate chemical modifications were carried out. With this in mind, it was apparent that the benzamide function of **18** must be changed in a way such that the nitrogen became basic. This was achieved by protecting the ketone in the form of its ethylene ketal (**20**) and then reducing the amide carbonyl with lithium aluminum hydride (Scheme III). This gave the ketal–benzyl amine (**21**), potentially an amino ketone of the type first studied in detail by Leonard and coworkers in their work on transannular interactions in eight-membered-ring systems.[8] Hydrolysis of **21** with aqueous hydrochloric or aqueous perchloric acid gave the hexahydropyrrolizinium salts **22a** and **b**, respectively, in which regeneration of the ketone has been followed immediately by transannular reaction. The intermediate ketone (**23**) was obtained by making the reaction mixture alkaline following hydrolysis of the ketal. Upon reaction with acid, **23** was converted into the hexahydropyrrolizinium salt (**22**). The infrared spectra of **23**, **22a**, and **22b** are consistent with the requirements of these molecules and with the observations of Leonard,[8] with one exception in the case of the hydroxyl absorption of the hydrochloride (**22a**). The carbonyl band of the ketone (**23**) is shifted to 1675 cm^{-1} (in chloroform), which agrees with the value (1683 cm^{-1}) observed by Leonard.[8] Absorption in the infrared due to a carbonyl is absent in both salts. A hydroxyl band is seen at 3290 cm^{-1} in the hexahydropyrrolizinium perchlorate (**22b**); however, no typical hydroxyl absorption is present in the infrared of the hydrochloride (**22a**). Instead the spectrum of **22a** shows a series of weak bands at 3000, 2760, 2720, 2620, and 2560 cm^{-1} in Nujol. This shift is apparently the result of hydrogen bonding, because the hydroxyl group in **22a** has been shown to be intact by the regeneration of the ketone (**23**) in alkaline solution. Reaction of the pyrrolizinium perchlorate (**22b**) with acetic anhydride gave the acetylated compound (**24b**). The infrared spectrum of **24b** supports the structure in that it has a typical ester carbonyl absorption band at 1750 cm^{-1}. Similarly, the hydrochloride (**22a**) formed an acetate (**24a**, carbonyl band at 1750 cm^{-1}) in acetic anhydride, which, because of its hygroscopic nature, was not further purified. Similar acetylations of

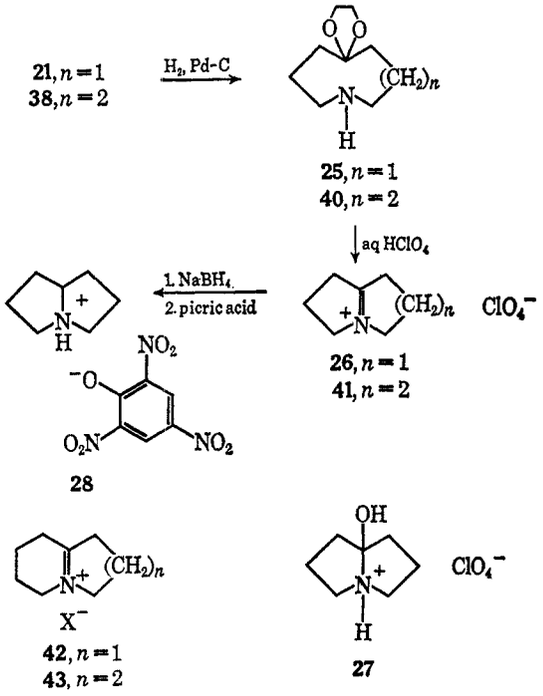
(7) N. J. Leonard, *Rec. Chem. Progr.*, **17**, 243 (1956); an appropriate distinction has been made between interactions and reactions, "the former signifying either partial orbital overlap or a field effect, and the latter, the generation of a full bond."

(8) N. J. Leonard, M. Ōki, and S. Chiavarelli, *J. Amer. Chem. Soc.*, **77**, 6234 (1955).

transannular carbinol–ammonium salts do not appear to have been reported. This reaction also served to support the conclusion that the hydroxyl group is present in the hydrochloride (**22a**). The above results provide proof that the major ketonic product from the bioconversion of **15** is 1-benzoylhexahydro-5(2H)-azocinone (**18**) as was anticipated.

It was of interest to continue with this series of compounds since removal of the benzyl group of **21** would provide a potential secondary amino ketone. Previous studies[7,9] of transannular interactions have dealt exclusively with tertiary amines. The hydrogenolysis of the benzyl group of **21** proceeded smoothly over palladium-on-charcoal catalyst, giving the ketal–amine (**25**) (Scheme IV). Reaction of **25** with aqueous perchloric acid gave a salt (**26**), which had a strong, sharp infrared absorption band at 1690 cm^{-1} while lacking any OH or NH absorption. The product (**26**) was found to contain a mole less of water than is required by the expected transannular salt (**27**). Dehydration of structure **27**, possibly formed as an intermediate, would explain the loss of water and suggested an iminium salt structure for **26**. The infrared absorption band at 1690 cm^{-1} in the spectrum of **26** is analogous to bands observed for a number of iminium salts.[10,11] The nmr spectrum of **26** confirms this structure. The spectrum has three multiplets, each integrating for four protons. Bands centered at δ 3.92, 2.95, and 2.55 ppm are assigned to the methylene groups $-CH_2-N^+(=)-CH_2-$, $-CH_2-C(=)-CH_2-$, and $2(-CH_2-CH_2-CH_2-)$, respectively. The nmr spectra of a series of 2,3,5,6,7,8-hexahydro-1H-indolizinium salts (**42**) and 1,2,3,4,6,7,8,9-octahydroquinolizinium salts (**43**) have been tabulated.[11] The signals for the $-CH_2-N^+(=)-$ and $-CH_2-C(=)-$ methylene groups were found in the ranges δ 4.12–4.30 and 3.18–3.20 ppm, respectively, when in the five-membered ring, and between 3.70–3.94 ppm and 2.74–2.90 ppm, respectively, when in the six-membered ring. Hydride reduction of iminium salts is a well-known reaction.[12] When **26** was reduced with sodium borohydride, hexahydro-1H-pyrrolizine (pyrrolizidine) was obtained and isolated as the picrate (**28**), mp 260–263° dec. Hexahydro-1H-pyrrolizine picrate (**28**) has been reported by several groups,[13] mp 260–262° dec. The pathway **18** → **20** → **21** → **25** → **26** described above provides a new synthetic route to iminium salts *via* transannular reactions.

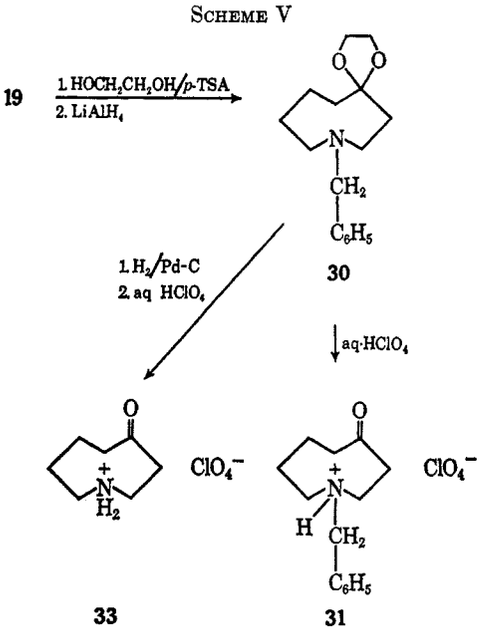
SCHEME IV



With the position of the ketone in the major bioconversion product (**18**) established at C-5, three possibilities remain for the position of the ketone in the minor product (**19**). The nmr spectrum of **19** (see Table I) bears out the prediction that this ketone will be found at C-4 of the heptamethylenimine ring. This spectrum has multiplet signals, corresponding to four protons each, centered at δ 3.42, 2.58, and 1.95 ppm, which are assigned to the methylene groups $-CH_2-N-CH_2-$, $-CH_2-C(=O)-CH_2-$, and $-CH_2-CH_2-$, respectively.

For comparative purposes, we carried out a sequence of chemical modifications of **19** (Scheme V) similar to those which were applied to the 5 ketone (**18**). In this series, transannular interactions[7] are expected to be reduced greatly while transannular reactions are not expected to occur. Consequently, **19** was converted into the ketal–amide (**29**), which was reduced, without purification, to the ketal–amine (**30**). Reaction of **30** with aqueous perchloric acid gave the perchlorate salt (**31**), in which the presence of a ketone was demonstrated by an absorption band at 1700 cm^{-1} in the infrared spectrum. Hydrogenolysis removed the benzyl group of **30**, giving **32**, which showed NH absorption at 3360 cm^{-1} in the infrared spectrum. The

SCHEME V



(9) M. G. Reineke, L. R. Kray, and R. F. Francis, *Tetrahedron Lett.*, 3549 (1965).
(10) N. J. Leonard and V. W. Gash, *J. Amer. Chem. Soc.*, **76**, 278 (1954).
(11) M. G. Reineke and L. R. Kray, *J. Org. Chem.*, **31**, 4215 (1966).
(12) N. J. Leonard, A. S. Hay, R. W. Fulmer, and V. W. Gash, *J. Amer. Chem. Soc.*, **77**, 439 (1955).
(13) (a) E. E. Schweizer and K. K. Light, *ibid.*, **86**, 2963 (1964); *J. Org. Chem.*, **31**, 870 (1966); (b) N. J. Leonard and W. E. Goode, *J. Amer. Chem. Soc.*, **72**, 5404 (1950).

final step in this sequence gave the ketone–amine salt (**33**, carbonyl absorption at 1695 cm^{-1}) when **32** was treated with aqueous perchloric acid. The infrared carbonyl absorptions of the two ketone–amine salts (**31** and **33**) in this series are found at the lower limits (with respect to wave numbers) usually given for normal ketones.[14] This may reflect a slight transannular interaction shifting the carbonyl bands in these molecules.

Octamethylenimine Series.—The bioconversion of 1-benzoyloctamethylenimine (**34**) with *S. sulfurescens* proceeded in a manner very similar to that of the heptamethylenimine series. Following oxidation of the isolated products, major (**35**) and minor (**36**) ketonic products (see Scheme II) were separated by chromatography. In view of recent results[1a] and now with the analogy of the heptamethylenimine series, the positions of oxygenation of the nine-membered ring (**34**) are predicted to be at C-5 (major product) and at C-4. The use of transannular reactions as a method of structure proof was applicable in this series also and the appropriate transformations which were carried out are described briefly below.

The ketal (**37**) of the major ketonic product (**35**) was first prepared and then was reduced to the ketal–amine (**38**) with lithium aluminum hydride. Transannular reaction followed the treatment of **38** with aqueous perchloric acid, giving **39** (no carbonyl absorption, OH absorption at 3420 cm^{-1} in the infrared spectrum). Hydrogenolysis of **38** gave the secondary amine–ketal (**40**). Reaction of **40** with aqueous perchloric acid resulted in ketal hydrolysis, transannular reaction, and dehydration of the molecule, giving the hexahydroindolizinium perchlorate (**41**), mp 227–228°, with an iminium salt absorption band at 1690 cm^{-1} in the infrared spectrum. The compound **41** has been prepared previously from the reaction of the product of mercuric acetate dehydrogenation of octahydroindolizine with perchloric acid and is reported[15] to have mp 218–219° dec while having an absorption band at 1689 cm^{-1} in its infrared spectrum. These results prove that the major product (**35**) from oxygenation of **34** is 1-benzoyloctahydro-5H-azonin-5-one. The minor ketonic product (**36**) was shown by its nmr spectrum (see Table I) to be the result of oxygenation of **34** at C-4. The important feature of this spectrum is the lack of a resonance signal which could be attributed to a methylene adjacent to both the nitrogen and the ketone group.

Experimental Section[16]

Biotransformation Process.—The culture used in these experiments was *Sporotrichum sulfurescens* V. Beyma (ATCC 7159). A medium of commercial dextrose (10 g/l.) and cornsteep liquor (20 g/l.) was prepared with tap water (to 1 l.) and adjusted to pH 5.0 with sodium hydroxide. Flasks of the sterilized medium were inoculated with spores of *S. sulfurescens*, which were grown on malt (wort) agar slants. The flasks were shaken for 48 hr (until heavy growth was apparent) and then used for seeding other flasks or tanks. The tanks used in this work were inoculated with five parts of the vegetative culture to 100 parts of fresh medium. On any scale the culture is aerated and agitated for the growth period. In stirred vessels, with good agitation, aeration with five to ten volumes of air per minute per 100 volumes of culture was satisfactory. The substance to be oxygenated was dissolved in dimethylformamide or acetone and was added after 24 hr of growth. The amount of solvent used for the substance was kept to a minimum (or less than one part solvent to 100 parts culture). The level of substrate was 0.2 to 0.3 g per liter. A conversion time of 72 hr was used. The products and residual substrate were removed from the "beer" by extraction with methylene chloride, using a volume of solvent one-fourth that of the "beer." The solvent was evaporated under reduced pressure.

1-Benzoyl-4-piperidinol (2).—The dry extract from the 10 l. bioconversion of 1-benzoylpiperidine (2.0 g) was dissolved in a minimum of methylene chloride and placed on a Florisil (200 g) column packed with Skellysolve B. Elution with 25% (v/v) acetone–Skellysolve B gave the product (0.407 g) as an oil: ν_{OH} 3420, $\nu_{C=O}$ 1620 cm^{-1} (neat).

Reaction with phenylisocyanate followed by chromatography on Florisil gave the phenylurethan, mp 184.5–186.5° (lit.[3] mp 184–185°).

1-Benzoyl-4-piperidone (4).—Jones reagent[2] was added until present in excess to a cold (ice bath) solution of 1-benzoyl-4-piperidinol (0.151 g, 0.000737 mol) in acetone. The excess oxidant was destroyed by addition of isopropyl alcohol and the mixture was allowed to evaporate to dryness. Water was added to the residue and the mixture was extracted with methylene chloride. After drying over sodium sulfate, the methylene chloride solution was concentrated to an oil: 0.137 g; $\nu_{C=O}$ 1715, 1625 cm^{-1} (neat).

Reaction with 2,4-dinitrophenylhydrazine gave the 2,4-dinitrophenylhydrazone (0.089 g). Recrystallization from ethanol gave the product, mp 196–198° (lit.[3] mp 196–198°).

1-*p*-Toluenesulfonylhexahydro-4H-azepin-4-one (8). A. From the Fermentation Beer Extracts.—The dry extract from the 2-l. bioconversion of 1-*p*-toluenesulfonylhexamethylenimine[17] (2.0 g) fermentation beer was chromatographed on Florisil by a gradient elution method. The residue was placed on a Florisil column with methylene chloride (75 ml). The column was eluted with Skellysolve B (4 l.) containing increasing proportions (from 0 to 30%) of acetone. Fractions of 105-ml volume were collected. Fractions 19–25, containing the product, were combined in acetone and oxidized with an excess of Jones reagent.[2] The resulting mixture was extracted with methylene chloride, and the extract was washed with water, dried over sodium sulfate, and concentrated by distillation of the solvent. Crystallization from ether gave 0.33 g of colorless product: mp 81°; $\nu_{C=O}$ 1700 cm^{-1} (in Nujol).

Anal. Calcd for $C_{13}H_{17}NO_3S$ (267.29): C, 58.41; H, 6.41; N, 5.24; S, 11.98. Found: C, 58.40; H, 6.47; N, 5.26; S, 12.24.

When the product was recrystallized from acetone–hexane a crystalline modification, mp 89–90°, was obtained. The infrared spectra of the two forms are identical when taken in chloroform solution. This latter form also is identical with 1-*p*-toluenesulfonylhexahydro-4H-azepin-4-one prepared synthetically as described below (part C).

B. From Ring Expansion of 1-*p*-Toluenesulfonyl-4-piperidone. Together with 6-*p*-Toluenesulfonyl-1-oxa-6-azaspiro[2.5]octane (10).—A solution of 1-*p*-toluenesulfonyl-4-piperidone[4] (4.41 g, 0.0174 mol) in methylene chloride (20 ml) and methanol (50 ml) was chilled in an ice–acetone bath and was treated with an ether solution (100 ml) containing an excess of diazomethane. The mixture was removed from the cold bath and left at room temperature for 20 min. The solvent was removed on a steam bath, applying reduced pressure during the latter stage. The treatment with diazomethane was repeated as just described. The residue was chromatographed on Florisil (200 g) by the gradient elution method, eluting with 8 l. of solvent. The solvent used was Skellysolve B plus an increasing proportion (0–30%) of acetone. Fractions of 225-ml volume were collected. By infrared spectroscopic examination, the fractions were pooled as follows: (A) fractions 9–11, (B) fractions 12–13, (C) fractions 14–16, (D) fraction 17, and (E) fractions 18–23.

Fraction A was recrystallized from acetone–Skellysolve B, giving 0.773 g (0.00289 mol, 16%) of **6-*p*-toluenesulfonyl-1-oxa-6-**

(14) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1958, pp 132–160.

(15) N. J. Leonard, W. J. Middleton, P. D. Thomas, and D. Choudhury, *J. Org. Chem.*, **21**, 344 (1956).

(16) Melting points were determined on a calibrated Fisher-Johns hot stage and are corrected. Magnesium sulfate was used as the drying agent unless indicated otherwise. 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-60 spectrometer, using tetramethylsilane as an internal standard.

(17) A. Muller and A. Sauerwald, *Monatsh. Chem.*, **48**, 727 (1927).

azaspiro[2.5]octane (10) as colorless crystals, mp 152–154°; no carbonyl bands were present in their spectrum in Nujol.

Anal. Calcd for $C_{13}H_{17}NO_3S$ (267.29): C, 58.41; H, 6.41; N, 5.24; S, 11.98. Found: C, 58.46; H, 6.22; N, 5.31; S; 12.19.

Fraction B was a mixture (1.10 g) of **10** and unchanged starting material. Fraction C was 0.97 g of unchanged starting material. Fraction D was a mixture of unchanged starting material and **8**. Fraction E contained **1-*p*-toluenesulfonylhexahydro-4H-azepin-4-one (8)** (1.36 g, 0.00509 mol, 29%), mp 89–90°. The infrared spectrum in Nujol is identical with that of the product obtained below.

C. From 1,4-Dioxa-8-azaspiro[4.6]undecane (14).—A solution of 1,4-dioxa-8-azaspiro[4.6]undecane (0.622 g, 0.00396 mol) in 16% aqueous sodium hydroxide was mixed with *p*-toluenesulfonyl chloride (0.786 g, 0.00413 mol). The mixture was shaken vigorously for several minutes, then warmed, and shaken more. The mixture was left at room temperature overnight and then was extracted with two 20-ml portions of ether. The ether solution was dried and concentrated to an oil. The oil was mixed with 2 *N* hydrochloric acid (10 ml) and acetone was added until solution was obtained. The acetone was removed on the steam bath, giving an oil, which was extracted with two 50-ml portions of ether. It was necessary to add some methylene chloride to the ether to prevent crystallization of the product. After drying the solution it was concentrated to an oil, which crystallized. Recrystallization from ether gave two crops of colorless needles, 0.506 g and 0.114 g (total 0.00232 mol, 58%), mp 91–92°. The mixture melting point with the sample obtained from diazomethane ring expansion of 1-*p*-toluenesulfonyl-4-piperidone was undepressed and their infrared spectra in Nujol are identical.

Isolation of 1-Benzoylhexahydro-4H-azepin-4-ol (11) and 1-Benzoylhexahydo-4H-azepin-4-one (12) from Bioconversion of 1-Benzoylhexamethylenimine.—The extract from the bioconversion of 1-benzoylhexamethylenimine[17,18] (2.0 g) with *Sporotrichum sulfurescens* was chromatographed on a Florisil (200 g) column packed with Skellysolve B. Elution with 25% (v/v) acetone–Skellysolve B gave unchanged starting material (0.25 g) and **1-benzoylhexahydro-4H-azepin-4-one (12)**: 0.25 g; $\nu_{C=O}$ 1700, 1625 cm^{-1} (on the oil). Elution with acetone gave a mixture of **12** and **1-benzoylhexahydro-4H-azepin-4-ol (11)**: ν_{OH} 3400, $\nu_{C=O}$ 1660 broad cm^{-1} (on the oil).

Oxidation of **11** with Jones reagent[2] gave an oil giving an infrared spectrum identical with that of **12**.

Oxidation and Isolation of Products from the Bioconversion of 1-Benzoylhexamethylenimine. 1-Benzoylhexahydro-4H-azepin-4-one (12).—The oily extract residue from the 125-l. bioconversion of 1-benzoylhexamethylenimine[17,18] (54.0 g, 0.266 mol) was dissolved in acetone (1 l.) and oxidized with an excess (cloudy mixture remained reddish brown) of Jones reagent.[2] The excess of oxidizing agent was consumed with isopropyl alcohol. The mixture was evaporated to dryness, the residue was diluted with water, and the mixture was extracted six times with methylene chloride. The methylene chloride solution was dried over sodium sulfate and concentrated to an oil. The oil was chromatographed on a Florisil column (2.5 kg) packed with Skellysolve B. The column was eluted with two fractions of Skellysolve B, three fractions of 10% (v/v) acetone–Skellysolve B, eleven fractions of 25% acetone–Skellysolve B, and two fractions of acetone. Fractions 11–17 contained one material as detected by thin layer chromatography and were pooled, giving 32.1 g (0.148 mol, 55%) of oily product. Attempted crystallization from acetone–Skellysolve B was unsuccessful; however, the oil partially crystallized after standing at room temperature for 2 months. A sample of the oil was distilled: bp 170–174° (0.3 mm) (a center cut of bp 173° was used for analysis); $\nu_{C=O}$ 1700, 1630, $\nu_{C=C}$ 1600, 1575, 1495, $\nu_{C_6H_5}$ 780, 730, 700 cm^{-1} (on the oil).

Anal. Calcd for $C_{13}H_{15}NO_2$ (217.26): C, 71.86; H, 6.96; N, 6.45. Found: C, 71.51; H, 7.25; N, 6.46.

1-Benzoylhexahydro-3H-azepin-3-one (12a).[6]—Chromatography of the oxidized products (200 g) obtained from a large-scale bioconversion of 1-benzoylhexamethylenimine on a Florisil (7 in. × 36 in.) column gave in addition to **12** (96.9 g), a second, crystalline ketonic product (12.57 g), mp 111.5–113°. Three recrystallizations from ethyl acetate–Skellysolve B gave 1-benzoylhexahydro-3H-azepin-3-one (**12a**) as colorless crystals: mp 113–114°; $\nu_{C=O}$ 1705, 1655, $\nu_{C=C}$ 1600, 1575, 1495, $\nu_{C_6H_5}$ 785, 750, 705 cm^{-1} (in Nujol).

Anal. Calcd for $C_{13}H_{15}NO_2$: C, 71.86; H, 6.96; N, 6.45. Found: C, 71.48; H, 6.75; N, 6.49.

8-Benzyl-1,4-dioxa-8-azaspiro[4.6]undecane (13) *via* the Ketal-Amide.—A mixture of 1-benzoylhexahydro-4H-azepin-4-one (11.957 g, 0.0551 mol) in benzene (200 ml) and *p*-toluenesulfonic acid hydrate (1.020 g, 0.00537 mol) in ethylene glycol (28 ml) was stirred and heated to the reflux temperature of benzene for 24 hr. The condensate was passed through a calcium carbide trap to remove water. Pyridine (3 ml) was added at the end of the reflux period and the mixture was cooled. The mixture was extracted with 5% aqueous sodium bicarbonate (100 ml) and with two 100-ml portions of water. The benzene layer was dried and concentrated under reduced pressure to a brownish yellow oil, $\nu_{C=O}$ 1630 cm^{-1} (on the oil).

After the above oil failed to crystallize, it was dissolved in ether (75 ml) and added to a mixture of lithium aluminum hydride (2.0 g) and ether (200 ml). The mixture was stirred and heated to reflux for 3 hr, stirred at room temperature over a weekend, and again heated to reflux for 2 hr before the excess lithium aluminum hydride was decomposed by the addition of ethyl acetate and water. The solids were removed by filtration and were washed with ether. The combined pale yellow ether solutions were dried and concentrated to a yellow oil. Simple distillation of the oil gave the product (9.696 g, 0.0392 mol, 71%) as a pale yellow oil: bp 120–121° (0.13 mm); $n^{25}D$ 1.5312; $\nu_{C=C}$ 1600, 1580, 1490, $\nu_{C_6H_5}$ 730, 695 cm^{-1} (neat).

Anal. Calcd for $C_{15}H_{21}NO_2$ (247.33): C, 72.84; H, 8.56; N, 5.66. Found: C, 73.22; H, 8.98; N, 6.11.

1,4-Dioxa-8-azaspiro[4.6]undecane (14).—A solution of 8-benzyl-1,4-dioxa-8-azaspiro[4.6]undecane (8.692 g, 0.0352 mol) in methanol (130 ml) was shaken with hydrogen and 5% palladium-on-carbon (2.50 g) in a Parr apparatus until the uptake of hydrogen ceased. The catalyst was removed by filtration and washed with methanol. The combined methanol solution was concentrated under reduced pressure to an oil. Simple distillation of the oil in a semimicro apparatus gave the product (5.047 g, 0.0321 mol, 91%) as a colorless oil: bp 58–60° (0.05 mm); $n^{25}D$ 1.4885; ν_{NH} 3330 cm^{-1} (neat).

Anal. Calcd for $C_9H_{15}NO_2$ (157.21): C, 61.12; H, 9.62; N, 8.91. Found: C, 61.17; H, 9.86; N, 8.85.

1-Benzoylhexahydro-5(2H)-azocinol (16) from the Bioconversion of 1-Benzoylheptamethylenimine.—The dry extract from the 125 l. of bioconversion of 1-benzoylheptamethylenimine[19] (25.0 g) was dissolved in a minimum of methylene chloride and placed on a Florisil column (2.5 kg) packed with Skellysolve B. Fractions (2 l.) were collected, beginning by elution with one fraction of Skellysolve B, followed by five fractions of 10% (v/v) acetone–Skellysolve B, six fractions of 25% acetone–Skellysolve B, nine fractions of 50% acetone–Skellysolve B, and four fractions of acetone. Fractions 11–13 (6.6 g) consisted of an oil: ν_{OH} 3450, $\nu_{C=O}$ 1710, 1650 cm^{-1} (on the oil). Fractions 15–18 (12.4 g) consisted of oily crystals: ν_{OH} 3460, $\nu_{C=O}$ 1710, 1625 cm^{-1} (on the oil). Crystals (1.5 g), mp 118–120°, were obtained from fraction 16 by washing with acetone. Two recrystallizations from acetone–Skellysolve B gave colorless crystals: mp 119–120°; ν_{OH} 3430, $\nu_{C=O}$ 1610, $\nu_{C=C}$ 1560, 1500, $\nu_{C_6H_5}$ 796, 737, 707 cm^{-1} (in Nujol).

Anal. Calcd for $C_{14}H_{19}NO_2$ (233.30): C, 72.07; H, 8.21; N, 6.00. Found: C, 71.98; H, 8.41; N, 5.84.

Oxidation and Isolation of the Products from Bioconversion of 1-Benzoylheptmethylenimine. 1-Benzoylhexahydro-4(1H)-azocinone (19) and 1-Benzoylhexahydro-5(2H)-azocinone (18).—The dry extract from the 125 l. bioconversion of 1-benzoylheptamethylenimine[19] (23.0 g) was dissolved in acetone (750 ml) and oxidized with Jones reagent as described above for **12**. The oily solid product was combined with crude ketone (7.2 g) from a previous run and was dissolved in methylene chloride (100 ml) and placed on a Florisil column (2.5 kg) packed with Skellysolve B. Fractions (2 l.) were collected, beginning by elution with two fractions of Skellysolve B, followed by three fractions of 10% (v/v) acetone–Skellysolve B, ten fractions of 25% acetone–Skellysolve B, and five fractions of acetone. Fractions 9, 10, and 11 contained 4.75 g of a brownish yellow

(18) (a) A. Muller, *Chem. Ber.*, **61**, 568 (1928). (b) G. S. Kolesnikov, T. V. Smirnova, L. I. Mizrakh, N. N. Mikhailovskaya, and L. I. Shcherbo, *Zh. Obshch. Khim.*, **27**, 3005 (1957); *J. Gen. Chem. USSR*, **27**, 3034 (1957).

(19) (a) R. Takamoto, *Yakugaku Zasshi*, **48**, 686 (1928); *Chem. Abstr.*, **23**, 387 (1929). (b) A. Muller, E. Srepel, E. Funder-Fritzsche, and F. Dicher, *Monatsh. Chem.*, **83**, 386 (1952).

crystalline material. Following decolorization with activated charcoal from an acetone solution, crystallization occurred from methylene chloride–Skellysolve B in the freezer. Colorless, slightly sticky crystals were obtained, mp 59–62°. Two recrystallizations from cold methylene chloride–Skellysolve B gave colorless crystals of **1-benzoylhexahydro-4(1H)-azocinone (19)**: mp 64–65°; $\nu_{C=O}$ 1700 ms, 1640 s, $\nu_{C_6H_5}$ 792 m, 754 mw, 716 m cm^{-1} (in Nujol).

Anal. Calcd for $C_{14}H_{17}NO_2$ (231.28): 72.70; H, 7.41; N, 6.06. Found: C, 72.75; H, 7.21; N, 6.39.

Fractions 15 through 18 gave 14.50 g of brownish oily crystals. Decolorization with activated charcoal and crystallization from acetone–Skellysolve B gave **1-benzoylhexahydro-5(2H)-azocinone (18)** as colorless crystals: mp 124–126°; $\nu_{C=O}$ 1685 ms, 1630 s, $\nu_{C_6H_5}$ 796 m, 748 m, 718 cm^{-1} (in Nujol).

Anal. Calcd for $C_{14}H_{17}NO_2$: C, 72.70; H, 7.41; N, 6.06. Found: C, 72.63; H, 7.59; N, 6.31.

1-Benzoylhexahydro-5(2H)-azocinone (18) from 1-Benzoylhexahydro-5(2H)-azocinol.—A solution of 1-benzoylhexahydro-5(2H)-azocinol (1.163 g, 0.00500 mol) in acetone was oxidized with Jones reagent[2] as described above except that the acetone solution of the product was filtered and concentrated. Addition of Skellysolve B resulted in crystallization of the product. Colorless crystals (0.594 g, 0.00257 mol, 51%) were collected, mp 126–129°. There was no depression in the mixture melting point with the sample obtained from the oxidized bioconversion products and their infrared spectra in Nujol are identical.

9-Benzoyl-1,4-dioxa-9-azaspiro[4.7]dodecane (20).—A mixture of 1-benzoylhexahydro-5(2H)-azocinone (39.883 g, 0.172 mol), *p*-toluenesulfonic acid hydrate (2.96 g, 0.0155 mol), ethylene glycol (75 ml, 83 g, 1.34 mol), and benzene (500 ml) was heated to reflux. The condensate was dried by passing it through a calcium carbide trap. The mixture was refluxed 30 hr. Pyridine (6 ml) was added to the cooled mixture; the benzene layer was extracted with three 100-ml 5% aqueous sodium bicarbonate and dried. Concentration of the benzene solution gave a viscous oil. An attempted distillation of this oil was stopped after the pot temperature reached 180° and the oil did not appear about to distil. As the cooled oil (42.95 g, 0.156 mol, 90%) was being transferred in ether, it crystallized, mp 72–74°. Two recrystallizations from ether–Skellysolve B gave colorless crystals: mp 72–73°; $\nu_{C=O}$ 1630 s cm^{-1} (in Nujol).

Anal. Calcd for $C_{16}H_{21}NO_3$ (275.34): C, 69.79; H, 7.69; N, 5.09. Found: C, 69.99; H, 7.84; N, 5.20.

9-Benzyl-1,4-dioxa-9-azaspiro[4.7]dodecane (21).—A solution of 9-benzoyl-1,4-dioxa-9-azaspiro[4.7]dodecane (40.9 g, 0.148 mol) in ether (300 ml) was added slowly to a stirred mixture of lithium aluminum hydride (6.0 g, 0.158 mol) and ether (200 ml). The resulting mixture was stirred at room temperature for 16 hr and at reflux temperature for 5 hr. The excess lithium aluminum hydride was decomposed with 1:1 acetone–water and with water. The inorganic solids were collected on a pad of Celite in a sintered glass funnel. The solids were washed three times with ether and the combined ether solution was dried and concentrated to an oil (38.26 g). Simiple distillation gave a colorless oil (33.72 g, 0.129 mol, 87%): bp 125–127° (0.3 mm); ν_{C-O} 1115 s cm^{-1} (on the liquid).

Anal. Calcd for $C_{16}H_{23}NO_2$ (261.35): C, 73.53; H, 8.87; N, 5.36. Found: C, 73.39; H, 8.31; N, 5.34.

1-Benzylhexahydro-5(2H)-azocinone (23). A. From Hydrolysis of 9-Benzyl-1,4-dioxa-9-azaspiro[4.7]dodecane.—A solution of 9-benzyl-1,4-dioxa-9-azspiro[4.7]dodecane (6.734 g, 0.0258 mol) in 0.75 *M* hydrochloric acid (40 ml) was stirred at room temperature for 3 hr. The solution was made alkaline with 1 *M* sodium hydroxide solution, causing an oil to precipitate. The oil was extracted with ether and the ether solution was dried and concentrated to a nearly colorless oil. Distillation of the oil in a semimicro still gave the following fractions: (1) colorless oil (1.00 ml), bp 110–112° (0.1 mm); (2) colorless oil, bp 112–115° (0.1 mm); and (3) colorless oil (1.15 ml), bp 115–117° (0.1 mm); total weight 6.293 g (0.0290 mol, 112%). A sample of fraction 2 was used for analysis: $\nu_{C=O}$ 1685 cm^{-1} (on the liquid), 1675 cm^{-1} (in chloroform solution).

Anal. Calcd for $C_{14}H_{19}NO$ (217.30): C, 77.38; H, 8.81; N, 6.45. Found: C, 77.21; H, 8.63; N, 6.49.

B. From Basification of 4-Benzylhexahydro-7a-hydroxy-1H-pyrrolizinium Chloride.—A solution of 4-benzoylhexahydro-7a-hydroxy-1H-pyrrolizinium chloride (0.146 g) in water (10 ml) was made alkaline (pH 10) with the addition of 1 *M* sodium hydroxide solution. The solution was extracted with three 15-ml portions of ether; the ether was dried and concentrated to an oil. The infrared spectrum of the oil is identical with that of the oil obtained by reduction of 9-benzoyl-1,4-dioxa-9-azaspiro[4.7]dodecane with lithium aluminum hydride.

4-Benzylhexahydro-7a-hydroxy-1H-pyrrolizinium Chloride (22a).—Addition of concentrated aqueous hydrochloric acid (4 ml) to a solution of 1-benzylhexahydro-5(2H)-azocinone (2.729 g, 0.0125 mol) in absolute ethanol (15 ml) caused an exothermic reaction. After 5 min ether was added to the solution until it became cloudy. Ethanol was added until a clear solution was obtained. Crystallization occurred slowly and gave 2.157 g (0.00850 mol, 68%) of colorless crystals, mp 205–212°. Three recrystallizations from ethanol–ether gave colorless crystals: mp 212–214°; ν_{OH}? 3000, 2760 w, 2720 w, 2620 w, 2560 w, $\nu_{C=C}$ 1565 w, 1495, 1490, $\nu_{C_6H_5}$ 735, 705 cm^{-1} (in Nujol).

Anal. Calcd for $C_{14}H_{20}NOCl$ (253.77): C, 66.26; H, 7.94; N, 5.52. Found: C, 66.13; H, 8.28; N, 5.60.

4-Benzylhexahydro-7a-acetoxy-1H-pyrrolizinium Chloride (24a).—A mixture of 4-benzylhexahydro-7a-hydroxy-1H-pyrrolizinium chloride (0.190 g, 0.000750 mol) and acetic anhydride (6 ml) was heated on a steam bath for 1.5 hr. Complete solution was obtained after 0.5 hr and the solution became yellow after 1 hr. Ether was added to the cooled solution causing precipitation of a crystalline solid. The solid was collected by filtration and was immediately placed in a desiccator when it was found to be hygroscopic. The infrared spectrum had bands at $\nu_{C=O}$ 1750 s, $\nu_{C_6H_5}$ 763 s, 708 s cm^{-1} (in Nujol).

4-Benzylhexahydro-7a-hydroxy-1H-pyrrolizinium Perchlorate (22b). A. From 9-Benzyl-1,4-dioxa-9-azaspiro[4.7]dodecane.—Aqueous (70%) perchloric acid (1 ml) was added dropwise to a solution of 9-benzyl-1,4-dioxa-9-azaspiro[4.7]dodecane (0.2 ml) in absolute ethanol (3 ml). A cloudy and warm solution was formed. Addition of ether gave a clear solution in which crystals began to form. Colorless needles were obtained, mp 144–145°. Two recrystallizations from ethanol–ether gave colorless crystals: mp 144–145°; ν_{OH} 3290 m, $\nu_{C_6H_5}$ 760 m, 715 ms, 705 ms cm^{-1} (in Nujol).

Anal. Calcd for $C_{14}H_{20}NO_5Cl$ (317.77): C, 52.91; H, 6.34; N, 4.41. Found: C, 52.78; H, 6.44; N, 4.62.

B. From 1-Benzylhexahydro-5(2H)-azocinone.—Aqueous (70%) perchloric acid (ten drops) was added to a solution of 1-benzylhexahydro-5(2H)-azocinone (0.298 g, 0.00137 mol) in absolute ethanol (3 ml). The soluion became warm and crystals began to form after several minutes. Ether (6 ml) was added and the product (0.347 g, 0.00109 mol, 80%), mp 142–144°, was collected after 1 hr. The infrared spectrum in Nujol is identical with that of the compound obtained from reaction of 9-benzyl-1,4-dioxa-9-azaspiro[4.7]dodecane with aqueous perchloric acid.

4-Benzylhexahydro-7a-acetoxy-1H-pyrrolizinium Perchlorate (24b).—A mixture of 4-benzylhexahydro-7a-hydroxy-1H-pyrrolizinium perchlorate (0.342 g, 0.00108 mol) and acetic anhydride (6 ml, 0.065 mol) was warmed on a steam bath for 0.5 hr, giving a light yellow solution. Addition of ether to the cooled solution gave an oily precipitate which rapidly crystallized (0.281 g, 0.000781 mol, 72%). Recrystallization from acetone–ether gave nearly colorless crystals, mp 190–195°. Two more recrystallizations from acetone–ether gave colorless crystals: mp 203–204°; $\nu_{C=O}$ 1750 s, $\nu_{C=C}$ 1580, 1495, $\nu_{C_6H_5}$ 760, 700 cm^{-1} (in Nujol).

Anal. Calcd for $C_{16}H_{22}NO_6Cl$ (359.81): C, 53.41; H, 6.16; N, 3.89. Found: C, 53.62; H, 6.18; N, 4.04.

1,4-Dioxa-9-azaspiro[4.7]dodecane (25).—A solution of 9-benzyl-1,4-dioxa-9-azaspiro[4.7]dodecane (24.642 g, 0.0945 mol) in absolute ethanol (150 ml) was shaken with 5% palladium on carbon (5.88 g) and hydrogen in a Parr hydrogenation apparatus. After 30 min the hydrogen uptake had stopped and totaled 28 lb (calculated, 27.4 lb). The catalyst was removed by a first filtration through Celite on a coarse porosity sintered-glass funnel and then by filtration through a medium porosity sintered-glass funnel. The catalyst was washed twice in each case with ethanol. The ethanol was removed by distillation through a 30-cm Vigreux column. The residual oil was purified by a simple distillation which gave a colorless oil (14.447 g, 0.0844 mol, 89%): bp 70–75° (0.2 mm); $n^{25}D$ 1.4835; ν_{NH} 3360 cm^{-1} (on the oil).

Anal. Calcd for $C_9H_{17}NO_2$ (171.23): C, 63.13; H, 10.00; N, 8.18. Found: C, 63.31; H, 10.02; N, 8.17.

The oil gives a white solid when left in contact with the atmosphere, presumably from reaction with carbon dioxide.

1,2,3,5,6,7-Hexahydropyrrolizinium Perchlorate (26).—Aqueous 70% perchloric acid (3 ml) was added to a solution of 1,4-dioxa-9-azaspiro[4.7]dodecane (1.070 g, 0.00625 mol) in ethanol (5 ml). The solution became hot. After cooling, ether was added, causing formation of colorless crystals (1.032 g, 0.00493 mol, 78%). Recrystallization from ethanol gave colorless crystals, mp 239–241° dec. A second recrystallization gave colorless plates: mp 238–240° slight dec; $\nu_{C=N^+}$ 1690 s cm^{-1} (in Nujol).

Anal. Calcd for $C_7H_{12}NO_4Cl$ (209.64): C, 40.10; H, 5.77; N, 6.68. Found: C, 40.23; H, 5.82; N, 6.87.

Hexahydro-1H-pyrrolizine Picrate (28).—A mixture of 1,2,3,-5,6,7-hexahydropyrrolizinium perchlorate (0.353 g, 0.00168 mol) and sodium borohydride (0.9 g) in absolute ethanol was heated on a steam bath for 3 hr. Water (10 ml) and 1 *M* aqueous sodium hydroxide solution (20 ml) were added and the mixture was filtered. The filtrate was extracted with three 50-ml portions of ether; the ether was dried and concentrated to a small quantity of oil. The oil was dissolved in ethanol (5 ml) and ethanolic picric acid (3–5 ml) was added in small portions. The solution became dark red in color. Cooling in the freezer gave dark red crystals, mp 145–160°, with some crystals remaining to 225°. Recrystallization gave olive-yellow crystals, mp 260–263° dec (lit.[13] mp 260–262° dec).

8-Benzoyl-1,4-dioxa-8-azaspiro[4.7]dodecane (29).—A mixture of 1-benzoylhexahydro-4(1H)-azocinone (16.487 g, 0.0712 mol), *p*-toluenesulfonic acid hydrate (1.35 g, 0.00710 mol), ethylene glycol (25 ml), and benzene (200 ml) was heated to reflux for 24 hr. The condensate was passed through a calcium carbide drying trap during this time. Pyridine (2 ml) was added and the mixture was cooled. The mixture was extracted with aqueous sodium bicarbonate solution and with three 100-ml portions of water. The benzene layer was dried and concentrated to a viscous oil which did not crystallize. This oil was used in the next reaction without further purification.

8-Benzyl-1,4-dioxa-8-azaspiro[4.7]dodecane (30).—The oily 8-benzoyl-1,4-dioxa-8-azaspiro[4.7]dodecane from the preceding reaction was partially dissolved in ether (2 l.) and slowly added to a stirred mixture of lithium aluminum hydride (3.0 g, 0.0790 mole) and ether (200 ml). The excess ether was allowed to boil off. The remaining oil, insoluble in ether, was dissolved in tetrahydrofuran and added to the reaction mixture. The mixture was stirred and refluxed 7 hr and then kept at room temperature overnight. The excess lithium aluminum hydride was destroyed by addition of ethyl acetate and water. The inorganic solids were removed by filtration through Celite and were washed with ether. The combined ether filtrates were dried and concentrated to a yellow oil. Distillation of the oil gave 8-benzyl-1,4-dioxa-8-azaspiro[4.7]dodecane (**30**) (5.700 g, 0.0218 mol, 30%) as a colorless oil: bp 140–150° (0.2 mm); $n^{26}D$ 1.5284; $\nu_{C=C}$ 1600 mw, 1580 w, 1490 s, $\nu_{C_6H_5}$ 725 s, 695 s cm^{-1} (on the oil).

Anal. Calcd for $C_{16}H_{23}NO_2$ (261.35): C, 73.53; H, 8.87; N, 5.36. Found: C, 73.33; H, 8.91; N, 5.14.

Distillation also gave a light yellow oil (4.768 g), bp 190–205° (0.2 mm), obtained by heating the distillation flask with a small flame.

1-Benzylhexahydro-4(1H)-azocinone Perchlorate (31).—Aqueous 70% perchloric acid (0.5 ml) was added to a solution of 8-benzyl-1,4-dioxa-8-azaspiro[4.7]dodecane (0.357 g, 0.00137 mol) in absolute ethanol (2 ml). Ether was added to the point of cloudiness and the mixture was allowed to cool. Oil drops precipitated and crystallized after cooling in the freezer, giving colorless crystals (0.301 g, 0.000949 mol, 69%), mp 103–142° dec. Three recrystallizations from ethanol–ether gave colorless crystals: mp 151–153°; ν_{NH^+} 3080 ms, $\nu_{C=O}$ 1700 s, $\nu_{C=C}$ 1495 m, $\nu_{C_6H_5}$ 745 ms, 700 ms cm^{-1} (in Nujol).

Anal. Calcd for $C_{14}H_{20}NO_5Cl$ (317.77): C, 52.91; H, 6.34; N, 4.41. Found: C, 52.91; H, 6.58; N, 4.17.

1,4-Dioxa-8-azaspiro[4.7]dodecane (32).—A solution of 8-benzyl-1,4-dioxo-8-azaspiro[4.7]dodecane (5.152 g, 0.0197 mol) in absolute ethanol (75 ml) was shaken with 5% palladium on carbon (1.5 g) and hydrogen in a Parr apparatus for 3 hr. Hydrogen uptake was complete after 1 hr. The catalyst was removed by filtration and was washed twice with methanol. The excess solvent was removed by distillation through a 30-cm Vigreux column under reduced pressure. Distillation of the residual oil gave a colorless oil (2.771 g, 0.0162 mol, 82%): bp 68–69° (0.1 mm); $n^{26}D$ 1.4853; ν_{NH} 3360 mw cm^{-1} (on the oil).

Anal. Calcd for $C_9H_{17}NO_2$ (171.23): C, 63.13; H, 10.00; N, 8.18. Found: C, 63.16; H, 10.04; N, 7.88.

Hexahydro-4(1H)-azocinone Perchlorate (33).—Aqueous (70%) perchloric acid (1.0 ml) was added to a solution of 1,4-dioxa-8-azaspiro[4.7]dodecane (0.269 g, 0.00157 mol) in absolute ethanol (2 ml). The reaction was exothermic. After the solution cooled, ether was added and the solution placed in the freezer. Crystals formed overnight and were recrystallized from ethanol–ether, giving colorless crystals: mp 55, 75–85°; $\nu_{OH,NH}$ 3600 m, 3150 ms, $\nu_{C=O}$ 1695 s cm^{-1} in Nujol. The crystals were dissolved in ethanol and the solution was refluxed for 45 min. Addition of ether gave colorless crystals, mp 87–89°. Recrystallization from ethanol–ether gave colorless flakes: mp 87–89°; ν_{NH} 3150 s, $\nu_{C=O}$ 1695 s cm^{-1} (in Nujol).

Anal. Calcd for $C_7H_{14}NO_5Cl$ (227.65): C, 36.93; H, 6.20; N, 6.15. Found: C, 37.04; H, 6.28; N, 6.40.

Oxidation and Isolation of the Products from the Bioconversion of 1-Benzoyloctamethylenimine. 1-Benzoyloctahydro-4H-azonin-4-one (36) and 1-Benzoyloctahydro-5H-azonin-5-one (35).—The dry extract from the bioconversion of 1-benzoyloctamethyleneimine (25.0 g) was dissolved in acetone (500 ml) and oxidized with excess Jones reagent[2] as described previously. The residual oily material was dissolved in methylene chloride (100 ml) and the solution was placed on a column of Florisil (2.5 kg) packed in Skellysolve B. The following 2-l. fractions were collected: 2 of Skellysolve B, 10 of 10% (v/v) acetone in Skellysolve B, 8 of 20% acetone in Skellysolve B, 5 of 50% acetone-Skellysolve B, and 3 of acetone. Fractions 15 through 18 gave 4.80 g of **1-benzoyloctahydro-4H-azonin-4-one (36)** as a yellow crystalline material and fractions 20 through 23 gave 11.62 g of **1-benzoyloctahydro-5H-azonin-5-one (35)** as a yellowish crystalline solid. Removal of color from these products with activated charcoal renders them sufficiently pure for most uses; however, for preparation of analytical samples, portions were rechromatographed on Florisil. A sample (1.60 g) of the less polar material was dissolved in methylene chloride and placed on a column of Florisil (80 g) packed with Skellysolve B. Elution with 10% (v/v) acetone in Skellysolve B gave colorless crystals. Two recrystallizations from cold methylene chloride–Skellysolve B gave **36** as colorless plates: mp 87–88°; $\nu_{C=O}$ 1700 ms, 1625 s, $\nu_{C_6H_5}$ 787 ms, 744, ms, 705 ms cm^{-1} (in Nujol).

Anal. Calcd for $C_{15}H_{19}NO_2$ (245.31): C, 73.44; H, 7.81; N, 5.71. Found: C, 73.30; H, 8.03; N, 5.80.

A sample (11.40 g) of the more polar material dissolved in methylene chloride was placed on a column of Florisil (600 g) packed with Skellysolve B. Elution with 25% (v/v) acetone in Skellysolve B gave colorless crystals. Recrystallization from acetone–Skellysolve B was achieved by placing the solution in the freezer and gave colorless crystals, mp 69–71°. A final recrystallization from acetone–Skellysolve B gave **35** as colorless crystals: mp 70–72°; $\nu_{C=O}$ 1700 ms, 1625 s, ν 800 m, 748 ms, 717 s cm^{-1} (in Nujol).

Anal. Calcd for $C_{15}H_{19}NO_2$: C, 73.44; H, 7.81; N, 5.71. Found: C, 73.54; H, 7.72; N, 5.94.

9-Benzoyl-1,4-dioxa-9-azaspiro[4.8]tridecane (37).—A solution of 1-benzoyloctahydro-5H-azonin-5-one (18.819 g, 0.0768 mol) and *p*-toluenesulfonic acid hydrate (1.46 g) in benzene (200 ml) was stirred with ethylene glycol (30 ml). The mixture was heated to the reflux temperature of benzene for 22 hr and the condensate was passed through a calcium carbide drying trap. Pyridine (3 ml) was added at the end of the reflux period; the mixture was cooled and then extracted with 5% aqueous sodium bicarbonate (100 ml) and with two 100-ml portions of water. The benzene layer was dried and was concentrated under reduced pressure, giving a viscous yellow oil. The infrared spectrum of the oil showed the presence of a carbonyl function (1730 w cm^{-1}) and so the oil was again dissolved in benzene (200 ml) and mixed with ethylene glycol (30 ml) and *p*-toluenesulfonic acid hydrate (3 g). The mixture was heated to reflux for 24 hr after which the work-up used above was repeated. The oil product retained the unusual carbonyl absorption in its infrared spectrum. A sample of the oil kept from the first work-up partially crystallized and when the oil product was mixed with these crystals, crystallization occurred. Recrystallization from ether–Skellysolve B gave two crops (13.682 g, 0.0473 mol, 61%) of light yellow crystals, mp 90–95°. Recrystallization from ether–Skellysolve B gave crystals, mp 98–100°. A final recrystallization preceded by decolorization with activated charcoal gave colorless needles: mp 99–101°; $\nu_{C=O}$ 1630 s, $\nu_{C_6H_5}$ 798 m, 737 m, 704 ms cm^{-1} (in Nujol).

Anal. Calcd for $C_{17}H_{23}NO_3$ (289.36): C, 70.56; H, 8.01; N, 4.84. Found: C, 70.50; H, 8.08; N, 5.16.

9-Benzyl-1,4-dioxa-9-azaspiro[4.8]tridecane (38).—A solution of 9-benzoyl-1,4-dioxa-9-azaspiro[4.8]tridecane (13.331 g, 0.0462 mole in ether (250 ml) was dribbled into a mixture of lithium aluminum hydride (2.0 g) in ether (100 ml). The mixture was stirred at room temperature for 16 hr and at reflux temperature for 4 hr. The excess hydride was decomposed by the cautious addition of water. The inorganic solids were removed by filtration and washed with ether. The combined ether solution was dried and concentrated to an oil. A simple distillation of the oil gave the product (10.779 g, 0.0392 mol, 85%) as a colorless oil: bp 145–147° (1.5 mm); $n^{26}D$ 1.5302; $\nu_{C=C}$ 1600 w, 1498 m, $\nu_{C_6H_5}$ 750 m, 715 ms, 701 ms cm^{-1} (on the oil).

Anal. Calcd for $C_{17}H_{25}NO_2$ (275.38): C, 74.14; H, 9.15; N, 5.09. Found: C, 74.77; H, 9.66; N, 5.04.

The oil crystallized when kept overnight in the refrigerator, mp 44–46°.

4-Benzyloctahydro-8a-hydroxyindolizinium Perchlorate (39).—Aqueous (70%) perchloric acid (2 ml) was added to a solution of 9-benzyl-1,4-dioxa-9-azaspiro[4.8]tridecane (0.491 g, 0.00178 mole) in ethanol (3 ml). The solution became hot After cooling, ether was added to the point of forming two phases. Crystals formed slowly. The mixture was cooled in the freezer. Colorless crystals (0.456 g, 0.00137 mol, 73%) were obtained, mp 143–145°. Two recrystallizations from ethanol–ether gave colorless crystals: mp 152–153°; ν_{OH} 3420 s, $\nu_{C_6H_5}$ 768 s, 710 s cm^{-1} (in Nujol).

Anal. Calcd for $C_{15}H_{22}NO_5Cl$ (331.80): C, 54.29; H, 6.68; N, 4.22. Found: C, 54.16; H, 6.85; N, 4.48.

1,4-Dioxa-9-azaspiro[4.8]tridecane (40).—A solution of 9-benzyl-1,4-dioxa-9-azaspiro[4.8]tridecane (9.451 g, 0.0344 mol) in methanol (150 ml) was shaken with 5% palladium on carbon (2.5 g) in hydrogen for 45 min at which time uptake of hydrogen appeared complete. The catalyst was removed by filtration and the colorless filtrate was stored overnight in the refrigerator. The solution, now yellow, was concentrated under reduced pressure. The yellow oil product crystallized as it cooled. The solid was dissolved in ether, decolorized with activated charcoal, filtered, and crystallized by addition of Skellysolve B to the ether and by cooling in the freezer. Colorless crystals (4.649 g, 0.0251 mole, 73%) were obtained, mp 55–57°. Recrystallization from ether–Skellysolve B gave colorless, chunky crystals: mp 55–57°; ν_{NH} 3400 mw cm^{-1} (in Nujol).

Anal. Calcd for $C_{10}H_{19}NO_2$ (185.26): C, 64.83; H, 10.34; N, 7.56. Found: C, 64.66; H, 10.40; N, 7.62.

2,3,5,6,7,8-Hexahydro-1H-indolizinium Perchlorate (41).—Aqueous (70%) perchloric acid (2 ml) was added to a solution of 1,4-dioxa-9-azaspiro[4.8]tridecane (0.483 g, 0.00261 mol) in absolute ethanol. The solution became hot and, after cooling, ether was added to the point of separation of two phases. The solution was placed in the freezer and crystals slowly formed. Colorless crystals (0.350 g, 0.00157 mol, 60%) were collected by filtration, mp 212–220°. Three recrystallizations from ethanol–ether gave colorless flakes: mp 227–228° dec (lit.[15] mp 218–219° dec); $\nu_{C=N^+}$ 1690 cm^{-1} (in Nujol) [lit.[15] 1689 cm^{-1} (in Nujol)].

Registry No.—**8,** 16803-02-4; **10,** 16803-03-5; **12,** 15923-40-7; **12a,** 16803-05-7; **13,** 16803-06-8; **14,** 16803-07-9; **16,** 16803-08-0; **18,** 16803-09-1; **19,** 16803-10-4; **20,** 16803-11-5; **21,** 16803-12-6; **22a,** 16853-06-8; **22b,** 16853-07-9; **23,** 16853-08-0; **24b,** 16353-09-1; **25,** 16803-13-7; **26,** 16853-10-4; **30,** 16803-14-8; **31,** 16803-15-9; **32,** 16853-11-5; **33,** 16803-16-0; **35,** 16803-17-1; **36,** 16803-18-2; **37,** 16803-19-3; **38,** 16803-20-6; **39,** 16853-12-6; **40,** 16803-21-7; **41,** 14594-57-1.

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The Microbiological Oxygenation of Some Azabicycloalkanes

ROY A. JOHNSON, MILTON E. HERR, HERBERT C. MURRAY, LESTER M. REINEKE, AND GUNTHER S. FONKEN

Biochemical Research Division, The Upjohn Company, Kalamazoo, Michigan 49001

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The microbiological oxygenation of 3-benzoyl-3-azabicyclo[3.3.1]nonane (**1**) and 3-benzoyl-3-azabicyclo[3.2.2]nonane (**11**) with *Sporotrichum sulfurescens* has been shown to give 3-benzoyl-*endo*-3-azabicyclo[3.3.1]nonan-6-ol (**2**) in the first case and a mixture of 3-benzoyl-*endo*-3-azabicyclo[3.2.2]nonan-6-ol (**12**) and 3-benzoyl-3-azabicyclo[3.2.2]nonan-6-one (**13**) in the second case. Reduction of ketone **13** with sodium borohydride gave 3-benzoyl-*exo*-3-azabicyclo[3.2.2]nonan-6-ol (**14**), which underwent acyl migration in acidic solution. The major and minor hydroxylated products obtained from microbiological oxygenation of 2-benzoyl-2-azabicyclo[2.2.2]octane (**23**) were assigned *endo*-5-ol (**24**) and *endo*-6-ol (**25**) structures, respectively, based on the patterns of oxygenation observed in the above and in related molecules.

The oxygenation of saturated organic molecules by microorganisms is of particular importance because of the introduction of functionality at positions inaccessable to many methods of organic chemistry. Examples of interest are the numerous oxygenations of the steroid nucleus by a variety of microorganisms.[1] Recent extensions include oxygenations of macrocyclic alcohols[2] and the amide derivatives of cyclic amines[3] by the microorganism, *Sporotrichum sulfurescens*. As yet there is considerable uncertainty as to the position of oxidative attack by microorganisms. A proposal that such attack by *S. sulfurescens* will occur at a saturated carbon approximately 5.5 Å from an electron-rich center, such as a ketone or an amide carbonyl oxygen,[2,3] provides a working hypothesis with which to consider this question. Continued expansion of the varieties of molecules submitted to microbiological oxygenation aids in testing this proposal as well as in determining the limits of the reaction with respect to chemical structure. The successful microbial oxygenation of the amides of azacycloalkanes[4] suggested the further extension to the amides of azabicycloalkanes. The oxygenation of 3-benzoyl-3-azabicyclo[3.3.1]nonane (**1**), 3-benzoyl-3-azabicyclo[3.2.2]nonane (**11**), and 2-benzoyl-2-azabicyclo[2.2.2]octane

(1) *Cf.* S. H. Eppstein, P. D. Meister, H. C. Murray, and D. H. Peterson, "Vitamins and Hormones," Vol. XIV, Academic Press Inc., New York, N. Y., 1956, pp 359–432.

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