Anal. Calcd for $C_{20}H_{20}H_6O_4 \cdot 0.1HCl \cdot 0.6H_2O$: C, 56.81; H, 5.08; Cl, 0.84; N, 19.87. Found: C, 56.79; H, 4.82; Cl, 0.98; N, 19.81.

Ethyl 4-Amino-5-nitro-6(1*H*)-oxy-2-pyridinecarbamate (16).—A solution of **15**[5] (1.00 g, 2.45 mmol) in 10% HBr in HOAc (15 ml) containing phenol (50 mg) was stirred at room temperature for 24 hr. The solid (0.55 g) that deposited was collected by filtration; the filtrate was evaporated to dryness, and the residue was washed with ether to give an additional amount of product (0.20 g). The combined crops were recrystallized from 4:3 ethanol–water to give white crystals: yield 0.43 g (73%); mp 293–294° dec; λ_{max}, nm ($\epsilon \times 10^{-3}$),[7a] pH 7, 261 (6.53), 341 (13.8).

Anal. Calcd for $C_8H_{10}N_4O_5$: C, 39.67; H, 4.16; N, 23.13. Found: C, 39.45; H, 3.98; N, 23.26.

In a large-scale run (148 g), the crude product was washed with boiling ethanol to give material suitable for use in the next step, yield 81.5 g (93%), mp 285–290° dec.

Registry No.—**1**, 30768-44-6; **2**, 30768-45-7; **3**, 30768-46-8; **4b**, 6502-04-1; **5**, 30768-47-9; **6a**, 30826-43-8; **6b**, 30768-48-0; **7a**, 30826-44-9; **8**, 30768-49-1; **9a**, 30768-50-4; **9b**, 30768-51-5; **10a**, 30826-45-0; **10b**, 30768-52-6; **11a**, 30826-46-1; **11b**, 30826-47-2 **12a**, 30768-53-7; **12b**, 30826-48-3; **13a**, 30768-54-8; **13b**, 30826-49-4; **14a**, 30826-50-7; **14b**, 30826-51-8; **16**, 30768-55-9.

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Microbiological Oxygenation of *cis*-5-Acetyl-5a,6,7,8,9,10,11,11a-octahydro-5*H*-cyclooct[*b*]indole with *Calonectria decora*

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Oxygenation of *cis*-5-acetyl-5a,6,7,8,9,10,11,11a-octahydro-5*H*-cyclooct[*b*]indole (**3**) with *Calonectria decora* (CBS) gave *cis*-5-acetyl-5,5a,6,7,8,10,11,11a-octahydro-9*H*-cyclooct[*b*]indol-9-one (**4**) as the major product and *cis*-5-acetyl-5,5a,6,7,9,10,11,11a-octahydro-8*H*-cyclooct[*b*]indol-8-one (**5**) as the minor product. Dehydrogenation of **4** and **5**, followed by deacetylation, gave isomeric hexahydrocyclooct[*b*]indolones **8** and **9**, respectively. The positions of the ketones in these compounds were shown to be at C-8 and C-9 by nmr analysis. Final structure assignments, including the cis-ring junction in the octahydro compounds, were based on an X-ray crystallographic structure determination of the *p*-bromophenylhydrazone (**10**) of major product **4**.

In connection with other work in these laboratories, we have examined the microbiological oxygenation of *cis*-5-acetyl-5a,6,7,8,9,10,11,11a-octahydro-5*H*-cyclooct-[*b*]indole (**3**). The substrate **3** was prepared by zinc–hydrochloric acid reduction[2] of 6,7,8,9,10,11-hexahydro-5*H*-cyclooct[*b*]indole (**1**),[3] followed by acetylation of the resulting product **2**. At the outset, the stereochemistry of the saturated ring juncture of **2** was unknown but was suspected to be cis on the basis of the nmr signals for the C-5a (δ 3.95, triplet, $W_{1/2}$ = 21 cps) and C-11a (δ 3.28, triplet, $W_{1/2}$ = 21 cps) protons. The $W_{1/2}$ expected for these protons if a trans ring juncture were present is 12–15 cps while a $W_{1/2}$ of 12–21 cps is expected for cis protons.

Oxygenation of **3** with the fungus *Calonectria decora* gave two ketones, **4** (38% yield) and **5** (7% yield), as the only isolable products. The presence of optical activity in the minor product **5** was demonstrated by a CD spectrum (see Experimental Section).[4] Product **4**, however, was devoid of optical activity by the same criterion. Nmr analysis at this point showed that oxygenation had not occurred at the C-6 or C-11 positions in either product, since the signals for the C-5a and C-11a protons remained as broad multiplets. Presence of a ketone at C-6 (or C-11) would eliminate two protons α to the bridgehead and thus simplify the nmr signal for the bridgehead proton at C-5a (or C-11a).

Dehydrogenation of **4** and **5** over palladium on carbon gave the *N*-acetylindoles **6** and **7**, respectively. It now was possible, on the basis of the nmr spectra of these two compounds, to eliminate positions C-7 or C-10 as sites of oxygenation in either compound. As above, a ketone at either position would again simplify the signal of the geminal protons (at C-6 or C-11) between the indole ring and the carbonyl group. However, such signals were not apparent in the spectra of **6** or **7** (see Experimental Section). This conclusion is confirmed by the nmr spectra of the indoles **8** and **9** obtained from deacetylation of **6** and **7**, respectively. Ultraviolet spectra (Experimental Section) of compounds **6–9** were consistent with the *N*-acetylindole (**6** and **7**) and indole **8** and **9** structures of these compounds.[5] The positions of the ketones in these compounds were thus narrowed to the C-8 and C-9 carbons.

An X-ray crystallographic analysis was sought in order to make a final decision as to the position of the ketones in the products and, at the same time, to clear up the uncertainty with regard to the stereochemistry of the ring juncture in the octahydro compounds. To this end, the *p*-bromophenylhydrazone (**10**) of the major ketonic oxygenation product was prepared.

The crystal structure of **10** was determined by the heavy-atom method. X-Ray results were used to pre-

(1) To whom correspondence should be addressed.

(2) N. K. Kochetkov, N. F. Kucherova, and I. G. Zhukova, *Zh. Obshch. Khim.*, **31**, 924 (1961).

(3) B. Witkop, J. B. Patrick, and M. Rosenblum, *J. Amer. Chem. Soc.*, **73**, 2641 (1951).

(4) We are indebted to Dr. W. C. Krueger for determination of this spectrum.

(5) *Cf.* A. I. Scott, "Interpretation of the Ultraviolet Spectra of Organic Molecules," Macmillan, New York, N. Y., 1964, p 172.

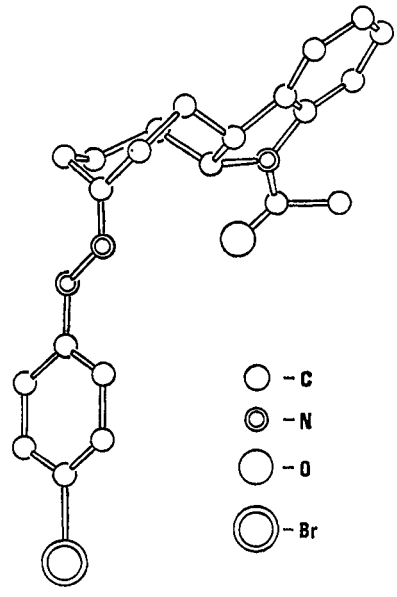


Figure 1.—Drawing from crystallographic data of 10. The molecule is viewed in an arbitrary direction in the crystal. Hydrogen atoms are not shown.

pare the drawing shown in Figure 1. The stereochemistry of the ring juncture is clearly cis. The C-9 carbon is the point of attachment of the *p*-bromophenylhydrazone and therefore the site of oxygenation. The crystals are centrosymmetric (and therefore racemic), confirming that both optical isomers are oxygenated by the organism.

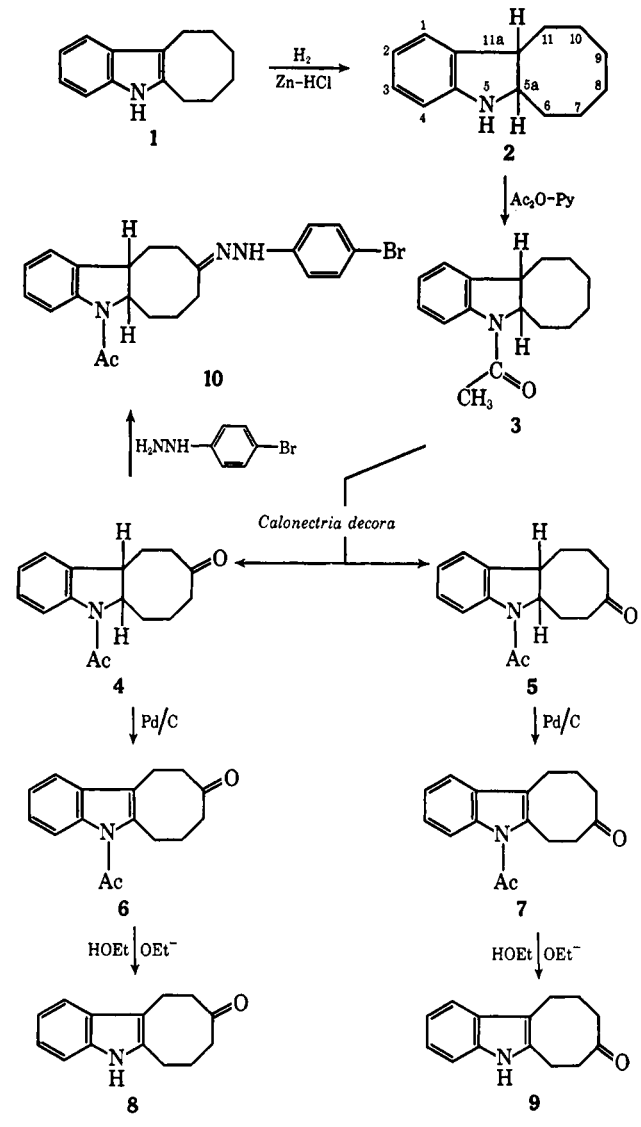
The optical activity of minor product **5** may arise from either stereoselectivity in the initial hydroxylation of the substrate (presumed to be the first step in formation of these ketones) or stereoselectivity in the dehydrogenation of the alcohol group.

Experimental Section

5a,6,7,8,9,10,11,11a-Octahydro-5*H*-cyclooct[*b*]indole (2).—Zinc dust (2.7 g) was added to a stirred mixture of 1 (3.98 g, 0.0200 mol), $HgCl_2$ (0.0372 g), and EtOH (28 ml); the mixture was heated on the steam bath and treated during 1 hr with 7.4 ml of concentrated HCl. Additional Zn dust and HCl were added to the mixture in this manner until a total of 28.0 g of Zn dust and 74.4 ml of HCl had been added during 10 hr. The mixture was kept at ambient temperature for 18 hr, diluted with water, and washed with Et_2O. The aqueous layer was cooled in an ice bath and treated with 50% NaOH until the initially precipitated zinc hydroxide had dissolved. This mixture was extracted with Et_2O. The extract was washed with water and brine, dried (K_2CO_3), and concentrated. The residue was crystallized from petroleum ether to give 1.12 g, mp 106–106.5°; 0.780 g, mp 100.5–102.5°; and 0.270 g, mp 91–95° of **2**. An analytical sample was obtained: mp 106.5–107°; uv (EtOH) end absorption; λ_{max} 243 mμ (ε 6700), 294 (2400); ir (Nujol) 3220 cm^{-1} (NH); nmr ($CDCl_3$) δ 3.26 (broad t, 1, *J* = 8.5 Hz, C-11a), 3.58 (s, 1, NH), 3.94 (broad t, 1, *J* = 8.5 Hz, C-5a).

Anal. Calcd for $C_{14}H_{19}N$: C, 83.53; H, 9.51; N, 6.96. Found: C, 83.76; H, 9.75; N, 7.20.

5-Acetyl-5a,6,7,8,9,10,11,11a-octahydro-5*H*-cyclooct[*b*]indole (3).—A mixture of **2** (2.01 g, 0.0100 mol), acetic anhydride (3.7 ml), and pyridine (94 ml) was stirred at ambient temperature for 18 hr, poured into water, and concentrated *in vacuo*. The residue was crystallized from EtOAc–Skellysolve B to give 1.93 g of **3**. An analytical sample was obtained: mp 119–120°; uv (EtOH) end absorption; λ_{max} 208 mμ (ε 22,350), 253 (14,150), 279 (4500), 288 (3750); ir (Nujol) 1650 cm^{-1} (C=O); nmr ($CDCl_3$) δ 2.28 (s, 3, $COCH_3$), 3.29 (broad t, 1, *J* = 8.5 Hz, C-11a), 4.51 (broad s, 1, C-5a).



Anal. Calcd for $C_{16}H_{21}NO$: C, 78.97; H, 8.70; N, 5.76. Found: C, 79.13; H, 8.53; N, 6.04.

Biotransformation Process.—The culture used in these experiments was *Calonectria decora* (CBS). The conditions described previously for fermentations with *Sporotrichum sulfurescens*[6] were used in the present experiments.

Oxygenation of *cis*-5-Acetyl-5a,6,7,8,9,10,11,11a-octahydro-5*H*-cyclooct[*b*]indole (3).—Two 125-l. bioconversions of **3** (25.0 g each, total 0.206 mol) were chromatographed separately on Florisil columns (10.5 × 50 cm). The columns were packed with Skellysolve B and eluted with 2-l. fractions of Skellysolve B containing increasing proportions of acetone. The products were eluted with 15–20% (v/v) acetone in Skellysolve B. From chromatography of the first bioconversion, there was obtained 7.07 g of a major product **4**, mp 201–204°, by crystallization from acetone–Skellysolve B. In the filtrate, rectangular crystals differing from the major product in appearance, were observed and some (0.170 g) were separated manually from the filtered second crop and had mp 175–178°. The remaining crystals and the filtrate were held for further separation. From chromatography of the second bioconversion, there was obtained two crops of the major product **4** (7.75 g, mp 198–204°, and 3.84 g, mp 188–195°) by crystallization from acetone–Skellysolve B. The filtrate and later fractions from the column were combined with the material held from above and chromatographed on silica gel (500 g) packed as a slurry in ethyl acetate. Elution with ethyl acetate (500-ml fractions) gave additional major product **4** (1.28 g, total 19.94 g, 0.0776 mol, 38%) in fractions 3 and 4, and two crops of the minor product (**5**, 3.28 and 0.30 g, mp 172–176°; total for both runs, 3.65 g, 0.0142 mol, 7%) in fractions 5–10. Two recrystalliza-

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

tions of the major product from acetone–Skellysolve B gave *cis*-**5-acetyl-5,5a,6,7,8,10,11,11a-octahydro-9*H*-cyclooct[*b*]indol-9-one** (**4**) as colorless crystals: mp 210–212°; $[\alpha]_D$ 0° ($CHCl_3$); $\nu_{C=O}$ 1700, 1650 cm^{-1} in Nujol; nmr ($CDCl_3$) δ 8.04 (broad, 1 H, aromatic), 7.14 (m, 3 H, aromatic), 4.07 (m, 1 H, NCH), 3.38 (m, 1 H, CHC_6H_4), 2.22 (s, 3 H, $COCH_3$).

Anal. Calcd for $C_{16}H_{19}NO_2$: C, 74.68; H, 7.44; N, 5.44. Found: C, 74.39; H, 7.38; N, 5.50.

Recrystallization of the minor product (0.170 g) from acetone–Skellysolve B gave *cis*-**5-acetyl-5,5a,6,7,9,10,11,11a-octahydro-8*H*-cyclooct[*b*]indol-8-one** (**5**) as colorless needles: mp 178–181°; CD (*c* 0.01019, methanol) λ ([θ]) 320 (0), 288 (+7500), 277 (0), 252 (+82,600), 232 (0), 217 (−60,000), 210 (0); $\nu_{C=O}$ 1700, 1655 cm^{-1} in Nujol; nmr ($CDCl_3$) δ 7.55 (broad, 1 H, aromatic), 7.13 (m, 3 H, aromatic), 4.95 (m, 1 H, NCH), 3.44 (m, 1 H, CHC_6H_4), 2.35 (s, 3 H, $COCH_3$).

Anal. Calcd for $C_{16}H_{19}NO_2$: C, 74.68; H, 7.44; N, 5.44. Found: C, 74.65; H, 7.38; N, 5.56.

5-Acetyl-5,6,7,8,10,11-hexahydro-9*H*-cyclooct[*b*]indol-9-one (**6**).—A solution of 12.9 g (0.05 mol) of *cis*-5-acetyl-5,5a,6,7,8,-10,11,11a-octahydro-9*H*-cyclooct[*b*]indol-9-one (**4**) in 150 ml of decalin was treated with 10.0 g of 10% Pd/C and heated under reflux in a N_2 atmosphere for 1.5 hr. The reaction mixture was stored at −10° overnight and filtered. The solid was extracted with boiling cyclohexane (1 l.) and filtered. The filtrate was cooled and the resultant precipitate collected and dried to give 6.8 g of **6**, mp 117–126°. Concentration of the mother liquor followed by chromatography on silica gel using ethyl acetate–cyclohexane (1:1) as eluting solvent gave an additional 0.73 g of **6**. Recrystallization from hot cyclohexane gave crystals: mp 119–127°; λ_{max} (95% EtOH) 246 nm (ϵ 14,250), 269 sh (8850), 275 sh (8400), 293 (5400), 303 (4950); nmr ($CDCl_3$) δ 3.08 (m, 4 H), 2.75 (s, 3 H, $COCH_3$), 2.75 (m, 2 H), 2.67 (m, 2 H), 1.97 (m, 2 H).

Anal. Calcd for $C_{16}H_{17}NO_2$: C, 75.24; H, 6.71; N, 5.49. Found: C, 75.46; H, 7.02; N, 5.87.

5-Acetyl-5,6,7,9,10,11-hexahydro-8*H*-cyclooct[*b*]indol-8-one (**7**).—A solution of 1.29 g (5 mmol) of *cis*-5-acetyl-5,5a,6,7,9,10,-11,11a-octahydro-8*H*-cyclooct[*b*]indol-8-one (**5**) in 17 ml of decalin was heated with 1.0 g of 10% Pd/C. The reaction mixture was heated under reflux in an N_2 atmosphere for 1.5 hr. The reaction mixture was cooled, Skellysolve B was added, and the reaction mixture was filtered. The solid was dissolved in hot cyclohexane and filtered to remove the Pd/C. The filtrate was cooled and the precipitate collected by filtration. This solid was combined with the decalin filtrate and chromatographed on 170 g of silica gel. The decalin was removed using cyclohexane to elute the column while 560 mg of **7** was recovered using 95% ethyl acetate–cyclohexane as eluent. The product was recrystallized twice from cyclohexane: mp 111–121°; λ_{max} (95% EtOH) 245 nm (ϵ 16,150), 266 sh (10,050), 275 sh (8850), 293 (5500), 302 (5250); nmr ($CDCl_3$) δ 3.63 (m, 1 H), 3.0–2.3 (7 H), 2.8 (s, 3 H, $COCH_3$), 1.88 (m, 2 H).

Anal. Calcd for $C_{16}H_{17}NO_2$: C, 75.24; H, 6.71; N, 5.49. Found: C, 75.13; H, 7.00; N, 5.81.

5,6,7,8,10,11-Hexahydro-9*H*-cyclooct[*b*]indol-9-one (**8**).—To a solution of 1.84 g (0.08 g-atom) of sodium in 190 ml of ethanol was added 6.8 g (0.207 mol) of 5-acetyl-5,6,7,8,10,11-hexahydro-9*H*-cyclooct[*b*]indol-9-one (**6**). The solution was stirred at 25°, under a N_2 atmosphere, for 1 hr. The reaction mixture was concentrated *in vacuo*, diluted with ice water, and cooled to 0°. The solid which resulted was collected by filtration to give 5.2 g of **8**, which could be recrystallized from ethyl acetate–cyclohexane: mp 160–161.5°; λ_{max} (95% EtOH) 224 nm (ϵ 35,000), 276 sh (7150), 284 (7750), 290 (6900); nmr ($CDCl_3$) δ 3.16 (m, 2 H), 2.9–2.2 (6 H), 1.77 (m, 2 H).

Anal. Calcd for $C_{14}H_{15}NO$: C, 78.84; H, 7.09; N, 6.57. Found: C, 78.68; H, 7.11; N, 6.40.

5,6,7,9,10,11-Hexahydro-8*H*-cyclooct[*b*]indol-8-one (**9**).—To a solution of 30 mg (1.25 mg-atoms) of sodium in 5 ml of ethanol was added 100 mg (0.31 mmol) of 5-acetyl-5,6,7,9,10,11-hexahydro-8*H*-cyclooct[*b*]indol-8-one (**7**). The solution was stirred for 1.5 hr at 25°, ice water was added, and the resulting solid was collected by filtration to give 66 mg of **9**. An analytical sample was prepared by recrystallization from ethyl acetate: mp 135–137°; λ_{max} (95% EtOH) 225 nm (ϵ 34,650), 276 (7750), 283 (8150), 291 (7000); nmr ($CDCl_3$) δ 3.1–2.2 (8 H), 1.83 (m, 2 H).

Anal. Calcd for $C_{14}H_{15}NO$: C, 78.84; H, 7.09; N, 6.57. Found: C, 78.57; H, 6.81; N, 7.04.

cis-**5-Acetyl-5,5a,6,7,8,10,11,11a-octahydro-9*H*-cyclooct[*b*]indol-9-one *p*-Bromophenylhydrazone** (**10**).—To a solution of 0.514 g (2 mmol) of *cis*-5-acetyl-5,5a,6,7,8,10,11,11a-octahydro-9*H*-cyclooct[*b*]indol-9-one (**4**) in 20 ml of ethanol were added 0.5 ml of acetic acid and 0.465 g (2.5 mmol) of *p*-bromophenylhydrazine. The reaction mixture was heated under reflux, in a N_2 atmosphere, for 3 hr and cooled, and the white solid was collected and dried, mp 213–216° dec. A sample of the product **10** was recrystallized from ethyl acetate–cyclohexane and from 95% ethanol, mp 205–211°.

Anal. Calcd for $C_{22}H_{24}BrN_3O$: C, 61.97; H, 5.67; N, 9.86; Br, 18.74. Found: C, 62.09; H, 5.58; N, 9.94; Br, 18.44.

Crystal Structure Determination of 10.—The crystals are triclinic with crystal data as follows: a = 10.880, b = 7.684, c = 12.531 Å; α = 84.77, β = 73.91, γ = 100.08°; V = 980.2 Å^3, Z = 2, space group $P\bar{1}$. Three-dimensional intensity data (3974 reflections) were collected on a computerized diffractometer using Cu Kα radiation. The heavy-atom method was used to obtain a trial structure. Three cycles of least-squares refinement (bromine anisotropic, all other atoms isotropic) using the 2540 largest data reduced the agreement index [$R = (\Sigma||F_o| - |F_c||)/\Sigma|F_o|$] to 0.091. Coordinates from this refinement were used to prepare Figure 1. Refinement is being continued with full data and more parameters. Further details and final results will be published later.

Registry No.—**2**, 30538-45-5; **3**, 30538-62-6; **4**, 30538-63-7; **5**, 30538-64-8; **6**, 30545-96-1; **7**, 30545-97-2; **8**, 30545-98-3; **9**, 30545-99-4; **10**, 30538-65-9.