resulting emulsion was well extracted with ether, and the ether extract was washed once with water and dried. The ether solution was made up to 650 ml and 100 ml of this was treated with ethereal HCl to precipitate the salt of **21**. This was recrystallized from methanol–ethanol–ether: yield, 0.744 g; mp 222° dec.

Anal. Calcd for $C_{15}H_{20}NOCl$: C, 67.78; H, 7.59; N, 5.27; Cl, 13.34. Found: C, 68.13; H, 7.77; N, 5.42; Cl, 13.17.

3-Azabicyclo[3.2.2]nonan-6-one Hydrochloride (22).—The ether solution of free base remaining from the above experiment was taken to dryness to yield 4.55 g of oil. This was dissolved in 90 ml of ethanol and shaken with 1.0 g of 10% palladium on carbon and hydrogen (50 psig) for 180 min. The catalyst was removed by filtration; the filtrate and wash were concentrated *in vacuo* to a small volume, diluted with ether, and treated with ethereal HCl. The hydrochloride of **22** was recovered, washed with ether, and dried: yield, 3.12 g; mp 218–220° dec. A sample from methanol–ether melted at 227–229° dec.

Anal. Calcd for $C_8H_{14}NOCl$: C, 54.70; H, 8.03; N, 7.98; Cl, 20.19. Found: C, 54.22; H, 8.14; N, 7.98; Cl, 20.64.

Bioconversion of 2-Benzoyl-2-azabicyclo[2.2.2]nonane (23). 2-Benzoyl-*endo*-2-azabicyclo[2.2.2]octan-5-ol (24) and 2-Benzoyl-*endo*-2-azabicyclo[2.2.2]octan-6-ol (25).—The methylene chloride extract residue from the bioconversion of **23** (25.0 g, 0.116 mol) was chromatographed over 1000 g of Florisil. Elution with 4 l. each of Skellysolve B containing 10, 15, and 20% acetone and with 12 l. of Skellysolve B containing 25% acetone by volume was carried out with collection of 800-ml fractions. The fractions were pooled as follows on the basis of tlc. Fractions 7–11 were 3.98 g (16%) of unchanged starting material. Fractions 17, and 18 gave, after recrystallization from acetone, 1.61 g (6.97 mmol, 6% **25**, mp 200–205°).

Anal. Calcd for $C_{14}H_{17}NO_2$: C, 72.70; H, 7.41; N, 6.03. Found: C, 72.70; H, 7.64; N, 5.82.

Fraction 19 was a mixture, 2.14 g (8%). Fractions 20–27 gave 12.16 g of solid. Recrystallization from acetone gave 10.62 g (0.0460 mol, 40%) of crystalline **24**, mp 146–148°.

Anal. Calcd for $C_{14}H_{17}NO_2$: C, 72.70; H, 7.41; N, 6.03. Found: C, 72.52; H, 7.19; N, 6.18.

2-Benzoyl-2-azabicyclo[2.2.2]octan-6-one (26).—2-Benzoyl-*endo*-2-azabicyclo[2.2.2]octan-6-ol (2 g) in 100 ml of acetone was oxidized by the Jones method[11] to give the ketone (1.95 g) as an oil which eventually crystallized: mp 67–72°; $\nu_{C=O}$ 1740, 1610 cm^{-1} in Nujol.

Anal. Calcd for $C_{14}H_{15}NO_2$: C, 73.34; H, 6.59; N, 6.11. Found: C, 72.82; H, 6.94; N, 6.08.

2-Benzoyl-2-azabicyclo[2.2.2]octan-5-one (27).—2-Benzoyl-*endo*-2-azabicyclo[2.2.2]octan-5-ol (300 mg) was oxidized[11] to the ketone which was recrystallized from acetone–hexane: mp 99–101°; $\nu_{C=O}$ 1740, 1610 cm^{-1} in Nujol.

Anal. Calcd for $C_{14}H_{15}NO_2$: C, 73.34; H, 6.59; N, 6.11. Found: C, 73.15; H, 6.70; N, 5.99.

Registry No.—**2**, 16780-54-4; **3**, 16780-67-9; **4**, 16780-68-0; **6**, 16780-69-1; **7**, 16780-70-4; **8**, 16780-71-5; **9**, 16780-72-6; **12**, 16780-73-7; **13**, 16780-74-8; semicarbazone of **13**, 16780-75-9; oxime of **13**, 16808-42-7; 2,4-dinitrophenylhydrazone of **13**, 16780-76-0; **14**, 16780-77-1; **15**, 16780-78-2; **16**, 16780-79-3; **17**, 16780-80-6; **18**, 16780-81-7; **19**, 16808-43-8; **20**, 16808-44-9; **21** HCl, 16808-45-0; **22**, 16808-46-1; **24**, 16785-68-5; **25**, 16785-69-6; **26**, 16785-70-9; **27**, 16808-47-2.

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The Microbiological Oxygenation of Acylated 1-Adamantanamines. Stereochemistry and Structural Determinations

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

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

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Microbiological oxygenation of N-acetyl-1-adamantanamine with *Sporotrichum sulfurescens* produced C-4 hydroxylation as the major reaction along with a minor quantity of C-3 hydroxylation. The reaction of the same organism with N-benzoyl-N-methyl-1-adamantanamine led to C-4 and C-6 dihydroxylation as the major conversion entity with a lesser quantity of C-4 monohydroxylation. Oxygenation occurred primarily on the methylene carbons and resulted in *trans* hydroxylation with respect to the N substituent; lipophilicity led to dihydroxylation, whereas hydrophilicity led to monohydroxylation. The products obtained from the biotransformations of some other N-acetylated adamantanamines are described.

In recent papers[1] we have described the microbiological oxygenation of macrocyclic alcohols,[1a] heterocyclic ring systems,[1b] and alicylic amides.[1c] When various substrates were dispersed in the active fermentation medium of *Sporotrichum sulfurescens*, oxygenation was shown to occur at an optimal distance of about 5.5 Å from an electron-rich center to the position of attachment at an unactivated methylene site. The authors have now studied the action of *S. sulfurescens* on some N-acylated 1-adamantanamines (Charts I, II, and III). The structures of the products, including the stereochemistry, have been determined by chemical and spectroscopic methods. The proposed enzyme–substrate model described previously[1a] was helpful in predicting the most favorable position for oxygenation of this rigid cage molecule, and the products obtained were compatible with the hypothesis.

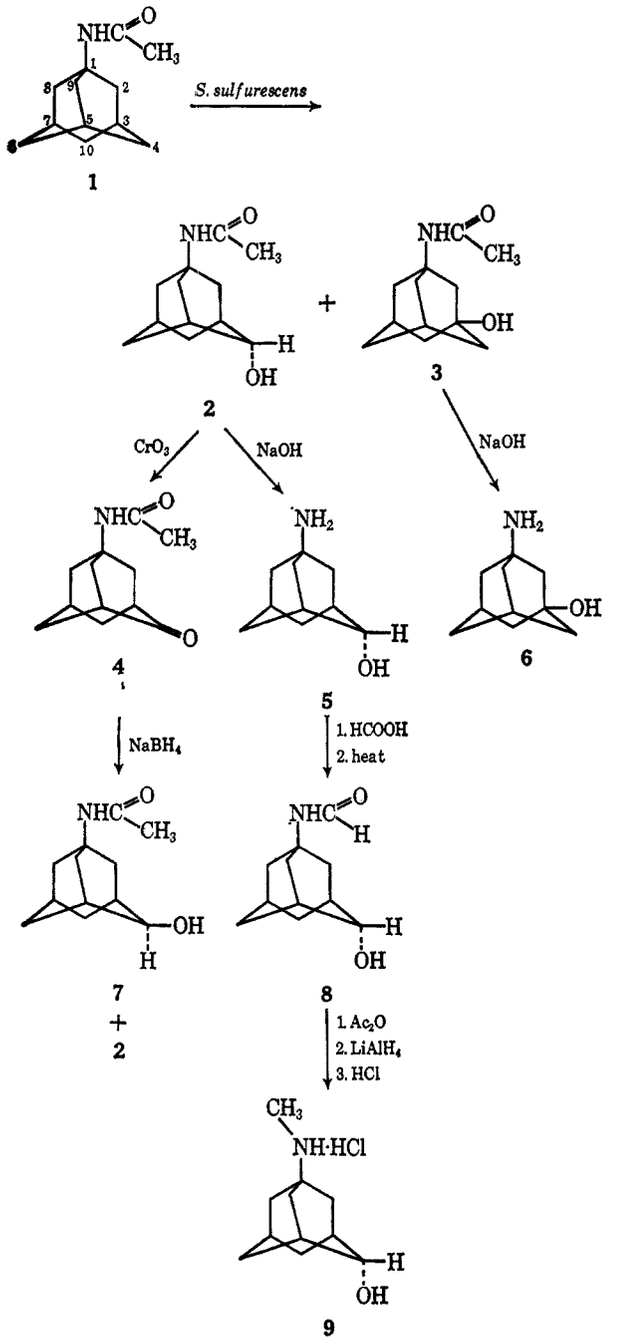
Bioconversion products of N-acetyl-1-adamantanamine[2] (**1**) (Chart I) unexpectedly were found to be quite water-soluble compounds and could not be extracted with methylene chloride. The compounds were readily absorbed on carbon from which they were recovered and further purified. Two monohydroxylated compounds were isolated from this conversion. The one produced in minor quantity could not be oxidized to ketone and was assigned a tertiary alcohol structure (**3**).[3] Heating at reflux in aqueous base produced the

(1) (a) G. S. Fonken, M. E. Herr, H. C. Murray, and L. M. Reineke, *J. Amer. Chem. Soc.*, **89**, 672 (1967); (b) R. A. Johnson, M. E. Herr, H. C. Murray, L. M. Reineke, and G. S. Fonken, *J. Org. Chem.*, **33**, 3195 (1968); (c) G. S. Fonken, M. E. Herr, H. C. Murray, and L. M. Reineke, *ibid.*, **33**, 3182 (1968).

(2) H. Stetter, M. Schwarz, and A. Hirschborn, *Chem. Ber.*, **92**, 1679 (1959).

(3) Since our isolation of this compound its chemical preparation has been reported: H. Stetter, J. Gartner, and P. Tacke, *Angew. Chem.*, **4**, 153 (1965).

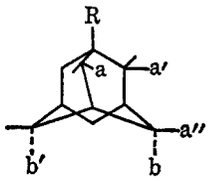
CHART I



hydroxy amine (**6**). The more important compound from this conversion on the basis of yield and uniqueness was the secondary alcohol (**2**). Oxidation of this product by the method of Jones, *et al.*,[4] readily produced a ketone (**4**); this could be expected from either a 2- or 4-hydroxy compound. Examination of the nmr spectrum of the keto amide (**4**) showed a downfield symmetrical signal centered at δ 2.60 ppm which integrated for two protons.[5] This signal could be attributed to the tertiary protons on carbon atoms 3 and 5 α to the C-4 carbonyl (compound **4**). However, it could be argued that if the ketone was at C-2 the long-range effect of the carbonyl function could cause a downfield shift of signal for the two protons on carbons 8 and 9 in closest proximity to the carbonyl. The latter possibility was ruled out by the observation that sodium borohydride reduction of the ketone gave a mixture of hydroxy amides, which was readily separated byc hromatography into two pure compounds, of mp 176–177° (**2**) and mp 206–207° (**7**), the former of which was identical with the major biotransformation product. Reduction of a C-2 ketone would have produced a racemic mixture of hydroxy amides on an asymmetric carbon which would not have been resolvable by chromatography. It will be shown in the discussion below that the major bioconversion product was in fact N-acetyl-1-adamantanamin-4α-ol (**2**),[6] and therefore the other product of the borohydride reduction was the corresponding 4β-ol (**7**). As in the case of the *t*-hydroxy amide (**3**) the C-4-hydroxy amide was readily hydrolyzed to C-4 hydroxy amine (**5**) in aqueous base.

We next turn to the discussion of the products obtained from the bioconversion of N-benzoyl-N-methyl-1-adamantanamine (**10**) (Chart II) with *S. sulfurescens*. The products in this case, unlike those described above, were more lipophilic and were extractable from the fermentation beer with methylene chloride. The residue from the extraction upon chromatography produced two hydroxylated products. The structures of these compounds were found to be interrelated with the structure of compound **2**. The major component of the microbiological oxidation proved to be a dihydroxy amide (**11**), and the minor component was found to be a monohydroxy amide (**12**).

The monohydroxy compound (**12**) upon reexposure to the action of *S. sulfurescens* was convertible into the same dihydroxy amide (**11**). This showed that compounds **11** and **12** had one hydroxyl function in common. The dihydroxy amide was readily oxidized by the Jones method[4] to a diketone, thus showing that both alcohol groups were secondary carbinols. The reaction of the diol with thionyl chloride produced a cyclic sulfite ester (**13**).[7] Consideration of a Dreiding model of adamantane showed that formation of the cyclic sulfite ester defined the stereochemical relationship of the two hydroxyl groups to each other. The possibility for formation of a cyclic product was limited to a situation where the OH functions protruded from the molecule in parallel proximity to each other. It did not, however, discriminate as to whether they were attached at a and a′, a′ and a″, or b and b′. Examination of the nmr spectrum of the diol in dimethylformamide-d_7 showed a symmetrical signal centered at 5.38 ppm for the two OH protons. The two tertiary protons attached to the carbons bearing the OH functions appeared as a symmetrical band centered at δ 3.93 ppm. The



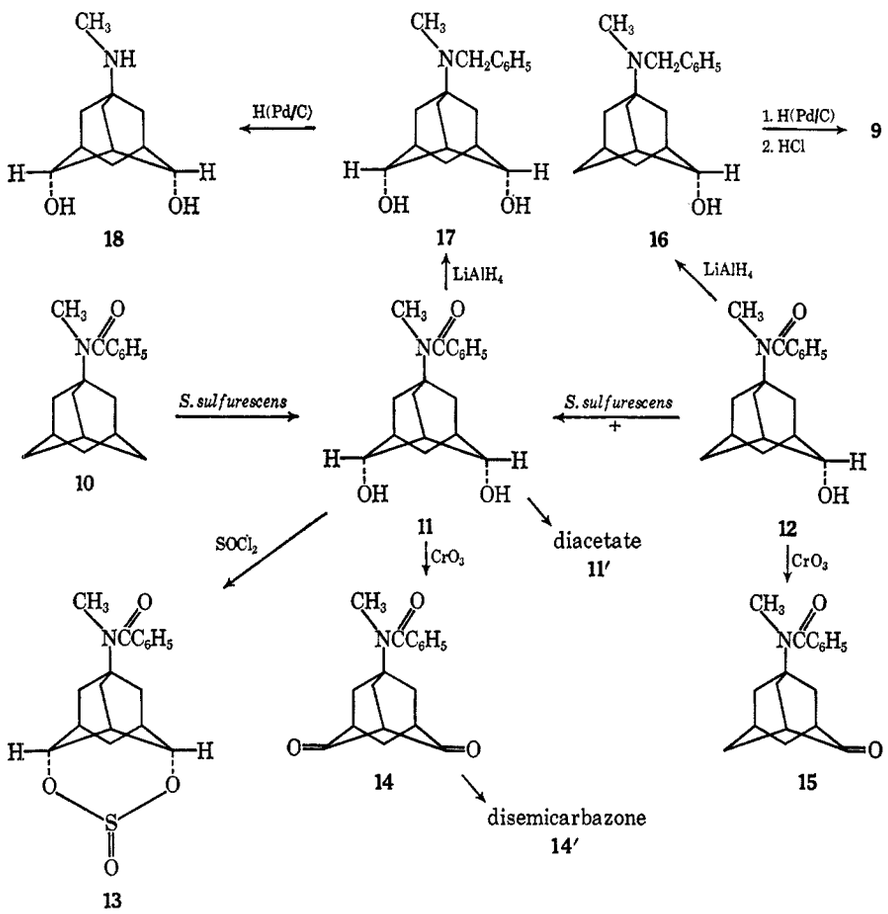
(4) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).

(5) Nuclear magnetic resonance spectra were determined at 60 Mcps on a Varian Model A-60 spectrometer with references to tetramethylsilane.

(6) We propose the following stereochemical notation for the C-4- (or C-6-) substituted 1-adamantanamines. The reference group is the C-1 carbon–nitrogen bond and a substituent at C-4 (or C-6) will be referred to as β or α according to whether it is *cis* or *trans* with respect to the general plane of the common six-membered ring.

(7) B. R. Brown, P. W. Trown, and J. M. Woodhous, *J. Chem. Soc.*, 2478 (1961).

CHART II



corresponding two protons of the cyclic sulfite ester produced a symmetrical signal centered at δ 4.68 ppm. These measurements showed that the two hydroxyl groups were attached to the molecule in a symmetrical pattern, thereby eliminating the a'a'' relationship.

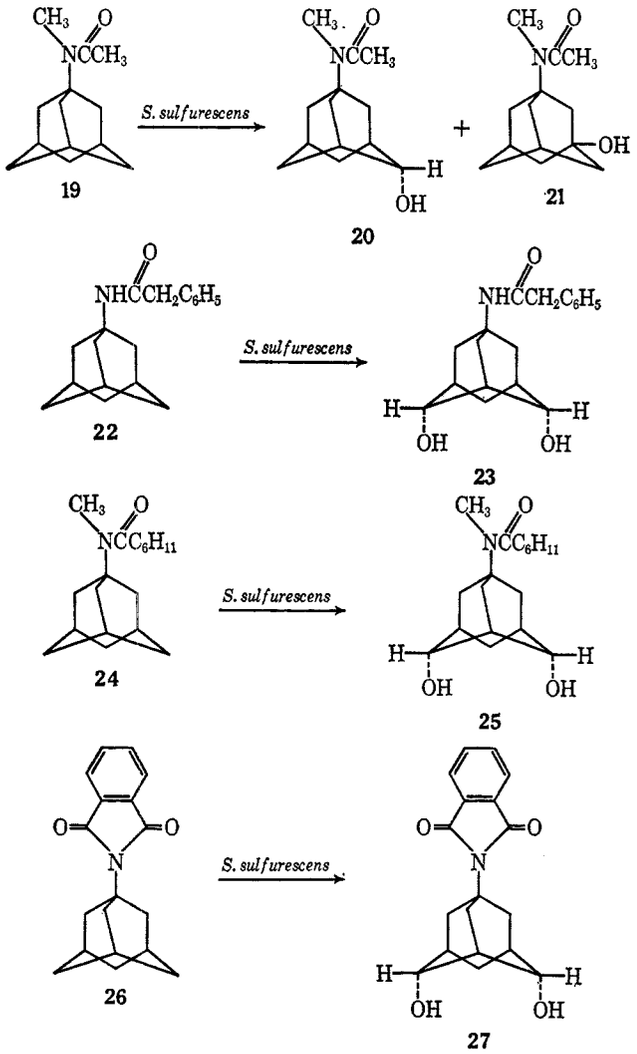
Oxidation of the monohydroxy amide (**12**) to the ketone (**15**) confirmed the presence of a secondary alcohol. Reduction of **12** with lithium aluminum hydride gave a hydroxy-N-methyl-N-benzyl amine (**16**) which upon catalytic hydrogenation produced a hydroxy-N-methyl amine, isolated as the hydrochloride salt (**9**). This salt was identical in all respects with a compound produced by the following series of reaction (shown in Chart I) on the previously described N-acetyl-1-adamantanamin-4-ol (**2**). The formic acid salt of the amine (**5**) was dehydrated to the N-formyl alcohol (**8**); the acetate of **8** upon hydride reduction gave an amine which upon treatment with hydrogen chloride produced the salt (**9**). It was thus shown that all three bioconversion products **2**, **11**, and **12** had one hydroxyl function in common. This therefore eliminated the possibility of the a and a' hydroxyl attachment sites since it was shown above that the hydroxyl group of product **2** must be at C-4 and not at C-2. This leaves only the bb' structure for the dihydroxy compounds and at the same time establishes the *trans* relationship of the hydroxyl groups to the amide nitrogen for compounds **2** and **12**.

Variation of the groups attached to the nitrogen of 1-adamantanamine produced other hydroxylated products as shown in Chart III. The structures of the two monohydroxy products obtained from the bioconversion of N-acetyl-N-methyl-1-adamantanamine (**19**) with *S. sulfurescens* were assigned on the basis of the following observations. The compound obtained as the minor product (*ca.* 7%) was shown to be a *t*-hydroxy amide (**21**) because it failed to oxidize with chromic acid. The other product obtained in *ca.* 35% yield was readily oxidized to a ketone, and the structure was determined to be **20** by chemical synthesis. The reaction of the free base of compound **9**, for which the position and orientation of the hydroxyl group is known (*vide supra*), with acetic anhydride in pyridine followed by treatment with base produced the same compound (**20**). Also comparison of the nmr spectrum ($CDCl_3$) of **20** with that of hydroxy amide **12** showed that the signals for the proton on the carbon bearing the hydroxy group appeared as identical in position and shape, centered at δ 3.96 ppm.

The remaining bioconversion products (Chart III) which were obtained from more lipophilic substrates (**22**, **24**, and **26**) were dihydroxylated compounds. We considered the nmr spectra in dimethylformamide-d_7, especially the positions and shapes of the signals for the protons in the carbons bearing the hydroxyl functions, as diagnostic when compared with similar protons of compound **11**. The dihydroxy amides (**23**, **25**, and **27**) all produced a symmetrical signal for these two protons which was centered between δ 3.80 and 4.20 ppm.

An observation of particular interest was that, in all cases where the action of *S. sulfurescens* has produced either mono or dihydroxy secondary alcohols, these groups have been introduced *trans* with references to the nitrogen substituent.[6]

CHART III



With regard to the hypothetical enzyme–substrate model described previously,[1a] the exact distance from the electron-rich site, in this case the amide carbonyl oxygen, to the position of hydroxylation on the methylene carbon cannot be measured because of the variable conformations which may be assumed by the amide function. However, the conformation of maximum distance between amide carbonyl and C-4(6) measures about 6.3 Å and that of minimum distance measures about 4.5 Å. Hydroxylation at the C-2 position was not predicted because this maximum to minimum distance varies from about 4.2 to 2.4 Å, depending on the amide conformation.

Experimental Section[8]

Fermentation Process.—The bioconversion process and description of the culture has been described previously[1a,b] with the following exceptions. When N-phenylacetyl-1-adamantanamine (**22**) and N-1-adamantanphthalimide (**27**) were added to the fermenter in N,N-dimethylformamide solution, essentially only starting material was recovered. The addition of 2.5 ml of the surfactant Ultrawet DS 30[9a] per liter of beer before adding the substrate led to almost complete dissimilation of these substrates. With the surfactant UCONLB 625[9b] was used as a defoaming agent.

The crude products were in each case extracted from the filtered beer with methylene chloride except for the isolation of the products from the fermentation of N-acetyl-1-adamantanamine (**1**) and N-(4α,6α-dihydroxy-1-adamantyl)phthalimide (**26**). These polar materials were separated from the beer by carbon adsorption.

Isolation of Products from the Bioconversion of N-Acetyl-1-adamantanamine.—The filtered beer and the mycelium wash from a 55-g bioconversion amounted to about 115 l. Granular CAL carbon[9c] (3 kg) was heated at 80–90° in deionized water, cooled to 25°, and packed into a column (10.8-cm diameter). The filtered beer was passed through the carbon. The column was stripped with 50 l. of methanol and a first dark 2-l. fraction was discarded after tlc examination showed that it contained no product. The remainder of the methanol eluate was concentrated under reduced pressure to dryness. The residue was stirred with 500 ml of methanol and filtered to remove some insoluble debris from the concentrate. The methanol filtrate was well mixed with 500 g of silica gel.[9d] This mixture was allowed to air dry and added to the top of a column (10.8-cm diameter) of 3 kg of silica gel which had been wet packed using ethyl acetate. The column was eluated as reported in Table I in fractions of 1 l. each.

TABLE I

Fractions	Amt, l.	Eluate
1–6	6	Ethyl acetate
7–12	6	2% methanol in ethyl acetate
13–18	6	5% methanol in ethyl acetate
19–24	6	8% methanol in ethyl acetate
25–30	6	12% methanol in ethyl acetate
31–35	6	15% methanol in ethyl acetate
36–41	6	18% methanol in ethyl acetate

The fraction residues were assayed on thin layer silica gel plates, developed with 10% methanol in ethyl acetate. Fractions 10–12 contained 9.16 g of unchanged substrate.

N-Acetyl-1-adamantanamin-4α-ol (2).—Fraction residues 15–26 from the above column weighed 27.32 g. Recrystallization from acetone gave 23.90 g: mp 173–175°; $\nu_{OH,NH}$ 3500, 3300, $\nu_{N-C=O}$ 1640 cm^{-1} in Nujol.

Anal. Calcd for $C_{12}H_{19}NO_2$: C, 68.86; H, 9.15; N, 6.69. Found: C, 68.87; H, 9.22; N, 6.83.

N-Acetyl-1-adamantanamin-3-ol (3).—Fraction residues 27–30 weighed 8.11 g and by tlc were found to be a mixture of **2** and **3**. Fraction residues 31–34 weighed 6.89 g and contained the C-3 alcohol. This was recrystallized from acetone: yield, 5.08 g; mp 223–225°; $\nu_{OH,NH}$ 3300, $\nu_{N-C=O}$ 1650 cm^{-1} in Nujol.

Anal. Calcd for $C_{12}H_{19}NO_2$: C, 68.86; H, 9.15; N, 6.69. Found: C, 69.00; H, 9.11; N, 6.82.

N-Acetyl-1-adamantanamin-4-one (4).—This oxidation of **2** was carried out by the method of Jones, *et al.*[4] The product was recrystallized from acetone: mp 175–177°; ν_{NH} 3330, $\nu_{C=O}$ 1730, $\nu_{N-C=O}$ 1650 cm^{-1} in Nujol.

Anal. Calcd for $C_{12}H_{17}NO_2$: C, 69.53; H, 8.27; N, 6.76. Found: C, 69.88; H, 8.51; N, 6.79.

N-Acetyl-1-adamantanamin-4α- and -4β-ol (2, 7).—Keto amide **4** (2 g) dissolved in 25.0 ml of methanol was treated with 1.0 g of sodium borohydride dissolved in 6.0 ml of 0.1 *N* sodium hydroxide solution, and the mixture was allowed to stand at room temperature for 18 hr. The mixture was chilled at 0° and carefully treated dropwise with 50% acetic acid until the pH was 6–7. The mixture was concentrated under reduced pressure, and the residue was triturated with 50 ml of tetrahydrofuran. The insoluble material was removed by filtration, and the filtrate was chromatographed over a column (1.8-cm i.d.) of 100 g of silica gel[9d] which had been prepared from a slurry of the silica gel in ethyl acetate–SSB[9e] (5:1). The column was eluted in fractions of 50 ml each with the same solvent mixture. The frac-

(8) Pertinent nmr assignment data are contained in the discussion and are not repeated in the Experimental Section. Melting points were determined on a Fisher-Johns block and are corrected. Infrared spectra were determined on a Perkin-Elmer Infracord. Tlc was on silica gel plates developed with the following solvent systems for the compounds indicated: **2** and **7**, ethyl acetate–SSB (5:1); **11**, **12**, and **25**, acetone–SSB (1:1); **20** and **21**, ethyl acetate–methanol (19:1); **27**, benzene–methanol (4:1).

(9) Trade name products: (a) an alkylaryl sulfonate detergent, Atlantic Chemical Co., Nutley, N. J.; (b) a polyalkylene glycol, Union Carbide Chemical Co., New York, N. Y., and (c) Pittsburg Activated Carbon Co., Pittsburg, Pa.; (d) no. 7734 (0.05–0.20 mm), E. Merck AG, Darmstadt, Germany; (e) SSB = Skellysolve B, a petroleum hydrocarbon fraction, bp 60–70°, Skelly Oil Co., Kansas City, Mo.; (f) a synthetic magnesium silicate product, The Floridin Co., Warren, Pa.

tions were examined by tlc.[8] Fraction residues 21–28 were combined and recrystallized from methanol–benzene to yield 0.76 g of **2**, mp 176–177°.

This was identical by melting point and infrared with the 4-hydroxy product isolated directly from the biotransformation of N-acetyl-1-adamantanamine; also the mixture melting point was not depressed.

Fraction residues 41–79 were combined and recrystallized from methanol–benzene to yield 0.72 g of product, mp 206–207°. This was the β-hydroxy isomer (**7**) (see discussion).

Anal. Calcd for $C_{21}H_{19}NO_2$: C, 68.86; H, 9.15; N, 6.69. Found: C, 68.90; H, 9.17; N, 6.53.

1-Adamantanamin-3-ol Hydrochloride (6′).—A mixture of 300 mg of hydroxy amide **3** and 20 ml of 10% aqueous sodium hydroxide solution was heated at reflux for 22 hr. The mixture was diluted with 10 ml of water and extracted with ether. The extract was dried over potassium hydroxide, and the solvent was removed to give 220 mg of crystalline free base. A sample recrystallized from ether–hexane melted at 267° in a sealed tube. The hydroxy amine (**6**, 100 mg) was dissolved in 50 ml of ether and treated with ethereal hydrogen chloride. The resulting amine salt was recovered by filtration, washed with ether, and recrystallized from methanol–methyl ethyl ketone, mp >300° dec.

Anal. Calcd for $C_{10}H_{18}NOCl$: C, 58.96; H, 8.90; N, 6.88; Cl, 17.41. Found: C, 59.07; H, 9.05; N, 7.21; Cl, 17.75.

1-Adamantanamin-4α-ol (5) and the Hydrochloride (5′).—The hydrolysis of **2** and the work-up was carried out as described above for the C-3 hydroxy amide. The free base melted at 248–250° in a sealed tube.

Anal. Calcd for $C_{10}H_{17}NO$: C, 71.81; H, 10.25; N, 8.38. Found: C, 71.67; H, 10.40; N, 8.60.

The hydrochloride salt melted at >300° dec.

Anal. Calcd for $C_{10}H_{18}NOCl$: C, 58.96; H, 8.90; N, 6.88; Cl, 17.41. Found: C, 58.79; H, 8.82; N, 6.73; Cl, 17.32.

N-Benzoyl-N-methyl-1-adamantanamine (10) was prepared from 1-adamantanamine *via* the sequence of preparation of HCOOH salt, dehydration to N-formylamide, reduction to N-methyl-1-adamantanamine, and finally reaction of this with benzoyl chloride and sodium hydroxide.

1-Adamantanamine Formic Acid Salt.—A solution of 4.0 g of adamantanamine in 50 ml of benzene was treated with 1 equiv of 98% formic acid to precipitate the formate salt. The mixture was diluted with ether, and the product was recovered, washed with ether, and dried: yield, 3.51 g; subl pt >200°.

Anal. Calcd for $C_{11}H_{19}NO_2$: C, 66.97; H, 9.71; N, 7.10. Found: C, 66.95; H, 9.79; N, 7.28.

N-Formyl-1-adamantanamine.—1-Adamantanamine formic acid salt (80 g) was mixed gently with 200 ml of acetic anhydride for several minutes. Heat was evolved and the solid went into solution. The mixture stood for 40 min, and then was stirred for 2 hr with 800 ml of water. The solids were recovered by filtration, washed with water, and dried: yield, 54.33 g; mp 130–136°. The filtrate was neutralized with 50% sodium hydroxide solution and allowed to stand to give a second crop, 15.75 g. The analytical sample, recrystallized from acetone–water, melted at 139–140°.

Anal. Calcd for $C_{11}H_{17}NO$: C, 73.70; H, 9.56; N, 7.81. Found: C, 73.90; H, 9.84; N, 7.98.

N-Methyl-1-adamantanamine Hydrochloride.—N-Formyl-1-adamantanamine (70 g) dissolved in 700 ml of dry tetrahydrofuran was added with stirring to a mixture of 40 g of lithium aluminum hydride in 2000 ml of dry ether. The mixture was then heated at reflux for 4 hr and then chilled in an ice–acetone bath; 150 ml of water was cautiously added to the stirred mixture. The solids were removed by filtration and washed well with ether. The combined filtrate and wash were dried ($MgSO_4$) and treated with ether containing 1 equiv of hydrogen chloride. The solid HCl salt was recovered by filtration, washed with ether, and dried: yield, 66.0 g; mp 248–250°. The analytical sample recrystallized from methanol–methyl ethyl ketone melted at 250°.

Anal. Calcd for $C_{11}H_{20}NCl$: C, 65.49; H, 9.99; N, 6.94; Cl, 17.58. Found: C, 65.32; H, 10.25; N, 6.86; Cl, 17.55.

N-Benzoyl-N-methyl-1-adamantanamine (10).—A mixture of 25.0 g of N-methyl-1-adamantanamine hydrochloride, 250 ml of 10% sodium hydroxide solution, and 25.0 ml of benzoyl chloride chilled at 0° was stirred vigorously for 2 hr. The product was recovered by filtration, washed with water, and dried: yield, 28.12 g; mp 117–119°. A sample recrystallized from aqueous acetone melted at 117–119°.

Anal. Calcd for $C_{18}H_{23}NO$: C, 80.25; H, 8.61; N, 5.20. Found: C, 80.03; H, 8.69; N, 5.30.

Isolation of Products from the Bioconversion of N-Benzoyl-N-methyl-1-adamantanamine.—The methylene chloride extract residue from an 18-g fermentation was placed on a column of Florisil[9f] (700 g) with 850 ml of methylene chloride followed by linear gradient elution in fractions of 350 ml each with 8 l. of solvent, SSB containing increasing proportions of acetone from 10 to 70%. This was followed with 4 l. of SSB + 70% acetone and finally 4 l. of acetone. After examination of the fraction residues by ir and tlc,[8] product fractions were pooled as reported in Table II.

TABLE II

Pool	Fractions	Wt, g
I	7–13	4.08
II	18–41	13.36

N-Benzoyl-N-methyl-1-adamantanamin-4α-ol (12).—Pool I was recrystallized from aqueous acetone: yield, 2.84 g, mp 179–181°; ν_{OH} 3400, $\nu_{N-C=O}$ 1610 cm^{-1} in Nujol.

Anal. Calcd for $C_{18}H_{23}NO_2$: C, 75.75; H, 8.12; N, 4.91. Found: C, 75.77; H, 8.35; N, 4.90.

N-Benzoyl-N-methyl-1-adamantanamine-4α,6α-diol (11).—Pool II was recrystallized from aqueous methanol: yield, 10.39 g; mp 223–226°; ν_{OH} 3430, 3200, $\nu_{N-C=O}$ 5190, 1570 cm^{-1} in Nujol.

Anal. Calcd for $C_{18}H_{23}NO_3$: C, 71.73; H, 7.69; N, 4.65. Found: C, 71.87; H, 8.02; N, 4.81.

Bioconversion of N-Benzoyl-N-methyl-1-adamantanamin-4α-ol (12) to N-Benzoyl-N-methyl-1-adamantanamine-4α,6α-diol (11).—The methylene chloride extract residue from a 2.0-g fermentation was chromatographed over 150 g of silica gel.[9d] The column was eluted in 50-ml fractions with ethyl acetate which had been saturated with water. Fractions 4–6 contained unchanged substrate; fractions 7–8 were a mixture; fractions 9–14 contained product. These later fractions were pooled and recrystallized from methanol–water: yield, 0.76 g; mp 223–226°. The infrared spectrum was identical with that of the diol described in the previous experiment. The mixture melting point of this product and the dihydroxyamide described above was not depressed.

N-Benzoyl-N-methyl-1-adamantanamine-4α,6α-diol Diacetate (11′).—Dihydroxy amide **11** (0.5 g), pyridine (5.0 ml), and acetic anhydride (2.0 ml) were mixed and warmed to dissolve the reactants and allowed to stand at room temperature for 17 hr. The mixture was poured onto ice and stirred for several minutes. The product was recovered by filtration, washed with water, and recrystallized from aqueous acetone, mp 141–142°.

Anal. Calcd for $C_{22}H_{27}NO_5$: C, 68.55; H, 7.06; N, 3.63. Found: C, 68.88; H, 7.35; N, 3.75.

N-Benzoyl-N-methyl-1-adamantanamine-4,6-dione (14).—Dihydroxy amide **11** (2 g) dissolved in acetone by heating was treated dropwise with a slight excess of chromic acid solution by the Jones method.[4] The resulting product was recrystallized from aqueous methanol: yield, 1.70 g. For spectral and elemental analysis it was necessary to dry a sample at its melt temperature, 157–160°, to remove water of crystallization: $\nu_{C=O}$ 1740, 1700, $\nu_{N-C=O}$ 1620 cm^{-1} in Nujol.

Anal. Calcd for $C_{18}H_{19}NO_3$: C, 72.70; H, 6.44; N, 4.71. Found: C, 72.79; H, 6.82; N, 5.07.

The Disemicarbazone (14′) had mp 280° dec.

Anal. Calcd for $C_{20}H_{25}N_7O_3$: C, 58.38; H, 6.12; N, 23.83. Found: C, 58.15; H, 6.07; N, 23.42.

N-Benzoyl-N-methyl-1-adamantanamine-4α,6α-diol Cyclic Sulfite Ester (13).—Diol **11** (0.5 g) was treated with 1.0 ml of thionyl chloride; immediate heat of reaction was noted and after 15 min the excess reagent was removed under reduced pressure. The residue was chromatographed over 100 g of Florisil by the linear gradient method, placing the material on the column with methylene chloride and eluting in fractions of 110 ml each, with 4 l. of solvent, SSB containing increasing proportions of acetone from 0 to 40%. Fractions 13–16 contained 0.35 g of product which was recrystallized from ether–hexane: white needles; mp 172–173°; ν_{OH} no peak, $\nu_{N-C=O}$ 1705 cm^{-1} in Nujol.

Anal. Calcd for $C_{18}H_{21}NO_4S$: C, 62.22; H, 6.09; N, 4.03; S, 9.23. Found: C, 62.28; H, 5.64; N, 3.73; S, 9.21.

N-Benzoyl-N-methyl-1-adamantanamine-4α,6α-diol Hydrochloride (17′).—Dihydroxy-N-methylbenzamide **11** (4 g) was reduced with a mixture of 4.0 g of lithium aluminum hydride in 100 ml of ether. Because of low solubility, the amide was placed into a Soxhlet and leached into the reaction mixture by the refluxing solvent. The mixture was worked up in the usual manner, and the solid residue of free base was taken up in ether. Addition of ethereal hydrogen chloride precipitated the hydrochloride salt which was recrystallized from methanol–methyl ethyl ketone: yield 3.0 g; mp 267–269°.

Anal. Calcd for $C_{18}H_{26}NO_2Cl$: C, 66.75; H, 8.09; N, 4.33; Cl, 10.95. Found: C, 66.71; H, 8.34; N, 4.36; Cl, 11.17.

N-Benzoyl-N-methyl-1-adamantanamin-4-one (**15**).—Hydroxybenzamide **12** (70 mg) dissolved in 10 ml of acetone was oxidized with chromic acid by the method of Jones, *et al.*[4] The product was recrystallized from aqueous acetone: mp 126–127°; $\nu_{C=O}$ 1720, $\nu_{N-C=O}$ 1620 cm^{-1} in Nujol.

Anal. Calcd for $C_{18}H_{21}NO_2$: C, 76.29; H, 7.47; N, 4.94. Found: C, 76.13; H, 7.54; N, 5.07.

N-Benzyl-N-methyl-1-adamantanamin-4α-ol (**16**).—Hydroxyamide **12** (4 g) in 150 ml of dry tetrahydrofuran was added with stirring to a mixture of 3.0 g of lithium aluminum hydride in 100 ml of anhydrous ether. The mixture was heated at reflux for 150 min; a cold bath was applied, and 20 ml of water was added with caution while stirring was continued for 30 min. The mixture was filtered and the insoluble material was washed well with ether. The filtrate and wash was dried over magnesium sulfate and the solvent removed to give 3.36 g of product. A sample was recrystallized from ether–hexane for analysis, mp 119–120°.

Anal. Calcd for $C_{18}H_{25}NO$: C, 79.66; H, 9.29; N, 5.16. Found: C, 79.44; H, 9.10; N, 5.07.

N-Methyl-1-adamantanamin-4α-ol and the Hydrochloride (**9**).—Benzyl amine **16** (3 g) was dissolved in 60 ml of ethanol. 10% palladium-on-carbon catalyst (0.5 g) was added, and the mixture was shaken wih hydrogen (42 psi) for 150 min. The mixture was freed of catalyst and concentrated to dryness under reduced pressure to a solid residue. A sample of 1.0 g was recrystallized from ether, mp 141–142°.

Anal. Calcd for $C_{11}H_{19}NO$: C, 72.88; H, 10.57; N, 7.73. Found: C, 72.82; H, 10.38; N, 7.49.

The remainder of the residue was dissolved in ether and treated with ethereal hydrogen chloride, and the resulting amine salt was recovered by filtration and washed with ether, yield 1.26 g. For analysis a sample was recrystallized from methanol–acetone, mp 220–221°. This compound was identical by infrared and melting point with the hydroxy amine hydrochloride prepared from 4-hydroxy-1-adamantanamine *via* the N-formylamine and hydride reduction; their mixture melting point showed no depression.

Anal. Calcd for $C_{11}H_{20}NOCl$: C, 60.67; H, 9.26; N, 6.43; Cl, 16.29. Found: C, 60.54; H, 9.50; N, 6.42; Cl, 16.42.

1-Adamantanamin-4α-ol Formic Acid Salt.—A solution of 3.5 g of hydroxy amine (**5**) in 1 l. of ether was treated with a slight excess of 98% formic acid. The resulting precipitate of formic acid salt was recovered by filtration and washed with ether: yield 3.60 g; mp 238–239°.

N-Formyl-1-adamantanamin-4α-ol (**8**).—Formic acid salt (1 g) was heated at 270° for 2 min and cooled. The residue was taken up in a small volume of ethyl acetate and chromatographed over a column prepared from a slurry of 100 g of silica gel[9d] and ethyl acetate saturated with water. The column was eluted with the same solvent in fractions of 50 ml each. Product fractions (14–19) were determined by infrared inspection of the residues (0.58 g). A sample for analysis was recrystallized from methanol–benzene, mp 141–142°.

Anal. Calcd for $C_{11}H_{17}NO_2$: C, 67.66; H, 8.78; N, 7.17. Found: C, 67.52; H, 8.79; N, 7.42.

N-Methyl-1-adamantanamin-4α-ol Hydrochloride (**9**).—A mixture of 0.5 g of hydroxyformamide (**8**), 2.0 ml of pyridine, and 1.0 ml of acetic anhydride was allowed to stand at 25° for 16 hr. The mixture was diluted with water and extracted with methylene chloride. The extract was washed with dilute sulfuric acid, water, and dried (Na_2SO_4). The solvent was removed under reduced pressure, and the residue was taken up in 25 ml of anhydrous tetrahydrofuran and added with stirring to a mixture of 0.5 g of lithium aluminum hydride in 25 ml of anhydrous ether. The mixture was heated at reflux for 2 hr and chilled at 0° during the addition of 5.0 ml of water. After stirring 30 min, the mixture was filtered, the solids were washed well with ether, and the combined filtrate and wash were dried ($MgSO_4$). The solution was filtered and treated with a slight excess of ethereal hydrogen chloride. The amine salt was recovered by filtration and recrystallized from methanol–acetone, mp 219–220°. This product was identical by infrared and melting point with the HCl salt prepared from N-benzoyl-N-methyl-1-adamantanamin-4α-ol *via* the benzyl amine. The mixture melting point showed no depression.

N-Acetyl-N-methyl-1-adamantanamine (**19**).—A solution of 6.5 g of N-methyl-1-adamantanamine hydrochloride (see above) in 100 ml of water was treated dropwise with a slight excess of 50% aqueous sodium hydroxide solution. The precipitated free base was recovered by filtration, washed with water, and dried: yield, 5.49 g.

The free base was dissolved in 25 ml of pyridine; 5 ml of acetic anhydride was added; and the mixture was allowed to stand at 25° for 56 hr. After diluting with 100 ml of water and chilling the product was recovered, washed well with water, and dried: yield, 3.65 g; mp 123–124°.

Isolation of Products from the Bioconversion of N-Acetyl-N-methyl-1-adamantanamine.—The methylene chloride extract residue from a 2.0-g fermentation was dissolved in methylene chloride and chromatographed over 100 g of Florisil. Elution was by the gradient method taking 100-ml fractions each using 4 l. of solvent, SSB containing increasing proportions of acetone from 0 to 30%. As indicated by tlc,[8] fraction residues were pooled as reported in Table III. Pool A and pool C were separate entities and pool B was a mixture of the two.

TABLE III

Pool	Fractions	Wt, g
A	25–32	0.982
B	33–35	0.210
C	36–39	0.212

N-Acetyl-N-methyl-1-adamantanamin-4α-ol (**20**).—Pool A was recrystallized from acetone–hexane: yield, 0.717 g; mp 151–154°; ν_{OH} 3360, $\nu_{N-C=O}$ 1610 cm^{-1} in Nujol.

Anal. Calcd for $C_{13}H_{21}NO_2$: C, 69.92; H, 9.48; N, 6.27. Found: C, 70.09; H, 9.57; N, 6.36.

This compound was readily oxidized to ketone by the Jones method:[4] mp 119–120°; $\nu_{C=O}$ 1740, $\nu_{N-C=O}$ 1640 cm^{-1} in Nujol.

N-Acetyl-N-methyl-1-adamantanamin-3-ol (**21**).—Pool C was recrystallized from acetone–hexane to yield 0.140 g of **21**: mp 155–156°; ν_{OH} 3310, $\nu_{N-C=O}$ 1610 cm^{-1} in Nujol.

Anal. Calcd for $C_{13}H_{21}NO_2$: C, 69.02; H, 9.48; N, 6.27. Found: C, 69.05; H, 9.26; N, 6.24.

This compound did not oxidize to ketone with chromic acid; unchanged starting material was recovered.

N-Phenylacetyl-1-adamantanamine (**22**).—A mixture of 20.0 g of 1-adamantanamine hydrochloride, 40 ml of 50% aqueous sodium hydroxide solution, 160 g of ice, and 20 ml of phenylacetylchloride was stirred vigorously for 1 hr and allowed to stand. The product was recovered by filtration and washed with water and air dried: yield, 20.23 g; mp 176–179°. An analytical sample recrystallized from acetone melted at 181–183°.

Anal. Calcd for $C_{18}H_{23}NO$: C, 80.25; H, 8.61; N, 5.20. Found: C, 79.89; H, 8.88; N, 5.04.

N-Phenylacetyl-1-adamantanamine-4α,6α-diol (**23**).—The methylene chloride extract residue from a 2-g fermentation of **22** was triturated with 50 ml of methylene chloride and filtered to obtain 1.05 g of solid product which was combined with that obtained from the chromatograph described below.

The filtrate was placed on a column of 100 g of Florisil. Elution was by the gradient method with 6 l. of solvent, SSB containing increasing proportions of acetone from 0 to 60%. Fractions of 55 ml each were collected. Fraction residues 78–83 (0.36 g) were identical with the material obtained by direct isolation. This combined product (1.41 g) was recrystallized from acetone after treatment with activated carbon (Darco G 60) in the same solvent: mp 201–202°; $\nu_{OH,NH}$ 3300, $\nu_{N-C=O}$ 1640, 1550 cm^{-1} in Nujol.

Anal. Calcd for $C_{18}H_{23}NO_3$: C, 71.73; H, 7.69; N, 4.65. Found: C, 71.74; H, 7.94; N, 4.62.

N-Cyclohexylcarbonyl-N-methyl-1-adamantanamine (**24**).—A mixture of 20.0 g of N-methyl-1-adamantanamine hydrochloride and 200 ml of 10% aqueous sodium hydroxide solution was chilled at 5° and treated with 20 ml of cyclohexanecarbonyl chloride during 120 min with vigorous stirring. The mixture

was extracted with methylene chloride; the extract was washed with water, dilute hydrochloric acid, 5% sodium bicarbonate solution, and dried over sodium sulfate. Evaporation of the solvent gave 20.20 g of solid product which was recrystallized from aqueous methanol, mp 91–93°.

Anal. Calcd for $C_{18}H_{29}NO$: C, 78.40; H, 10.61; N, 5.09. Found: C, 78.29; H, 10.90; N, 5.04.

N-Cyclohexylcarbonyl-N-methyl-1-adamantanamine-4α,6α-diol (25).—The methylene chloride extract residue from a 2.0-g fermentation of **24** was chromatographed over 100 g of Florisil. The column was eluted by the gradient method with 4 l. of solvent, SSB containing increasing amounts of acetone from 0 to 40%. Fractions of 110 ml each were collected, and the residues were examined by tlc.[8] Fractions 25–29 were pooled and recrystallized from acetone to yield 0.174 g of **25**: mp 190–191°; ν_{OH} 3400, $\nu_{N-C=O}$ 1620 cm^{-1} in Nujol.

Anal. Calcd for $C_{18}H_{29}NO_3$: C, 70.32; H, 9.51; N, 4.56. Found: C, 70.31; H, 9.51; N, 5.35.

Fractions 33–37 gave another material which also analyzed for a diol. This structure has not been determined.

N-(1-Adamantyl)phthalimide (26).—A mixture of 7.5 g of 1-adamantanamine, 10.0 g of phthalic anhydride, and 100 ml of pyridine was heated at 90° for 15 min; 100 ml of acetic anhydride was added; and the mixture was again heated at 90° for 1 hr. After cooling and stirring with 500 ml of water for 1 hr the product was recovered by filtration, washed with water, and crystallized from methanol: yield, 2.13 g; mp 140–143°.

Anal. Calcd for $C_{18}H_{19}NO_2$: C, 76.84; H, 6.81; N, 4.98. Found: C, 76.75; H, 7.02; N, 5.01.

N-(4α,6α-Dihydroxy-1-adamantyl)phthalimide (27).—The filtered beer (10 l.) from the conversion of 2.0 g of **26** was poured over a column of 300 g of CAL carbon.[9c] The column was eluted first with 10 l. of methanol, followed with 5 l. of ethyl acetate, and finally 5 l. of chloroform. Thin layer chromatography[8] showed that the chloroform eluate contained the product. The residue therefrom was chromatographed over 100 g of Florisil. Elution was by the linear gradient method with 4 l. of solvent SSB containing increasing amounts of acetone from 0 to 40%; cuts were *ca.* 110 ml each. The product eluted in fractions 23–28 was recrystallized from acetone–hexane: mp 218–220°.

Anal. Calcd for $C_{18}H_{19}NO_4$: C, 68.99; H, 6.11; N, 4.47. Found: C, 68.81; H, 6.39; N, 4.21.

Registry No.—**2**, 16790-57-1; **3**, 778-10-9; **4**, 16790-59-3; **5**, 16790-60-6; **5′**, 16790-61-7; **6′**, 16790-62-8; **7**, 16790-63-9; 1-adamantanamine formic acid salt, 16790-64-0; N-formyl-1-adamantanamine, 3405-48-9; N-methyl-1-adamantanamine hydrochloride, 3717-39-3; **8**, 16790-67-3; free base of **9**, 16790-68-4; **10**, 16790-69-5; **11**, 16790-70-8; **11′**, 16790-71-9; **12**, 16790-72-0; **13**, 16790-73-1; **14**, 16790-74-2; **14′**, 16790-75-3; **15**, 16790-76-4; **16**, 16790-77-5; **17′**, 16790-78-6; **19**, 3717-37-1; **20**, 16790-80-0; **21**, 16790-81-1; **22**, 16790-82-2; **23**, 16790-83-3; **24**, 16790-84-4; **25**, 16790-85-5; **26**, 16808-41-6; **27**, 16790-86-6.

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The Microbiological Hydroxylation of 1-Benzoyl-*trans*-decahydroquinoline. Determination of Structure, Stereochemistry, and Absolute Configuration of the Products

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

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

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Microbiological hydroxylation of (±)-1-benzoyl-*trans*-decahydroquinoline [(±)-**2**] with *Sporotrichum sulfurescens* has been shown to give (4a*S*,5*S*,8a*R*)-1-benzoyl-*trans*-decahydroquinolin-5-ol (**3**), (±)-1-benzoyl-*trans*-decahydroquinolin-6-ol (**4**), and (4a*S*,7*S*,8a*S*)-1-benzoyl-*trans*-decahydroquinolin-7-ol (**5**) in a total yield of 80–90%. Under the same conditions hydroxylation of (+)-**2** gave optically pure (+)-**5** and (4a*S*,6*S*,8a*S*)-1-benzoyl-*trans*-decahydroquinolin-6-ol [(+)-**4**] in a ratio of 35:65. Hydroxylation of (−)-**2** gave optically pure (−)-**3** and (4a*R*,6*R*,8a*R*)-1-benzoyl-*trans*-decahydroquinolin-6-ol [(−)-**4**] in a ratio of 87:13. Various chemical modifications of these products were carried out in order to determine their structures and stereochemistry and included the conversions of **3**, (+)-**5**, and (+)-**4** into (4a*S*,8a*R*)-*trans*-decahydroquinolin-5-one (**24**), (4a*S*,8a*S*)-*trans*-decahydroquinolin-7-one (**26**), and (4a*S*,8a*S*)-*trans*-decahydroquinolin-6-one (**25**), respectively. Application of the octant rule to the optical rotatory dispersion curves of the latter compounds allowed assignment of absolute configurations to the hydroxylation products. The absolute configurations of the parent molecules, (−)-*trans*-decahydroquinoline [(−)-**1**] and (+)-*trans*-decahydroquinoline [(+)-**1**], can be assigned as (4a*R*,8a*S*)-*trans*-decahydroquinoline and (4a*S*,8a*R*)-*trans*-decahydroquinoline, respectively.

The increasing number of substrates which are hydroxylated by the microorganism *Sporotrichum sulfurescens* provides an opportunity to explore further the relationships of the substrate molecules to the enzymic hydroxylation site. A recent proposal[1] has suggested that an electron-rich center of the substrate molecule provides an attachment site for the hydroxylating enzyme and thus facilitates oxygenation at some point in a saturated portion of the molecule. The approximate distance of this point from the attachment site was suggested to be 5.5 Å. Among the electron-rich centers which have been found useful are the alcohol[1] and the amide[2–4] functional groups. It seemed possible that additional information concerning the stereochemical relationship of the substrate molecule to the site of oxygenation could be obtained from examination of the oxygenated products. Some information concerning the stereochemistry of hydroxylation of the steroid nucleus has been gathered.[5] It is known,

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