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Lipophilicity determination of some monoamine oxidase inhibitors: the effect of methanol and ammonium chloride

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ABSTRACT

The lipophilicity (R_M value) of seventeen monoamine oxidase inhibitory drugs was determined by reversed-phase thin-layer chromatography, and the effect of salt concentration on the reversed-phase retention was studied by adding ammonium chloride to the eluent. Each drug exhibited regular retention behaviour, its R_M value linearly decreasing with increasing concentration of methanol in the eluent. Ammonium chloride decreased the retention: the effect was higher at lower salt concentrations, which indicates that the phenomenon is of saturation character. The influence of ammonium chloride depended on the concentration of methanol (on the dielectric constant of the eluent) suggesting that methanol suppresses the dissociation of ammonium chloride resulting in a modified salting-in effect.

INTRODUCTION

In the past three decades, quantitative structure–activity relationship (QSAR) methods have been frequently applied to the design of new drugs, pesticides, etc. [1,2] and to elucidate the impact of the various physicochemical parameters that influence biological activity [3–6]. A wide range of molecular parameters have been examined in QSAR studies [7,8], and many of these parameters can readily be determined by various chromatographic techniques [9]. Chromatographic methods have several advantages: they are rapid and relatively simple; very small amounts of the substances are required; and the compounds need not be very pure.

The biological activity of a compound is controlled by many factors, one of the most important being its lipophilicity as its penetration through the membrane of the target organism is governed chiefly by the molecular lipophilicity. In addition, interaction with receptors may also sometimes depend on lipophilicity. Reversed-phase thin-layer chromatography (RPTLC) has been frequently used to determine the lipophilicity of various bioactive molecules [10–12]. The R_M value (related to molecular lipophilicity) determined by RPTLC generally depends on the concentration of the organic modifier in the eluent [13]. In most cases the correlation is linear. In order to increase the accuracy of the lipophilicity determination, the R_M value extrapolated to zero organic component concentration (R_{M0}) has been calculated from the linear correlation between the actual R_M value and the concentration of organic modifier in the eluent [14]. The slope

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value of the correlation has been regarded as characteristic of the specific hydrophobic surface area of the compound [15]. In case of a homologous series of compounds, the slope and the R_{M0} values exhibited a significant linear correlation [16], but for a non-homologous series both parameters were needed to describe the lipophilicity accurately [17]. However, with peptitides [18,19], quaternary amino steroids [20] and crown ether derivatives [21,22], no linear correlation was found between the R_M valuc and the concentration of the organic mobile phase component. Salts are in a more or less dissociated state in the eluents. The dissociated ions markedly modify the retention (lipophilicity) of bioactive compounds containing one or more polarizable substructures [23,24]. This phenomenon can be explained by the capacity of ions to suppress dissociation [25], resulting in increased retention, and their salting-out or salting-in effects. As the biological processes always occur in ionic environment, the ionic effect discussed above may have a considerable impact on the strength of electrostatic interactions between the target organism and the bioactive molecules [26,27], which may modify the biological activity.

The objectives of our work were to determine the lipophilicity of some monoamine oxidase (MAO) inhibitors [28,29] for further QSAR studies and to elucidate how the relationship between the salt and organic component of the mobile phase affects the retention.

EXPERIMENTAL

Silcoplat plates (Labor MIM, Budapest, Hungary) were impregnated by overnight predevelopment in *n*-hexane–paraffin oil (95:5, v/v). Silcoplat plates are plastic sheets (20 × 20 cm) precoated with a 0.25-mm layer of silica. The structures of the MAO inhibitors are listed in Table I. They were separately dissolved in methanol to give a concentration of 5 mg/ml, and 2 μl of solution were spotted onto the plates. The developments were carried out in sandwich chambers (22 × 22 × 3 cm) at room temperature, and the running distance was *ca.* 15 cm. The chambers were not presaturated. The eluent contained 0–80 vol.% methanol in steps of 10 vol.%. The ammonium chloride concentration of the eluent varied between 0 and 5 *M*. As the drugs remained on the start line in pure water and in aqueous ammonium chloride solutions, the eluent always contained a mixture of methanol and ammonium chloride in various ratios. This experimental design allowed the extrapolation of R_M values to zero organic phase and to zero ammonium chloride concentration. We used ammonium chloride because of its high solubility in the eluents. After development the plates were dried at 105°C, and the spots were detected with iodine vapour. Each determination was run in quadruplicate.

The R_M values were calculated from eqn. 1:

$$R_M = \log (1/R_F - 1) \quad (1)$$

The dependence of the R_M value on the eluent composition was calcualted from eqn. 2:

$$R_M = R_{M0} + b_1C_1 + b_2C_1 \log C_2 \quad (2)$$

where R_M is the actual R_M value of a compound determined at C_1 vol.% methanol and C_2 *M* ammonium chloride concentrations, and R_{M0} is the lipophilicity value of a compound extrapolated to zero salt and methanol concentrations.

The inclusion of the combined independent variable in eqn. 2 was motivated by the following theoretical considerations.

(1) The effect of salts is of saturation character that can be described by a logarithmic function.

(2) The apparent dissociation constant of salts strongly depends on the dielectric constant of the solvent [30], which decreases with increasing methanol concentration in the eluent. This effect lessens the concentration of the dissociated ions responsible for the modified retention.

When the coefficient of variation (C.V.) between the parallel determinations was higher than 5%, the data were omitted from the calculations.

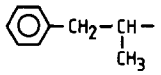
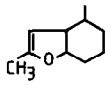
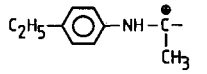
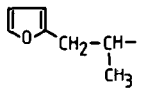
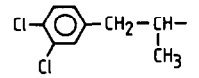
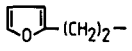
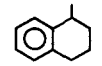
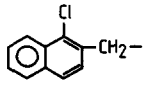
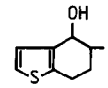
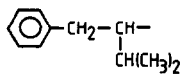
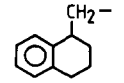
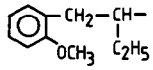
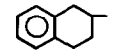
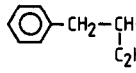
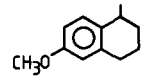
To elucidate the homogenous or inhomogenous character of the compounds, a linear correlation was calculated between the R_{M0} and b_1 values of eqn. 2:

$$R_{M0} = a + bb_1 \quad (3)$$

TABLE I

MOLECULAR STRUCTURES OF MONOAMINE OXIDASE INHIBITORS

General structure: R_1–N(–R_2)–CH_2–C≡CH

Compound No.	R_1	R_2	Compound No.	R_1	R_2
1 (+)		$-CH_3$			
2 (−)		$-CH_3$	10		$-CH_3$
3		$-CH_3$	11		$-H$
4		$-H$	12		$-CH_3$
5		$-CH_3$	13		$-CH_3$
6		$-CH_3$	14		$-CH_3$
7		$-CH_3$	15		$-CH_3$
8		$-CH_3$	16		$-CH_3$
9		$-C_4H_7$	17		$-CH_3$

RESULTS AND DISCUSSION

The drugs remained on the start line in water and in aqueous salt solution, therefore it was not possible to compare the calculated and measured R_{MO} values. We are well aware that the determination of the octanol/water log P values and the comparison of log P and R_{MO} values may enhance the information content of our experiments. Because several studies have proved that the log P and R_{MO} values show excellent correlation [31,32], and we accepted the validity of the reversed-phase chromatographic experiments, we did not determine the octanol/water partition of the drugs.

The R_M value of each compound decreased linearly with increasing concentration of methanol in the eluent (Fig. 1), and no anomalous retention behaviour was observed that would render invalid the extrapolation to zero organic phase concentration. The retention of the compounds decreased in the presence of ammonium chloride. This observation can be explained either by the salting-in effect or by a decreased retention capacity resulting from the competition between the dissociated ions of ammonium chloride and the solutes for the free silanol groups on the silica surface. The effect of salt depended on the molecular structure of the solute, and the retention order changed at higher salt concentrations. Very

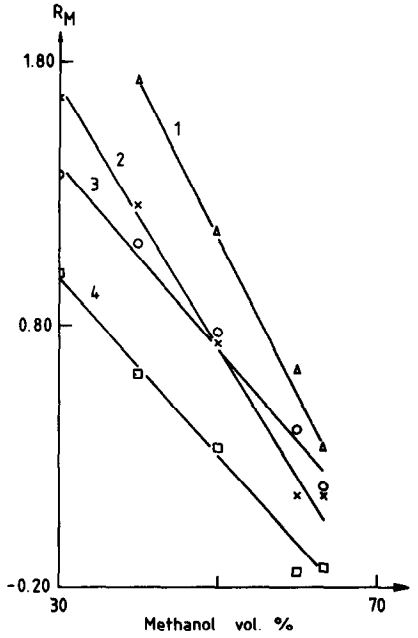


Fig. 1. Dependence of R_M value of some monoamine oxidase inhibitors on methanol concentration in the eluent. Curves: 1 = compound 12, 0.05 *M* NH_4Cl; 2 = compound 12, 0.50 *M* NH_4Cl; 3 = compound 17, 0.05 *M* NH_4Cl; 4 = compound 17, 0.50 *M* NH_4Cl.

low concentrations (0.025 *M*) of ammonium chloride drastically decreased the retention of MAO inhibitors (Fig. 2), but concentrations higher than 1 *M* did not cause a further decrease

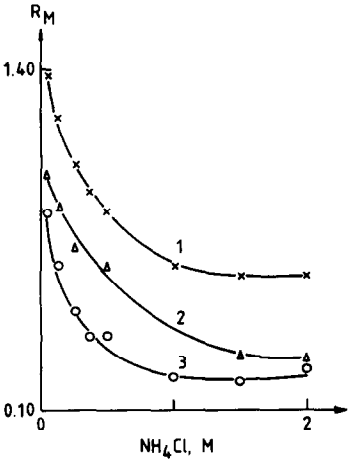


Fig. 2. Dependence of the R_M value of some monoamine oxidase inhibitors on the ammonium chloride concentration in water–methanol (6:4). Curves: 1 = compound 7; 2 = compound 8; 3 = compound 16.

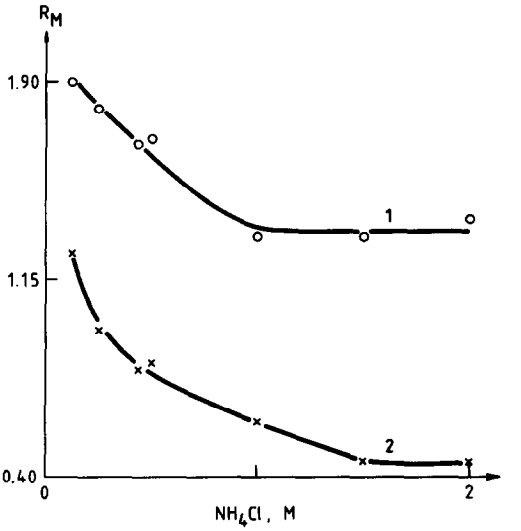


Fig. 3. Dependence of R_M value of compound 9 on the ammonium chloride concentration in the eluent. Curves: 1 = 40 vol.% methanol; 2 = 60 vol.% methanol.

in the retention. This observation can be explained by the supposition that the ions occupy the free silanol groups, thus enhancing the desorption rate of the solutes that leads to increased mobility. When the silanol groups are saturated with the ions, further increase of salt concentration has no effect on the retention. The non-linear dependence of retention on the concentration of ammonium chloride prevails at each methanol concentration (Fig. 3).

The parameters of eqn. 2 are compiled in Table II. The results of the calculations entirely support our previous qualitative conclusions. The equation fits well to the experimental data, and the significance level wās in each case higher than 99.9% (see $F_{calc.}$ values). The two independent variables explained the overwhelming majority of the change of R_M values (see r^2 values). Increasing the methanol concentration caused a linear decrease in the R_M valuc (sec b_1 values), and its impact on the retention was higher than that of the combined independent variable (see path coefficient *b* percentage values). The significant contribution of the combined variable to the change of R_M value (see b_2 values) proves that the effect of salt can be described with a logarithmic

TABLE II

PARAMETERS OF THE RELATIONSHIPS BETWEEN THE R_M VALUE OF MONOAMINE OXIDASE INHIBITORS AND THE METHANOL (C_1) AND AMMONIUM CHLORIDE (C_2) CONCENTRATIONS

Numbers refer to monoamine oxidase inhibitors in Table I. $R_M = R_{M0} + b_1C_1 + b_2C_1 \log C_2$. C_1 = methanol concentration in the eluent (vol.%); C_2 = ammonium chloride concentration in the eluent (M); n = number of observations; R_{M0} = R_M value of a drug extrapolated to zero methanol and ammonium chloride concentration; b_1 = change of R_M value caused by unit change (vol.%) of methanol concentration in the eluent; b_2 = change of R_M value caused by the change of the combined independent variable $C_1 \cdot \log C_2$; s_{bi} = standard deviation of the corresponding b (slope) value; b_i (%) = impact in percent of the corresponding independent variable on the R_M value irrespectively of the dimensions of the independent variable; r^2 = the ratio of total variance explained by the independent variables; $F_{calc.}$ = indicator of the fitness of equation to the experimental data.

Compound No.	n	R_{M0}	b_1	$s_{b_1} \cdot 10^2$	b_2	$s_{b_2} \cdot 10^2$	b_1 (%)	b_2 (%)	r^2	$F_{calc.}$
1	44	1.09	−2.70	7.08	−0.77	6.97	77.57	22.43	0.9927	729.1
2	44	1.09	−2.60	6.95	−0.79	6.83	76.49	23.51	0.9716	701.1
3	44	1.23	−2.51	10.7	−0.80	10.5	75.51	24.49	0.9305	274.6
4	44	0.87	−2.85	10.8	−0.66	10.6	80.90	19.10	0.9454	274.6
5	44	1.39	−2.86	10.6	−0.84	10.5	77.06	22.94	0.9468	364.6
6	35	3.72	−5.20	19.5	−0.42	5.31	77.28	22.72	0.9591	375.0
7	48	1.99	−3.28	9.22	−0.88	5.94	70.56	29.44	0.9658	635.8
8	44	1.48	−2.93	7.51	−0.82	7.39	77.92	22.08	0.9739	765.6
9	37	3.02	−3.94	16.6	−1.00	5.32	55.93	44.07	0.9482	310.8
10	44	1.40	−2.77	8.60	−0.86	8.46	76.17	23.83	0.9621	364.6
11	44	1.45	−3.31	14.0	−0.75	13.8	81.38	18.62	0.9359	285.1
12	41	2.86	−4.55	26.9	−0.75	10.4	69.98	30.02	0.8823	142.5
13	43	1.59	−2.32	7.90	−0.99	7.67	69.47	30.53	0.9559	433.1
14	44	1.19	−2.95	11.3	−0.66	11.2	81.42	18.58	0.9441	346.2
15	44	1.77	−2.38	10.0	−0.89	9.86	72.37	27.63	0.9324	282.8
16	41	1.75	−3.33	8.83	−1.01	8.32	75.71	24.29	0.9740	713.0
17	49	1.88	−3.48	8.76	−0.83	5.84	73.64	26.36	0.9720	798.0

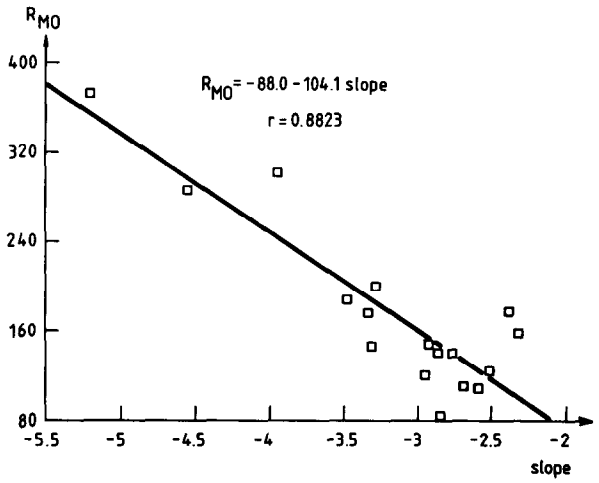


Fig. 4. Relationship between the lipophilicity (R_{M0}) and specific hydrophobic surface area (slope) of some MAO inhibitors. Numbers refer to inhibitors in Table I.

correlation, *i.e.* the effect probably is of saturation character. However, the influence of salt is related to the concentration of methanol (on the dielectric constant of the eluent). This result entirely supports the theoretical considerations outlined in the Introduction, *i.e.* the dissociated ions account for the modification of the retention, and the number of dissociated ions depends not only on the concentration of ammonium chloride in the eluent but also on the composition of the eluent.

A highly significant (significance level over 99.9%) linear correlation was found between the R_{M0} and b_1 values of eqn. 2 (Fig. 4). This finding indicates that the lipophilicity and specific hydrophobic surface area of these MAO inhibitors are intercorrelated, and they form a homologous se-

ries of compounds. This result is somewhat surprising because the drugs have chemically related structures only in the propargylamine group. The data suggest that the effect of the common propargylamine group prevails and markedly influences the retention of each MAO inhibitory drug. However, we must emphasize that the correlation is not strong enough to substitute the parameters for each other in QSAR calculations. Their information content is somewhat different, therefore their separate application in the further calculations is proposed.

ACKNOWLEDGEMENT

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