Journal of Chromatography, 218 (1981) 639–646
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,103

GROUP-CONTRIBUTION APPROACH TO THE BEHAVIOUR OF 2-PHENYLETHYLAMINES IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

High-performance liquid chromatography retention data are reported for 2-phenylethylamine and 30 of its derivatives, including examples of CH_3, OH, OCH_3, COOH, NH_2 and N-oxide substitution. An octadecyl-silica column was used with an eluent of 10% methanol containing an amine–phosphate buffer (pH 3.15) to limit the interference of the silica support with chromatographic retention. The effects of individual substituents on retention are quantified by using group-contribution values (τ) and are discussed with reference to the position of substitution and the intramolecular interactions with other groups.

INTRODUCTION

2-Phenylethylamine can be regarded as the parent compound of a large group of drugs known as the sympathomimetic amines. The structure is modified by substitution of the aromatic ring, the α- and β-carbon atoms and the terminal amino group to yield compounds with a wide range of pharmacological activity[1]. Many of the drugs undergo extensive modification *in vivo*. These reactions can include dealkylation of the amino group, hydroxylation of the aromatic ring or β-carbon or nitrogen atom and methylation of aromatic hydroxyl groups. Thus, there are a vast number of 2-phenylethylamine drugs and metabolites with closely related structures, which pose an enormous separation challenge to pharmaceutical, clinical and forensic laboratories. High-performance liquid chromatography (HPLC) offers an attractive approach to this problem, as all these compounds can be chromatographed without derivatization.

Chromatographic analysis can sometimes be limited by the absence of an authentic sample of the drug or metabolite for comparison of retention data. Many metabolites of 2-phenylethylamine drugs are difficult to obtain and in such instances, the prediction of retention data from molecular structure can be particularly valuable. The identification of unknown chromatographic peaks obtained during metabolic studies of new drugs or in toxicological studies may therefore be facilitated by the use of such data.

A group-contribution approach has been suggested for the prediction of

0021-9673/81/0000–0000/$02.50

HPLC retention data of compounds having a common parent structure (Tomlinson and co-workers[2–5]; Horváth and co-workers[6,7]). The chromatographic retention of a compound is described in terms of the retention of the parent structure with modifications for each of the substituents. Experimental data for a series of model compounds are required such that the group-contribution value (τ) for each substituent can be measured. Various factors can influence the magnitude of τ for a given substituent including temperature, eluent composition and stationary phase[2–7]. Further, the position of substitution within a molecule can also be important[6]. Nevertheless, for a given stationary phase and eluent, previous studies have indicated that this approach has potential for predicting chromatographic data.

Surprisingly, there have been few studies that have explored the effects of simple substituents on the chromatographic properties of a given structure. The present study seeks to expand our knowledge on the chromatographic behaviour of 2-phenylethylamine derivatives and contains data for 31 compounds on octadecyl-silica (ODS-silica). The effects of various substituents are discussed with reference to the positions of substitution and intra-molecular interactions with other substituents. Molnár and Horváth[6] have considered some substituent effects within a group of hydrophilic phenylethylamines, but no data were presented for hydrophobic compounds of this type.

EXPERIMENTAL

Apparatus

HPLC was performed with a Waters M6000 pump, a Rheodyne 7120 injection valve (fitted with a 20-μl loop) and a Perkin-Elmer LC-75 variable-wavelength UV detector operated at 250 nm. The column (25 cm × 5 mm I.D.) was of stainless steel and packed with 5-μm ODS-Hypersil (Shandon Southern Products, Runcorn, Great Britain) by a slurry procedure using isopropanol for dispersing the packing material and hexane as the pressurising solvent.

Materials

Methanol, sodium hydroxide and orthophosphoric acid were AnalaR grade (BDH, Poole, Great Britain). Diethylamine was puriss grade obtained from Fluka (Fluorochem, Glossop, Great Britain).

N,N-Dimethylphenylethylamine was prepared by reductive amination of phenylacetaldehyde with dimethylamine, using sodium cyanoborohydride following the method of Lane[8]. N,N-Dimethylphenylethylamine N-oxide was prepared by oxidation of N,N-dimethylphenylethylamine in 30% hydrogen peroxide at room temperature. N-Hydroxymethylamphetamine was prepared as the oxalate salt by the reaction of benzylmethylketone with N-hydroxymethylamine in the presence of sodium cyanoborohydride following the procedures of Morgan and Beckett[9].

Eluent

Orthophosphoric acid (25.5 ml; 87%; 0.40 mole) was added to distilled water (1700 ml), and diethylamine (21.0 ml; 0.20 mole) was added. The pH was adjusted to 2.9 with concentrated sodium hydroxide solution and the total volume was adjusted to 1800 ml. Methanol (200 ml) was then added to give an eluent that had a pH of 3.15.

A flow-rate of 2.5 ml/min was used, and the eluent was recycled through the HPLC column for at least 1 h before retention measurements were made. All measurements were made at a temperature of 23° ± 2°C.

Evaluation of substituent effects

Retention data were expressed as capacity ratios, k', which are defined by

$$k' = (t_R - t_0)/t_0$$

where t_R and t_0 are the retention times of the substance under investigation and a non-retained compound, respectively.

The effects of individual substituents on retention were quantified by using group-contribution values, τ, defined by

$$\tau = \log k'_1 - \log k'_2$$

where k'_1 and k'_2 are the capacity ratios of two compounds that differ by a single substituent (k'_2 is the capacity ratio of the unsubstituted compound). Hence, a positive value indicates that an increase in retention occurs on substitution.

RESULTS AND DISCUSSION

The eluent in this study contained diethylamine to ensure that chromatographic retention was not influenced by the silica matrix of the packing material. Without the addition of such masking agents, basic compounds often give broad and asymmetric peaks arising from a dual mechanism of hydrophobic interactions with bonded hydrocarbonaceous ligands and polar interactions with accessible silanol groups on the silica support[10–14]. The masking agent must show strong binding to the silanol groups and must be present at a concentration sufficient to compete with the solute. Previous studies on substituent effects using the group-contribution approach have not attempted to mask the silanophilic interactions of packing materials.

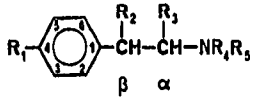
Table I lists the 2-phenylethylamine derivatives considered in this study, together with details of their structures and measured capacity ratios (k'). All compounds showed satisfactory peak shapes with the chosen eluent. It is envisaged that, at the pH of the eluent, the terminal amino groups of 2-phenylethylamines will be protonated. Further, the aromatic hydroxyl substituents will be non-ionised and the carboxyl groups of phenylalanine and tyrosine will be partially ionised (pK_a values 2.16 and 2.20, respectively)[15].

Table I contains two pairs of compounds that have identical molecular formulae (*viz.*, ephedrine and pseudoephedrine, and phenylpropanolamine and norpseudoephedrine). The data show that both pairs can be separated on the present HPLC system. Each structural formula has two non-identical asymmetric carbon atoms, and hence diastereoisomers can exist[16]. Fig. 1 gives Newman projections indicating the stereochemical structures of the four compounds. The chromatographic data show that the "A" isomers have the shorter retention times (*i.e.*, ephedrine is eluted before pseudoephedrine, and phenylpropanolamine before norpseudoephedrine). The explanation probably rests with the intra-molecular hydrogen bonding that

TABLE I

HPLC RETENTION DATA FOR 31 2-PHENYLETHYLAMINES

Eluent: diethylamine (0.1 *M*) and orthophosphoric acid (0.2 *M*) in 10% (v/v) methanol adjusted to pH 3.15 with sodium hydroxide. Column: Hypersil-ODS; 25 cm × 5 mm I.D.



Compound	*Substituents*						*HPLC capacity ratio (k')*
	R_1	R_2	R_3	R_4	R_5	*Other*	
Amphetamine	H	H	CH_3	H	H		8.48
N,N-Dimethylamphetamine	H	H	CH_3	CH_3	CH_3		11.08
N,N-Dimethylphenylethylamine	H	H	H	CH_3	CH_3		4.82
N,N-Dimethylphenylethylamine N-oxide	H	H	H	CH_3	CH_3	N-oxide	10.78
Ephedrine	H	OH	CH_3	CH_3	H		5.68
Hordenine	OH	H	H	CH_3	CH_3		2.00
p-Hydroxyamphetamine	OH	H	CH_3	H	H		2.24

5-Hydroxydopamine	OH	H	H	H	H	3-OH, 5-OH	0.05
p-Hydroxyephedrine	OH	OH	CH_3	CH_3	H		0.73
N-Hydroxymethylamphetamine	H	H	CH_3	CH_3	OH		33.53
Mescaline	OCH_3	H	H	H	H	3-OCH_3, 5-OCH_3	16.82
p-Methoxyamphetamine	OCH_3	H	CH_3	H	H		14.95
o-Methoxyphenamine	H	H	CH_3	CH_3	H	2-OCH_3	32.17
p-Methoxyphenylethylamine	OCH_3	H	H	H	H		5.55
Methylamphetamine	H	H	CH_3	CH_3	H		10.52
β-Methylphenylethylamine	H	CH_3	H	H	H		8.55
N-Methylphenylethylamine	H	H	H	CH_3	H		4.23
Noradrenaline	OH	OH	H	H	H	3-OH	0.10
Norpseudoephedrine	H	OH	CH_3	H	H		4.39
Octopamine	OH	OH	H	H	H		0.18
Oxedrine	OH	OH	H	CH_3	H		0.27
Phenelzine	H	H	H	NH_2	H		5.91
Phentermine	H	H	CH_3	H	H	α-CH_3	19.46
Phenylalanine	H	H	COOH	H	H		2.46
Phenylethanolamine	H	OH	H	H	H		1.63
Phenylethylamine	H	H	H	H	H		3.64
Phenylpropanolamine	H	OH	CH_3	H	H		3.87
Pseudoephedrine	H	OH	CH_3	CH_3	H		5.90
p-Tolylethylamine	CH_3	H	H	H	H		12.68
Tyramine	OH	H	H	H	H		0.81
Tyrosine	OH	H	COOH	H	H		0.74

TABLE II

EFFECT OF SUBSTITUENTS ON THE CHROMATOGRAPHIC RETENTION OF 2-PHENYLETHYLAMINES

Substituent	*Position*	*Parent compound*	*Substituted compound*	*τ value*
Methyl	α	Oxedrine	*p*-Hydroxyephedrine	+0.43
	α	Tyramine	*p*-Hydroxyamphetamine	+0.44
	α	*p*-Methoxyphenylethylamine	*p*-Methoxyamphetamine	+0.43
	α	Phenylethanolamine	Phenylpropanolamine/norpseudoephedrine	+0.38/+0.43
	α	N-Methylphenylethylamine	Methylamphetamine	+0.40
	α	Phenylethylamine	Amphetamine	+0.37
	α	N,N-Dimethylphenylethylamine	N,N-Dimethylamphetamine	+0.36
	α	Amphetamine	Phentermine	+0.36
	β	Phenylethylamine	β-Methylphenylethylamine	+0.37
	p-Ar	Phenylethylamine	*p*-Tolylethylamine	+0.54
	N	Phenylpropanolamine	Ephedrine	+0.17
	N	Norpseudoephedrine	Pseudoephedrine	+0.13
	N	Amphetamine	Methylamphetamine	+0.09
	N	Phenylethylamine	N-Methylphenylethylamine	+0.07
	N	N-Methylphenylethylamine	N,N-Dimethylphenylethylamine	+0.06
	N	Methylamphetamine	N,N-Dimethylamphetamine	+0.02
Hydroxyl	β	Phenylethylamine	Phenylethanolamine	−0.35
	β	Amphetamine	Phenylpropanolamine/norpseudoephedrine	−0.34/−0.29
	β	Methylamphetamine	Ephedrine/pseudoephedrine	−0.27/−0.25
	p-Ar	Ephedrine	*p*-Hydroxyephedrine	−0.89
	p-Ar	Phenylethylamine	*p*-Tyramine	−0.65
	p-Ar	Amphetamine	*p*-Hydroxyamphetamine	−0.58
	p-Ar	Phenylalanine	Tyrosine	−0.52
	p-Ar	N,N-Dimethylphenylethylamine	Hordenine	−0.38
	N	Methylamphetamine	N-Hydroxymethylamphetamine	+0.50
Methoxyl	*p*-Ar	Phenylethylamine	*p*-Methoxyphenylethylamine	+0.18
	p-Ar	Amphetamine	*p*-Methoxyamphetamine	+0.25
	o-Ar	Methylamphetamine	*o*-Methoxyphenamine	+0.49
Carboxyl	α	Tyramine	Tyrosine	−0.04
	α	Phenylethylamine	Phenylalanine	−0.17
Amino	N	Phenylethylamine	Phenelzine	+0.21
N-oxide	N	N,N-Dimethylphenylethylamine	N,N-Dimethylphenylethylamine N-oxide	+0.35

can occur between the terminal amino group and the β-hydroxyl group. Models show that this hydrogen bonding is destabilised in the "A" isomers as a result of steric interactions between the methyl group on the α-carbon atom and the phenyl group of the β-carbon atom (see Fig. 1). In contrast, the "B" isomers show no steric crowding and can form strong hydrogen bonds. Thus, the polar groups of the "B" isomers are less accessible for interaction with the eluent and so increase retention. These examples emphasise the limitations of the group-contribution approach for predicting chromatographic retention and the need to consider intra-molecular interactions between substituents.

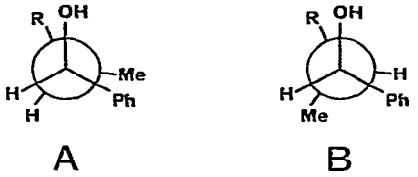


Fig. 1. Stereochemical structures of phenylpropanolamine (A, R = NH_2), norpseudoephedrine (B, R = NH_2), ephedrine (A, R = NHMe) and pseudoephedrine (B, R = NHMe). Me = Methyl; Ph = phenyl.

The data in Table I have been used to assess the quantitative effects of substituents. Table II contains τ values calculated for pairs of compounds that differ by a single substituent. Those compounds with $k' < 0.25$ (*i.e.*, 5-hydroxydopamine, noradrenaline and octopamine) have not been used, as the accuracy of such small capacity ratios is not expected to be high. Values of τ shown in Table II represent the replacement of a hydrogen atom on the phenylethylamine "core" by the given substituent. However, the N-oxide substitution is of a different type, the oxygen atom being added without replacement of hydrogen. Where substitution can produce two alternative diastereoisomers (*e.g.*, α-methylation of phenylethanolamine), both τ values are included in Table II. Values of τ for further substitution of diastereoisomers (*e.g.*, N-methylation of phenylpropanolamine; *p*-hydroxylation of ephedrine) are only included when the substituted compound has the same stereochemistry at the asymmetric carbon atoms.

The attachment of a methyl group to the molecule causes an increase in retention (τ positive) irrespective of the position of substitution. The values for α-carbon substitution are in the range +0.36 to +0.44, and the one example of β-carbon substitution also lies in this range. Methyl substitution on the aromatic ring causes a larger change (+0.54), whereas substitution on the nitrogen atom has a smaller effect than elsewhere. Further, it appears that the effect of the first methyl substitution on nitrogen is greater than the second.

Hydroxyl substitution on carbon leads to a decrease in retention (τ negative) in all instances. Values for β-carbon substitution fall in the range −0.25 to −0.35, and *p*-aromatic substitution causes larger decreases in retention (range −0.38 to −0.89). The low values for β-carbon substitution probably reflect the hydrogen bonding that can occur with the terminal amino group.

In contrast, hydroxyl substitution on nitrogen causes an increase in retention (τ = +0.50). It is interesting to compare this result with that from the other example of N-oxidation included in this study (*i.e.*, N-oxide formation) where again an increase

in retention is observed ($\tau = +0.35$). This observation is supported by literature data for other N-oxides. Jensen[17] records that the N-oxides of amitriptyline and imipramine are more strongly retained than the free bases (ODS-silica; 60% acetonitrile; pH 3). Under acidic conditions, both hydroxylamines and N-oxides protonate to give cations. Nitrogen-protonation of a hydroxylamine and oxygen-protonation of a N-oxide give species with equivalent cationic centres (*i.e.*, tetracoordinate nitrogen bearing one hydroxyl group). Thus, when the protonated forms of "parent" and "substituted" compounds are compared, it can be seen that a N–H bond is replaced by a N–OH bond for both hydroxylamine formation and N-oxide formation. The results indicate that cations bearing the N–OH group are more hydrophobic than the protonated parent amines. The attachment of an amino group to nitrogen also gives an increase in retention (see Table II).

Table II shows that the substitution of methoxyl groups on the aromatic ring increases chromatographic retention. Substitution at the 2-position gives the larger effect and again this probably indicates hydrogen bonding between the terminal amino group and the introduced substituent. A τ value of $+0.66$ is calculated for the simultaneous introduction of three methoxyl groups into phenylethylamine to give mescaline (see Table I). This is about three times the value for the substitution of one methoxyl group in the *p*-position (Table II) and suggests that the τ values for methoxyl groups in the *m*- and *p*-positions are equivalent and independent. The small changes in retention observed for substitution with a carboxyl group at the α-carbon atom are in agreement with the results of Molnár and Horváth[6].

The present data provide a useful framework for understanding the behaviour of 2-phenylethylamine derivatives in reversed-phase HPLC. Further, the group-contribution values, τ, could prove valuable as an aid to the prediction of chromatographic data for the metabolites of sympathomimetic drugs.

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