Alteration of Relative Affinities toward Myocardial and Vascular β Adrenoceptors Induced by Side-Chain Substitution of Aryloxypropanolamines[1]

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Several conformationally defined aryloxypropanolamines of the type $ArOCH_2\overset{*}{C}H(OH)\overset{*}{C}H(R)NHR_1$ have been synthesized and tested in vivo for β-adrenoceptor blockade. Key intermediates in the syntheses were the appropriate cis- and trans-disubstituted olefins. Epoxidation of the olefins, followed by amination of the resulting *cis*- and *trans*-epoxides, yielded the desired diastereomeric model compounds with a defined threo and erythro stereochemistry, respectively. All active compounds in this series exhibit a simple, bimolecular, competitive antagonism at β adrenoceptors. Proper substitutions of the alkanolamine side chain result in vascular selective or cardioselective β-adrenoceptor antagonists, probably as a consequence of the sterically altered ability to interact with β_1 and β_2 adrenoceptors. *dl-erythro*-1-Phenoxy-3-[(3,4-dimethoxyphenethyl)amino]butan-2-ol is a cardioselective β-adrenoceptor antagonist with a selectivity ratio significantly higher than that of practolol ($\beta_1/\beta_2 > 40$ vs. $\beta_1/\beta_2 = 22$) but of equal potency (pA_2 values = 6.66 and 6.64, respectively). Phenyl substitution at C-3 of the alkanolamine side chain drastically reduces affinity to both types of β adrenoceptors ($pA_2 < 5.0$), thus representing a cutoff point. It is concluded that steric factors, as manifested by bulk tolerance at various parts of the aryloxypropanolamine side chain, are major determinants of affinity toward β-adrenoceptor subtypes. β-Adrenoceptor blockade is unrelated to the lipophilic character of the test compounds.

β-Adrenoceptor blocking drugs of the aryloxypropanolamine type can be endowed with a significant degree of specificity for myocardial β_1 receptors by proper substitutions either at the aromatic nucleus or at the terminal amino group. In the series of 1-(substituted phenoxy)-3-isopropyl- (or *tert*-butyl-) aminopropan-2-ols, aryl substitution leading to cardioselectivity is exclusively confined to the para position. This pattern of substitution is exemplified by practolol [1-(4-acetamidophenoxy)-3-isopropylaminopropan-2-ol] and related *p*-carbamoyl-substituted analogues.[2-4] Cardioselectivity in the aryloxypropanolamine series has also been achieved by replacing the "traditional" isopropyl or *tert*-butyl groups on the amino nitrogen with bulkier substituents such as 4-carboxamidophenoxyethyl[5] (tolamolol) or 3,4-dimethoxyphenethyl[6] groups. Pharmacological evaluation of aryloxypropanolamines substituted at positions other than the aromatic nucleus and the terminal amino nitrogen, namely, at the side-chain carbon atoms (C-1–C-3), has been tried on a limited scale only in the cases of propranolol [1-(1-naphthoxy)-3-isopropylaminopropan-2-ol] and practolol.[7,8] Methyl-substituted propranolol and practolol analogues (mixtures of erythro and threo diastereomers) were substantially weaker β blockers than the parent compounds. There is an apparent lack of parallelism between the pharmacologic consequences of side-chain methylation in the arylethanolamine series which gives rise to vascular selectivity in β blockade [e.g., *dl-erythro*-1-(4-methylphenyl)-2-isopropylaminopropan-1-ol, H35/25; vascular selective β blocker[9]] and in the aryloxypropranolamine series which fails to induce any organ selectivity (e.g., C-3 methylpropranolol; nonselective β blocker). In view of this, it was decided to analyze the β-adrenoceptor blocking properties of erythro and threo C-3-substituted 1-phenoxy-3-substituted aminopropan-2-ols and compare them with those of the "parent compound", 1-phenoxy-3-isopropylaminopropan-2-ol (**9**). The present report describes the synthesis and pharmacologic activity of several conformationally defined erythro and threo C-3-substituted 1-phenoxy-3-substituted aminopropan-2-ols. The results of this study stress the importance of steric factors and of a proper balance between side-chain substituents on C-3 and the amino nitrogen in directing the compounds toward β_1- or β_2-adrenoceptor subtypes.

Chemistry. Introduction of a substituent at C-3 of the alkanolamine side chain of an aryloxypropanolamine results in erythro and threo forms of the compound. In order to obtain pure positional isomers of such C-3-substituted compounds with a defined erythro or threo sterochemistry and without resorting to separation procedures, syntheses of the final products employed properly disubstituted cis and trans olefins as starting materials. The synthetic route is outlined in Scheme I.

trans-1-Phenoxy-2-butene (**1**),[10] the starting material for *dl-erythro*-1-phenoxy-3-isopropylaminobutan-2-ol (**12**) and for *dl-erythro*-1-phenoxy-3-[(3,4-dimethoxyphenethyl)-amino]butan-2-ol (**13**), was prepared from the commercially available *trans*-1-chloro-2-butene and phenol refluxed in 2-butanone in the presence of anhydrous K_2CO_3. Under the same experimental conditions, *trans*-3-chloro-1-phenylpropene (prepared from cinnamyl alcohol and thionyl chloride) yielded *trans*-3-phenoxy-1-phenylpropene (**3**),[11] which served as the starting material for *dl-erythro*-1-phenoxy-3-isopropylamino-3-phenylpropan-2-ol (**15**). *cis*-1-Phenoxy-2-butene (**2**), the starting material for *dl-threo*-1-phenoxy-3-isopropylaminobutan-2-ol (**14**), was prepared in three steps from the commercially available 2-butyn-1-ol. Reaction of the alcohol with phosphorus tribromide gave 1-bromo-2-butyne which was converted to 1-phenoxy-2-butyne[12] by reacting with phenol in refluxing 2-butanone in the presence of anhydrous K_2CO_3. The acetylenic compound was finally subjected to selective hydrogenation of the triple bond to a cis double bond by employing 5% palladium on barium sulfate poisoned by pyridine.

Olefins **1–3** were subjected to epoxidation which is a stereospecific reaction, leading to a cis addition of the oxygen atom to the double bond. The resulting *cis*- and *trans*-epoxides **6–8** were subsequently converted to the corresponding *threo*- and *erythro*-amino alcohols by amination with the appropriate primary amines. Opening of an epoxide ring by nucleophiles proceeds with inversion of configuration at the carbon atom attacked. Therefore, the two-step procedure of epoxidation followed by amination results in an overall trans addition to the double bond[13] and enables the preparation of pure *threo*- and *erythro*-amino alcohol diastereomers from the corresponding cis and trans olefins. Unlike the monosubstituted epoxides **4** and **5**, which underwent quantitative amination under mild conditions of room temperature, the disub-

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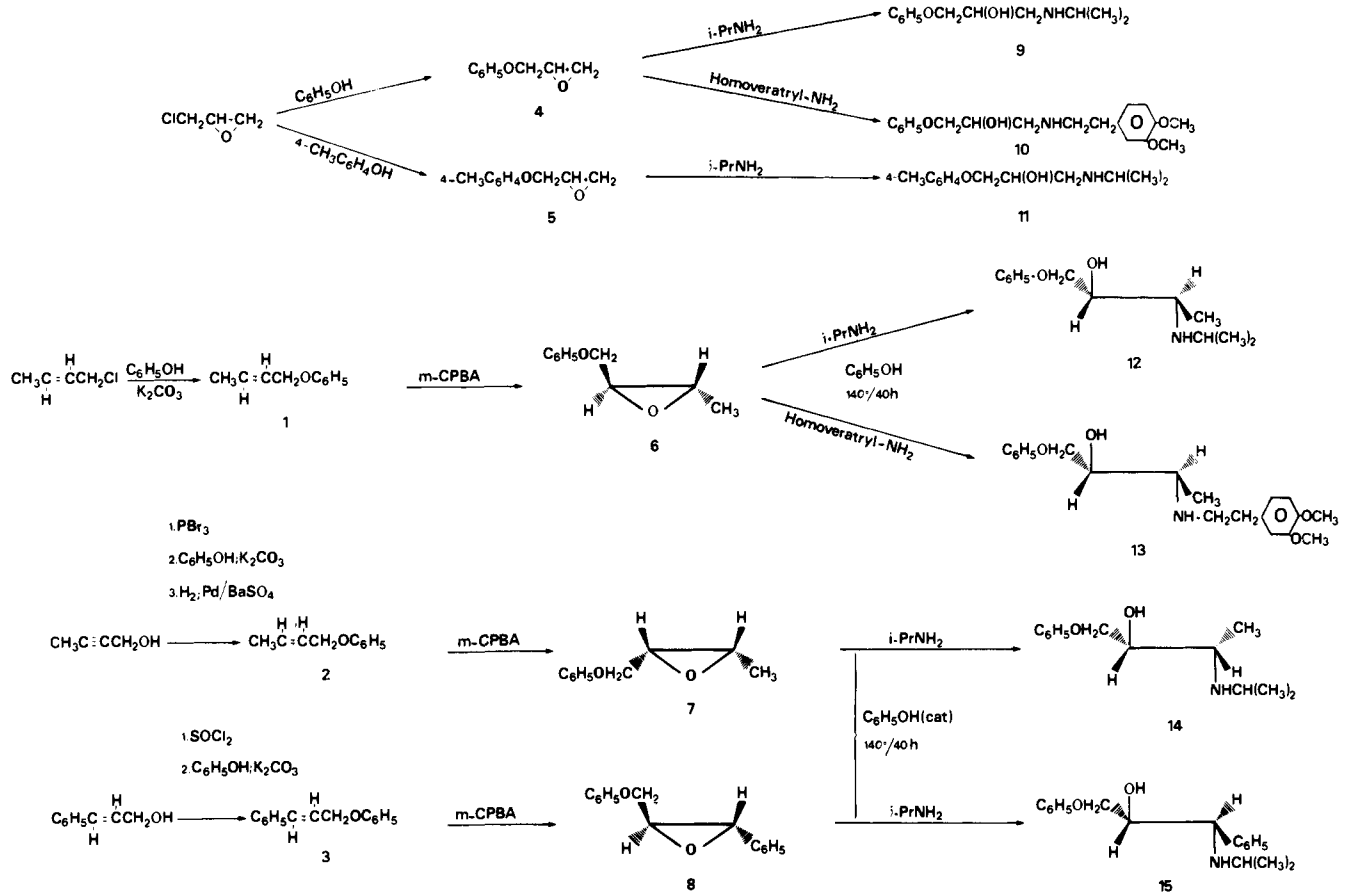
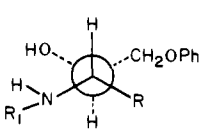
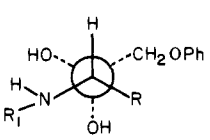
Scheme I



stituted epoxides 6–8 required forcing conditions for ring opening, and the general method of Colclough et al.[14] was therefore adopted. According to this method, equimolar quantities of the epoxide and the amine interact at 140 °C for ca. 40 h in the presence of phenol as a catalyst. The direction of ring opening of the epoxides on reaction with the amines was checked by mass spectrometry of the resulting final products. All products exhibited an intensive ion (100% rel abundance) in their mass spectra corresponding to the fragment, $CH(R)=^{+}NHR_1$, thus confirming the desired structure of the secondary alcohol and terminal amino group. This structure is derived from a nucleophilic attack of the amine on the disubstituted epoxide ring at the less hindered carbon atom carrying either Me (6 and 7) or Ph (8). Similar mass spectral studies have been reported before.[15] Another prominent peak in the mass spectra of all C-3-substituted aryloxypropanolamines was $M^+ - C_2H_4O$, thus lending further support to a previous report on the expulsion of acetaldehyde directly from the molecular ion.[16] Even at high dilution (10^{-4} M) in CCl_4, all aryloxypropanolamines exhibited a significant intramolecular hydrogen-bonded OH···N stretching bands at ca. 3350 cm^{-1}. As expected on steric grounds and after examining space-filling CPK models, such intramolecular hydrogen bonding should contribute to the stability of those rotamers where the two bulkiest vicinal groups, namely, phenoxymethyl and substituted amine, are anti.



threo for R = Me, Ph



erythro for R = Me, Ph

Pharmacological Evaluation. β-Adrenoceptor blocking potency was determined in vivo against isoproterenol-induced tachycardia and fall in diastolic pressure in chloralose-anesthetized, vagotomized cats. Details of the technique as well as the derivation of pA_2 values were described previously.[17] pA_2 values derived from whole animals experiments are assumed to be analogous to pA_2 values derived from isolated organ preparations and to be related to the concentration of the antagonist at its site of action. The results of the pharmacological tests are summarized in Table I. All active compounds with the exception of the C-3-phenyl-substituted 1-phenoxy-3-isopropylaminopropan-2-ol (15), which was practically devoid of β-blocking activity ($pA_2 < 5.0$), produced parallel rightward shifts of the log dose–response curve to isoproterenol, with no depression of the maximal response. Additionally, slopes of the plots of log (dose ratio − 1) vs. −log [antagonist] were close to the theoretical value of −1. Active compounds reported herein thus exhibit the characteristics of simple, bimolecular, competitive antagonism. In this connection it should be noted that the C-3 methyl analogue of propranolol, when tested in dogs, yielded a slope value equivalent to 1 only on isoproterenol-induced drop in diastolic pressure, whereas for the positive chronotropic response to isoproterenol a slope value of 0.61 was obtained.[8]

Discussion and Structure–Activity Relationships. The present investigation was aimed at elucidating the effect of structural variation of the alkanolamine side chain in aryloxypropanolamines on their β-adrenoceptor blocking properties. As a reference point for this study the observation that side-chain methylation at the α position of phenylethanolamines converted nonselective β blockers to vascular (β_2) selective ones[9] was used.

Table I. Physical and Biological Properties of β-Adrenoceptor Antagonists

Compd no.	Structure	Mp, °C	Crystn solvent	Emp formula	Analyses	pA_2,[a] heart rate (β_1)	Slope	pA_2,[a] diastolic pressure (β_2)	Slope	Potency ratio, cardiac/vascular	Partition coeff[b]
9	(±)-$C_6H_5OCH_2CH(OH)CH_2NH$-*i*-Pr[c]	92–93	Cyclohexane	$C_{12}H_{19}NO_2$	C, H, N, M$^+$	7.35	1.04	6.69	1.10	5	0.73
10	(±)-$C_6H_5OCH_2CH(OH)CH_2NH$-homoveratryl[d]	152	MeOH	$C_{19}H_{25}NO_4 \cdot HCl$	C, H, N, M$^+$	7.20	0.95	6.16	1.00	10	50
11	(±)-4-$CH_3C_6H_3OCH_2CH(OH)CH_2NH$-*i*-Pr[e]	91	Benzene	$C_{13}H_{21}NO_2$	C, H, N, M$^+$	6.63	0.95	6.63	1.08	1	2.65
12	(±)-*erythro*-$C_6H_5OCH_2CH(OH)CH(CH_3)NH$-*i*-Pr	77–78	*n*-Hexane	$C_{13}H_{21}NO_2$	C, H, N, M$^+$	6.30	1.10	6.82	1.02	0.3	0.57
		206	EtOH–AcOEt	$C_{13}H_{21}NO_2 \cdot HCl$	C, H, N						
13	(±)-*erythro*-$C_6H_5OCH_2CH(OH)CH(CH_3)NH$-homoveratryl	152–153	*i*-PrOH	$C_{20}H_{27}NO_4 \cdot HCl$	C, H, N, M$^+$	6.66	1.07	<5.00		>40	46
14	(±)-*threo*-$C_6H_5OCH_2CH(OH)CH(CH_3)NH$-*i*-Pr	58–59	*n*-Hexane	$C_{13}H_{21}NO_2$	C, H, N, M$^+$	5.66	1.05	6.20	0.99	0.3	0.57
		124	EtOH–AcOEt	$C_{13}H_{21}NO_2 \cdot C_2H_2O_4$	C, H, N						
15	(±)-*erythro*-$C_6H_5OCH_2CH(OH)CH(C_6H_5)NH$-*i*-Pr	126	*n*-Hexane	$C_{18}H_{23}NO_2$	C, H, N, M$^+$	<5.00		<5.00		125	
16	(±)-*erythro*-4-$CH_3C_6H_4CH(OH)CH(CH_3)NH$-*i*-Pr (H35/25)[f]	171–172	EtOH–AcOEt	$C_{18}H_{23}NO_2 \cdot HCl$	C, H, N	<5.00		5.47	1.04	<0.3	1.41
17	(±)-Practolol[g]					6.64	1.05	5.30	0.97	22	0.09

[a] Values represent the average of three determinations or more. SEM were ± 0.1 or less. [b] Values represent the mean of two determinations. Duplicates agreed within 5% or less. [c] See ref 18. [d] See ref 6. [e] See ref 23. [f] See ref 24. [g] See ref 25.

A representative case is the pair of β-adrenoceptor blockers: 1-(4-methylphenyl)-2-isopropylaminoethanol (H29/50), a nonselective blocker, and its α-methyl analogue, 1-(4-methylphenyl)-2-isopropylaminopropan-1-ol (H35/25), a vascular, (β_2) selective blocker.[9]

Extrapolation of this finding to the aryloxypropanolamine series, and thereby its validation, would require introduction of alkyl substituents at C-3 of the side chain of the "parent compound" **9**. This has not been done yet; instead, C-3 methylation of propranolol failed to shift its nonselective β-blocking property toward vascular selectivity.[8] Against this rather inconsistent background information, the findings reported herein, based on the general structure of 1-phenoxy-3-substituted aminopropan-2-ol, allow certain conclusions to be made regarding the effect of side-chain substitutions on β-adrenoceptor blockade.

Firstly, the parent compound itself (**9**) is a moderately cardioselective β-adrenoceptor antagonist ($\beta_1/\beta_2 = 5$). Cardioselectivity of **9** becomes even more pronounced ($\beta_1/\beta_2 = 25$) when tested in vitro, comparing its relative affinities to guinea pig right atria and trachea.[18] Introduction of a methyl substituent into the side chain of **9**, at C-3, produced two diastereomeric methyl analogues, *erythro*-**12** and *threo*-**14**, both exhibiting vascular selectivity to the same extent ($\beta_2/\beta_1 = 3$). Side-chain methylation of **9** thus *reverses* its selectivity in β blockade from cardiac to vascular receptors, exclusively due to perturbation of fit to myocardial receptors. This perturbation of fit most likely reflects limited bulk tolerance at the β_1-adrenoceptor site for C-3 substitution on the alkanolamine side chain. However, the possibility of repulsion interaction between C-3 hydrophobic substituents and a complementary polar region at the receptor site leading to perturbation of fit should also be considered.

The *erythro*-methyl analogue **12** exhibits the same affinity for vascular β adrenoceptors as the parent compound (pA_2 values 6.82 and 6.69, respectively), whereas its affinity for myocardial receptors is reduced to one-tenth of that of the parent compound (pA_2 values 6.30 and 7.35, respectively). Of the two positional isomers, *erythro*-**12** and *threo*-**14**, **12** is about four times more potent as a β blocker than **14** on both types of β adrenoceptors. A similar stereochemical relationship has been reported for the 1-aryl-2-aminopropan-1-ol series.[7] The observed shift in selectivity from myocardial to vascular β-adrenoceptors upon side-chain methylation at C-3 of **9** parallels and amplifies a similar change from nonselectivity to vascular selectivity in the series of phenylethanolamines.[9] Bulk tolerance for C-3 substituents at both cardiac and vascular β adrenoceptors is exceeded by phenyl substitution. The C-3 phenyl analogue **15** is practically devoid of β-blocking activity ($pA_2 < 5.0$) and thus represents a cutoff point in steric fit.

The effect of C-3 substitution of aryloxypropanolamines on their relative affinities toward either myocardial or vascular β adrenoceptors is profoundly affected by the nature of the substituent on the amino nitrogen. This is clearly illustrated when the *N*-isopropyl group of the parent compound **9** is replaced by a bulkier, 3,4-dimethoxyphenethyl substituent. 1-Phenoxy-3-(3,4-dimethoxyphenethyl)aminopropan-2-ol (**10**) is a potent, cardioselective β blocker ($\beta_1/\beta_2 = 10$), approaching the selectivity ratio observed for practolol ($\beta_1/\beta_2 = 22$).[2] Compared to **9**, the presence of the bulkier 3,4-dimethoxyphenethyl group on the amino nitrogen reduces the affinity of **10** to β_2-vascular adrenoceptors by a factor of 3, without affecting its affinity to β_1-myocardial receptors.

The in vivo potency ratio of myocardial to vascular β blockade for **10** compares favorably with the in vitro value of 22 reported earlier.[6] Introduction of a methyl substituent at C-3 of **10** further enhances its cardioselectivity, the potency ratio β_1/β_2 of β blockade for the methyl analogue **13** being >40. This is in contrast with the observed shift from cardioselectivity to vascular selectivity following side-chain methylation of **9**. The simultaneous introduction of methyl and 3,4-dimethoxyphenethyl groups at C-3 and the terminal amino group, respectively, of the side chain of 1-phenoxy-3-aminopropan-2-ol severely perturbs the fit of **13** to vascular β_2 adrenoceptors ($pA_2 <$ 5.0) while permitting efficient interaction with myocardial β_1 receptors ($pA_2 = 6.66$). The net result is thus the emergence of an aryloxypropanolamine analogue carrying side-chain substitution, **13**, exhibiting a marked in vivo cardioselective β blockade with a potency equal to that of practolol. Finally, the contribution of the oxymethylene group inserted between the substituted enthanolamine side chain and the aryl nucleus to the affinity of the β blockers is clearly evident when comparing the pA_2 values of *dl-erythro*-1-(4-methylphenyl)-2-isopropylaminopropan-1-ol (**16**) (pA_2 on $\beta_2 = 5.47$) and **12** (pA_2 on $\beta_2 = 6.82$). While both compounds exhibit vascular selectivity, **12** is 22 times more potent at a β_2 blocker. The direct involvement of the ethereal oxygen in binding of aryloxypropanolamine molecules (with unsubstituted side chains) to their receptors has been suggested previously.[19]

For strict comparison, the *p*-methyl substitution in **16** should also be considered when comparing its pA_2 value on vascular β_2 receptors with those of the aryloxypropanolamine compounds. It is therefore noteworthy that *p*-methyl substitution of **9** does not affect at all its affinity to vascular adrenoceptors: pA_2 values for **9** and **11** are 6.69 and 6.63, respectively. Affinity to myocardial receptors, however, is considerably reduced in the *p*-methyl analogue **11** ($pA_2 = 6.63$ compared to $pA_2 = 7.35$ for **9**), in keeping with in vitro data on guinea pig atrial preparation.[20]

There is thus an adverse steric effect for β-adrenoceptor interaction at position 4 of the aromatic nucleus of certain aryloxypropanolamine compounds. In the case of 4-methyl substitution (**11**), affinity toward myocardial β adrenoceptors is reduced with concomitant loss of cardioselectivity observed in the parent compound **9**. With increasing length of the para substituent, this adverse steric effect expresses itself more strongly on vascular β_2 adrenoceptors, leading in the case of the 4-acetamido analogue (practolol, **17**)[2] to a cardioselective β-adrenoceptor antagonist.

Apparent partition coefficient measurements in 1-octanol/buffer, pH 7.4, (Table I) reveal that the lipid solubility of **9** is only slightly affected by the introduction of a methyl substitution on the side chain (**12**, **14**). The expected increase in lipophilicity ($\pi \approx 0.5$), however, is observed when the methyl group is attached to the aromatic ring (**11**). The highest values of lipophilicity are attained when aromatic substituents such as phenyl (**15**) or homoveratryl (**10**, **13**) are present in the molecule.

In the case of the methyl substituent, the electronic character of the region in which it is placed seems to have a profound effect on its π value. Thus, the neighboring charged center of the amine in **12**–**14** completely negates the hydrophobic contribution of the methyl group. With the much more hydrophobic aromatic substituents (compounds **10**, **13**, and **15**), however, this effect is not observed.

Considering the base strength of the amine function in the series of aryloxypropanolamines ($pK_a \sim 9.3$),[18] the amino alcohols are present predominantly as protonated species (~99.9%) at physiological pH. The uncharged species is therefore of very little importance in partitioning in 1-octanol/buffer, pH 7.4.

In conclusion, proper side-chain substitution at C-3 and the amino nitrogen of 1-phenoxy-3-aminopropan-2-ol leads to either vascular selective or cardioselective β blockers, due to the perturbed fit of the resulting analogues to either myocardial (β_1) or vascular (β_2) β-adrenoceptors. Steric factors, with the exclusion of lipophilicity, appear to determine the relative affinities toward either myocardial or vascular adrenoceptors of the β blockers reported herein. A high degree of in vivo cardioselectivity, together with a substantial β-blocking potency, has been achieved in the case of *dl-erythro*-1-phenoxy-3-[(3,4-dimethoxyphenethyl)amino]butan-2-ol (**13**).

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are corrected. IR spectra were recorded either in CCl_4 solutions, in KBr disks, or as liquid films on a Perkin-Elmer Infracord Model 177 spectrophotometer equipped with a NaCl prism. NMR spectra were taken on a Varian A-60 spectrometer for 5–10% solutions in either $CDCl_3$ or Me_2SO-d_6 containing Me_4Si as an internal standard. Mass spectra were taken with a Hitachi Perkin-Elmer RMU-6 instrument, the samples being introduced directly into the ion source through a vacuum-lock, electron energy 70 eV, electron current 20 μA, source temperature 170–200 °C, secondary electron multiplier as the detector. TLC's were performed on silica gel G or alumina precoated plates containing fluorescence indicator, layer thickness 0.25 mm (Riedel-De Haen, AG, Germany), and spots detected by UV and exposure to I_2 vapor. Reaction products were checked routinely by IR, NMR, and mass spectrometry and by TLC. All compounds showed the expected spectral characteristics. GC analysis of the aryloxypropanolamines (**9**–**15**) was carried out on a Packard Model 417 Beker gas chromatograph (flame ionization detector), with a 1 m × 4 mm i.d. glass column packed with 10% OV-101 coated on Gas Chrom Q (100–120 mesh). Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.2% of the theoretical values.

Partition coefficients (*P*) were measured by a modified Hansch procedure, employing a 1-octanol/phosphate buffer (0.02 M, pH 7.4) system. Partitioning was carried out at 20 ± 1.0 °C. Partition coefficients were calculated according to the following equation.

$$P = \frac{V_{\text{buffer}}}{V_{\text{octanol}}} \cdot \left(\frac{C_{\text{buffer, i}}}{C_{\text{buffer, f}}} - 1 \right)$$

The first factor in this equation is the ratio of buffer to octanol volume used for partitioning of the sample. The second factor contains the ratio of initial to final sample concentrations in the aqueous buffer layer, as determined by absorbance readings in the UV. A standard curve (A vs. C_{buffer}) of at least four points was used for each sample to determine the aqueous solutions' concentrations.

General Synthetic Methods. Olefins. *trans*-1-Phenoxy-2-butene (**1**)[10] was prepared from *trans*-crotyl chloride in 70% yield: bp 98 °C (14 mm) [lit.[10] bp 95–98 °C (12 mm)]; n^{20}_D 1.5220; IR (neat) 1675 (C=C), 1245 and 1220 (aryl ether), and 970 cm^{-1} (=CH–); ^1H NMR ($CDCl_3$) δ 1.53–1.78 (m, 3 H, $-CH_3$), 4.38–4.50 (m, 2 H, $-CH_2-$), 5.35–6.10 (m, 2 H, –CH=CH–), 6.65–7.45 (m, 5 H, Ph).

***cis*-1-Phenoxy-2-butene** (**2**) was prepared from 2-butyn-1-ol via 1-phenoxy-2-butyne,[12] followed by catalytic hydrogenation over $Pd/BaSO_4$.

To a well-stirred mixture of 3.50 g (50 mmol) of 2-butyn-1-ol, in Et_2O (12 mL) and pyridine (0.25 mL), was slowly added with cooling (–30 °C) and under a slow stream of N_2 4.32 g (16 mmol) of PBr_3. After completion of the PBr_3 addition, the temperature was maintained at –30 °C for 2 h and then allowed to rise gradually to 20 °C over a period of 2 h. The reaction mixture was finally

warmed to 40 °C for 30 min and then poured into 25 mL of a saturated NaCl solution. After shaking, the upper organic layer was separated. The aqueous layer was extracted with small portion of Et_2O, and the combined ethereal solutions were dried over anhydrous $MgSO_4$. The ether was distilled off at normal pressure, and the residue was carefully distilled in vacuo. **1-Bromo-2-butyne** was obtained as a colorless liquid: bp 95 °C (145 mm) [lit.[21] bp 60 °C (80 mm)]; yield 80%.

A mixture of 2.66 g (20 mmol) of 1-bromo-2-butyne, 1.88 g (20 mmol) of phenol, 2.76 g (20 mmol) of anhydrous K_2CO_3, and NaI (100 mg) in 2-butanone (100 mL) was refluxed for ca. 24 h. The reaction mixture was then filtered, the filtrate evaporated, and the residue taken up in CH_2Cl_2. The organic phase was extracted with 0.1 N NaOH, washed with H_2O, and dried ($MgSO_4$). After evaporation of the solvent, the residue was distilled in vacuo. **1-Phenoxy-2-butyne** was obtained as a colorless liquid: bp 65 °C (0.5 mm) [lit.[12] bp 65 °C (0.5 mm)]; yield 60%; R_f 0.27 (homogenous on TLC) (hexane); IR (neat) 2230 (–C≡C–), 1245 and 1220 cm^{-1} (aryl ether); ^1H NMR ($CDCl_3$) δ 1.84 (t, J = 1.8 Hz, 3 H, $-CH_3$), 4.63 (q, J = 1.8 Hz, 2 H, $-CH_2-$), 6.84–7.46 (m, 5 H, Ph).

A well-stirred mixture of 1-phenoxy-2-butyne (1.46 g, 10 mmol) and 10% Pd/$BaSO_4$ (150 mg) in pyridine (10 mL) was hydrogenated at atmospheric pressure at room temperature. Partial reduction of the acetylenic bond to an olefinic bond (absorption of 10 mmol of H_2) was completed in 23 h. The reduction stopped sharply at the olefin stage. The mixture was then diluted with Et_2O and filtered to remove the catalyst. The filtrate was extracted with 1 N HCl to remove the pyridine, washed with H_2O, and dried ($MgSO_4$). The Et_2O extract was evaporated, and the residue was distilled in vacuo. ***cis*-1-Phenoxy-2-butene (2)** was obtained as a colorless liquid: bp 80 °C (0.5 mm); yield 95%; n^{20}_D 1.5260; R_f 0.40 (homogenous on TLC) (hexane); IR (neat) 1660 (C=C), 1415 (=CH–), 1245 and 1220 cm^{-1} (aryl ether); ^1H NMR ($CDCl_3$) δ 1.73 (br d, J = 2.7 Hz, 3 H, $-CH_3$), 4.50–4.70 (m, 2 H, $-CH_2-$), 5.60–6.00 (m, 2 H, –CH=CH–), 6.80–7.45 (m, 5 H, Ph). Anal. ($C_{10}H_{12}O$) C, H.

***trans*-3-Phenoxy-1-phenylpropene (3)**[11] was prepared from cinnamyl alcohol via the chloride: mp 69–70 °C (cyclohexane) (lit.[11] mp 68–69.7 °C).

Epoxides. Epoxides 4 and 5 were prepared by reacting epichlorohydrin in large excess with phenol and *p*-cresol, respectively. The appropriate phenol (5 mmol), dissolved in 0.6 M NaOH (ca. 10 mL), was added slowly into epichlorohydrin (100 mmol) dissolved in MeOH (ca. 15 mL), with stirring, at room temperature. At the end of the reaction, excess epichlorohydrin and MeOH were removed by evaporation in vacuo, and the residue was taken into EtOAc. Evaporation of the dried extract, followed by fractional distillation, furnished the analytically pure products in high yields (80–90%).

***dl*-1-Phenoxy-2,3-epoxypropane (4)**:[22] bp 125–126 °C (15 mm) [lit.[22] bp 115 °C (3 mm)].

***dl*-1-(4-Methylphenoxy)-2,3-epoxypropane (5)**: bp 164–165 °C (21 mm); n^{20}_D 1.5270. Anal. ($C_{10}H_{12}O_2$) C, H.

Epoxides 6–8 were prepared from the corresponding olefins 1–3 by reaction with a slight excess (10%) of 90% *m*-chloroperbenzoic acid (mCPBA) in CH_3CN, at 4 °C, for 5 days. To the appropriate olefin (20 mmol) dissolved in CH_3CN (50 mL) was added dropwise with stirring and cooling mCPBA (22 mmol) dissolved in CH_3CN (60 mL). The reaction mixture was left at 4 °C for 5 days and the *m*-chlorobenzoic acid which precipitated was filtered off. The filtrate was evaporated to about one-third of the original volume and diluted with H_2O. The epoxides were extracted with CH_2Cl_2 and the organic extracts washed consecutively with 10% Na_2SO_3, 10% $NaHCO_3$, and H_2O and dried ($MgSO_4$). The epoxides were finally chromatographed on alumina (neutral, grade III) columns and eluted with *n*-hexane–benzene (9:1). Yields of the epoxidation reaction were in the range of 80%.

***dl-trans*-(2*SR*,3*SR*)-1-Phenoxy-2,3-epoxybutane (6)**: bp 75 °C (0.2 mm); n^{20}_D 1.5189; ^1H NMR ($CDCl_3$) δ 1.23 (d, J = 5 Hz, 3 H, CH_3), 2.72–3.40 (m, 2 H, epoxy CH–), 3.62–4.26 (m, 2 H, $-CH_2-$), 6.75–7.55 (m, 5 H, Ph). Anal. ($C_{10}H_{12}O_2$) C, H.

***dl-cis*-(2*RS*,3*SR*)-1-Phenoxy-2,3-epoxybutane (7)**: bp 100 °C (0.4 mm); n^{20}_D 1.5219; ^1H NMR ($CDCl_3$) δ 1.16–1.53 (m, 3 H, $-CH_3$), 3.00–3.50 (m, 2 H, epoxy CH–), 4.13 (d, J = 5 Hz, 2 H, $-CH_2-$), 6.83–7.70 (m, 5 H, Ph). Anal. ($C_{10}H_{12}O_2$) C, H.

***dl-trans*-(2*SR*,3*SR*)-1-Phenoxy-3-phenyl-2,3-epoxypropane (8)**: mp 113–114 °C (benzene). Anal. ($C_{15}H_{14}O_2$) C, H, M^+.

Aryloxypropanolamines. Amination of the monosubstituted epoxides 4 and 5 with an excess of i-$PrNH_2$ proceeded smoothly at room temperature to give the desired amino alcohols 9 and 11. The epoxides 4 and 5 in i-PrOH were added slowly into a large excess of i-$PrNH_2$, while stirring (amine/epoxide molar ratio, 50–100/1). The reaction mixture was left at room temperature for ca. 24 h and then evaporated to dryness in vacuo. The resulting amino alcohols 9 and 11 were crystallized to constant melting points. Yields were almost quantitative.

Amination under Forcing Conditions. Epoxides 4 and 6–8 were reacted with equimolar quantities of either homoveratrylamine or i-$PrNH_2$ in the presence of phenol as a catalyst. Molar ratios of epoxide/amine/phenol were 1:1:0.1. The reaction mixtures were heated in a sealed autoclave at 140 °C for ca. 40 h, treated with ethanolic HCl (10 N), and evaporated to dryness to give the crude amino alcohols as the hydrochloride salts. Yields of the amination reaction were almost quantitative. The resulting amino alcohols **10**, **12**, **13**, and **15** were recrystallized as the HCl salts and **14**, after generating the free base, as the oxalate. Amino alcohols as free bases were subsequently generated from the salts. Purity of all products was checked by elemental analyses, TLC, and GC.

TLC (alumina) R_f values for the stereoisomers of 1-phenoxy-3-isopropylaminobutan-2-ol are as follows: **12**, 0.29, PhH–AcOH (10:1), 0.28, *n*-PrOH–NH_4OH (9:1); **14**, 0.36, PhH–AcOH (10:1), 0.39, *n*-PrOH–NH_4OH (9:1).

A mixture of **12** and **14** displayed a depression of melting point.

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An ab Initio Study of Electronic Factors in Metabolic Hydroxylation of Aliphatic Carbon Atoms

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The monooxygenase-mediated hydroxylations of aliphatic carbon atoms are known to be regioselective for positions α to heteroatoms or to π systems (aromatic rings, carbon–carbon double bonds, carbonyl groups). Ab initio calculations (STO-3G and in some cases 4-31G) were performed on model molecules, indicating that the Mulliken overlap populations (taken as indices of electron bond densities) of C_α–H bonds being regioselectively hydroxylated are larger than C_β–H and C_γ–H overlap populations. These results support the hypothesis that metabolic C-hydroxylations occur by insertion of an activated oxygen species of electrophilic nature, probably oxene.

Carbon atoms in drugs and other xenobiotics undergo monooxygenase-mediated oxidations;[2] many questions regarding the basic mechanisms of these reactions cannot be answered convincingly at present, while it is certain that innumerable other questions remain unformulated.

In the past years, much effort has been directed toward an understanding of oxygen activation by monooxygenases.[3] These enzymes act by cleaving one molecule of oxygen and transferring an activated oxygen species to the substrate. There is now converging[4-7] but not definitive[8] evidence that cytochrome P-450 monooxygenases transfer a highly electrophilic oxenoid species to the substrate. A scheme summarizing our current understanding of cytochrome P-450 mediated oxygen activation, oxygen cleavage, and oxene transfer has been published.[9] Flavin-cofactored monooxygenases also act as oxenoid reagents.[10,11]

The mechanism of activated oxygen transfer from monooxygenases to aliphatic carbon atoms in drugs and other xenobiotics has been investigated by stereochemical studies[12-14] (for reviews, see also ref 2 and 15). The results have shown that the reaction occurs by a frontside displacement involving the insertion of an oxygen atom in the C–H bond. Isotope kinetic studies confirm that the cleavage of the C–H bond is often rate limiting in the overall hydroxylation reaction[16-19] (for a review, see also ref 20). A transition state involving approach of the activated oxygen atom toward the C–H bond and creation of a 2-electron 3-center bond has also been postulated in N-deethylation reactions.[21] Recently, an elegant technique utilizing intramolecular competition has shown unexpectedly high primary kinetic isotope effects in benzylic hydroxylation catalyzed by cytochrome P-450.[22] These results are fully compatible with, and provide strong evidence for, the oxene model.

A significant structural feature of biotransformation processes is their regioselectivity; this is particularly marked with aliphatic carbon hydroxylations. Indeed, it is consistently found that aliphatic carbons in position α to a heteroatom (N, O, S), an aromatic ring (benzylic position), a carbon–carbon double bond (allylic position), or a carbonyl group undergo preferred hydroxylation as compared to carbon atoms in β, γ, or other positions. The topic of regioselective biotransformation has been extensively reviewed.[23] Recent studies of interest include the first report of enzymic hydroxylation α to an acetylenic group[24] and a careful investigation of the metabolism of 1-methylcyclohexene and related terpinoids in mammals showing high regioselectivities for allylic positions.[25] The biotransformation of *N*-*n*-propylamphetamine by rat liver homogenates yielded ten times more N-dealkylated metabolites (resulting from C_α-hydroxylation) than C_β-hydroxylated metabolites.[26]

A recent quantum mechanical study[9] using the EHT method has pointed to a direct correlation between the electron density at the C–H bond and the regioselectivity of metabolic oxidation. Indeed, the C–H electron density at the benzylic position and at carbon atoms α to nitrogen and oxygen atoms was found to be larger than the C–H electron density at other positions. In the present study, these preliminary results are confirmed by ab initio calculations (STO-3G and 4-31G) and extended to include other types of C_α positions.

Computations. The molecules investigated belong to three groups: the *N*-alkyl derivatives [ethylamine (**1**), *n*-propylamine (**2**), protonated *n*-propylamine (**2**$^+$-H), isopropylamine (**3**), and *N*-ethylaniline (**4**)]; the *O*-alkyl derivatives [ethyl methyl ether (**5**), *n*-propyl methyl ether (**6**), isopropyl methyl ether (**7**), and phenethole (**8**)], and the π-alkyl group [ethylbenzene (**9**), 1-butene (**10**), 1-butyne (**11**), and propionamide (**12**)]. Their oxidative metabolism is known either for the molecules as such or as fragments of larger molecules.[2] Standard geometries were used,[27,28] and no geometry optimization was undertaken. The molecules were set in low-energy conformations as depicted (**1**–**12**). The neutral (nonprotonated) state was considered for the amines, with the exception of **2**$^+$-H taken for comparison purposes. Kinetic studies have indeed revealed that the basic amines undergoing N-demethylation react in the neutral state with microsomal enzymes.[29]

The ab initio SCF–MO calculations[30] were carried out on the CDC-CYBER 7326 computer of the Federal Institute of Technology and of the University of Lausanne using the GAUSSIAN 70 program.[31] In this treatment, each molecular orbital is represented as a linear combination of a set of basis functions. Current computations replace the Slater-type (exponential) functions with linear combinations of Gaussian functions. When the basis functions correspond just to those shells which are fully or partly populated in the atomic ground state, the basis set is described as minimal. The most popular basis set is

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