

In Vitro Stereoselective Metabolism of the Psychotomimetic Amine, 1-(2,5-Dimethoxy-4-methylphenyl)-2-aminopropane. An Apparent Enantiomeric Interaction

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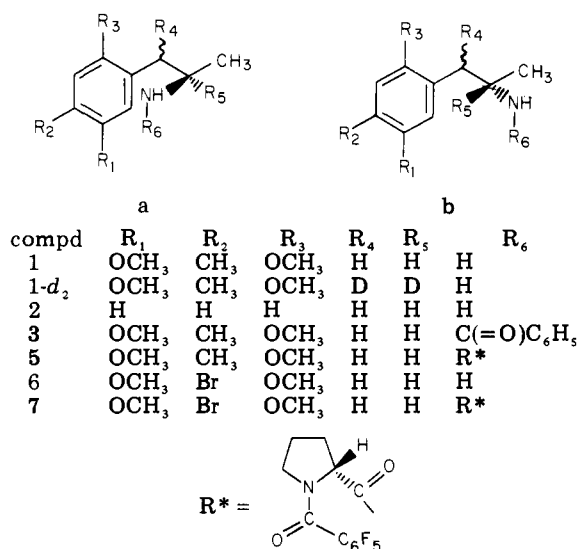
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Received February 27, 1976

The stereoselective metabolism [R/S (metabolized) < 1] of the psychotomimetic amine (R,S)-1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane in 10000g rabbit liver homogenate supernatant and 100000g microsomal fractions has been demonstrated with the aid of the chiral reagent (S)- N -pentafluorobenzoylpropyl-1-imidazolide and GLC analyses. In contrast to the enantiomeric discrimination observed with racemic amine, the individual isomers were metabolized at approximately the same rate. This apparent enantiomeric interaction illustrates the fact that racemates should be viewed as unique chemical species with pharmacodynamic and toxicologic profiles potentially different from the individual antipodes.

Variations in the biological activities and dispositions of drugs with the configurations about chiral centers are well documented.¹ The human psychotomimetic properties of the title compound, 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (STP, DOM, 1),² have been shown to be sensitive to the configuration about its chiral center with the R isomer **1a** being twice as active as racemic **1** (**1a,b**); the S isomer **1b** is devoid of activity at the doses studied.³ A similar stereochemical dependence is associated with this amine's ability to disrupt conditioned avoidance behavior in rats⁴ and to cause hyperthermia in rabbits.⁵ The serotonin agonist activity of **1b**, however, is reported to be significantly greater than that of **1a**.⁶ In acute toxicity studies in mice, Zweig obtained the same LD₅₀ values for the two enantiomers (115 mg/kg) but a considerably lower value (80 mg/kg) for **1a,b**.⁷ These considerations have led us to undertake an investigation of the stereochemical course followed in the metabolism of amine **1** as part of our program to evaluate potential relationships between biological activity and metabolic fate of psychotomimetic 1-phenyl-2-amino-
propanes.⁸⁻¹⁰

In a previous study we showed that following intraperitoneal administration of **1a,b** to rabbits, more **1a** is excreted in the urine than **1b**.⁸ A similar metabolic stereochemical preference for (S)-amphetamine (**2b**) has been reported in man following administration of either racemic drug (**2a,b**)¹¹ or the individual enantiomers.¹² Rabbit liver microsomal preparations of amphetamine, however, metabolize pure (R)-amphetamine (**2a**) about five times faster than pure (S)-amphetamine (**2b**).¹³ This paper reports our results on the extent to which **1a,b**, **1a**, and **1b** are metabolized in rabbit liver 10000g supernatant fractions at fairly high initial substrate concentrations (250–770 μ M).¹⁴ Additionally, we have examined the rates of metabolism of these compounds in 100000g microsomal preparations

at low initial substrate concentrations (7.5–30 μ M).



Results and Discussion

Our initial studies with the 10000g supernatant fractions employed **1a,b**-1-¹⁴C as substrate⁸ and a radiochemical isotope dilution analysis on the N -benzoyl derivative **3** of **1** to estimate the overall percent metabolism. The enantiomeric composition of **1** in the postincubate was determined by derivatization with (S)- N -pentafluorobenzoylpropyl-1-imidazolide (**4**)¹⁶ to form the diastereomeric amides **5a** and **5b** from **1a** and **1b**, respectively, followed by GLC analysis employing a flame ionization detector. In order to study the individual isomers in this series, the liver homogenate from a single animal was divided into three equal portions in which **1a**, **1b**, and **1a,b**-¹⁴C were incubated separately. The overall percent

Table I. Comparison of the in Vitro Metabolism of 1a, 1b, and 1a,b by 10 000g Rabbit Liver Homogenate Supernatant Fractions

Expt	Compd	Initial concn, μ M	% metabolized ^a	μ mol metabolized ^b			R/S (1a/1b) metabolized
				1a,b	1a	1b	
1	1a,b	770	30	3.9	1.5	2.4	0.64 ^c
	1a	510	43 ^c		3.6		
	1b	510	43 ^c			3.6	1.0 ^d
2	1a,b	370	55	3.5	1.1	2.4	0.44
	1a,b	370	56	3.5	1.0	2.5	0.38
3	1a	370	65 ^c		4.0		
	1b	370	59 ^c			3.6	1.1 ^d
4	1a,b	310	61	3.2	0.8	2.4	0.35
5	1a,b	280	67	3.1	0.9	2.2	0.41
6	1a,b	250	63	2.6	0.5	2.1	0.24
	1a,b	250	58 ^e	2.4	0.6	1.8	0.33
7	1a	250	56 ^e		2.3		
	1b	250	64 ^e			2.7	0.88 ^c

^a Racemic incubations determined by radiochemical isotope dilution analysis of 1 as its *N*-benzoyl derivative 3. ^b Calculated from the experimentally determined enantiomeric composition of 1a,b in the postincubate and the percent of 1 metabolized. ^c Determined by GLC analysis of the prolylamides 5a and 5b. ^d Calculated from percent metabolized. ^e Determined by CI-MS stable isotope dilution analysis.

metabolism for the individual isomers was estimated by adding to each postincubation mixture measured amounts of the opposite enantiomer which served as an internal standard in the subsequent GLC assay. In one experiment the percent metabolism was estimated by a direct insertion probe chemical ionization mass spectral (CIMS) stable isotope dilution analysis^{14,17} with the dideuterio derivative 1a,b-*d*₂¹⁴ serving as internal standard.

Table I summarizes the results from the incubations with the 10000g rabbit liver homogenate supernatant fractions. In those experiments employing various initial concentrations of 1a,b, it was apparent that 1b was more extensively metabolized than 1a. In general, the enantiomeric ratio of 1a/1b metabolized decreased with decreasing initial substrate concentration (from 770 to 250 μ M), 1a showing decreased consumption while 1b was metabolized to a similar extent over the entire concentration range.

This in vitro stereoselectivity was consistent with our in vivo rabbit studies⁸ but opposite from the stereoselectivity reported for the rabbit liver microsomal metabolism of the individual enantiomers of amphetamine.¹² In order to be able to compare more directly our results with those reported for amphetamine, we examined the metabolism of the individual enantiomers of 1. At all concentrations studied (see Table I) the individual enantiomers were metabolized approximately to the same extent [*R/S* metabolized = 0.99 ± 0.09 ($n = 3$)], a result clearly different from the stereoselectivity observed in the racemic case [*R/S* metabolized = 0.40 ± 0.12 ($n = 7$)]. Racemization of either enantiomer during the incubation or work-up was shown not to have occurred since GLC analyses of postincubates of 1a and 1b with the prolyl reagent revealed the presence of only the amide corresponding to the substrate amine.

The relative extents of metabolism of 1a and 1b with the racemic drug vs. the individual isomers indicated that an enantiomeric interaction was influencing the metabolic fate of 1a,b. The high substrate concentration used in these experiments could have led to substrate inhibition of the mixed function oxidase enzyme system as reported for the aromatic hydroxylation of amphetamine.¹⁸ Additionally, these experiments could have involved complications resulting from stereoselective interactions of substrate with the soluble protein fraction present in the 10 000g liver preparation. These considerations led us to undertake a second series of experiments to determine the

ratio of the disappearance rates of 1a to 1b in microsomal preparations.¹⁵ In order to avoid enzyme saturation or inhibition, we worked in the initial substrate concentration range of 7.5–30 μ M. These concentrations are well below the *K*_m values reported for metabolic conversions such as the demethylation of codeine,¹⁹ the demethylation of (+)- and (–)-benzphetamine,^{13b} and the aromatic methyl oxidation of tolbutamide.²⁰

In order to work at these low substrate concentrations, it was necessary to develop an assay sensitive at the picomole level. The electron capture (EC) sensitive nature of the prolylamides 5a and 5b¹⁶ allowed us to use an EC detector which provided the required sensitivity for our GLC assay. The amides 5a and 5b were synthesized and used extensively in the development of the assay. An analogue of 1, the bromo compound 6b, served as internal standard in all analyses. Under the optimized derivatization conditions established, essentially 100% conversion of mixtures of 1a, 1b, and 6b to 5a, 5b, and 7b, respectively (comparison of peak areas after injection of pure 5a and 5b vs. peak areas obtained with reaction mixtures), and near baseline resolutions (SP 2250 column) were achieved.

A standard curve was prepared by carrying through the entire procedure measured amounts of 1a,b using liver microsomal suspensions kept cold and devoid of NADPH (to prevent loss of 1a and 1b by metabolism). The plot of peak height or peak area ratios (5a/7b and 5b/7b) gave equally satisfactory straight lines and therefore the more convenient peak height ratios have been used throughout. Periodically, the standard curve was redetermined and coefficients of determination²¹ of 0.98 or better were routinely obtained. Corresponding standard curves also were employed for studies of the individual enantiomers. Protein concentrations were determined for all the microsomal incubates by the method of Sutherland et al.²² When incubations were carried out with boiled liver homogenates or microsomal systems not supplemented with NADPH, no evidence of metabolism was observed (100% recovery of unchanged amine by radioisotope dilution and GLC analyses). In order to establish that the amount of NADPH added to the incubation mixture was not limiting the metabolism of 1 during the time interval studied, fresh 1b was added to a 30 μ M postincubation mixture. Since the rate of disappearance of the newly added 1b was not dramatically different from the initial rate, the NADPH concentration was shown to be sufficient.

The results from these studies are summarized in Table

Table II. Kinetic Comparison of the in Vitro Metabolism of 1a, 1b, and 1a,b by 100 000g Rabbit Liver Microsomal Fractions

Expt	Substrate	Initial concn, μM	mg of microsomal protein/ml ^a	Nonnormalized disappearance rate constants		Normalized half-lives, min		Ratio of normalized half-lives, R/S (1a/1b)
				k_{1a}	k_{1b}	1a	1b	
1	1a,b	30	2.44*	0.20	0.30	8.4	5.7	1.5
2	1a,b	30	1.76	0.087	0.20	14	6.2	2.3
3	1a,b	30	1.04	0.054	0.11	13	6.4	2.1
4	1a,b	30	0.713	0.042	0.065	12	7.6	1.6
5	1a,b	30	0.623	0.047	0.049	9.2	8.8	1.1
6	1a,b	15	2.32*	0.33	0.48	4.9	3.4	1.5
7	1a,b	15	1.61	0.17	0.25	6.6	4.5	1.5
8	1a	30	2.13*	0.22		6.8		
9	1a	30	2.28*	0.23		6.8		
10	1b	30	2.03*		0.22		6.5	1.0
11	1b	30	2.32*		0.22		7.4	
12	1a	15	1.64	0.22		4.9		
13	1a	15	2.09*	0.29		5.0		1.1
14	1b	15	1.64		0.18		6.2	
15	1b	15	2.28*		0.34		4.7	
16	1a	7.5	2.16*	0.44		3.4		
17	1a	7.5	2.25*	0.46		3.4		0.84
18	1b	7.5	2.47*		0.37		4.6	
19	1b	7.5	2.30*		0.46		3.4	

^a Incubations done with microsomes pooled from three rabbits are asterisked. The remainder were done with microsomes from individual rabbits.

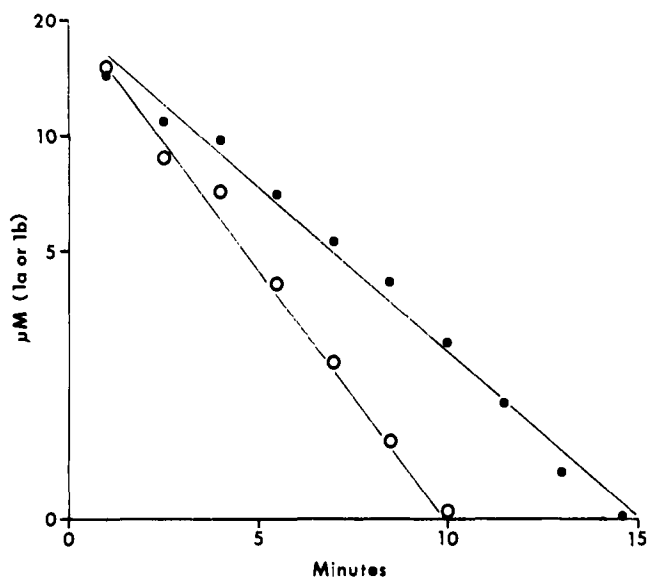


Figure 1. Plots of the disappearance of 1a (—●—) and 1b (—○—) from incubation of 1a,b (experiment 2) together with their least-squares regression lines.

II. Microsomal protein concentrations, which were used to normalize the kinetic data, also are listed. Figure 1 shows representative semilog disappearance plots of the results from the incubation of 30 μM 1a,b (experiment 2). Figure 2 shows plots of the results from the incubations of 15 μM 1a and 15 μM 1b (experiments 12 and 14, respectively). In each figure the calculated least-squares regression lines have been drawn. From these semilog plots, apparent first-order disappearance rate constants were calculated using the relationship $k = 0.693/t_{1/2}$. Since the various incubations were run at different microsomal protein concentrations, each disappearance rate constant was normalized by expressing it per milligram of microsomal protein from which the normalized half-life value was obtained. The R/S ratios of these half-lives are also listed in Table II.

The rates of disappearance of 1a vs. 1b in microsomal incubates containing initially racemic compound appear

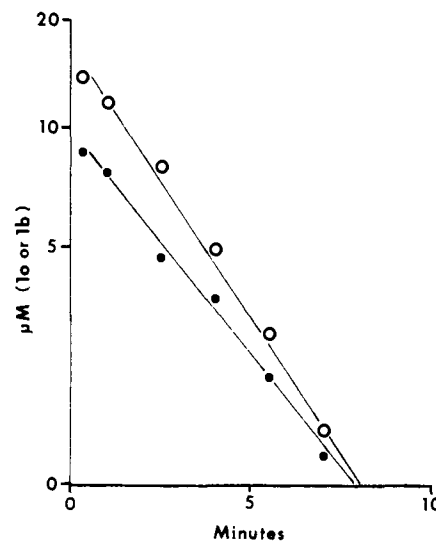


Figure 2. Plots of the disappearance of 1a (—●—) and 1b (—○—) from incubation of the individual enantiomers 1a (experiment 12) and 1b (experiment 14) together with their least-squares regression lines.

to follow simple first-order kinetics (Figure 1). These rate data consistently showed that the half-life of the R enantiomer 1a is greater than that of the S enantiomer 1b, irrespective of initial substrate concentration or microsomal protein concentration. It is noteworthy, however, that the ratios of normalized half-lives approach unity as the microsomal protein concentrations decrease at constant initial substrate concentration (experiments 2-5, Table II). These results could mean that the quantitative importance of the metabolic pathways to which 1 is subject (aromatic methyl oxidation,¹⁴ O-demethylation,¹⁰ N-hydroxylation,⁹ and side-chain oxidation¹⁴) varies with the ratio of substrate to microsomal protein concentrations. Since we know from product analysis that different metabolic pathways experience different stereochemical control,^{9,10,14} the dominance of a stereoselective pathway at higher enzyme/substrate concentration ratios could be lost at lower ratios. Further studies on the enantiomeric com-

position and quantities of metabolites formed at different enzyme/substrate concentration ratios will be required to evaluate this possibility.

Consistent with the results obtained after 1-h incubation of the separate enantiomers (Table I), the ratio of individually determined half-lives of **1a**/**1b** was found to be unity at all concentrations studied (Table II). Thus the evidence obtained from both sets of experiments indicates that the enantiomeric discriminations witnessed in incubations of racemic drug are dependent on some type of chiral interaction, presumably at the level of the enzymes responsible for the metabolism of these compounds. The major enantiomeric effect appears to be an increase in the half-life of **1a** in the presence of **1b** since under comparable conditions the half-lives of **1b** in the presence and absence of **1a** are nearly equal. It appears likely therefore that the metabolism of **1a** is preferentially inhibited by **1b** or a metabolite of **1b**.

A similar type of enantiomeric interaction has been reported for methadone.²³ The ratio of *S*/*R* (metabolized) was smaller with the racemic drug than the corresponding value for the individual enantiomers. Enantiomeric differences in the metabolism of several drugs, including narcotic analgesics,^{23,24} barbiturates,^{25,26} 1-phenyl-2-aminopropanols,²⁷ 1-phenyl-2-aminopropanes,¹³ and the experimental antidepressant, muscle relaxant nefopam,²⁸ have been documented. Except for the present report and the methadone study, enantiomeric interactions in the metabolism of racemates have not been investigated. In the case of nefopam, however, the demethylation of the racemate at high substrate concentrations by 9000g rabbit liver homogenate supernatant fractions was slower than the demethylation of either isomer alone. The nature of this enantiomeric interaction is not known since enantiomeric composition of the metabolites of racemic nefopam was not determined. The generality of interactions, as cited above, remains to be explored.

The extent to which metabolic enantiomeric interactions may influence the pharmacological and toxicological properties of racemic drugs remains unknown although Anders et al.²⁹ have speculated that a pharmacologically inactive enantiomer could be employed as an inhibitor of drug metabolism to prolong the action of the active isomer. If the *in vitro* results obtained in the present study are reflected in the *in vivo* metabolic disposition of amine **1**, then higher brain levels of the pharmacologically active *R* enantiomer might be achieved when **1a** is coadministered with **1b**. Preliminary results in our laboratory have shown that the ratios of **1a**/**1b** are as high as 1.6 to 1 (whole rat brains) and 3 to 1 (rat livers) following intraperitoneal administration of **1a,b**. Currently we are attempting to determine the brain levels achieved following administration of comparable doses of the individual enantiomers. The results should allow us to evaluate the potential significance of *in vivo* metabolic enantiomeric interactions for this drug.

Experimental Section

The mass spectra were obtained on an AEI MS 902 double focus mass spectrometer equipped with a direct inlet system and modified for chemical ionization analyses. Isobutane was used as the reactant gas at 0.7 Torr with a source temperature of 210 °C. GLC analyses were performed on a Varian Aerograph 2100 Life Sciences gas chromatograph equipped with a hydrogen flame ionization detector using U-shaped 2 m × 2 mm i.d. glass columns packed with 3% OV 17 or 3% OV 25 on acid-washed DMCS-treated Chromosorb W, mesh 100–120, or on a Hewlett-Packard Model 5713A gas chromatograph equipped with a ⁶³Ni electron capture detector using coiled 6 ft × 2 mm i.d. glass columns packed with 3% SP 2250 on Supelcoport, mesh 100–120. Column

temperatures used were 240 °C for OV 17, 255 °C for OV 25, and 250 °C for SP 2250. Retention times for **1a** and **1b**, respectively, were 9.7 and 10.1 min on OV 17, 6.5 and 7.4 min on OV 25, and 12.8 and 14.8 min on SP 2250. GLC peak intensities were measured on a Du Pont Curve Resolver Model 310, by cutting and weighing, or by determination of peak heights. Liquid scintillation counting of samples in 10 ml of Aquasol (New England Nuclear) was performed on a Packard-Tricarb Model 3375. All values were corrected for efficiency by toluene-¹⁴C internal standard or by automatic external standard. NMR spectra were taken on a Varian Associates A-60A spectrometer and chemical shifts are reported in parts per million (δ) downfield relative to Me₄Si as internal standard. Elemental analyses were performed by the Microanalytical Laboratory of the University of California, Berkeley.

Chemistry. The synthesis, resolution, and absolute configuration assignments⁸ of 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (**1**) and the synthesis of **1a,b**-*1*-¹⁴C have been previously reported.⁸ The dideuterioamine 1-*d*₂ used as internal standard in the CI mass spectral stable isotope dilution analysis was prepared by reduction of 1-(2,5-dimethoxy-4-methylphenyl)-2-nitropropene (4.74 g, 20.0 mmol) with LiAlD₄ (>99% enriched, 2.2 g, 52.0 mmol) in anhydrous THF. The pure amine hydrochloride (3.0 g, 71%) had mp 185–188 °C and an isotope enrichment of greater than 99% (by CI mass spectral analysis). The NMR spectrum of 1-*d*₂ in CDCl₃ shows a singlet at δ 1.1 ppm (side chain CH₃) and two broad bands at δ 2.5 and 2.7 ppm, each integrating for 0.5 proton, corresponding to two unique C-1 protons. The remaining signals account for the aromatic and aromatic substituent resonances.³⁰ This spectrum is consistent with a 50:50 diastereomeric mixture of erythro and threo forms of compound 1-*d*₂. The integration of the spectrum confirmed the presence of the two deuterium atoms.

N¹-Pentafluorobenzoyl-N²-[(*R*)- β -(2,5-dimethoxy-4-methylphenyl)isopropyl]-(*S*)-prolylamide (5a**).** To a solution of **1a** (60 mg, 0.287 mmol) in 25 ml of dry benzene was added a solution of **4** (95 mg, 0.287 mmol) in 30 ml of dry benzene and 4 ml of dichloromethane. This solution was stirred magnetically under nitrogen at room temperature overnight. The organic solution was then washed with 1 N NaOH (2 × 25 ml) followed by water (2 × 25 ml), dried (MgSO₄), and evaporated under nitrogen. The residue was recrystallized from benzene and sublimed (bath 160 °C, 15 μ) to give 65 mg (45%) of **5a**: mp 83–84 °C. Diastereomeric purity was confirmed by the GLC method described below. Anal. (C₂₄H₂₃F₅N₂O₄) C, H, N.

N¹-Pentafluorobenzoyl-N²-[(*S*)- β -(2,5-dimethoxy-4-methylphenyl)isopropyl]-(*S*)-prolylamide (5b**).** To a solution of **1b** (21.8 mg, 0.104 mmol) in 50 ml of dry benzene was added a solution of **4** (34.6 mg, 0.104 mmol) in 18 ml of dry benzene and 3 ml of dichloromethane. This solution was stirred magnetically under nitrogen at room temperature overnight. The organic solution was then washed with 1 N NaOH (2 × 25 ml) and water (2 × 25 ml), dried (MgSO₄), and evaporated under nitrogen. The residue was recrystallized from benzene–pentane to give 12.5 mg (24%) of **5b**: mp 150–151 °C. Diastereomeric purity was confirmed by the GLC method described below. Anal. (C₂₄H₂₃F₅N₂O₄) C, H, N.

Incubations. Male Dutch rabbits 6–10 months old and weighing 1.5–2.5 kg were stunned by a blow to the neck and decapitated, and their livers were immediately removed and rinsed in ice-cold 1.15% KCl. For the 10000g experiments, 5-g portions of the livers were scissor minced and homogenized in 6 vol of ice-cold 0.05 M Tris-HCl buffer (pH 7.4) containing 0.25 M sucrose with a Potter-Elvehjem homogenizer. For the microsomal preparations, 8-g portions of liver were minced and homogenized in 4 vol of ice-cold 0.01 M phosphate buffer (pH 7.4) containing 1.15% KCl. The homogenates were centrifuged at 10000g for 20 min in a Sorvall RCZ-B refrigerated centrifuge at 4 °C and the supernatant fractions were either used for incubations or further centrifuged at 100000g for 1 h in a refrigerated Spinco Model L centrifuge at 4 °C to yield microsomal pellets. The 10000g supernatant incubation mixtures contained substrate at a level of 250–770 μ M, 14 ml of homogenate supernatant (equivalent to 2 g of liver), 1.1 mM NADPH, and 9.6 mM MgCl₂. The microsomal pellets formed on centrifugation at 100000g were suspended in 14 ml of the Tris-HCl buffer (pH 7.4) containing

0.25 M sucrose. The microsomal incubations contained substrate at a level of 7.5–30 μ M, 14 ml of microsomal suspension (yielding in total incubate, 0.62–2.5 mg of microsomal protein per milliliter), 1.1 mM NADPH, and 9.6 mM $MgCl_2$. The final total volume for both incubation preparations was 16.6 ml. The 10000g supernatant fraction mixtures were incubated under air for 1 h and the microsomal fraction mixtures were incubated under air for 7–20 min, all in a metabolic shaker held at 37 °C.

Determination of Enantiomeric Composition for the 10000g Supernatant Incubations. Aliquots of the 10000g supernatant fraction incubations were treated with 1 ml of 10% NaOH and then extracted with 3 \times 25 ml portions of hexane in a separatory funnel with gentle shaking. The combined hexane extracts were concentrated in vacuo and the residue in 20 ml of anhydrous benzene was dried by azeotropic distillation. Drying agents like $MgSO_4$ absorbed the amine and therefore could not be used. The dry residue was dissolved in 0.5 ml of anhydrous benzene and treated with 1 mg of 4. After about 15 min at room temperature, the mixture was analyzed by FID–GLC.

Quantitative Estimations for 10000g Supernatant Incubations. (a) Radiochemical Isotope Dilution Analysis. Analyses were done with the benzoyl derivative of 1 as previously described⁸ using 2-ml aliquots of the 10000g supernatant fraction incubation mixture and 20 mg of cold carrier 1. Two or three recrystallizations of the benzamide derivative yielded a constant specific activity.

(b) GLC Analysis. To the incubation mixture of 1a or 1b was added 0.75 mg of the opposite enantiomer. Work-up and derivatization with the prolyl reagent 4 proceeded as described above. GLC analyses on OV 17 or OV 25 provided ratios of amides 5a and 5b from which the concentrations of the amine were calculated.

(c) Stable Isotope Dilution Analysis. Aliquots (2 ml) of the incubation mixtures containing 1a, 1b, and 1a,b were taken and to each was added 0.125 mg of internal standard, 1-d₂. After adjusting the pH to 12 with 10% NaOH, the mixtures were extracted with 3 \times 5 ml of Et_2O . The combined extracts were evaporated to dryness and the residues made anhydrous by azeotropic distillation with dry benzene. The resulting residues were transferred with $EtOH$ to the direct insertion probe of the mass spectrometer. Repeated scans of the MH^+ ions, 210 and 212 corresponding to 1-d₀ and 1-d₂, respectively, were obtained during evaporation of the sample (probe temperature 200–230 °C). Variation of the 210/212 mass ratio was less than 5%. The concentration of 1 was then calculated on the basis of this ratio. The remaining incubates containing 1a and 1b were combined. Enantiomeric composition determinations were made on the resulting mixture and on the remaining incubate containing 1a,b.

Determination of Enantiomeric Composition and Quantitation in Microsomal Fraction Incubations. Aliquots (0.5 ml) were taken at minute or longer intervals and transferred to tubes containing 1 ml of 30% K_2CO_3 (pH 13) and 3.75 or 7.5 nmol of internal standard 6b. These were then extracted with benzene and an aliquot was added to a dichloromethane solution of the prolyl reagent 4 in at least a 300 M excess. After heating at 70 °C for 1 h, the solvent was evaporated on a steam cone and the residue was taken up in 0.1 N NaOH and the amides were extracted into 0.3 ml of benzene and analyzed by EC–GLC. Comparison of the resultant chromatographic peak heights of the prolylamides of 5a, 5b, and 7b, by referring to a standard curve, yielded the enantiomeric composition and levels of 1a and 1b remaining in the incubation at the time of the sampling.

Acknowledgment. The authors are grateful to Dr. A. T. Shulgin for a gift of (S)-(+)-1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane hydrochloride (6b·HCl), to Professor R. Weinkam for providing the mass spectra, and

to Mr. R. Rapoport for his technical assistance. This research was supported by U.S. Public Health Service Grant MH 21219 and U.S. Public Health Service Training Grant 3920.

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