Metabolism of Imipramine by Microorganisms

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Abstract □ The microbial metabolism of imipramine was studied using selected fungal organisms. The major microbial metabolites were isolated, and their structures were established by spectroscopic analyses (particularly ^{13}C-NMR) and by comparison with authentic samples. The microbial metabolites identified included 2-hydroxyimipramine, 10-hydroxyimipramine, iminodibenzyl, imipramine-*N*-oxide, and desipramine; these metabolites also have been found in mammalian metabolism studies.

Keyphrases □ Imipramine—microbial metabolism, identification of metabolites, fungi □ Metabolites, microbial—imipramine, identification, fungi □ Microbial metabolism—imipramine, identification of metabolites, fungi

Microorganisms possess adaptive enzymes that can be raised to high levels under suitable culture conditions or by induction. These enzymes are capable of carrying out numerous types of chemical reactions and have been utilized for microbial transformations on a wide variety of substrates (1–3).

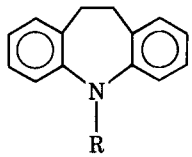
The concept of microbial models of mammalian metabolism involves the observation that microbial metabolism often parallels mammalian metabolism (4, 5). The use of microorganisms for the study of biotransformations of drugs has been reviewed (6, 7). Numerous microbial transformation studies have involved the production of derivatives of natural product drugs with increased biological activities and/or decreased toxicities. The use of microorganisms to study drug metabolism has received far less attention, even though its potential has been noted (4–7).

Imipramine (I) is a widely used tricyclic antidepressant whose metabolism in mammals has been studied thoroughly (8–12). The metabolism of I using microorganisms was considered in this study and compared with human and mammalian metabolic studies.

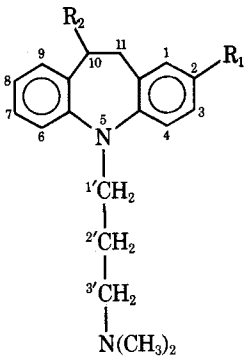
RESULTS AND DISCUSSION

Imipramine (I) was subjected to screening using 47 fungi. Of these fungi, 14 organisms were capable of metabolizing I to one or more metabolites (Table I) as indicated by TLC. Four microorganisms, *C. blakesleeana* (8688a), *M. griseo-cyanus* (1207a), *F. oxysporum* f. sp. *cepae* (11711), and *A. flavipes* (16795), were selected for preparative-scale study.

Incubation of I with stirred cultures of *C. blakesleeana* resulted in the production of two more polar metabolites, which were separated by chromatography on silica gel. Both metabolites had a molecular formula of $C_{19}H_{24}N_2O$ (mass spectrometry), and their identities were established mainly by ^{13}C-NMR. The ^{13}C-NMR assignments for I were made previously (13). Compound I shows only seven distinct ring carbon signals since there is an effective plane of symmetry (13), whereas each metabolite shows 14 distinct signals, indicating that the metabolites differ from imipramine in the ring carbons (the signals for the side-chain carbons are essentially the same) (Table II). One metabolite shows a singlet at 152.9 ppm, suggesting that it is hydroxylated in the aromatic ring. The most logical site of hydroxylation would be at C-2, and the ^{13}C-NMR data are consistent with formulation of this metabolite as 2-hydroxyimipramine (II). The other metabolite shows a doublet at 70.0 ppm and a triplet at 40.2 ppm, suggesting that it is 10-hydroxyimipramine (III). The



I: R = $(CH_2)_3N(CH_3)_2$
IV: R = H
V: R = $(CH_2)_3NO(CH_3)_2$
VI: R = $(CH_2)_3NHCH_3$



II: R_1 = OH, R_2 = H
III: R_1 = H, R_2 = OH

identities of both metabolites were confirmed by direct comparisons with authentic samples of II and III.

Preparative-scale fermentation of I using stirred cultures of *F. oxysporum* f. sp. *cepae* resulted in the production of one metabolite that was readily identified as iminodibenzyl (IV); this identification was confirmed by direct comparison with an authentic sample. Metabolism of I using shaken cultures of *A. flavipes* (16795) on a preparative scale resulted in one metabolite that was identified as imipramine-*N*-oxide (V) by comparison with an authentic sample.

The fourth microorganism chosen for preparative-scale study (stirred cultures of *M. griseo-cyanus*) produced three metabolites, two of which were identical to metabolites isolated previously (III and V). The third metabolite was shown to be desipramine (VI) by direct comparison of its hydrochloride salt with an authentic sample. These preparative-scale experiments using the four fungi allowed isolation and identification of the major microbial metabolites (II–VI) of I. The same metabolites also were detected by TLC in the screening-scale fermentation studies with the 10 additional microorganisms.

High-pressure liquid chromatographic (HPLC) procedures also were developed for the identification of the metabolites in cultures not subjected to preparative-scale study and quantitation of the metabolites in all 14 microorganisms. The retention properties of I and the metabolite standards were characterized using retention times relative to I and a retention index scale (14, 15) using HPLC System A (Table III). The retention index of drug metabolites can be estimated (15, 16) from:

$$I_m = 200\pi_x + I_{ref} \qquad \text{(Eq. 1)}$$

where I_m is the retention index observed for the metabolite, I_{ref} is the retention index of the reference or parent drug, and π_x is the sum of the Hansch substituent constants (17) for the metabolite. Using this method, retention indexes of 789, 725, and 846 were calculated for II, III, and VI, respectively. These calculated values were in good agreement with the experimentally observed values (Table III). The retention indexes of IV and V were more difficult to calculate because the loss of the ionized amino group produces a retention index shift that is pH dependent.

0022-3549/81/0200-0151$01.00/0

Table I—Fungi Used for Screening Imipramine

Fungus (Number)[a]	Metabolite Production[b]
Alternaria solani (11078)	−
Aspergillus flavipes (1030)	+
Aspergillus flavipes (11013)	+
Aspergillus flavipes (16795)	+
Aspergillus flavus (9170)	−
Aspergillus flavus (24741)	−
Aspergillus flavus (22947)	−
Aspergillus niger (10549)	−
Aspergillus niger (11394)	−
Aspergillus niger (16888)	−
Aspergillus ochraceus (18500)	−
Aspergillus parasiticus (15517)	−
Beauveria bassiana (13144)	+
Beauveria sulfurescens (7159)	−
Botrytis allii (9435)	−
Calonectria decora (14767)	−
Chaetomium cochloides (10195)	+
Cladosporium resinae (22712)	−
Cladosporium resinae f. avelleneum (22711)	−
Cunninghamella blakesleeana (8688a)	+
Cunninghamella echinulata (NRRL 3655)	+
Cunninghamella echinulata (9244)	+
Cunninghamella echinulata (11585a)	+
Cunninghamella echinulata (11585b)	−
Cunninghamella elegans (9245)	−
Curvularia lunata (12017)	−
Curvularia lunata (13633)	−
Cylindrocarpon radicicola (11011)	−
Fomes pinicola (15341)	−
Fusarium javanicum (12575)	−
Fusarium oxysporum (7601)	−
Fusarium oxysporum f. sp. *cepae* (11711)	+
Fusarium solani var. *coeruleum* (24389)	+
Mucor griseo-cyanus (1207a)	+
Penicillium adametzi (10407)	−
Penicillium chrysogenum (9480)	−
Penicillium chrysogenum (10002)	−
Penicillium frequentans (10444)	−
Penicillium oxalicum (24784)	+
Penicillium spinulosum (16348)	−
Rhizopus arrhizus (11145)	+
Rhizopus stolonifer (6227b)	−
Rhizopus stolonifer (15441)	−
Septomyxa affinis (6737)	−
Syncephalastrum racemosum (18192)	−
Whetzelinia sclerotiorum (18015)	−
Whetzelinia sclerotiorum (24156)	−

[a] All numbers refer to the American Type Culture Collection in Rockville, Md., unless otherwise specified. [b] Metabolite production denoted by a + indicates one or more metabolites were produced, as evidenced by TLC.

Table II—^{13}C-NMR Data of Imipramine and Metabolites[a]

Position Assignment[b]	I[c]	II	III	IV[c]	V	VI
C-1	129.7 d	116.1 d	130.7 d[1]	130.6 d	130.0 d	129.9 d
C-2	120.0 d	152.9 s	120.4 d[2]	119.4 d	120.0 d	120.1 d
C-3	126.3 d	113.2 d	126.8 d[3]	126.7 d	126.7 d	126.4 d
C-4	122.3 d	121.7 d[1]	122.3 d[4]	118.0 d	123.0 d	122.5 d
C-4a	148.4 s	140.7 s	148.9 s	142.5 s	147.8 s	148.5 s
C-5a	148.4 s	148.9 s	146.7 s	142.5 s	147.8 s	148.5 s
C-6	122.3 d	121.6 d[1]	123.2 d[4]	118.0 d	123.0 d	122.5 d
C-7	126.3 d	126.4 d	127.9 d[3]	126.7 d	126.7 d	126.4 d
C-8	120.0 d	119.0 d[1]	119.9 d[2]	119.4 d	120.0 d	120.1 d
C-9	129.7 d	130.5 d	130.3 d[1]	130.6 d	130.0 d	129.9 d
C-9a	134.2 s	132.7 s	132.2 s[5]	128.6 s	134.2 s	134.4 s
C-10	32.3 t	33.0 t[2]	70.0 d	34.9 t	32.2 t	32.4 t
C-11	32.3 t	31.7 t[2]	40.2 t	34.9 t	32.2 t	32.4 t
C-11a	134.2 s	137.2 s	134.9 s[5]	128.6 s	134.2 s	134.4 s
C-1′	48.9 t	48.9 t	48.7 t	—	47.9 t	48.8 t[1]
C-2′	26.2 t	25.7 t	26.2 t	—	22.4 t	28.3 t
C-3′	57.6 t	57.6 t	57.6 t	—	69.3 t	50.0 t[1]
N-CH_3	45.4 q	45.0 q	45.2 q	—	57.9 q	36.5 q

[a] All data were obtained in deuterochloroform as the free bases. [b] Based on predicted chemical shifts, literature comparisons, and single-frequency off-resonance decoupling. Assignments bearing the same numerical superscript may be interchanged. [c] The assignments for I and IV are given in Ref. 13, but the data listed are from the present study.

Table III—Chromatographic Properties of Imipramine and Its Metabolites on HPLC System A

Compound	t_R[a]	Retention Index	A_{254}/A_{280}[b]
III	0.31	720	2.53
II	0.38	769	2.06
VI	0.43	807	1.83
V	0.48	827	1.78
IV	0.72	871	0.197
I	1.00	1003	1.78

[a] Retention time relative to I (21 min). [b] Ratio of absorbances at 254 and 280 nm.

In complex samples, such as those obtained with the simple solvent extractions of the screening fermentation samples, it is possible that some constituents of the medium might have the same retention time as one metabolite. To minimize errors associated with this possibility, the absorbance ratio at 254 and 280 nm was measured for each standard (Table III), and these values were compared to the A_{254}/A_{280} values obtained for each chromatographic peak in the fermentation extracts. With this method, the chance of an error in the identification of a metabolite peak is greatly reduced (18). Compounds I, V, and VI all had A_{254}/A_{280} values near 1.79 (Table III and Fig. 1), which is consistent with their common UV chromophores. The two hydroxylated metabolites (II and III) had higher absorbance ratios, while IV showed a decrease in the ratio and a marked increase in the absorbance at 280 nm (Fig. 1).

To confirm the metabolite identifications made using HPLC System A, the metabolite extracts also were analyzed using HPLC System B, which was a normal-phase silica system. The metabolite standards generally were well resolved, and the elution order (Fig. 2) was very different from that of the reversed-phase system. Metabolite II could not be resolved from the substrate peak if the extract contained a large residual amount of the substrate.

All extracts obtained from the fermentation screening study were examined using both HPLC systems. The chromatogram of the extract of the *R. arrhizus* culture shown in Fig. 3 was typical of the results obtained with the other organisms. The yields of II, III, V, and VI in *R. arrhizus* were between 4 and 11%, and none of these chromatographic peaks was detected in the culture control. On examination of the same extract using System B (Fig. 2), the same metabolite peaks were detected. Metabolite IV was detected in the cultures of *R. arrhizus* using Systems A and B;

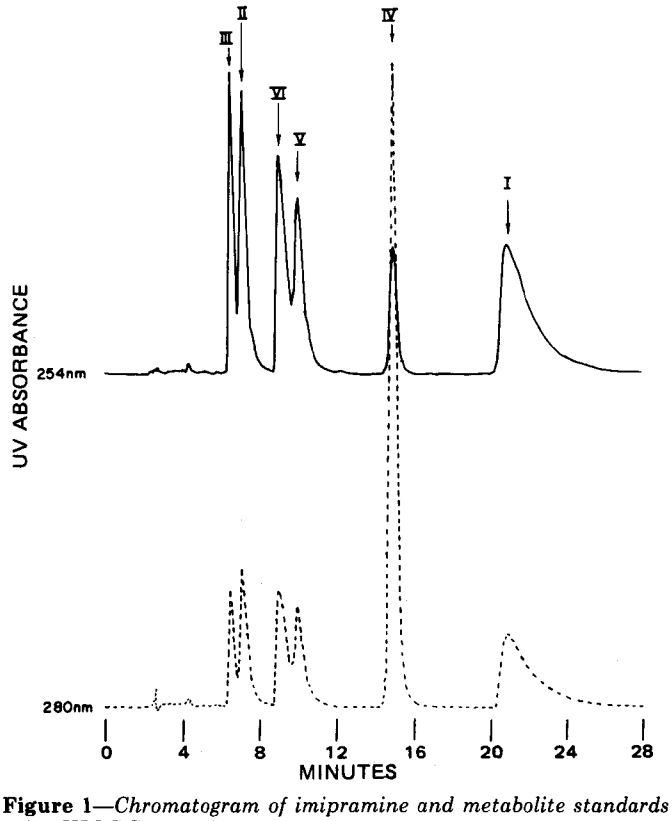


Figure 1—*Chromatogram of imipramine and metabolite standards using HPLC System A.*

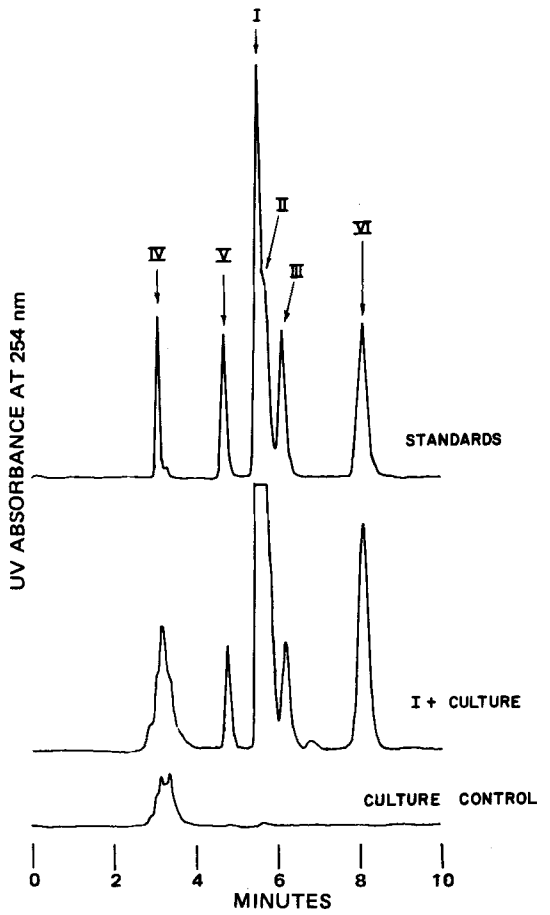


Figure 2—*Chromatograms of the extract of* R. arrhizus *and standards using HPLC System B. The top detector response curve was obtained from a mixture of the reference standards. The middle detector response curve was obtained from the extract of the culture containing I. The bottom detector response curve was obtained from the extract of a control culture to which I had not been added.*

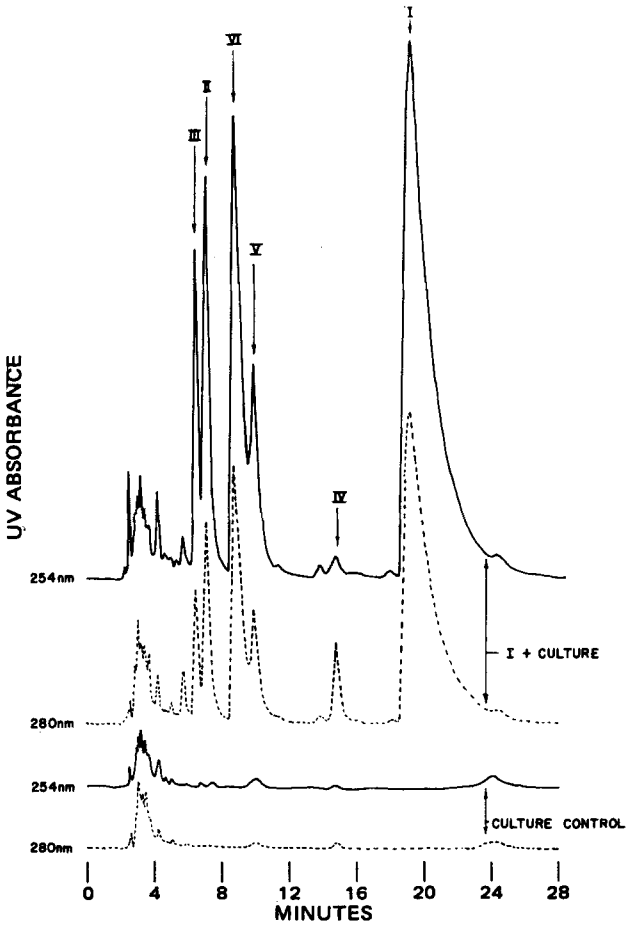


Figure 3—*Chromatogram of the extract of* R. arrhizus *using HPLC System A. The top two UV detector response curves were obtained from the extract of the culture containing I. The bottom two UV detector response curves were obtained from the extract of a control culture to which I had not been added.*

however, the yield of IV was not significantly greater than that found in the substrate control. Furthermore, IV was not present in the samples of I used to prepare the fermentation samples. The low levels of IV that were found in the cultures of *R. arrhizus* and most of the other organisms probably were largely due to chemical decomposition rather than metabolism. Iminodibenzyl IV also has been reported to be a microbial metabolite of *Aspergillus petrakii* (19); but since substrate controls were not conducted and since the yields were quite low, it is likely that the IV isolated here resulted from degradation and not from metabolism by *A. petrakii* (Table IV). However, in the studies with *F. oxysporum* f. sp. *cepae*, IV was consistently found at markedly higher levels than the controls, which indicates that IV was produced as a metabolite by this organism.

Using System A to quantitate metabolite production (Table IV), *F. solani*, *A. flavipes* (11013), and *A. flavipes* (16795) gave nearly quantitative conversion to the *N*-oxide (V). Moderate yields of the desmethyl metabolite also were found in the studies with *M. griseo-cyanus*, *R. arrhizus*, and *C. echinulata* (9244). Many of the organisms produced both hydroxylated metabolites; however, *M. griseo-cyanus* produced III almost exclusively, and *C. blakesleeana* produced II in the largest proportion.

An unidentified metabolite (Y) was detected in the cultures of *C. cochloides*, *C. echinulata* (3655), and *C. echinulata* (11585a); it had a retention index of 484 on System A and a retention time of 9.2 min on System B. This chromatographic peak was not detected in the substrate control or the culture control. A retention index of 529 was calculated for 10-hydroxydesmethylimipramine using Eq. 1, which was consistent with the retention index observed for Y. An A_{254}/A_{280} value of 2.65 was observed for Y, which is consistent with hydroxylation at the 10-position (A_{254}/A_{280} = 2.53 for III) and with *N*-demethylation (which produces no change in the ratio). Using the normal-phase HPLC system (System B), Y had a slightly longer retention time than the *N*-desmethyl metabolite (VI), and the shift in the retention time was similar to that observed between I and the 10-hydroxy metabolite (III). In the extract of the cultures of *C. cochloides*, *C. echinulata* (3655), and *C. echinulata*

Table IV—Yields of Metabolites of Imipramine Using Microorganisms in the Screening-Scale Fermentation Studies [a]

Microorganism	Yield, %					
	I	II	III	IV	V	VI
A. flavipes (1030)	33	—	—	0.9	8.8	—
A. flavipes (11013)	—	—	—	1.2	81	—
A. flavipes (16795)	—	—	—	1.3	87	—
B. bassiana	72	2.9	—	0.7	1.9	2.5
C. cochloides [b,c]	—	0.9	1.9	1.4	1.6	1.3
C. blakesleeana	72	10	6.5	0.7	3.2	1.4
C. echinulata (3655) [b]	—	16	28	—	3.9	3.8
C. echinulata (9244)	15	7.1	4.1	0.3	—	7.8
C. echinulata (11585a) [b]	—	6.9	15	0.1	1.6	1.6
F. oxysporum f. sp. *cepae*	22	—	—	9.3	0.8	—
F. solani var. *coeruleum*	—	—	—	1.1	101	—
M. griseo-cyanus	80	—	5.6	1.1	—	9.7
P. oxalicum	7.1	—	3.2	0.7	0.8	—
R. arrhizus	45	5.4	4.1	0.7	3.7	11
Substrate control	98	—	—	1.2	—[d]	—

[a] Yields are expressed as a percent conversion on a mole basis. [b] Using HPLC System A, an unknown metabolite (Y) was found having a retention index of 484 and an A_{254}/A_{280} value of 2.65. The metabolite also was observed at a retention time of 9.2 min on HPLC System B. Using both HPLC systems, the metabolite peak was not detected in the culture control or the substrate control. [c] Using HPLC System A, an unknown metabolite (Z) was found having a retention index of 867 and an A_{254}/A_{280} value of 1.67. The chromatographic peak was not detected in the culture control or the substrate control. [d] A trace of V was detected using HPLC System A; however, the presence of V could not be confirmed using HPLC System B.

(11585a), Y was one of the major components, the substrate was exhausted, and only moderate levels of the *N*-desmethyl and 10-hydroxylated metabolites were present. Although these findings were consistent with Y being 10-hydroxydesmethylimipramine, this hypothesis could not be verified because an authentic reference standard was not available for a direct comparison.

An additional unidentified metabolite (Z) was detected in the extract of *C. cochloides;* it was not found in either the substrate control or the culture control. Using System A, Z had a retention index of 967 (A_{254}/A_{280} = 1.67). Because of the high value for the retention index and the essentially unchanged absorbance ratio, it was concluded that Z did not represent a ring hydroxylation and that it probably was modified in the side chain. Since Z was found in relatively low yields in only one microorganism, no additional studies were done with this metabolite.

CONCLUSIONS

The microbial metabolism of imipramine (I) was studied systematically using a variety of fungi. The major metabolites identified included 2-hydroxyimipramine (II), 10-hydroxyimipramine (III), iminodibenzyl (IV), imipramine-*N*-oxide (V), and desipramine (VI). These microbial metabolites also correspond to major human and mammalian metabolites of imipramine (8–12, 20). The results of this study clearly suggest that microorganisms can be used to study drug metabolism (biotransformation), with the added advantage of producing large quantities of metabolites so that complete biological evaluation as well as complete and rigorous structural elucidation studies can be obtained.

EXPERIMENTAL[1]

Fermentation Screening Procedures—Stock cultures were maintained on mycophil (BBL) agar. Preliminary screening for biotransformation products was carried out on gyrotory shakers operating at 200 rpm (room temperature) in erlenmeyer flasks (125 ml) holding 25 ml of medium. A two-stage fermentation procedure was utilized as described previously (21, 22). The fermentation medium consisted of 20 g of dextrose, 5 g of yeast extract, 5 g of peptone, 5 g of sodium chloride, and 5 g of dibasic potassium phosphate in 1000 ml of water. Imipramine hydrochloride (5 mg/culture) was added as a 5% solution in dimethylformamide.

Culture controls consisted of fermentation blanks, in which organisms were grown under identical conditions but without the substrate. Substrate controls consisted of sterile media containing the same amount of substrate and incubated under the same conditions.

Fermentation Sampling and TLC Analysis—The fermentations were sampled by withdrawing 5 ml of culture broth, adjusting to pH 8, and extracting with three 5-ml portions of chloroform. The chloroform layers were evaporated, and the residue was redissolved in chloroform and spotted on precoated silica gel G and aluminum oxide TLC plates (0.25 mm). The silica gel G plates were developed in ethyl acetate–methanol–ammonium hydroxide (81:15:4) and sprayed with diazotized *p*-nitroaniline followed by concentrated hydrochloric acid. The R_f values were: I, 0.58 (blue); II, 0.47 (red); III, 0.40 (yellow); IV, 0.91 (yellow green); V, 0.15 (blue); and VI, 0.27 (blue). The aluminum oxide plates were developed in 2.5% methanol–chloroform and sprayed with diazotized *p*-nitroaniline followed by concentrated hydrochloric acid. The R_f values were: I, 0.80 (blue); II, 0.18 (red); III, 0.46 (yellow); IV, 0.98 (green black); V, 0.08 (blue); and VI, 0.24 (blue).

HPLC Analysis—All quantitative analyses of the culture extracts were accomplished using HPLC System A. The mobile phase for this system was prepared using 3.3 g of dibasic potassium phosphate, 4.2 g of monobasic potassium phosphate, 0.88 ml of acetic acid, 1.2 liters of water, and 2.8 liters of methanol. A 4.6 × 250-mm octadecyl bonded phase column[2] (5-μm particles) and a mobile phase flow rate of 1.0 ml/min were used. An HPLC pump[3] and a microsyringe-loaded loop injector[4] also were used. Two UV detectors[5] were used in series (254-nm detector followed by 280-nm detector) and were calibrated using a morphine reference standard (18).

The HPLC retention indexes were made using System A, and the method was essentially the same as that reported previously (14, 15). The retention index scale was based on the relative retention of a series of 2-keto alkanes[6] (C_3+C_{23}). The retention index of a given 2-keto alkane standard was, by definition, equal to 100 times the number of carbons in the compound. Thus, 2-butanone was assigned a value of 400. The HPLC retention index of I and its metabolites was determined by interpolation of the logarithm of the capacity factors observed for the test compound and the standards.

HPLC System B consisted of a 3.9-mm × 30-cm silica column[7] with 10-μm particles. The mobile phase was prepared using methanol, 2 *N* ammonia, and 1 *N* ammonium nitrate (27:2:1); the flow rate was 1.0 ml/min.

The quantitative analyses of the microbial culture extracts were accomplished using HPLC System A. The chloroform extracts from the screening-scale fermentation studies were evaporated and then redissolved in 400 μl of methanol. Then 4 μl of the solution was chromatographed, and the peak heights were compared to those of a mixture of the standards to calculate the amount of metabolites present. The identity of each metabolite peak was verified by a comparison of the observed A_{254}/A_{280} value to that of the standards and by analysis of the extracts using HPLC System B.

Preparative-Scale Metabolism of Imipramine (I) Using *C. blakesleeana* (ATCC 8688a)—*C. blakesleeana* was grown in 5 liters of culture medium contained in a 7.5-liter fermentor jar[8]. After a 48-hr incubation, I·HCl (1 g in 10 ml of dimethylformamide) was added to the culture. The incubation (stir rate of 200 rpm, air flow of 2 liters/min, room temperature) was continued for 20 days (no imipramine by TLC). The culture was filtered (büchner funnel), and the culture broth was adjusted to pH 7–8 and extracted with chloroform (4 × 800 ml). The combined chloroform layers were dried (sodium sulfate) and evaporated (40°) to leave 992 mg of residue. This residue was redissolved in 10 ml of chloroform and extracted thoroughly with 1% HCl (3 × 10 ml).

The combined aqueous acid extracts were adjusted to pH 8 with ammonia and extracted with chloroform (3 × 15 ml). The evaporated solution gave 591 mg of residue, which was chromatographed over silica gel (59 g, benzene–methanol–triethylamine, 93:1:6) and gave Fraction A (127 mg, pure III by TLC) and Fraction B (196 mg, pure II by TLC). It was found in later experiments that II and III could be separated more conveniently on alumina[9] using 2% methanol in chloroform.

Fraction B was crystallized from ether to give II as nearly colorless chunks (88 mg), mp 133–134° [lit. (23) mp 134–135°]; mass spectrum: M^+ at *m/z* 296.1901 ($C_{19}H_{24}N_2O$ requires 296.1902). The ^{13}C-NMR data are given in Table II. Direct comparison of II with an authentic sample of 2-hydroxyimipramine (regenerated from the hydrochloride salt) showed the two samples to be identical (melting point, mixed melting point, superimposable IR spectra, TLC, and co-TLC).

Fraction A was purified further by chromatography over 122 g of alumina[9] with 1% methanol–chloroform as the eluent. A total of 30 mg of crystalline residue (III) (mp 84–86°) was obtained, $[\alpha]_D^{24}$ +3.3° (*c*, 0.7 in chloroform); mass spectrum: M^+ at *m/z* 296. The ^{13}C-NMR data are given in Table II. Direct comparison of III with an authentic sample of 10-hydroxyimipramine (mp 86–88°) showed the two samples to be identical (TLC and co-TLC on silica and alumina, superimposable IR spectra, and identical ^{13}C-NMR spectra).

Preparative-Scale Metabolism of Imipramine (I) Using *F. oxysporum* f. sp. *cepae* (ATCC 11711)—A total of 1 g of I·HCl was subjected to metabolism using *F. oxysporum* f. sp. *cepae* under the same fermentation conditions as described for *C. blakesleeana.* After 15 days, the culture was filtered, and the broth was adjusted to pH 7–8 and extracted with chloroform (10 × 500 ml). The combined chloroform extracts were evaporated to dryness, and the residue (326 mg) was chromatographed over alumina[9] (30 g) with benzene as the eluent. The iminodibenzyl (IV) eluted in the first few fractions followed by I (189 mg, identified by TLC and HPLC).

The fractions containing IV were combined (48 mg), and crystallization from methanol–water gave 7 mg of IV as needles, mp 106–107° [lit. (19) mp 105–107°]. Direct comparison of IV with an authentic sample of iminodibenzyl showed the two samples to be identical (melting point, mixed melting point, TLC, co-TLC, and superimposable IR spectra). The

[1] Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. IR spectra were run in potassium bromide or chloroform using a Perkin-Elmer 281b spectrophotometer. The ^{13}C-NMR spectra (15.03 MHz) were recorded in deuterochloroform on a JEOL-FX60 FT NMR spectrometer with tetramethylsilane as the internal standard. Iminodibenzyl was obtained from Aldrich Chemical Co. and was purified by crystallization from methanol–water (mp 105–107°). The melting points of the authentic samples received were determined and were: II·HCl, 186–188°; III, 86–88°; IV, 77–79°; and VI·HCl, 212–213°.

[2] Partisil PXS 5/25 ODS column, Whatman, Clifton, N.J.

[3] Model M-6000, Waters Associates, Milford, Mass.

[4] Model U6-K, Waters Associates, Milford, Mass.

[5] Model 440, Waters Associates, Milford, Mass.

[6] Analabs Inc., North Haven, Conn.

[7] μ-Porasil, Waters Associates, Milford, Mass.

[8] New Brunswick model FS 307.

[9] Woelm neutral grade I.

^{13}C-NMR data for IV are listed in Table II.

Preparative-Scale Metabolism of Imipramine Using *A. flavipes* (ATCC 16795)—*A. flavipes* was grown in 4.0 liters of medium contained in 40 500-ml erlenmeyer flasks. After a 24-hr incubation of the Stage II cultures, a total of 800 mg of I·HCl was distributed evenly among the cultures; incubation was continued for 13 days (200 rpm, room temperature). The cultures were harvested by homogenization of the whole culture, followed by filtration (through büchner funnel). The aqueous culture filtrate (pH 8) was extracted with 10 500-ml portions of chloroform, and the combined chloroform layers were dried (sodium sulfate) and evaporated *in vacuo* to leave 663 mg of a brown oily residue.

Preparative TLC of 330 mg of the residue was carried out using silica gel G plates (2.0 mm thick, 20 × 20 cm) developed in ethyl acetate–methanol–ammonium hydroxide (81:15:4). The major band, corresponding to the *N*-oxide, was located by UV light and scraped off, and the silica gel was extracted exhaustively with 10% methanol–chloroform. Evaporation of the solvent afforded 112 mg of crude V, which was crystallized from benzene–hexane as white needles, mp 76–78° [lit. (24) mp 75–79°]. Direct comparison of V with an authentic sample of imipramine-*N*-oxide showed the two samples to be identical (melting point, mixed melting point, TLC, co-TLC, and superimposable IR spectra).

Preparative-Scale Metabolism of Imipramine (I) Using *M. griseo-cyanus* (ATCC 1207a)—A total of 1 g of I·HCl was fed to *M. griseo-cyanus* as described for *C. blakesleeana* and worked up as described for *F. oxysporum*. The chloroform residue (664 mg) was chromatographed over alumina[9] (90 g) using chloroform followed by an increasing percentage of methanol in chloroform. A total of 496 mg of I and 45 mg of III was obtained with chloroform as the eluent (identities established by TLC and HPLC). Elution with 1% methanol–chloroform gave 30 mg of V (TLC and HPLC); 2% methanol–chloroform gave 49 mg of VI, which was converted to the hydrochloride salt (acidic ether) and crystallized from acetone as needles (10 mg), mp 211–212° [lit. (24) mp 206–208° and 214–218°]. Direct comparison of VI·HCl with an authentic sample of desipramine hydrochloride showed the two samples to be identical (melting point, mixed melting point, TLC, co-TLC, and superimposable IR spectra). The ^{13}C-NMR data for VI are listed in Table II.

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ACKNOWLEDGMENTS

Supported in part by the Committee on Faculty Research, University of Mississippi, and the Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi.

The authors are grateful for the samples of 2-hydroxyimipramine hydrochloride, 10-hydroxyimipramine, and imipramine-*N*-oxide provided by Ciba-Geigy Corp. and desipramine hydrochloride provided by Merrell-National Laboratories. They also thank Ms. Ann Calloway and Ms. Sue Johnson for technical assistance.

Metabolism of Phencyclidine by Microorganisms

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Received June 13, 1980, from the **Department of Pharmacognosy and the* ‡*Department of Medicinal Chemistry, School of Pharmacy, University of Mississippi, University, MS 38677.* Accepted for publication July 18, 1980.

Abstract □ A number of microorganisms were screened for their ability to metabolize phencyclidine. Two microorganisms, *Beauveria sulfurescens* and *Cunninghamella echinulata*, produced hydroxylated metabolites, which were identified as 1-(1-phenylcyclohexyl)-4-hydroxypiperidine and 4-phenyl-4-piperidinocyclohexanol by high-pressure liquid chromatographic analysis.

Keyphrases □ Phencyclidine—microbial metabolism, identification of metabolites □ Metabolites, microbial—phencyclidine, identification □ Microbial metabolites—phencyclidine, identification of metabolites

Phencyclidine (I) is a commonly abused drug whose metabolism has received limited attention in mammals. Ober *et al.* (1) conducted a study of the metabolism of I in rhesus monkeys and reported some unchanged I but larger amounts of metabolites. The major metabolite present in the urine was identified as a nonphenolic dihydroxyphencyclidine. Specific structures were not proposed, but a later report (2) gave structures for those metabolites, although details were not provided. Glazko (2) also reported considerable species variation of these metabolites,

0022-3549/81/0200-0155$01.00/0