Cytochrome P-455 nm Complex Formation in the Metabolism of Phenylalkylamines. 8. Stereoselectivity in Metabolic Intermediary Complex Formation with a Series of Chiral 2-Substituted 1-Phenyl-2-aminoethanes

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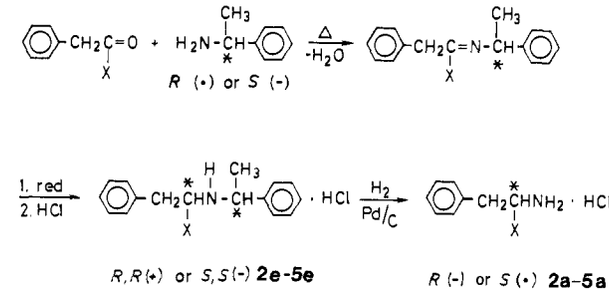
The formation of cytochrome P-450 metabolic intermediary (MI) complexes from the enantiomers of four 2-alkyl-substituted 1-phenyl-2-aminoethanes was investigated during reduced nicotinamide adenine dinucleotide phosphate (NADPH) dependent metabolism in liver microsomes from phenobarbital-pretreated rats. The 2-alkyl substituents were methyl (amphetamine), ethyl, *n*-propyl, and *n*-butyl groups. The chiral amines were prepared from the corresponding alkyl benzyl ketones by asymmetric hydrogenolytic transamination. Circular dichroism analysis showed that all the amines possessed the *S*-(+) and *R*-(−) configuration. The maximal velocity ($V_{max(obsd)}$) of complex formation increased with increasing size of the alkyl group, and for each series of enantiomers a good correlation was obtained between log $V_{max(obsd)}$ and the logarithm of the octanol/buffer partition coefficient of the substrates. With increasing lipophilicity, the *S*-(+) enantiomers became more active than the *R*-(−) isomers in generating the complex. The rates of complex formation for the faster *S*-(+) enantiomers coincided with those of the previously investigated racemates, indicating that the *R*-(−) enantiomers do not act as competitive enzyme inhibitors in the rat liver preparations. In agreement with two previous studies, the results from the present investigation establish a stereoselectivity in cytochrome P-450 MI complex formation by 1-phenyl-2-aminoethanes. However, detection of such differences are dependent on the intrinsic activity of the compound.

The capacity of amphetamine and a number of related aliphatic amines to form so-called cytochrome P-450 metabolic intermediary (MI) complexes during NADPH-dependent microsomal metabolism is now well established.[1-5] Such complexes are formed more readily from the N-oxidized congeners of the amines—the *N*-hydroxylamines and *C*-nitroso compounds.[5-9] N-Oxidation is considered a prerequisite for complex formation, and nitroso compounds are proposed as the ultimate ligands.[2,8,9]

Most studies on N-hydroxylation and MI complex formation using amines with asymmetric centers have so far been performed with racemic substrate mixtures. With the development of the technology needed for the preparation of chiral substrates and to measure enantiomeric products, an increased interest has recently been devoted to enantiomeric differences in the pathways of drug transformation. This is because a better understanding of enantioselectivity of a specific drug, not only at its site of action but also in its biotransformation, could be an important determinant in the action of the drug as a whole. Studies on the influence of the chiral center of amphetamine on metabolism and complex formation in rabbit liver microsomes have indicated that the *R*-(−) enantiomer is a better substrate than the *S*-(+) enantiomer, and enantiomeric interactions between the two antipodes have been reported.[10-12] Chiral interactions in MI complex formation were also noted with the psychotomimetic amine 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane in rabbit liver microsomes.[13]

Scheme I. Synthetic Pathway



We have recently investigated the structure–activity relationships in cytochrome P-455 nm complex formation in rat liver microsomes with a homologous series of racemic 2-alkyl-substituted 1-phenyl-2-aminoethanes.[5] It then appeared of interest to see how the chirality of these substrates affected the formation of the MI complexes. In the previous study,[5] the $V_{max(obsd)}$ values for ligand formation by the racemic amines increased markedly with increasing size of the 2-alkyl group, and good correlation was obtained between log $V_{max(obsd)}$ and the logarithm of the octanol/buffer partition coefficient of the substrates. Eight optical isomers of four representative racemates from the previous study have now been synthesized. Their absolute configuration was determined, and their ability to generate the MI complexes during NADPH-dependent metabolism in liver microsomes from phenobarbital-pretreated rats were investigated.

Results and Discussion

Synthesis and Assignment of Absolute Configuration. Synthesis of optically active open-chain amines from ketones by asymmetric hydrogenolytic transamination is well documented.[14] Nichols et al.[15] and Standridge and co-workers[16] indicated a general applicability of this re-

(1) Franklin, M. R. *Xenobiotica* **1974**, *4*, 133.
(2) Jonsson, J.; Lindeke, B. *Acta Pharm. Suec.* **1976**, *13*, 313.
(3) Franklin, M. R. *Pharmacol. Ther., Part A* **1977**, *2*, 227.
(4) Mansuy, D. *Biochimie* **1978**, *60*, 969.
(5) Lindeke, B.; Paulsen-Sörman, U.; Hallström, G.; Khuthier, A.-H.; Cho, A. K.; Kammerer, R. C. *Drug Metab. Disp.* **1982**, *10*, 700.
(6) Franklin, M. R. *Mol. Pharmacol.* **1974**, *10*, 975.
(7) Mansuy, D.; Rouer, E.; Bacot, C.; Gans, P.; Chottard, J.-C.; Leroux, J.-P. *Biochem. Pharmacol.* **1978**, *27*, 1229.
(8) Mansuy, D.; Gans, P.; Chottard, J.-C.; Bartoli, J.-F. *Eur. J. Biochem.* **1977**, *76*, 607.
(9) Paulsen-Sörman, U.; Lundkvist, G.; Khuthier, A.-H.; Lindeke, B. *Chem.-Biol. Interact.*, in press.
(10) Gal, J.; Wright, J.; Cho, A. K. *Res. Commun. Chem. Pathol. Pharmacol.* **1976**, *15*, 525.
(11) Beckett, A. H.; Al-Sarraj, S. *J. Pharm. Pharmacol.* **1972**, *24*, 174.
(12) James, R. C.; Franklin, M. R. *Biochem. Pharmacol.* **1975**, *24*, 835.
(13) McGraw, N. P.; Castagnoli, N., Jr. *J. Med. Chem.* **1981**, *24*, 299
(14) Weinges, K.; Graab, G. *Chem. Ztg., Chem. Appar.* **1970**, *94*, 728.
(15) Nichols, D. E.; Barfknecht, C. F.; Rusterholz, D. B.; Benington, F.; Morin, R. D. *J. Med. Chem.* **1973**, *16*, 480.

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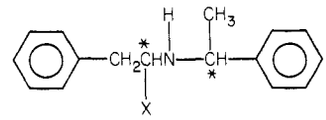
Table I. Diastereomeric Composition of *N*-(1-Phenylethyl)-1-phenyl-2-aminoalkanes Prepared by Various Reduction Methods as Crude Bases and After Recrystallization of Their Hydrochloride Salts



compd	X	reducing agent[a]	form (no. of recrystn's)	*RR/SR* or *SS/RS* ratio[b]
(*R*)-2e	Me	$NaBH_4$	base[c]	67:33
(*S*)-2e		$NaBH_4$	HCl (1)	94:6
		$NaBH_4$	HCl (2)	100:0
		$H_2/Pt^{IV}O_2$	base	87:13
		$H_2/Pt^{IV}O_2$	HCl (1)	98:2
		$H_2/Pt^{IV}O_2$	HCl (2)	100:0
		H_2/Raney Ni	base	97:3
		H_2/Raney Ni	HCl (1)	100:0
(*R*)-3e	Et	$NaBH_4$	base[d]	52:48
(*S*)-3e		$NaBH_4$	HCl (1)	90:10
		$NaBH_4$	HCl (2)	95:5
		$NaBH_4$	HCl (3)	99:1
		$H_2/Pt^{IV}O_2$	base	69:31
		$H_2/Pt^{IV}O_2$	HCl (1)	83:17
		$H_2/Pt^{IV}O_2$	HCl (2)	97:3
		H_2/Raney Ni	base	56:44
		H_2/Raney Ni	HCl (1)	97:3
		H_2/Raney Ni	HCl (2)	99:1
(*R*)-4e	*n*-Pr	$NaBH_4$	base[e]	62:38
(*S*)-4e		$NaBH_4$	HCl (1)	100:0
		$H_2/Pt^{IV}O_2$	base	59:41
		$H_2/Pt^{IV}O_2$	HCl (1)	98:2
		$H_2/Pt^{IV}O_2$	HCl (2)	100:0
		H_2/Raney Ni	base	55:45
		H_2/Raney Ni	HCl (1)	91:9
		H_2/Raney Ni	HCl (2)	97:3
(*R*)-5e	*n*-Bu	$NaBH_4$	base[f]	77:23
(*S*)-5e		$NaBH_4$	HCl (1)	96:4
		$NaBH_4$	HCl (2)	100:0
		$H_2/Pt^{IV}O_2$	base	70:30
		$H_2/Pt^{IV}O_2$	HCl (1)	97:3
		$H_2/Pt^{IV}O_2$	HCl (2)	100:0
		H_2/Raney Ni	base	60:40
		H_2/Raney Ni	HCl (1)	81:19
		H_2/Raney Ni	HCl (2)	95:5

[a] For details, see Experimental Section. [b] Peak height ratios between diastereomers as determined by GC. [c] GC: col temp 184 °C; t_R = 4.6/4.0 min. [d] GC: col temp 184 °C; t_R = 5.7/5.0 min. [e] GC: col temp 184 °C; t_R = 7.1/6.6 min. [f] GC: col temp 177 °C; t_R = 10.9/10.0 min. For additional GC conditions, see Experimental Section

action to the synthesis of various ring-substituted 1-phenyl-2-aminopropanes and analogues of mescaline.

The chiral 2-alkyl-substituted 1-phenyl-2-aminoethanes reported in this present study (Table III) were synthesized by modification of the method by Nichols et al.[15] (Scheme I), but we found the method to be stereoselective rather than stereospecific for the amines containing 2-alkyl substituents larger than methyl. Thus, investigation by gas chromatographic (GC) analysis (Figure 1) of the diastereomeric composition of the crude amines, which were obtained by reduction of the intermediary imines, showed a low degree of diastereomeric purity (Table I). However, a few recrystallizations of the hydrochloride salts of the secondary amines afforded pure diastereomers (Table I and II and Figure 1). When reductions with H_2 were run over $Pt(IV)O_2$,[17] instead of the Raney Ni used by Nichols et al.,[15] the stereoselectivity increased somewhat. $NaBH_4$ reduction in absolute ethanol[18] was of no advantage (Table I).

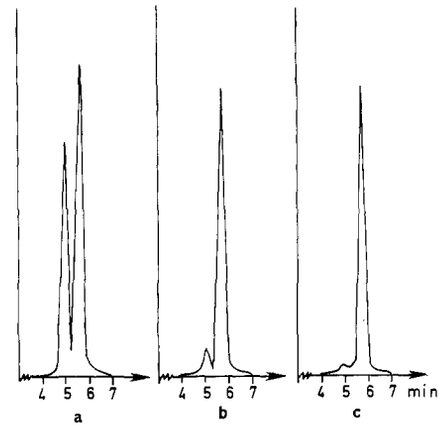


Figure 1. GC chromatograms showing the diastereomeric composition of *N*-(1-phenylethyl)-1-phenyl-2-aminobutane (**3e**) as crude reaction product and after purification. The intermediary imine was reduced with H_2 over Raney/Ni. The various chromatograms was obtained from (a) the crude base, (b) the base after one recrystallization of its hydrochloride salt, and (c) the base after two recrystallizations of its hydrochloride salt. For further details, see Experimental Section and Table I.

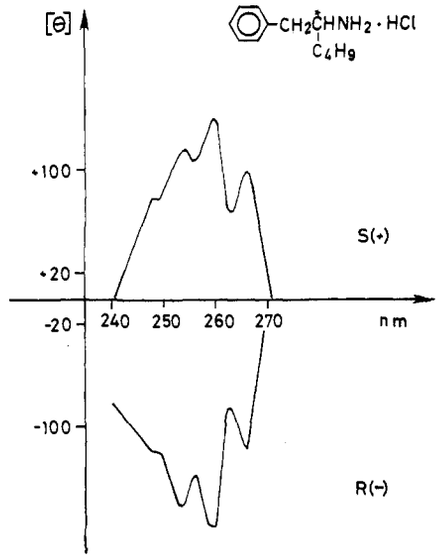


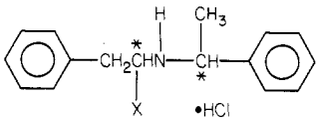
Figure 2. Circular dichroism curves in the range 240–270 nm of (*R*)-(−)- and (*S*)-(+)-1-phenyl-2-aminohexane hydrochloride (**5a**) in *i*-PrOH at 25 °C. UV and CD data of the various 1-phenyl-2-aminoalkane hydrochloride salts are available on request.

The relatively poor yields of pure secondary amines (Table II) were due to the necessity of repeated recrystallization of their hydrochloride salts, and decomposition into highly colored byproducts, which initially occurred upon treatment of the amines with hydrochloride-saturated ether, was also a contributing factor. The hydrochloride salts were filtered and recrystallized immediately after formation in order to minimize decomposition.

Data on the various primary amine hydrochloride salts, obtained by hydrogenolytic cleavage of the corresponding secondary amine hydrochloride salts, are given in Table III. Since the reductive cleavage of the secondary amines occurs with complete retention of configuration,[15] the optical purity of the *R* and *S* primary amines **2a**–**5a** must be

(16) Standridge, R. T.; Howell, H. G.; Gylys, J. A.; Partyka, R. A.; Shulgin, A. T. *J. Med. Chem.* **1976**, *19*, 1400.
(17) McDermed, J. D.; McKenzie, G. M.; Phillips, A. P. *J. Med. Chem.* **1975**, *18*, 362.
(18) House, H. O. In "Modern Synthetic Reactions"; W. A. Benjamin: Menlo Park, CA, 1972; p 73.

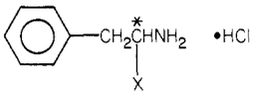
Table II. Physical Data[a] for *N*-(1-Phenylethyl)-1-phenyl-2-aminoalkane Hydrochloride Salts



compd	X	isomer	$[\alpha]^{20}_D$, deg (MeOH)	mp,[b] °C	optical purity,[c] %	formula[d]	final yield,[e] %
(*R*)-2e	Me	*R,R*-(+)	+21.50[f]	231-232[f]	98	$C_{17}H_{22}ClN$	12
(*S*)-2e	Me	*S,S*-(−)	−22.89	229-230	100		14
(*R*)-3e	Et	*R,R*-(+)	+23.41	233-235	100	$C_{18}H_{24}ClN$	14
(*S*)-3e	Et	*S,S*-(−)	−22.73	230-232.5	99		27
(*R*)-4e	*n*-Pr	*R,R*-(+)	+31.15	267-270	100	$C_{19}H_{26}ClN$	24
(*S*)-4e	*n*-Pr	*S,S*-(−)	−29.77	266-267	100		27
(*R*)-5e	*n*-Bu	*R,R*-(+)	+28.65	220-222	100	$C_{20}H_{28}ClN$	23
(*S*)-5e	*n*-Bu	*S,S*-(−)	−29.54	224-226	100		28

[a] Data on ^1H NMR and mass spectra are available on request. [b] Recrystallization solvent: MeOH/EtOH (25:75)/Et_2O. [c] Determined as peak height ratios between the diastereomers in GC analysis. For GC conditions, see Experimental Section. [d] All new compounds were analyzed for C, H, and N. The analyses were correct within ±0.4% of the theoretical values. [e] After recrystallization to a diastereomeric purity of ≥98% as determined by GC. [f] Literature[15] $[\alpha]^{20}_D$ (MeOH) +21.0°; mp 233.5-234.5 °C (corrected).

Table III. Physical Data[a] for the 1-Phenyl-2-aminoalkane Hydrochloride Salts



compd	X	isomer	$[\alpha]^{20}_D$, deg (H_2O)	mp,[b] °C	formula[c]	yield, %
(*R*)-2a	Me	*R*-(−)	−24.20[d]	149-151[d]	$C_9H_{14}ClN$	30
(*S*)-2a	Me	*S*-(+)	+23.21	147-150		35
(*R*)-3a	Et	*R*-(−)	−33.64	148-149.5	$C_{10}H_{16}ClN$	70
(*S*)-3a	Et	*S*-(+)	+33.16	147-148		53
(*R*)-4a	*n*-Pr	*R*-(−)	−19.74	157-159	$C_{11}H_{18}ClN$	67
(*S*)-4a	*n*-Pr	*S*-(+)	+20.04	153-155		50
(*R*)-5a	*n*-Bu	*R*-(−)	−16.42	162-163	$C_{12}H_{20}ClN$	30
(*S*)-5a	*n*-Bu	*S*-(+)	+14.95	161-162		59

[a] Data on ^1H NMR and mass spectra are available on request. [b] Recrystallization solvent: *i*-PrOH/Et_2O. [c] All new compounds were analyzed for C, H, and N. The analyses were correct within ±0.4% of the theoretical values. [d] Literature[15] $[\alpha]^{20}_D$ (H_2O) −27.2°; mp 157-158 °C (corrected).

considered to be the same as for the *R* and *S* compounds **2e–5e**, respectively, i.e., at least 98% (Table II).

Circular dichroism (CD) analysis showed that all the primary 2-alkyl-substituted 1-phenyl-2-aminoethane hydrochloride salts in the present study possessed multiple Cotton effects in the range 240–270 nm. The curves from the (+) enantiomers were exact mirror images of those from the (−) isomers (Figure 2) and the enantiomers were all comparable by their UV spectra. Furthermore, the wavelengths of the CD maximum and CD minimum obtained, together with the molecular ellipticity [θ] values, were in good agreement with those expected.[19]

It is well established that the base or salt of (*S*)-(+)-1-phenyl-2-aminopropane[20] exhibits positive multiple Cotton effects in the range 240–270 nm.[19,21] When comparing the sign of the CD curves of the amines in the present study with those from commercial (*S*)-(+)- and (*R*)-(−)-amphetamine, dextrorotatory **3a–5a** gave curves of the same sign as (*S*)-(+)-amphetamine sulfate. Accordingly, the amines **3a–5a** could be described the *S*-(+) and the *R*-(−) configuration. Moreover, in the preparation of secondary 1-phenyl-2-aminopropanes, according to Nichols et al.[15] (cf. Scheme I), the absolute configuration of the product amine was reported to preferentially coincide with that of the 1-phenyl-1-aminoethane employed.

(19) Smith, H. E.; Willis, T. C. *J. Am. Chem. Soc.* **1971**, *93*, 2282.
(20) Leithe, W. *Chem. Ber.* **1932**, *65*, 660.
(21) Craig, J. C.; Chan, R. P. K.; Roy, S. K. *Tetrahedron* **1967**, *23*, 3573.

Table IV. Biochemical Interactions with the 1-Phenyl-2-aminoalkane Hydrochloride Salts[a]

compd	$V_{max(obsd)}$[b]	$K_{m(app)}$, μM	concn range, μM
(*R*)-2a	~1.5	~270	500-4000
(*S*)-2a	~1.6	~280	500-4000
(*R*)-3a	3.2 ± 0.5 (3)	23 ± 5 (3)	25-250
(*S*)-3a	4.0 ± 0.5 (3)	30 ± 3 (3)	25-250
(*R*)-4a	5.1 ± 0.4 (3)	28 ± 6 (3)	10-250
(*S*)-4a	9.1 ± 0.5 (3)	32 ± 4 (3)	10-250
(*R*)-5a	5.7 ± 0.1 (3)	16 ± 3 (3)	5-250
(*S*)-5a	10.5 ± 0.3 (3)	21 ± 5 (3)	5-250

[a] Data are means plus or minus standard errors, followed by *N* in parentheses. [b] $\Delta A_{455-490}$ cm^{-1} min^{-1} (mM cytochrome P-450)$^{-1}$.

Formation of the MI Complexes. Both enantiomers of all the primary amines (**2a–5a**) formed cytochrome P-450 MI complexes during NADPH-dependent metabolism. As for the racemic amines,[5] double-reciprocal plots of the highest observed rate vs. substrate concentration gave linear relationships over a defined substrate range from which the observed maximal velocity ($V_{max(obsd)}$) of complex formation and the half-saturating substrate concentration ($K_{m(app)}$) could be calculated (Table IV). The $V_{max(obsd)}$ values increased with increasing size of the alkyl group for each group of enantiomers, while the $K_{m(app)}$ values obtained did not. However, the *S*-(+) enantiomers of **4a** and **5a** produced the chromophores at a significantly higher rate than the *R*-(−) enantiomers, as exemplified by **4a** in Figure 3, while there was no significant difference

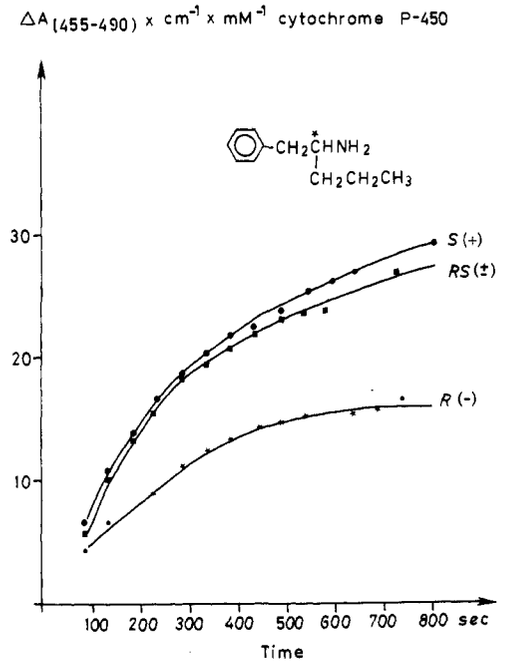


Figure 3. Cytochrome P-450 complex formation by the *R*-(−) and *S*-(+) enantiomers of 1-phenyl-2-aminopentane [(*R*)- and (*S*)-**4a**] and the racemate [(*RS*)-**4a**] at a concentration of 500 μM.

Table V. *S*-(+)/*R*-(−) Ratio in the Extent of Cytochrome P-450 MI Complex Formation for the 1-Phenyl-2-aminoalkane Hydrochloride Salts[a]

compd	*S*-(+)/*R*-(−) ratio	time,[b] s	concn,[c] μM
(*R*)-2a (*S*)-2a	1.05 ± 0.02 (3)	300	500
(*R*)-3a (*S*)-3a	1.10 ± 0.01 (3)	400	100
(*R*)-4a (S)-4a	1.74 ± 0.01 (3)	300	100
(*R*)-5a (*S*)-5a	1.80 ± 0.07 (3)	400	100

[a] Data are means plus or minus standard errors, followed by *N* in parentheses. [b] Time when a constant *S*(+)/*R*(−) ratio is reached. [c] Concentration above which a constant *S*(+)/*R*(−) ratio is obtained.

in the rate of complex formation between the enantiomers of **2a** and **3a** (Table IV). Similar relationships were also seen in the extent of complex formation (Table V).

A correlation between the octanol/buffer partition coefficient (log K_D) and $V_{max(obsd)}$ was obtained (Figure 4), but with two slopes, one for each group of isomers. Thus, with increasing lipophilicity, the *S*-(+) enantiomer becomes more active relative to the *R*-(−) enantiomer in generating the ligand. The rates of complex formation from the enantiomers of **2a** and **3a** did not appear different from those of the corresponding racemates,[5] while complex formation from the racemates of **4a** and **5a** were statistically different from that of (*R*)-(−)-**4a** and -**5a** but similar to that of (*S*)-(+)-**4a** and -**5a** (Figures 3 and 4).

In one of the early reports on cytochrome P-450 MI complex formation in liver microsomes from phenobarbital-pretreated rats, Franklin[1] reported that (*S*)-(+)-amphetamine was about twice as active as the *R*-(−) enantiomer. We were unable to detect any significant differences between the enantiomers of **2a** in our system, although numerous experiments were run at substrate concentrations of 0.5–4 mM. However, when small differences were seen in a particular experiment, (*S*)-(+)-amphetamine was always more active.

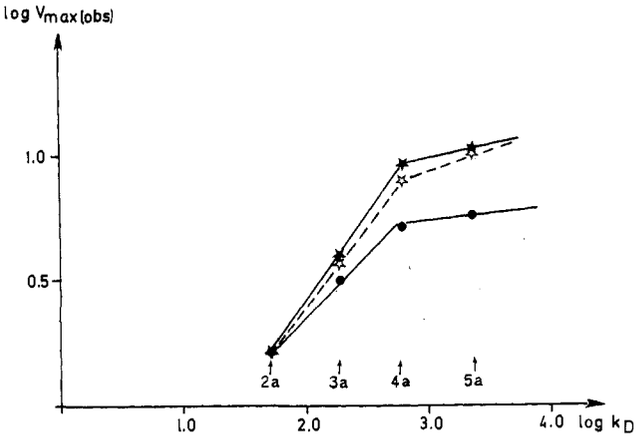


Figure 4. Correlation between the octanol/buffer partition coefficient (log K_D) and rate of cytochrome P-450 MI complex formation (log $V_{max(obsd)}$) for *R* (●) and *S* (★) compounds **2a–5a** and their racemates (☆) (cf. ref 5). For statistics, see Table IV.

Amphetamine exhibits substantial species differences in both its in vivo and in vitro metabolism.[22-24] Such differences are seen also in the cytochrome P-450 MI complex formation, since rabbit liver microsomes, in contrast to rat liver microsomes, show a stereochemical preference for the *R*-(−) over the *S*-(+) enantiomer.[12] The initial rate of complex formation by the *R*-(−) isomer of the lipophilic amphetamine analogue 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane with rabbit liver microsomes was reported to be five times as fast as that of the *S*-(+) isomer.[13] Complex formation by the racemic compound was also investigated in the latter study and was shown to equal that of the slower *S*-(+) isomer.

The results from the present study establish, in agreement with the cited reports,[1,12,13] a stereoselectivity in the rate and extent of cytochrome P-450 MI complex formation from 2-alkyl-substituted 1-phenyl-2-aminoethanes. However, the differences are only discernible when the intrinsic activity of the compound is sufficiently high (cf. Figure 4).

When enantioselectivity was noted, the *S*-(+) enantiomers were more active in rat liver microsomes in the present as well as in the previous study.[12] The similar rates of complex formation by *S*-(+) compounds **2a–5a** and their corresponding racemates indicate that the *R*-(−) enantiomers are not competitively inhibiting the *S*-(+) conversion in the rat liver preparation. This result is in contrast to that reported by McGraw and Castagnoli Jr.,[13] for rabbit liver microsomes, in which the *S*-(+) enantiomer of their substrate generated the ligand at a rate similar to that of the racemate, but where the *R*-(−) enantiomer was considerably more active. Moreover, complex formation by the R-(−) enantiomer was inhibited by the *S*-(+) enantiomer.[13]

Since N-oxidation is considered to be a prerequisite for complex formation by 1-phenyl-2-aminoalkanes[5,13] and is likely to be the rate-limiting step in the overall reaction,[5] enantioselectivity, when noted, should reflect differences in the N-oxidizing capacity of the enzymes. Although the enantioselectivity in the N-oxidation of compounds **3a–5a** has yet to be established, the results with the *R* and *S* enantiomers of **2a** are consistent with the notion that (*S*)-(+)- and (*R*)-(−)-amphetamine are similar as substrates

(22) Dring, L. G.; Smith, R. L.; Williams, R. T. *Biochem. J.* **1970**, *116*, 425.
(23) Jenner, P.; Testa, B. *Drug Metab. Rev.* **1973**, *2*, 117.
(24) Caldwell, J. *Drug Metab. Rev.* **1976**, *5*, 219.

for N-oxidation in rat liver preparations.[11] Complex formation data from two previous studies[12,13] suggest that N-oxidation of amphetamine derivatives is considerably more extensive in rabbit than in rat liver preparations and has a preference for the *R*-(−) enantiomers.[11,25] Thus, all data so far available are consistent with the notion that complex formation by primary amphetamine analogues appears to follow the species differences seen in N-oxidation.

Experimental Section

Melting points were determined in an electrically heated metal block and are uncorrected. The microanalyses were carried out at the Department of Chemistry, Swedish University of Agricultural Sciences, Uppsala, and are correct within ±0.4% of the theoretical value. ^{1}H NMR and mass spectra were recorded for identification purposes on a JEOL FX 90Q spectrometer and a LKB Model 9000 mass spectrometer, respectively, and were all in accordance with the assigned structures. The mass spectra were obtained by the direct probe technique at ionizing potentials of 70 and 12 eV, a trap current of 60 μA, and an accelerating voltage of 3.5 kV. A Beckman Model 25 spectrophotometer was used for recording the UV spectra and for quantitative determination of the protein and cytochrome P-450. Optical rotations were measured with a Perkin-Elmer Model 241 spectropolarimeter, and circular dichroism spectra were obtained at 25 °C in a JASCO J-41A spectropolarimeter. GC analyses were performed in a Pye-Unicam 104 gas chromatograph equipped with a H_2 flame ionizing detector with N_2 as the carrier gas, at a flow rate of 40 mL/min. A 1.8 m × 0.2 cm glass column packed with 3% OV 17 on Gas Chrom Q (Supelco) was used. The injector and detector temperatures were 270 and 250 °C, respectively, and the column temperatures are given in Table I.

Microsomal complex formation was determined by difference spectroscopy in an Aminco Model DW-2 or in a Varian Cary 219 spectrophotometer.

(*S*)-(−)-1-Phenyl-1-aminoethane and (*R*)-(+)-1-phenyl-1-aminoethane, benzyl methyl ketone, the various nitriles, W-2 Raney Ni, and phenobarbital were obtained from FLUKA AG, Buchs, Switzerland, $NaBH_4$ was from Merck, Darmstadt, Germany, and NADPH was from Sigma Chemical Co., St. Louis, MO, $Pt^{IV}O_2$ was purchased from Kebo-Grave AB, Sweden. The various ketones were prepared as previously described.[5]

(*R*,*R*)-(+)- or (*S*,*S*)-(−)-*N*-(1-Phenylethyl)-1-phenyl-2-aminoalkane Hydrochloride Salts [(*R*)- or (*S*)-2e–5e]. The appropriate ketone (0.03 mol) and (*R*)-(+)- or (*S*)-(−)-1-phenyl-1-aminoethane (3.3 g, 0.027 mol) were dissolved in 50 mL of dry toluene, and the mixture was refluxed in a Dean–Stark apparatus for 24 h with continuous removal of water. After evaporation of the solvent, the resulting imine was dissolved in 50 mL of 99% EtOH and immediately reduced to the corresponding secondary amine.

Three sets of conditions were used for the reductions: method **1**, hydrogenation over EtOH-washed W-2 Raney Ni (0.5 g) for 24 h and an initial pressure of 50 psig; method **2**, hydrogenation over $Pt^{IV}O_2$ (81% Pt; 0.2 g) for 15 h and atmospheric pressure; and method **3**, reflux together with $NaBH_4$ (1.53 g, 0.04 mol) for 15 h.

After complete reduction, the catalyst was removed by filtration, or the $NaBH_4$ complex was destroyed by the addition of 1 M HCl. The solvent was then evaporated, and the residue was dissolved in 1 M NaOH (pH 10) and extracted with ether (3 × 20 mL). The ether layer was separated and dried, and the amine was isolated as the hydrochloride salt. The salts were recrystallized from MeOH/99% EtOH (25:75)/dry Et_2O until a diastereomeric purity of ≥98% (as measured by GC) was obtained.

(*R*)-(−)- or (*S*)-(+)-1-Phenyl-2-aminoalkane Hydrochloride Salts [(*R*)- or (*S*)-2a–5a]. The secondary amine hydrochloride salts [(*R*)- or (*S*)-**2e–5e**; 0.006 mol] were added to a slurry of 10% Pd on charcoal (0.4 g) in MeOH plus H_2O (50 mL + 10 mL). The mixture was shaken at 35 psig in a Parr flask, and the reduction was complete within 30 h. After filtration, the solvent was evaporated, and the residue recrystallized from *i*-PrOH/Et_2O.

Determination of the Absolute Configuration. The various primary amine hydrochloride salts [(*R*)- and (*S*)-**2a–5a**] were dissolved in 30% EtOH or *i*-PrOH (two sets of experiments) at a concentration of 5 mg in 0.2 mL. The CD absorption in the range 240–270 nm were measured and expressed in molecular ellipticity units [θ]. Commercial (*S*)-(+)- and (*R*)-(−)-amphetamine sulfate were used as references. In addition, UV spectra from all the solutions were recorded.

Microsomal Preparations and Incubations. Liver microsomes were prepared from phenobarbital-pretreated male Sprague–Dawley rats weighing 200–240 g [three daily intraperitoneal (ip) injections of 80 and 40 mg/kg on the 4th day]. The microsomes were isolated by gel filtration[26] to ensure complete removal of hemoglobin.[27]

The MI complex formation was determined at 25 °C by difference spectroscopy, either by repetitive scanning or by continuous monitoring of the absorbance difference between 455 and 490 nm. The protein concentration was 1 mg/mL in 50 mM potassium phosphate buffer, pH 7.5, containing the substrate and 10 mM $MgCl_2$ in a total volume of 6 mL. The reaction was started by the addition of 1 mg of NADPH in 0.1 mL water to the sample cuvette, and, when applicable, 0.1 mL of water was added to the reference cuvette.

The rate of change of absorbance $\Delta A_{455-490}$ (μmol of P-450)$^{-1}$ as a function of time was determined. Protein[28] and cytochrome P-450 concentrations[29] were measured according to standard procedures.

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Registry No. (*R*)-**2a**, 156-34-3; (*R*)-**2a**·HCl, 41820-21-7; (*S*)-**2a**, 51-64-9; (*S*)-**2a**·HCl, 1462-73-3; (*R*,*R*)-**2e**, 87923-30-6; (*R*,*R*)-**2e**·HCl, 50505-64-1; (*S*,*R*)-**2e**, 87923-31-7; (*S*,*S*)-**2e**, 87923-32-8; (*S*,*S*)-**2e**·HCl, 87923-38-4; (*R*,*S*)-**2e**, 87923-33-9; (*R*)-**3a**, 30543-89-6; (*R*)-**3a**·HCl, 87923-45-3; (*S*)-**3a**, 30543-90-9; (*S*)-**3a**·HCl, 87923-46-4; (*R*,*R*)-**3e**, 87923-34-0; (*R*,*R*)-**3e**·HCl, 87923-39-5; (*S*,*R*)-**3e**, 87923-35-1; (*S*,*S*)-**3e**, 87923-36-2; (*S*,*S*)-**3e**·HCl, 87923-40-8; (*R*,*S*)-**3e**, 87923-37-3; (*R*)-**4a**, 87982-79-4; (*R*)-**4a**·HCl, 87982-78-3; (*S*)-**4a**, 87982-81-8; (*S*)-**4a**·HCl, 87982-80-7; (*R*,*R*)-**4e**, 87923-47-5; (*R*,*R*)-**4e**·HCl, 87923-41-9; (*S*,*R*)-**4e**, 87923-48-6; (*S*,*S*)-**4e**, 87923-49-7; (*S*,*S*)-**4e**·HCl, 87923-42-0; (*R*,*S*)-**4e**, 87923-50-0; (*R*)-**5a**, 87982-83-0; (*R*)-**5a**·HCl, 87982-82-9; (*S*)-**5a**, 87982-85-2; (*S*)-**5a**·HCl, 87982-84-1; (*R*,*R*)-**5e**, 87923-51-1; (*R*,*R*)-**5e**·HCl, 87923-43-1; (*S*,*R*)-**5e**, 87923-52-2; (*S*,*S*)-**5e**, 87923-53-3; (*S*,*S*)-**5e**·HCl, 87923-44-2; (*R*,*S*)-**5e**, 87923-54-4; (*R*)-(+)-$H_2NCH(CH_3)C_6H_5$, 3886-69-9; (*S*)-(−)-$H_2NCH(CH_3)C_6H_5$, 2627-86-3; $C_6H_5CH_2COMe$, 103-79-7; $C_6H_5CH_2COEt$, 1007-32-5; $C_6H_5CH_2COPr$-*n*, 6683-92-7; $C_6H_5CH_2COBu$-*n*, 25870-62-6.

(25) Low, L. K.; Castagnoli, N., Jr. *Annu. Rep. Med. Chem.* **1978**, *13*, 304.
(26) Tangen, O.; Jonsson, J.; Orrenius, S. *Anal. Biochem.* **1973**, *54*, 597.
(27) Mansuy, D.; Chottard, J.-C.; Chottard, G. *Eur. J. Biochem.* **1977**, *76*, 617.
(28) Geiger, P. J.; Bessman, S. P. *Anal. Biochem.* **1972**, *49*, 467.
(29) Omura, T.; Sato, R. *J. Biol. Chem.* **1964**, *239*, 2370.