MICROBIAL BIOTRANSFORMATIONS. I. O-DEMETHYLATION OF 7,8-DIMETHOXY-2-METHYL-1-(4'-METHOXYBENZYL) 1,2,3,4-TETRAHYDROISOQUINOLINE BY *CUNNINGHAMELLA BLAKESLEEANA*

Joy B. Reighard[1], Paul L. Schiff, Jr., David J. Slatkin and Joseph E. Knapp

Department of Pharmacognosy, University of Pittsburgh School of Pharmacy, Pittsburgh, PA 15261

Abstract.—The microbial biotransformation of the alkaloid 7,8-dimethoxy-2-methyl-1-(4'-methoxybenzyl)1,2,3,4-tetrahydroisoquinoline by *Cunninghamella blakesleeana* was studied; two major metabolites were isolated and identified. These two metabolites were identified as 7-hydroxy-8-methoxy-2-methyl-1-(4'-methoxybenzyl) 1,2,3,4-tetrahydroisoquinoline and 7,8-dimethoxy-2-methyl-1-(4'-hydroxybenzyl)1,2,3,4-tetrahydroisoquinoline.

In recent years, the microbial biotransformation of alkaloids has become an area of increasing interest. Studies have been conducted on alkaloids of various groups, including indole derivatives (1,2), benzylisoquinolines (3), bisbenzylisoquinolines (4,5), and aporphines (6,7).

The most common transformations which occur with these compounds include N- and O- dealkylations (1,3,4,6,7,8) and hydroxylations (2), but other transformations such as dimerizations (9), and oxidative benzylic cleavage (5), have been reported.

The biotransformations which have been reported on the isoquinoline group of alkaloids have been observed primarily in the more commonly occurring 6,7 substituted alkaloids. This paper describes the O-dealkylation of an isoquinoline alkaloid of the less common 7,8 substitution pattern, by *Cunninghamella blakesleeana*.

7,8-Dimethoxy-2-methyl-1-(4'-methoxybenzyl)1,2,3,4-tetrahydroisoquinoline (**1**) was synthesized by an unambiguous route in these laboratories (10). The alkaloid was a white crystalline material which was a racemic mixture.

Initial small-scale screening experiments were conducted to determine which of several micro-organisms were capable of metabolizing the starting material, as well as when the peak metabolite production occurred.

Three organisms were chosen based on previous reports of their capabilities to O-dealkylate various substrates (3,7,11). Of these organisms, *Cunninghamella blakesleeana* (ATCC 8688a) was chosen for further study based on its quantitative production of various metabolites. *Streptomyces griseus* (UI-1158) and *Aspergillus alliaceus* (ATCC 1024) also metabolized the substrate, but not as efficiently as did *Cunninghamella blakesleeana*.

Two metabolites were isolated from the preparative scale fermentations with *Cunninghamella blakesleeana*. Both of these were O-desmethyl products and could be identified on the basis of changes which occurred in the nmr and mass spectra.

The mass spectral fragmentation of this group of isoquinoline alkaloids is quite diagnostic in the identification of these metabolites. Fragmentation occurs by cleavage beta to the nitrogen, which separates the isoquinoline portion of the molecule from the benzylic portion (See figure 1) (12).

---

[1]Present address: Temple University School of Pharmacy, 3307 N. Broad Street, Philadelphia, PA 19140.

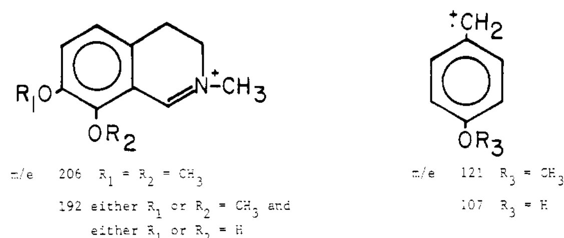


FIG. 1. Prominent fragment ions of the metabolites.

Both Metabolite A and Metabolite B exhibited parent ions at *m/e* 313, fourteen mass units lower than the parent ion of the starting material. This indicated the probable loss of a methyl group from the molecule.

For Metabolite A, the ion equivalent to the isoquinoline portion of the molecule occurred at *m/e* 192, fourteen mass units lower than that of the starting material, indicating that the loss of the methyl group occurred at either position 7 or position 8.

For Metabolite B, the ion equivalent to the benzylic portion of the molecule occurred at *m/e* 107, fourteen mass units lower than that of the starting material, indicating the loss of the methyl group at the 4' position.

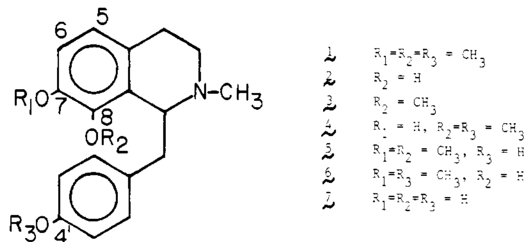
In chemical O-demethylation studies conducted on the starting material in this laboratory, it was noted that the various demethylated products formed could be divided into two groups based on the substituent at position 8, and that these two groups exhibited quite differing nmr and mass spectral characteristics (10).

The Series A alkaloids (**2**) possess a hydroxyl group at position 8, while the Series B alkaloids (**3**) have a methoxyl functionality at that position.

For the Series A (**2**) alkaloids, the nmr spectrum showed a doublet for the protons on C_5 and C_6, while the Series B (**3**) alkaloids showed a singlet for these protons.

This information was used in the identification of the two metabolites produced by microbial biotransformation.

Metabolite A showed a singlet in the nmr for the two aromatic protons at C_5 and C_6 indicating an intact methyl group in position 8. This indicated the loss of the methyl group at position 7, and identifies Metabolite A as 7-hydroxy-8-methoxy-2-methyl-1-(4'-methoxybenzyl)1,2,3,4-tetrahydroisoquinoline (**4**).



Metabolite B also showed a two-proton singlet for the C_5 and C_6 protons. This confirms the presence of a methyl group at position 8. Metabolite B is 7,8-dimethoxy-2-methyl-1-(4'-hydroxybenzyl)1,2,3,4-tetrahydroisoquinoline (**5**).

The structures for Metabolite A and Metabolite B were confirmed by direct comparison with products formed by chemical O-demethylation (10).

It is interesting to note that the major product formed in chemical O-demethylation, 7-methoxy-8-hydroxy-2-methyl-1-(4'-methoxybenzyl)1,2,3,4-tetrahydroisoquinoline (**6**), was produced, but in quantities too small to isolate, in the microbial biotransformation. The two metabolites isolated were those that were obtained in the lowest yield by chemical demethylation (10).

EXPERIMENTAL[2]

STARTING MATERIAL.—The starting material used in this study was provided by Dr. Mohamed M. El-Azizi, Department of Pharmacognosy, University of Pittsburgh School of Pharmacy, Pittsburgh, Pennsylvania 15261, and was synthesized by an unambiguous route (10). The HCl salt was a white crystalline material (mp 178–179°) and showed no optical activity: $[\alpha]^{26}D=0$ (c, 0.83, $CHCl_3$); ir ν max (KBr) 2915, 2815, 2410, 1605, 1580, 1510, 1490, 1440, 1420, 1370, 1350, 1300(sh), 1280, 1250, 1240, 1220(sh), 1180, 1165, 1155, 1130, 1110, 1090, 1060, 1025, 1010(sh), 985, 960, 940(sh), 880, 835, 825, 805, 795, 780, 760(sh), 730, 720, 690, 650(weak), 640(weak) cm^{-1}; uv λ max (MeOH) 235nm (log ϵ3.30), 280 (3.05) with no observed shift in acid or base; nmr (free base, $CDCl_3$) δ 2.31(3H, s, $-NCH_3$), 2.4–3.5(6H, $-CH_2-$), 3.75(3H, s, C_4 $-OCH_3$), 3.82(3H, s, C_7 $-OCH_3$), 3.87(3H, s, C_8 $-OCH_3$), 3.98(1H, q, $J=4$ Hz, C_1 -CH-), 6.75 (2H, s, C_5 and C_6 H), 6.8 and 7.2 (4H, dd, $J=8.5$ Hz, $C_{2'}$, $C_{3'}$, $C_{5'}$ and $C_{6'}$ H); ms, M^+ *m/e* 327 (0.1%) consistent with $C_{20}H_{25}NO_3$, with other fragment ions at *m/e* 326(M^+-1) (0.28), 313(0.11), 207(14), 206(100), 191(2), 190(13), 162(5), 148(4), 121(4), 120(2), 57(8), 56(2), 55(8), 43(9), 29(2).

FERMENTATION PROCEDURE.—The cultures used in this study are maintained at the University of Pittsburgh on Mycophil agar or sporulation agar and stored at either 0° (fungi) or at 4° (Streptomycetes).

The liquid medium used consisted of glucose (40 g), yeast extract (5 g), soybean meal (5 g), NaCl (5 g), K_2HPO_4 (5 g), Tween-80 (0.1 ml), and sufficient distilled water to make 1000 ml. The pH was adjusted to 7.0 with 0.1N HCl.

A two-stage fermentation procedure as described by Rosazza (6) was employed. Stage I cultures (100 ml of broth in 500 ml Erlenmeyer flasks) were shaken at 250 rpm at 27° for 72 hours. Stage II cultures were inoculated with 10 ml of the Stage I cultures and shaken at 250 rpm at 27° for 24 hours. The alkaloid was then added as the hydrochloride salt dissolved in water.

PRELIMINARY WORK.—In the time-course study, the conversion of starting material to metabolites was followed via thin-layer analysis.

In the first study, the exposure of compound to the organism was 72 hours. After thin-layer analysis, no starting material remained, and there was little evidence of any alkaloid material remaining. It was believed that the unstable trihydroxy compound (7), was formed and probably decomposed during the isolation procedure (10).

In the second study, thin-layer analysis was conducted at 7, 23 and 49 hours. Complete degradation was again noted as early as 32 hours.

The procedure was repeated and thin-layer analysis was conducted at 1, 2, 4 and 5 hours. Peak metabolite production was found to occur at four hours.

Controls were used in this preliminary work to ensure the metabolites were not artifacts.

SCALE-UP WORK.—For the scale-up, 40 Stage II cultures were inoculated, and, after 24 hours of growth, 25 mg of the alkaloid was added to each flask. At the end of five hours, the broth was combined and filtered. The mycelium was extracted with acetone (1 liter); the acetone then evaporated leaving an aqueous extract which was combined with the filtered broth. The broth was then acidified to pH 2 with concentrated HCl and extracted three times with an equal volume of petroleum ether, which was then discarded. The broth was basified to pH 10 with ammonium hydroxide then extracted three times with ether and two times with chloroform to ensure complete extraction of the alkaloids. The ether and chloroform solutions were dried over anhydrous sodium sulfate and evaporated to yield a combined extract of 1.42 g.

CHROMATOGRAPHY OF THE ALKALOIDS.—The extract, dissolved in chloroform, was placed on a column of Silica Gel G: acid washed Celite (3:1) and eluted with chloroform-methanol-am-

[2]Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Nmr spectra were determined in $CDCl_3$ with tetramethylsilane as internal standard on a Hitachi Perkin-Elmer model R-24 high resolution spectrometer (60 MHz) and recorded in δ(ppm) units. Ir spectra were taken in KBr on a Perkin-Elmer model 257 Recording Spectrophotometer. Uv spectra were taken in methanol on a Perkin-Elmer model 202 Recording Spectrophotometer. Optical rotations were measured on a Perkin-Elmer model 241 Automatic Polarimeter. Thin-layer and column chromatography were performed on Silica Gel G(CAMAG). Solvents were evaporated *in vacuo* on a Buchler Rotatory evaporator at a temperature not exceeding 40°.

monium hydroxide (95:5:0.1) to give 133 fractions of 500 drops each, which were pooled according to thin-layer analysis to give nine fractions. The third fraction contained a mixture of Metabolite A and starting material. The fifth fraction contained solely Metabolite B.

METABOLITE A (7-HYDROXY-8-METHOXY-2-METHYL-1-(4'-METHOXYBENZYL)1,2,3,4-TETRAHYDROISOQUINOLINE) (**4**).—This alkaloid was obtained by preparative thin-layer chromatography of the third fraction in chloroform-methanol (9:1) containing five drops of ammonium hydroxide per 100 ml. Metabolite A was obtained as a yellow oily residue (20 mg); Rf 0.66 [benzene-acetone-ammonium hydroxide (16:16:3)]. The hydrochloride salt of (4) had a mp of 115°; ir ν max (KBr) 3400(broad), 2945(sh), 2920, 2845, 1600, 1570, 1555, 1510, 1505(sh), 1465(weak), 1455(weak), 1450(sh), 1400(weak, broad), 1380, 1365(broad), 1300, 1175, 1125, 1080(weak), 1025, 815 cm^{-1}; uv λ max (MeOH) 230nm (log ϵ 4.20), and 280(3.70); λ max (0.1N KOH in MeOH) 228(4.22), 250(3.93), 280(3.75), 300(3.60). The nmr (free base, $CDCl_3$) showed δ 2.40(3H, s, $-NCH_3$), 3.78(3H, s, C_4 $-OCH_3$), 3.80(3H, s, C_8 $-OCH_3$), 6.70(2H, s, C_5 and C_6 H), 6.72 and 7.12(4H, dd, $J=8$ Hz $C_{2'}$, $C_{3'}$, $C_{5'}$, and $C_{6'}$ H); ms M^+ *m/e* 313(0.1%) consistent with $C_{19}H_{23}NO_3$, with other fragments at *m/e* 312 (M^+-1) (0.36), 206(2), 193(13), 192(100), 191(3), 190(3), 177(27), 150(2), 148(3), 129(3), 121(7), 120(2), 97(4), 91(2), 83(6), 72(10), 71(6), 69(11), 60(8), 57(11), 43(15), 42(5), 41(13), 29(6).

METABOLITE B (7,8-DIMETHOXY-2-METHYL-1-(4'-HYDROXYBENZYL)1,2,3,4-TETRAHYDROISOQUINOLINE) (**5**).—This alkaloid was obtained from the fifth fraction as a yellow oily residue (47.5 mg); Rf 0.59 [benzene-acetone-ammonium hydroxide (16:16:3)]. The hydrochloride salt of (**5**) showed ir ν max (KBr) 3400(broad), 3200(broad), 2920, 2840, 2700(broad), 2610(broad), 1610, 1510, 1495, 1450, 1440, 1420, 1380, 1360(weak), 1340, 1280, 1230, 1170, 1125, 1085, 1065, 1025, 990, 960, 820, 810 cm^{-1}; uv λ max (MeOH) 232nm (log ϵ 4.13), 282(3.86) and λ max (0.1N KOH in MeOH), 234(4.10), 285(3.67), 303(3.60); nmr (free base, $CDCl_3$) δ 2.40 (3H, s, $-NCH_3$), 3.86 (3H, s, C_7 $-OCH_3$), 3.91 (3H, s, C_8 $-OCH_3$), 6.60 and 7.07 (4H, dd, $J=8$ Hz $C_{2'}$, $C_{3'}$, $C_{5'}$, and $C_{6'}$ H), 6.87 (2H, s, C_5 and C_6 H); ms showed *m/e* 313 (M^+) (0.1%) consistent with $C_{19}H_{23}NO_3$, with other fragments at *m/e* 312(M^+-1)(0.4), 207(27), 206(100), 192(23), 191(11), 190(26), 177(5), 163(10), 148(10), 107(10), 91(2), 77(7), 43(1), 42(4), 41(7), 29(2).

ACKNOWLEDGMENTS

The authors are grateful to Mr. John Naworal, Graduate School of Public Health, University of Pittsburgh, for determining the mass spectra and to Dr. John P. Rosazza, College of Pharmacy, The University of Iowa, for a culture of *Streptomyces griseus* (UI 1158). The mass spectrometry facility was supported by Research Grant RR-00273 to the University of Pittsburgh from the National Institutes of Health.

Received 29 January 1981

LITERATURE CITED

1. M. M. Chien and J. P. Rosazza, *J. Nat. Prod.*, **42**, 643 (1979).
2. M. M. Chien and J. P. Rosazza, *Drug Met. Dispos.*, **7**, 211 (1979).
3. J. P. Rosazza, M. Kammer, L. Youel, R. V. Smith, P. W. Erhardt, D. H. Thuong and S. W. Leslie, *Xenobiotica*, **7**, 133 (1977).
4. P. J. Davis, D. R. Wiese and J. P. Rosazza, *Lloydia*, **40**, 239 (1977).
5. T. Nabih, P. J. Davis, J. F. Caputo and J. P. Rosazza, *J. Med. Chem.*, **20**, 914 (1977).
6. J. P. Rosazza, A. W. Stocklinski, M. A. Gustafson and J. Adrian, *J. Med. Chem.*, **18**, 791 (1975).
7. P. J. Davis, D. Wiese and J. P. Rosazza, *J. Chem. Soc., Perkin I*, 1 (1977).
8. C. D. Hufford, C. C. Collins and A. M. Clark, *J. Pharm. Sci.*, **68**, 1239 (1979).
9. T. Nabih, L. Youel and J. P. Rosazza, *J. Chem. Soc., Perkin I*, 757 (1978).
10. M. M. El-Azizi, Ph.D. Dissertation, Department of Pharmacognosy, School of Pharmacy, University of Pittsburgh, Pittsburgh, Pennsylvania 15261 (1979). Manuscript in preparation.
11. P. J. Davis and J. P. Rosazza, *J. Org. Chem.*, **41**, 2548 (1976).
13. H. Budzikiewicz, C. Djerassi and D. H. Williams, "*Structure Elucidation of Natural Products* by *Mass Spectrometry*", Vol. I: Alkaloids, Holden-Day, Inc., San Francisco, CA, 1964, pp. 173-175.