Inhibition of Monoamine Oxidases A and B by Simple Isoquinoline Alkaloids: Racemic and Optically Active 1,2,3,4-Tetrahydro-, 3,4-Dihydro-, and Fully Aromatic Isoquinolines

Michael E. Bembenek,† Creed W. Abell,*,† Linda A. Chrisey,‡ Maria D. Rozwadowska,‡ Wieslaw Gessner,‡ and Arnold Brossi‡

Division of Medicinal Chemistry, College of Pharmacy, University of Texas at Austin, Austin, Texas 78712-1074, and Medicinal Chemistry Section, Laboratory of Analytical Chemistry, NIDDK, National Institutes of Health, Bethesda, Maryland 20892. *Received February 21, 1989*

A series of 1,2,3,4-tetrahydro-, 3,4-dihydro-, and fully aromatic isoquinolines were tested as substrates and/or inactivators of highly purified human monoamine oxidase A and B (MAO A and B). None were found to be a substrate for either enzyme, but many of these isoquinolines could selectively inhibit either MAO A or B. Stereoselective competitive inhibition of MAO A was found with the *R* enantiomer of all the stereoisomers tested, including salsolinol (K_i = 31 μM), salsoline (K_i = 77 μM), salsolidine (K_i = 6 μM), and carnegine (K_i = 2 μM). As a class, the 3,4-dihydroisoquinolines were the most potent inhibitors tested (K_i = 2–130 μM), and the fully aromatic isoquinolines had intermediate activity (K_i = 17–130 μM) against MAO A. In contrast, only a few of these compounds markedly inhibited MAO B. 1,2,3,4-Tetrahydroisoquinoline, its 2-methyl derivative, and *o*-methylcorypalline gave apparent K_i values of 15, 1, and 29 μM, respectively, and two 3,4-dihydroisoquinolines (compounds **22** and **25**) showed substantial inhibition of MAO B (K_i = 76 and 15 μM, respectively). These results support the concept that the topography of the inhibitor binding site differs in MAO A and B.

The observation that a potent neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), causes irreversible parkinsonism in humans[1] has stimulated a search for structurally similar neurotoxic substances in the environment. Several investigators have proposed that endogenous and/or environmental chemical species could contribute to the etiology of idiopathic Parkinson's disease.[2] Isoquinoline alkaloids represent potential candidates because they contain similar structural features (aromatic ring, *N*-methyl group) as MPTP and are found in a variety of natural sources, including several plant species.[3,4] Furthermore, some isoquinolines have also been found in humans. These include salsolinol when blood alcohol levels are elevated,[5] norcoclaurine-1-carboxylic acid in patients with phenylketonuria,[6] and norlaudanosoline-1-carboxylic acids in Parkinson's patients undergoing L-DOPA therapy.[7] Recently, Nagatsu and Yoshida[8] reported that administration of tetrahydroisoquinoline produced parkinsonism in marmosets with concomitant reductions in brain tyrosine hydroxylase activity, dopamine concentrations, and total biopterin levels.

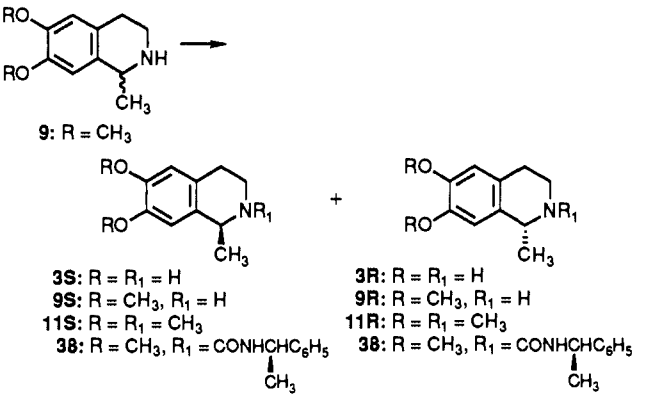
Isoquinolines comprise one of the most abundant groups of alkaloids.[4,9] The compounds studied here and referred to as "simple isoquinolines" are unsubstituted and 1-methyl-substituted representatives. In this series, tetrahydroisoquinolines with two or more methoxy groups are Cactus alkaloids,[10] whereas catecholic congeners present in mammals and obtained by condensation of dopamine with formaldehyde or acetaldehyde are often referred to as "mammalian alkaloids".[11]

The purpose of this study was to examine systematically a large number of "simple isoquinolines" as potential substrates and/or inhibitors of highly purified human monoamine oxidase A and B (MAO A and B) to gain insight into the structure–activity relationships of 1,2,3,4-tetrahydro-, 3,4-dihydro-, and aromatic isoquinolines.

Chemistry

Since most of the isoquinolines investigated in this study are known compounds, references for their preparation are given in Tables I–III. The new compounds that were prepared in our laboratories have been fully characterized, and the details of their preparation are given below.

Scheme I



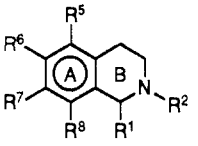
From racemic salsolidine (**9**),[13] both the (+)-(1*R*)- and (−)-(1*S*)-salsolidine isomers **9R** and **9S** were obtained in

(1) Davis, G. C.; Williams, A. C.; Markey, S. P.; Ebert, M. H.; Caine, E. D.; Reichert, C. M.; Kopin, I. J. *Psychiatry Res.* **1979**, *1*, 249. Langston, J. W.; Ballard, P.; Tetrud, J. W.; Irwin, I. *Science* **1983**, *219*, 979.
(2) Testa, B.; Naylor, R.; Costall, B.; Jenner, P.; Marsden, C. D. *J. Pharm. Pharmacol.* **1985**, *37*, 679. Ramsden, D. B.; Williams, A. C. *Lancet* **1985**, 215. Ohkubu, S.; Hirano, T.; Oka, K. *Lancet* **1985**, 1272.
(3) Menachery, M. D.; Lavanier, G. L.; Wetherly, M. D.; Guinaudeau, H.; Shamma, M. *J. Nat. Prod.* **1986**, *49*, 745.
(4) Lundström, J. Simple Isoquinoline Alkaloids. In *The Alkaloids*; Brossi, A., Ed.; Academic Press: New York, 1983; Vol. 21, pp 255–327.
(5) Melchoir, C.; Collins, M. A. *CRC Rev. Toxicol.* **1982**, *9*, 313. Sjoquist, B.; Johnson, H. A.; Borg, S. *Drug Alcohol Depend.* **1985**, *16*, 241. Ung-Chhun, N.; Cheng, B. Y.; Pronger, D. A.; Serrano, P.; Chavez, B.; Perez, R. F.; Morales, J.; Collins, N. A. *Prog. Clin. Biol. Res.* **1985**, *183*, 125.
(6) Lasala, J. M.; Coscia, C. J. *Science* **1979**, *203*, 283.
(7) Coscia, C. J.; Burke, W.; Jamroz, G.; Lasala, J. M.; McFarlane, J.; Mitchell, J.; O'Toole, M. M.; Wilson, M. L. *Nature (London)* **1977**, *269*, 617.
(8) Nagatsu, T.; Yoshida, M. *Neurosci. Lett.* **1988**, *178*, 182.
(9) Shamma, M. *The Isoquinoline Alkaloids*; Academic Press: New York, 1972; pp 1–594. Shamma, M. *Isoquinoline Alkaloids Research 1972–1977*; Plenum Press: New York, 1978; pp 1–405. Kametani, T. *The Chemistry of the Isoquinoline Alkaloids*; *Elsevier Publishing Co.*: New York, 1969; pp 1–265. Kametani, T. *The Chemistry of the Isoquinoline Alkaloids*; The Sendai Institute of Heterocyclic Chemistry: Sendai, Japan, 1974; Vol. 2, pp 1–359. Lundström, J., see ref 4.

† University of Texas at Austin.
‡ NIDDK, National Institutes of Health.

0022-2623/90/1833-0147$02.50/0 © 1989 American Chemical Society

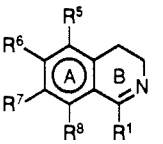
Table I. Inhibition of MAO A and B by 1,2,3,4-Tetrahydroisoquinolines



no.	form	R^1	R^2	R^5	R^6	R^7	R^8	common name	K_i, μM MAO A	K_i, μM MAO B	ref
1	oil	H	H	H	H	H	H	1,2,3,4-tetrahydroisoquinoline	210[a]	15[a]	Aldrich
2	HCl	H	CH_3	H	H	H	H	2-methyl-1,2,3,4-tetrahydroisoquinoline	27[a]	1[a]	18, 19
3R	HBr	CH_3	H	H	OH	OH	H	(+)-(*R*)-salsolinol	31[a]	*c*	16
3S	HBr	CH_3	H	H	OH	OH	H	(−)-(*S*)-salsolinol	284[a]	*c*	16
4	HBr	H	CH_3	H	OH	OH	H	2-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline	1300[b]	*c*	3
5	base	CH_3	CH_3	H	OH	OH	H	(+/−)-*N*-methylsalsolinol	36[a]	*c*	16
6	HCl	CH_3	H	H	OCH_3	OH	H	(+/−)-isosalsoline	34[a]	*c*	15
7R	HCl	CH_3	H	H	OH	OCH_3	H	(+)-(*R*)-salsoline	77[a]	*c*	15
7S	HCl	CH_3	H	H	OH	OCH_3	H	(−)-(*S*)-salsoline	160[a]	*c*	15
8	HCl	CH_3	CH_3	H	OH	OCH_3	H	(+/−)-*N*-methylsalsoline	70[b]	2300[a]	3
9R	HCl	CH_3	H	H	OCH_3	OCH_3	H	(*R*)-(+)-salsolidine	6[a]	*c*	14
9S	HCl	CH_3	H	H	OCH_3	OCH_3	H	(*S*)-(−)-salsolidine	186[a]	*c*	12, 14
10	HBr	H	CH_3	H	OCH_3	OCH_3	H	*o*-methylcorypalline	27[a]	29[a]	3
11R	HCl	CH_3	CH_3	H	OCH_3	OCH_3	H	(*R*)-(−)-carnegine	2[a]	*c*	3, 4
11S	HCl	CH_3	CH_3	H	OCH_3	OCH_3	H	(*S*)-(+)-carnegine	102[a]	1600[b]	3, 4, 12, 16
12	fumarate	CH_3	H	H	OCH_3	OCH_3	OCH_3	(+/−)-*o*-methylanhalonidine	170[a]	*c*	20
13	fumarate	CH_3	CH_3	H	OCH_3	OCH_3	OCH_3	(+/−)-*o*-methylpellotine	160[a]	*c*	4
14	HCl	H	H	OCH_3	OCH_3	OCH_3	OCH_3	norweberine	700[b]	580[b]	22
15	HCl	H	CH_3	OCH_3	OCH_3	OCH_3	OCH_3	weberine	260[a]	1900[a]	3, 22
16	HCl	CH_3	H	OCH_3	OCH_3	OCH_3	OCH_3	(+/−)-1-methylnorweberine	1000[a]	*c*	22
17	methiodide	H	$(CH_3)_2$	OCH_3	OCH_3	OCH_3	OCH_3	weberine methiodide	*c*	*c*	*d*
18	HCl	CH_3	CH_3	OCH_3	OCH_3	OCH_3	OCH_3	(+/−)-1-methylweberine	*c*	*c*	22
19	nonsalt	CH_3/CO_2H	H	H	OH	OH	H	(+/−)-salsolinol-1-carboxylic acid	*c*	*c*	23
20	nonsalt	CH_3/CO_2H	H	H	OCH_3	OH	H	(+/−)-salsoline-1-carboxylic acid	*c*	*c*	24
21	base	CH_3/CO_2CH_3	H	H	OH	OCH_3	H	methyl (+/−)-salsoline-1-methyl carboxylate	*c*	*c*	14

[a] Competitive inhibition. [b] Mixed inhibition. [c] No inhibition at 1 mM. [d] See the Experimental Section.

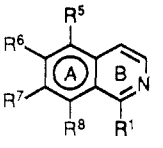
Table II. Inhibition of MAO A and B by 3,4-Dihydroisoquinolines



no.	form	R^1	R^5	R^6	R^7	R^8	K_i, μM MAO A	K_i, μM MAO B	ref
22	methiodide	H	H	H	H	H	130[a]	76[a]	18
23	base	CH_3	H	OCH_3	OH	H	13[a]	*b*	25
24	HCl	CH_3	H	OH	OCH_3	H	80[a]	*e*	26
25	methiodide	H	H	OCH3	H	H	4[a]	15[a]	26
26	methiodide	H	H	OCH_3	OCH_3	H	6[a]	*d*	27
27	base	CH_3	H	OCH_3	OCH_3	H	4[a]	*b*	3, 4
28	fumarate	CH_3	H	OCH_3	OCH_3	OCH_3	2[a]	*c*	28
29	HCl	CH_3	OCH_3	OCH_3	OCH_3	OCH_3	10[a]	*c*	22

[a] Competitive inhibition. [b] No inhibition at 0.1 mM. [c] No inhibition at 0.2 mM. [d] No inhibition at 0.3 mM. [e] No inhibition at 0.5 mM.

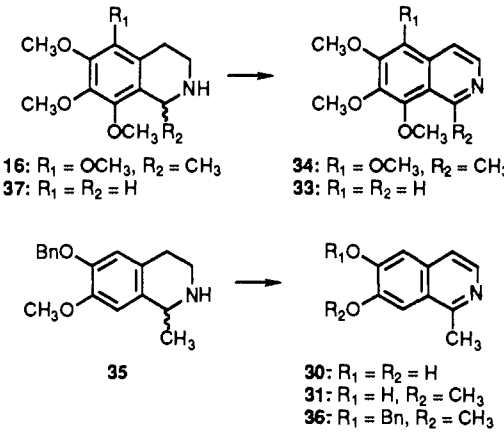
Table III. Inhibition of MAO A and B by Aromatic Isoquinolines



no.	form	R^1	R^5	R^6	R^7	R^8	K_i, μM MAO A	K_i, μM MAO B	ref
30	HBr	CH_3	H	OH	OH	H	130[b]	*c*	*d*
31	base	CH_3	H	OH	OCH_3	H	130[a]	*c*	*d*
32	methosulfate	CH_3	H	OH	OCH_3	H	150[b]	*c*	*d*
33	HCl	H	H	OCH_3	OCH_3	OCH_3	41[a]	*c*	*d*
34	HCl	CH_3	OCH_3	OCH_3	OCH_3	OCH_3	17[a]	*c*	*d*

[a] Competitive inhibition. [b] Mixed inhibition. [c] No inhibition at 0.5 mM. [d] See the Experimental Section.

Scheme II



65% yield and high optical purity (Scheme I). Salsolidine was reacted with (+)-(1*R*)-phenylethyl isocyanate to produce a mixture of the two diastereomeric ureas, which were easily separated by fractional crystallization[14]. (1*R*)-2-[(*R*-1-Phenylethyl)carbamoyl]salsolidine (**38**) was recovered as a crystalline material of higher optical purity than the previously reported compound.[14] Alcoholysis of the ureas occurred smoothly in refluxing sodium butoxide/butanol to yield pure (+)-(1*R*)-**9R** and (−)-(1*S*)-salsolidine (**9S**), which were identical with the natural alkaloid and its antipode.[14] Optically pure (1*R*)- and (1*S*)-salsoline (**7R** and **7S**, respectively) were prepared according to the literature procedure.[15]

Optically pure (1*R*)-(−)- and (1*S*)-(+)-carnegine (**11R** and **11S**, respectively) have been prepared from **9R** and **9S** via reductive methylation using Raney nickel and formaldehyde under H_2, and optically pure (1*R*)-(+)- and (1*S*)-(−)-salsolinol (**3**) were obtained from **9R** and **9S**, respectively, in refluxing 48% HBr.[16]

Preparation of the novel aromatic isoquinolines described in Table III (**31**, **33**, **34**) was achieved by Pd/C-catalyzed dehydrogenation of the corresponding 1,2,3,4-tetrahydroisoquinolines (**35**, **37**, **16**) in either toluene or xylene following a published procedure applied to other tetrahydroisoquinolines (Scheme II).[17] Ether cleavage of **31** to yield compound **30** was accomplished in refluxing 48% HBr, and the water-soluble methosulfate **32** was prepared from **31** by treatment with dimethyl sulfate in refluxing acetonitrile. Similarly, quaternization of weberine (**15**) with methyl iodide in tetrahydrofuran provided the methiodide **17**.

Results

A series of isoquinoline alkaloids were tested as substrates with purified human MAO A and B or as inhibitors with kynuramine as substrate. None were found to be a substrate for MAO A and B (see Biochemical Methods), but many compounds selectively inhibited either MAO A or B (Table I). With MAO A, the range of apparent K_i's was from 15 to 1300 μM for these 1,2,3,4-tetrahydroisoquinolines. Compounds that produced apparent K_i's below 100 μM were 2-methyl-1,2,3,4-tetrahydroisoquinoline (**2**), (*R*)-salsolinol (**3R**), *N*-methylsalsolinol (**5**), isosalsoline (**6**), (*R*)-salsoline (**7R**), *N*-methylsalsoline (**8**), (*R*)-salsolidine (**9R**), *N*-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**10**), and (*R*)-carnegine (**11R**).

Stereoselective competitive inhibition of MAO A was observed with the *R* enantiomer of all of the stereoisomers tested, including salsolinol, salsoline, salsolidine, and carnegine. The *R* stereoisomer of salsolinol (**3R**) was found to be about 10-fold more potent than the *S* stereoisomer (**3S**) (K_i of 31 μM versus 284 μM). A K_i of 77 μM was obtained for (*R*)-salsoline (**7R**), whereas the K_i of the *S* enantiomer (**7S**) was 160 μM. (*R*)-Salsolidine (**9R**) was 30 times more potent than the *S* stereoisomer (K_i of 6 μM versus 186 μM), and the *R* stereoisomer of carnegine (**11R**) was about 50-fold more potent than the *S* enantiomer (K_i of 2 μM versus 102 μM).

Racemic isosalsoline, **6**, was also found to be an effective competitive inhibitor with a K_i of 34 μM. Other potent inhibitors of MAO A were N-methylated derivatives of the 1,2,3,4-tetrahydroisoquinolines. Comparable competitive inhibition was found with compounds **2** (K_i = 27 μM), **5** (K_i = 36 μM), and **10** (K_i = 27 μM). Compound **8** gave a mixed pattern of inhibition that was 2–3 times less potent than these other N-methylated compounds (K_i = 70 μM).

Intermediate inhibition of MAO A activity was observed with the trimethoxy-substituted 1,2,3,4-tetrahydroisoquinolines **12** and **13** (competitive inhibition with K_i's of about 150 μM) and the parent compound of the series, **1** (competitive inhibition with a K_i of 210 μM). Addition of a carboxyl group at the R^1 position gave amino acids or an ester derivative (**19–21**) that were inactive at 1 mM concentrations.

Compounds **4** and **14–16** were found to be relatively weak inhibitors of MAO A. Weberine and its analogues (**14–16**) were competitive inhibitors with K_i's above 0.25 mM. Increasing the degree of substitution produced alkaloids that were inactive (compounds **17** and **18**).

In contrast to the wide range of compounds that inhibited MAO A, only a few 1,2,3,4-tetrahydroisoquinolines (**1**, **2**, **10**) were found to be potent inhibitors of MAO B (Table I). The most effective were 1,2,3,4-tetrahydroisoquinoline (**1**) and 2-methyl-1,2,3,4-tetrahydroisoquinoline (**2**), producing competitive inhibition with apparent K_i's of 15 and 1 μM, respectively. The only other isoquinoline tested that gave comparable inhibition was compound **10** (K_i = 29 μM). The differences in patterns of inhibition appeared to be strongly influenced by the type and extent of substitutions on the ring.

Evaluation of the 3,4-dihydroisoquinolines revealed that these compounds were more potent competitive inhibitors of MAO A than the 1,2,3,4-tetrahydroisoquinolines with K_i's ranging from 2 to 130 μM (Table II). Equipotent activity in the range of 2–6 μM was found with compounds **25–28**. These included structural analogues containing a methyl group at C-1 or N-2 and mono-, di-, and trimethoxysubstituents. Similar inhibitory activity was observed with compounds **23** and **29**, which gave K_i's of 13 and 10 μM, respectively. The least potent inhibitors in this series were compounds **22** and **24** with K_i's of 130 and 80 μM, respectively.

Inhibition of MAO B by these 3,4-dihydroisoquinolines was highly selective (Table II). The monomethoxy *N*-methyl analogue, **25**, showed competitive inhibition with

(10) Reti, L. *The Alkaloids*; Manske, R. H. F., Ed.; Academic Press: New York, 1954; Vol. 4, pp 23–29.
(11) Collins, M. A. *The Alkaloids*; Brossi, A., Ed.; Academic Press: New York, 1983, Vol. 21, pp 329–358. Bringmann, G.; Schneider, S.; Hille, A. *Nachr. Chem. Tech. Lab.* **1986**, *34*, 223.
(12) Battersby, A.; Edwards, T. *J. Chem. Soc.* **1960**, 1214.
(13) Racemic salsolidine was prepared by reduction of 6,7-dimethoxy-1-methyl-3,4-dihydroisoquinoline with $NaBH_4$ in methanol as described in ref 29.
(14) Schönenberger, B.; Brossi, A. *Helv. Chim. Acta* **1986**, *69*, 1486.
(15) Teitel, S.; O'Brien, J.; Pool, W.; Brossi, A. *J. Med. Chem.* **1974**, *17*, 134.
(16) Teitel, S.; O'Brien, J.; Brossi, A. *J. Med. Chem.* **1972**, *15*, 845.
(17) Buchs, P.; Brossi, A. *Helv. Chem. Acta* **1981**, *64*, 681.

a K_i of 15 μM. Another *N*-methyl derivative, **22**, displayed weaker competitive inhibition (apparent K_i of 76 μM). The remaining isoquinolines within this series showed no inhibitory activity against MAO B within the concentration range tested.

Fully aromatic isoquinoline derivatives produced inhibition of MAO A but not MAO B (Table III). Interestingly, the compound (**34**) with the greatest degree of substitution gave the lowest K_i (17 μM). Comparable competitive inhibition (K_i = 41 μM) was found with compound **33**, the trimethoxy-substituted isoquinoline. The remaining isomers of the fully aromatic isoquinolines produced equipotent K_i's of approximately 150 μM. For compounds **30–32**, the extent of methylation had no apparent effect on inhibitory activity. Compounds **30** and **32** produced mixed patterns of inhibition whereas compound **31** was a competitive inhibitor of MAO A.

Discussion

Much of the ongoing work on parkinsonism has focused on whether environmental or endogenous compounds whose structural features are similar to those of MPTP can undergo biotransformation to form neurotoxins. Although several chemically synthesized analogues of MPTP have been identified,[30] only one naturally occurring counterpart (1,2,3,4-tetrahydroisoquinoline) has been found thus far.[8] The results of our studies, which tested a series of natural and synthetic isoquinoline alkaloids, did not uncover any previously unrecognized substrates for MAO A or B. These findings are in agreement with previous reports in the literature. Gibb et al.[31] were unable to show that salsolinol can be oxidized by crude mitochondrial preparations of MAO. Also, Naoi et al.[32] found that *N*-methylisoquinoline and *N*-methylquinoline can competitively inhibit MAO A, but these compounds apparently do not undergo additional biotransformation.

Many of the compounds tested in our study were found to inactivate MAO A and a few were effective against MAO B. In assessing the inhibitory action of isoquinolines, two molecular features for interaction with these enzymes are important to consider: the aromatic benzene ring A and the basic nitrogen atom that have different basicity depending on the oxidation state.[33]

Stereoselective inhibition of MAO A activity was observed with the stereoisomers of salsolinol (**3**), salsoline (**7**), salsolidine (**9**), and carnegine (**11**). In all cases, the *R* isomer was the strikingly more potent inhibitor of the two stereoisomers. This effect might be explained by steric hindrance in the *S* isomers of the lone-pair electrons on the nitrogen by the 1-methyl group. Binding is not impaired in the equally basic 3,4-dihydroisoquinolines, which are achiral. In fully aromatic isoquinolines, binding at this site may be low because the nitrogen has low basicity.

MAO A is markedly inhibited by 3,4-dihydroisoquinolines that possess a basic nitrogen and substituents in ring A that provide a hydrophobic region. However, the weberines (**14–18**), which are tetraoxygenated in ring A, apparently are too hydrophilic and sterically congested to allow efficient interaction with the enzyme. The high potency of the quaternary salt **25** against MAO A and B suggests that these macromolecules will tolerate a positively charged nitrogen. (*S*)-Carnegine, **11S**, was about 50-fold less inhibitory against MAO B than its desmethyl analogue, **10**, indicating further constraints on the C-1 position of these alkaloids. The inhibition constant produced by compound **22** was about 5 times greater than the inhibitory activity of **25** and some 70 times less potent than the tetrahydroisoquinoline analogue, **2**. This result suggests that a positively charged amine is not well tolerated within the active site of MAO B unless the charge is offset by hydrophobic substituents on the phenyl ring. Therefore, it appears that the active site of MAO B is selective for isoquinolines possessing extensive hydrophobic character. This interpretation is supported by recent studies on the amino acid sequences of MAO A and B that were deduced from their cloned cDNA seqeunces.[34] Hydropathy plots of these enzymes show a small but significant difference in amino acid residues 270–290 in which MAO B exhibits a more hydrophobic character.

A comparison of the potency of methylated analogues versus their nonmethylated congeners show that methylation increases inhibitory activity. Furthermore, these findings illustrate that methoxy-substituted tetrahydroisoquinolines are more potent inhibitors than the corresponding catechol isoquinolines. These results also suggest that the active site of MAO A is less restrictive and hydrophobic in nature than that of MAO B. Additional studies are necessary to confirm these conclusions and to aid in mapping the isoquinoline binding sites on MAO A and B.

Salsolinol and a few related isoquinolines have previously been shown to inhibit MAO from crude mitochrondrial preparations and other enzymes involved in neurotransmitter metabolism, including catechol-*O*-methyltransferase and phenylethanolamine *N*-methyl transferase.[35-39] In general, MAO A was inhibited more exten-

(18) Leonard, N. Y.; Leubner, G. W. *J. Am. Chem. Soc.* **1949**, *71*, 3408.
(19) Mizra, R. *J. Chem. Soc.* **1957**, 4400.
(20) Karady, S. *J. Org. Chem.* **1962**, *27*, 3720.
(21) Brossi, A.; Blount, J.-F.; O'Brien, J.; Teitel, S. *J. Am. Chem. Soc.* **1971**, *93*, 6248.
(22) Takahashi, K.; Brossi, A. *Heterocycles* **1982**, *19*, 691.
(23) Hahn, G.; Stiehl, K. *Chem. Ber.* **1936**, *69*, 2627.
(24) Hahn, G.; Rumpf, F. *Chem. Ber.* **1938**, *71*, 2141.
(25) Bruderer, H.; Brossi, A. *Helv. Chim. Acta* **1965**, *48*, 1945.
(26) Whaley, W. M.; Govindachari, T. R. Synthesis of Isoquinolines I In *Organic Reactions*; Adams, R., Ed.; John Wiley & Sons: New York, 1951, Vol. 6, pp 74–150.
(27) Knabe, J. *Arch. Pharm.* **1959**, *292*, 652.
(28) Späth, E. *Monatsch. Chem.* **1921**, *42*, 97.
(29) Brossi, A.; Dolan, L. A.; Teitel, S. In *Organic Syntheses*; Büchi, J. H., Ed.; John Wiley: New York, 1977; Vol. 56, pp 3–6.
(30) Wilkening, D.; Vernier, V. G.; Arthaud, L. E.; Treacy, G.; Kenney, J. P.; Nickolson, V. J.; Clark, R.; Smith, D. H.; Boswell, G. *Brain Res.* **1986**, *368*, 239. Fuller, R. W.; Henrick-Leuke, S. K. *Res. Commun., Chem. Pathol. Pharmacol.* **1986**, *53*, 167. Fuller, R. W.; Robertson, D. W.; Hemrick-Leuke, S. K. *J. Pharmacol. Exp. Ther.* **1987**, *240*, 415. Youngster, S. K.; Sonsalla, P. K.; Heikkila, R. E. *J. Neurochem.* **1987**, *48*, 929. Harik, S. I.; Schemdley, J. W.; Iacofano, L. A.; Blue, P.; Arora, P. K.; Sayre, L. M. *J. Pharmacol. Exp. Ther.* **1987**, *241*, 669. Kindt, M. V.; Heikkila, R. E.; Nicklas, W. J. *J. Pharmacol. Exp. Ther.* **1987**, *241*, 669. Kindt, M. V.; Heikkila, R. E.; Nicklas, W. J. *J. Pharmacol. Exp. Ther.* **1987**, *242*, 858.
(31) Gibb, C.; Willoughby, J.; Glover, V.; Sandler, M.; Testa, B.; Jenner, P.; Marsden, C. D. *Neurosci. Lett.* **1987**, *48*, 709.
(32) Naoi, M.; Hirata, Y.; Nagatsu, T. *J. Neurochem.* **1987**, *48*, 709.
(33) Albert, A. In *Physical Methods in Heterocyclic Chemistry*; Katritzky, A. R., Ed.; Academic Press: New York, 1963; pp 2–55.
(34) Bach, A. W. J.; Lan, N. C.; Johnson, D. L.; Abell, C. W.; Bembenek, M. E.; Kwan, S.-W.; Seeburg, P. H.; Shih, J. C. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4934.
(35) Collins, A. C.; Cashaw, J. L.; Davis, V. E. *Biochem. Pharmacol.* **1973**, *22*, 2337.
(36) Giovine, A.; Renis, M.; Bertolini, A. *Parmacol.* **1976**, *14*, 86.
(37) Meyerson, L. R.; McMurtrey, K. D.; Davis, V. E. *Biochem. Pharmacol.* **1976**, *25*, 1013.
(38) Grunewald, G. L.; Sall, D. J.; Monn, J. A. *J. Med. Chem.* **1988**, *31*, 824.

sively than MAO B, a finding that is in agreement with the results reported here. The ability of isoquinolines to inhibit these enzymes in vitro suggests that selected alkaloids have the potential to interfere with mammalian neurotransmitter metabolism.

Experimental Section

Chemical Synthesis and Analysis. The following instruments were used. Melting point: Fisher-Johns melting point apparatus. Optical rotation: Perkin-Elmer 241 MC polarimeter. UV spectra: Hewlett Packard 845A. IR spectra: Beckman IR 4230. ^{1}H NMR spectra: Varian XL 300 (300 MHz). MS: Finnegan 1015D instrument (CI). TLC: silica gel GHLF plates from Analtech, Inc. CC: silica gel 60 (Merck), 230–400 mesh, 60 A (flash chromatography was performed with CH_2Cl_2/MeOH (10:1) as eluent).

(+)-(*R*)-Salsolidine Hydrochloride (9R) and (-)-(*S*)-Salsolidine Hydrochloride (9S). The two optical isomers were prepared from racemic salsolidine (**9**) by separation as diastereomeric (1-phenylethyl)ureas by fractional crystallization.[14]

(1*S*)-2-[((*R*)-1′-Phenylethyl)carbamoyl]salsolidine (38): mp 224–225 °C (lit.[14] mp 199–202 °C); $[\alpha]^{23}$ represents (c = 0.72, $CHCl_3$) {lit.[14] $[\alpha]^{rt}$ = +48.0 (c = 0.7, $CHCl_3$)}; CIMS m/z 355 (M^+ + 1, 12), 208 (100). This urea was identical on TLC with an authentic sample, but represents improvement in the enantiomeric purity of the previously reported compound.[14]

(1*R*)-2-[((*R*)-1′-Phenylethyl)carbamoyl]salsolidine (39): $[\alpha]^{23}$ -101.5 (c = 0.63, $CHCl_3$) {lit.[10] $[\alpha]^{rt}$ -101.6° (c = 0.9, $CHCl_3$)}; CIMS m/z 355 (M^+ + 1, 56), 208 (100); identical on TLC with an authentic sample of (1*S*)-2-[((*R*)-1′-phenylethyl)carbamoyl]-salsolidine (**38**).

(-)-(1*S*)-Salsolidine hydrochloride (9S): mp 242 °C (lit.[14] mp 238–240 °C); $[\alpha]^{22}$ -23.9° (c = 0.43, H_2O) {lit.[14] $[\alpha]^{rt}$ -25.6° (c = 2.1, H_2O)}; CIMS m/z 208 (M^+ + 1, 100).

(+)-(1*R*)-Salsolidine hydrochloride (9R): mp 241 °C (lit.[10] mp 240–242 °C); $[\alpha]^{23}$ +23.2° (c = 0.5, H_2O) {lit.[14] $[\alpha]^{rt}$ +24.1° (c = 1.8, H_2O)}; CIMS m/z 208 (M^+ + 1, 100).

(-)-(1*R*)-Carnegine Hydrochloride (11R). (1*R*)-(+)-Salsolidine hydrochloride (**9R**) (148 mg, 0.61 mmol) was dissolved in 20 mL of MeOH to which 0.3 mL of 40% HCHO (4 mmol) was added. Raney Ni (50 mg) was added and the mixture was shaken under 50 psi H_2 for 5 h at ambient temperature. After removal of the catalyst by filtration, the solvent was removed by evaporation and the resulting residue taken up in 2% HCl. The acidic solution was washed (ethyl ether) and then made alkaline with KOH. The basic solution was extracted with EtOAc and then the organic layer was dried ($MgSO_4$) and the solvent evaporated. (1*R*)-(-)-Carnegine hydrochloride (**11R**) was crystallized from EtOH, yielding colorless crystals: mp 202–203 °C [lit.[3] mp 210 °C (rac)]; $[\alpha]^{22}$ -5.1° (c = 0.3, H_2O); CIMS m/z 222 (M^+ + 1, 100). Anal. ($C_{13}H_{20}NO_2Cl$) C, H, N.

(+)-(1*S*)-Carnegine Hydrochloride (11S). Compound **11S** was prepared from **9S** exactly as described for **11R**: mp 203 °C [lit.[3] 210 °C (rac)]; $[\alpha]^{22}$ +5.3° (c = 0.345, H_2O); CIMS m/z 222 (M^+ + 1, 100). Anal. ($C_{13}H_{20}NO_2Cl$) C, H, N.

***N*-Methyl-5,6,7,8-tetramethoxy-1,2,3,4-tetrahydroisoquinoline Methiodide (17).** Weberine (60 mg) was dissolved in 1 mL of warm THF. CH_3I (1.05 mmol) was added and the mixture was stirred at room temperature until a pale yellow precipitate formed. After filtration and washing with Et_2O, pure weberine methiodide was obtained (80 mg, 87%): mp 208–209 °C; CIMS m/z 282 (M^+ - HI), 268 (100); ^{1}H NMR (CD_3OD) δ 4.54 (s, 1 H, H-C(1)), 3.90–3.88 (m, 12 H, 4 $ArOCH_3$), 3.66 (br m, 2 H, H- and H′-C(2)), 3.22 (br s, 6 H, $(CH_3)_2N$), 3.1 (br m, 2 H, H- and H′-C(4)). Anal. ($C_{15}H_{24}NO_4I$) C, H, I.

6-Hydroxy-7-methoxy-1-methylisoquinoline (31) and 6-(Benzyloxy)-7-methoxy-1-methylisoquinoline (36). A suspension of 10% Pd/C (0.55 g) in toluene (30 mL) was stirred under Ar at reflux for 30 min. A solution of *O*-benzylsalsoline (**35**, 1.09 g, 3.85 mmol) in toluene (95 mL) was added and the mixture was stirred under reflux for 1 h. Toluene (50 mL) was added and the hot mixture was filtered through Celite. The catalyst was washed with boiling toluene (50 mL) and then solvent was evaporated under vacuum. The resulting solid (0.585 g, 80%) was recrystallized from 95% EtOH to give 0.455 g of crystalline **31**: mp 240–250 °C dec; UV (EtOH) 238 nm (ϵ 37900), 282 (4000), 312 (2090), 326 (3100), 365 (1250); CIMS m/z 190 (M^+ + 1, 100); ^{1}H NMR ($CDCl_3$) δ 1.51 (s, 1 H, disappears upon addition of D_2O, OH), 2.82 (s, 3 H, CH_3-C(1)), 4.01 (S, 3 H, Ar-OCH_3), 7.13, 7.23 (2 s, 2 H, Ar-H), 7.28, 8.18 (2 d, J = 6 Hz, 2 H, Ar-H). Anal. ($C_{11}H_{11}NO_2$) C, H, N. After flash chromatography of the mother liquors, compound **36** (20 mg) was obtained: mp 148–149 °C; CIMS m/z 280 (M^+ + 1, 100), 108 (38); ^{1}H NMR ($CDCl_3$) δ 2.89 (s, 3 H, CH_3-C(1)), 4.04 (S, 3 H, Ar-OCH_3), 5.29 (s, 2 H, Ar-CH_2), 7.09 (s, 1 H, Ar-H), 7.26–7.52 (m, 7 H, Ar-H), 8.27 (d, J = 6 Hz, 1 H, Ar-H). Anal. ($C_{18}H_{17}NO_2$) C, H, N.

6-Hydroxy-7-methoxy-1-methylisoquinoline Methosulfate (32). Compound **31** (0.145 g, 0.77 mmol) and dimethyl sulfate (0.23 mL) in acetonitrile (16 mL) were refluxed under Ar for 5 h. After cooling, crystals of pure **32** (140 mg, 58%) were collected: mp 260 °C dec; CIMS m/z 204 (M^+ + 1, 100); ^{1}H NMR (D_2O) δ 2.96 (s, 3 H, CH_3-C(1)), 3.95 (s, 3 H, CH_3N), 4.26 (s, 3 H, Ar-OCH_3) 7.02, 7.29 (2 s, 2 H, Ar-H), 7.68, 8.08 (2 d, J = 7 Hz, 2 H, Ar-H). Anal. ($C_{13}H_{17}NO_6S$) C, H, N.

6,7-Dihydroxy-1-methylisoquinoline (30). Compound **31** (0.189 g, 1 mmol) and 48% HBr (6.2 mL) were refluxed under Ar for 5 h. After cooling, crystalline **30** (0.245 g, 96%) was obtained as the HBr salt and was recrystallized from 95% EtOH: mp 269–270 °C dec; CIMS m/z 176 (M^+ + 1, 100), 160 (10); ^{1}H NMR (CD_3OD) δ 3.01 (s, 3 H CH_3-C(1)), 7.38, 7.63 (2 s, 2 H, Ar-H), 7.90, 8.02, (2 d, J = 6 Hz, 2 H, Ar-H). Anal. ($C_{10}H_{10}NO_2Br$) C, H, N. The hydrobromide salt was converted into the base by dissolving it in water and neutralizing with 3% NH_4OH, filtration of solids, and crystallization from EtOH: mp 235–240 °C dec; UV (EtOH) 237 nm (ϵ 36800), 275 (5500), 331 (3900), 359 (3600); CIMS m/z 176 (M^+ + 1, 100), 160 (9); ^{1}H NMR (DMSO-d_6) δ 2.68 (s, 3 H, CH_3-C(1)), 7.04, 7.30 (2 s, 2 H, Ar-H), 7.31, 7.98 (2 d, J = 6 Hz, 2 H, Ar-H).

6,7,8-Trimethoxyisoquinoline Hydrochloride (33). Prepared from 6,7,8-trimethoxytetrahydroisoquinoline (**37**) as described for compound **31**, except that the solvent used was xylene. The HCl salt was crystallized from MeOH–EtOAc: mp 175–176 °C; UV (EtOH) 240 nm (ϵ 34500), 258 (27100); CIMS m/z 220 (M^+ + 1, 100); ^{1}H NMR ($CDCl_3$) δ 4.01 (s, 3 H, Ar-OCH_3), 4.13 (s, 3 H, OCH_3), 4.22 (s, 3 H, Ar-OCH_3), 7.05 (s, 1 H, Ar-H), 7.88, 8.31 (2 d, J = 6.5 Hz, 2 H, Ar-H), 9.47 (s, 1 H, Ar-H). Anal. ($C_{12}H_{14}NO_3Cl$) C, H, N.

5,6,7,8-Tetramethoxy-1-methylisoquinoline Hydrochloride (34). Prepared from the tetrahydroisoquinoline **16** as described for **31**. After flash chromatography, a crystalline material was isolated: mp 144–145 °C; UV (EtOH) 242 nm (ϵ 53800), 263 (43600); CIMS m/z 264 (M^+ + 1, 100); ^{1}H NMR ($CDCl_3$) δ 3.45 (s, 3 H, Ar-OCH_3), 4.02 (s, 3 H, Ar-OCH_3), 4.06 (s, 3 H, Ar-OCH_3), 4.19 (s, 3 H, Ar-OCH_3), 8.13–8.20 (m, 2 H, Ar-H). Anal. ($C_{14}H_{18}NO_4Cl$) C, H, N.

Biochemical Methods. Isolation of Human Placental MAO A and Human Liver MAO B. Human placental MAO A was prepared according to the method of Weyler and Salach[40] with modifications. Briefly, the mitochondria from seven to eight placentas were resuspended in 160 mL of 0.1 mM PMSF, 0.1 M TEA, pH 7.4, using a loose-fitting Teflon homogenizer. The protein concentration was adjusted to 20–30 mg/mL and then calcium chloride was added to a final concentration of 25 mM. For every 500 mg of mitochondrial protein in the suspension, 1 mg and 670 units of phospholipase C and A were added, respectively. The mixture was stirred at room temperature for 1.5 h while the pH was maintained at 7.4 with 2 M ammonium hydroxide. After lipase treatment, the suspension was centrifuged at 45000*g* for 30 min at 4 °C. The pellet was resuspended in 0.1 mM PMSF, 0.1 M TEA, pH 7.4, and centrifuged as before. The resulting mitochondrial pellet was finally resuspended in 0.1 mM PMSF, 0.1 M TEA, pH 7.4, and then extracted with Triton X-100 (1 mg of detergent/3 mg of protein). The suspension was stirred at room temperature for 30 min. After centrifugation at 45000*g* for 20 min at 4 °C, the supernatant was collected and the pellet

(39) Gheng, B. Y.; Origitano, T. C.; Collins, M. A. *J. Neurochem.* **1987**, *48*, 779.

(40) Weyler, W.; Salach, J. I. *J. Biol. Chem.* **1985**, *260*, 13199.

was extracted a second time as described above. The supernatants were pooled and then dialyzed overnight against 2 L of 0.1 mM PMSF, 20 mM NaPi, pH 7.4, containing 50 g of SM-2 Bio-Beads to remove the Triton X-100. For every 8 mL of dialyzed solution, 0.5 g of dextran (MW 500 000) and 0.4 g of polyethylene glycol (MW 8000) were added. The suspension was stirred at room temperature until both solids were dissolved and then centrifuged in a swinging bucket rotor at 10000g for 15 min at 10 °C. The top phase (polyethylene glycol) was removed, solid ammonium sulfate was slowly added to make a 12% (w/v) solution, and the suspension was stirred at 4 °C for 30 min. After centrifugation at 20000g for 20 min at 4 °C, the pellet was resuspended in 5–10 mL of 20% glycerol (w/v), 25 mM NaPi, pH 7.4, 1 mM EDTA, 3 mM 2-ME, and 0.1 mM PMSF and dialyzed overnight at 4 °C against 2 L of the same buffer. The sample was then passed through a CM-cellulose column previously equilibrated with 20% glycerol, 20 mM NaPi, pH 6.4, 3 mM 2-ME, 1.25 mM EDTA, and 0.1 mM PMSF. Fractions of MAO A activity (assayed versus kynuramine) that eluted in the void volume were pooled and β-D-octyl glucoside was added to a final concentration of 0.8% (w/v). This sample was immediately loaded onto a DEAE-Sepharose Cl-4B column previously equilibrated with 20% glycerol, 20 mM NaPi, pH 7.4, 3 mM 2-ME, 1 mM EDTA, and 0.8% β-D-octyl glucoside. The fraction with MAO A activity was then eluted by using a linear gradient of 20–200 mM NaPi, pH 7.4, containing 20% glycerol, 0.8% β-D-octyl glucoside, 1 mM EDTA, and 3 mM 2-ME. The enzyme fraction with activity that elutes in the last third of the gradient was then divided into various fractions and dialyzed versus 2 L of 20 mM NaPi, pH 7.4, 20% glycerol, 3 mM 2-ME, and 1 mM EDTA to remove β-D-octyl glucoside. The enzyme fractions containing the highest specific activity were then concentrated. After the volume was reduced to a protein concentration of 3–7 mg/mL, glycerol was added to a final concentration of 50% (v/v) and the purified MAO A stored at −70 °C.

Human liver MAO B was prepared from human autopsy liver according to the method of Patel et al.[41] with minor modifications. Briefly, isolated human liver mitochondria were homogenized in 10 volumes of ice-cold water and then pelleted at 105000g for 15 min. The pellets were homogenized in 1.5% Triton X-100 (v/v), 0.1 M NaPi, pH 8.0, and stirred for 1.5 h. The suspension was then centrifuged at 105000g for 1.5 h. The supernatant was incubated overnight with an excess amount of a monoclonal antibody specific for MAO B (MAO B-1C2) and then passed through a protein A Sepharose column (Pharmacia). The column was washed with 10 bed volumes of 0.5% Triton X-100, 25 mM NaPi, pH 8.0, and MAO B:MAO B-1C2 was eluted by washing the protein A column with 0.5% Triton X-100, 25 mM NaPi-citrate, pH 5.7. After neutralizing the fractions with 1 M NaPi, pH 10.0, the peak of activity (assayed versus benzylamine) was pooled and treated with 40 g of SM-2 Bio-Beads to remove Triton X-100. The solution was separated from the Bio-Beads by filtration and dialyzed against 10 mM NaPi, pH 7.4, until MAO B:MAO B-1C2 precipitated. The enzyme antibody complex was collected by centrifugation and resuspended in 50% glycerol (v/v), 25 mM NaPi, pH 7.4, 0.1 mM dithiothreitol. MAO B:MAO B-1C2 retains full catalytic activity for 10–12 months when stored at −20 °C.

Enzyme Assays. Assays were carried out in a DU-7 HS Beckman spectrophotometer. For inhibitor studies, the appropriate enzyme (MAO A or MAO B) at a concentration of between 5 and 7 μg was incubated versus various concentrations of test compound in 0.5 mL of 50 mM NaPi, pH 7.6. Kynuramine was added at a concentration of twice the K_m value, and the initial rates of the reaction were monitored in duplicate at 314 nm (4-hydroxyquinoline, ϵ = 12 600/M cm) for 3 min at 30 °C. The maximum concentration of a particular inhibitor within the dihydro- and fully aromatic isoquinoline classes was limited by its overlapping absorption spectral value at 314 nm. Therefore, the upper concentration limits for these particular test compounds are listed in the legends of Tables II and III. I_{50} values were determined for each test compound and used as initial concentrations for K_i studies (data not shown). MAO A or B was then mixed with various concentrations (0.5–2 K_m) of kynuramine (for MAO A, K_m = 33 μM; for MAO B, K_m = 34 μM) to obtain initial reactions rates in the absence of any inhibitor. Increasing concentrations of individual alkaloids were added to each enzyme at various fixed concentrations of kynuramine. Duplicate values of rate measurements varied no more than ±10% in all experiments. Double reciprocal ($1/v$ versus $1/[S]$) and Hanes-Woolf plots ($[S]/v$ versus v) were analyzed on an IBM-PC to determine the type of inhibition (Eric Gordon, personal program). The K_i value for each of the test substances was obtained by replotting the data collected over a 3–4-fold concentration range as described by Segel.[42] The curves produced from these plots had linear correlation coefficients of 0.98 or greater. Test compounds were classified as noninhibitory if the activity of the enzyme did not fall below 80% of the activity in the absence of the test substance (concentration of kynuramine was 2 K_m).

Determination of the ability of these isoquinolines to act as substrates for MAO A and B was assessed by two methods. For non-catecholic compounds, an indirect assay was used that measured the production of hydrogen peroxide by a colorimetric method.[43,44] A second method used direct spectral scanning of the test substance in the presence and absence of MAO.

Incubations were carried out for as long as 1 h over temperature and pH ranges of 30–37 °C and 7.5–9.5, respectively, at concentrations of compound as low as 2 K_i and as high as 10 mM. The level of sensitivity of these methods would permit detection of as little as approximately 5% oxidation of the test compound.[44] None of the isoquinoline alkaloids tested were found to serve as substrates for MAO A or B (data not shown).

(Abbreviations: MAO A and B, monoamine oxidase A and B; NaPi, sodium phosphate; 2-ME, 2-mercaptoethanol; EDTA, ethylenediaminetetraacetic acid; TEA, triethanolamine; PMSF, phenylmethanesulfonyl fluoride.)

Acknowledgment. This work was generously supported by Grant NS-24932 from the National Institutes of Health, DHHS (C.W.A.), and a BRSG grant through the University Research Institute of the University of Texas at Austin (M.E.B.).

Registry No. 1, 91-21-4; **2**, 53112-33-7; **3R**, 38221-22-6; **3S**, 38221-21-5; **4**, 57553-18-1; **5**, 123535-89-7; **6**, 98321-33-6; **7R**, 51424-33-0; **7S**, 881-26-5; **8**, 123438-40-4; **9R**, 110691-65-1; **9S**, 883-87-4; **10**, 123463-05-8; **11R**, 123438-41-5; **11S**, 65140-22-9; **12**, 123463-06-9; **13**, 123463-07-0; **14**, 82261-01-6; **15**, 74947-49-2; **16**, 123537-27-9; **17**, 123438-42-6; **18**, 123535-90-0; **19**, 88598-22-5; **20**, 94885-90-2; **21**, 110691-59-3; **22**, 3947-78-2; **23**, 4602-71-5; **24**, 4667-12-3; **25**, 123438-43-7; **26**, 30045-07-9; **27**, 4721-98-6; **28**, 123438-44-8; **29**, 82261-03-8; **30**, 123463-08-1; **30** (base), 123438-49-3; **31**, 57229-60-4; **32**, 123438-45-9; **33**, 123438-46-0; **34**, 93627-72-6; **35**, 123438-47-1; **36**, 123438-48-2; **37**, 642-30-8; weberine, 74046-24-5; monoamine oxidase, 9001-66-5.

(41) Patel, N.; Fritz, R. R.; Abell, C. W. *Biochem. Biophys. Res. Commun.* **1984**, *125*, 748.

(42) Segel, I. H. In *Biochemical Calculations*, 2nd ed.; John Wiley & Sons: New York, 1968; pp 208–272.

(43) Majkic-Singh, N.; Conteh, B.; Stojanov, M.; Berkers, I. *Enzyme* **1983**, *29*, 120.

(44) Brossi, A.; Gessner, W. P.; Fritz, R. R.; Bembenek, M. E.; Abell, C. W. *J. Med. Chem.* **1986**, *29*, 444.