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Use of TLC and multivariate mathematical statistical methods to study the interaction of monoamine oxidase inhibitory drugs with amino acids*

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Abstract: The interaction of 17 monoamine oxidase inhibitory drugs (propargylamine derivatives) with amino acids was studied by charge-transfer chromatography. The data set was evaluated by principal component analysis (PCA). To assess the effect of the information loss caused by normalization, PCA was separately carried out on the covariance and on the correlation matrix. The strength and selectivity of interaction was separated by the spectral mapping technique. Calculation proved that the amino group of the drugs interacted with the second carboxyl group of dicarboxylic amino acids, and that the interaction was of electrostatic character. This finding made probable the direct interaction between the drugs and the target enzyme or enzymes. The results of both PCA methods were similar. However, coordinates of the spectral map showed only slight correlation with the corresponding coordinates of the two-dimensional nonlinear maps proving the different information content of the methods.

Keywords: *Principal component analysis; spectral mapping; stepwise regression analysis.*

Introduction

Various substituted propargylamine derivatives are promising therapeutic compounds as monoamine oxidase inhibitory drugs [1, 2], however, their exact mode of action has not been elucidated in detail [3]. It is possible that they bind directly to the enzyme thereby modifying its activity.

In recent research, charge-transfer chromatography has been applied to study biologically important molecular interactions [4–6]. To avoid the application of nonpolar organic solvents in the study of charge-transfer interactions, reversed-phase thin-layer chromatography (RPTLC) was applied using aqueous eluents [7].

The objectives of our work were to elucidate the possible role of individual amino acids in the binding of some monoamine oxidase inhibitory drugs to enzymes by determining the drug–amino acid interactions. Charge-transfer chromatography and various multivariate mathematical statistical methods helped the elucidation of the character of interaction.

Experimental

Silufol, UV_{254} (Kavalier, Brno, Czechoslovakia) plates were impregnated by overnight predevelopment in *n*-hexane–paraffin oil (95:5, v/v). The L-amino acids of analytical purity were purchased from REANAL Fine Chemicals (Budapest, Hungary). The chemical structures of the monoamine oxidase inhibitory drugs are listed in Table 1. The drugs were dissolved separately in methanol to give a concentration of 5 mg ml^{-1}, and 2 μl of solution was spotted onto the plates. The eluents were methanol–water (1:1, v/v) mixtures containing 25 mM amino acid. Amino acid free methanol–water (1:1, v/v) mixtures served as control. The spots were detected with iodine vapours. Each determination was run in quadruplicate. The R_M values were separately calculated for each drug and for each amino acid.

To find the similarities and dissimilarities between the effect of amino acids taking into consideration simultaneously the strength and selectivity of interaction, PCA was applied [8].

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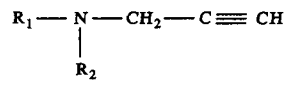
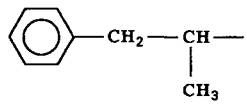
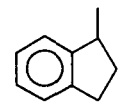
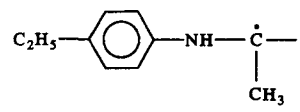
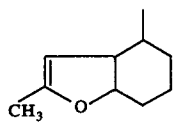
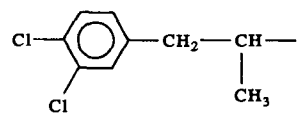
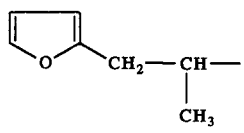
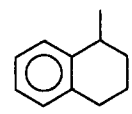
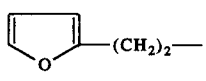
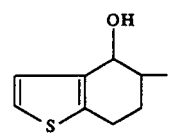
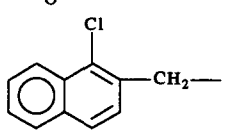
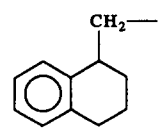
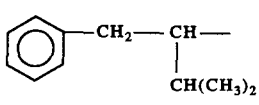
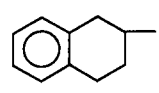
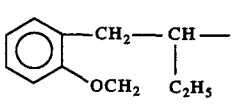
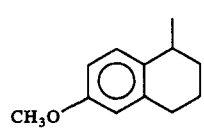
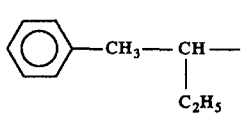
Table 1
Chemical structure of monoamine oxidase inhibitors



General structure

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

PCA, nonlinear mapping and spectral mapping softwares were developed at the Plant Protection Institute of Hungarian Academy of Sciences (Budapest, Hungary) by Dr B. Bordás. The 17 drugs served as variables and the amino acids were the observations. The two-dimensional nonlinear map of PC loadings and variables was also calculated [9]. To elucidate the effect of data normalization on the results of PCA, the calculation was carried out both on the covariance and on the correlation matrices and linear correlations were calculated between the corresponding coordinates of the nonlinear maps. The potency (strength of interaction taking into consideration simultaneously all interacting compounds) and spectral map (selectivity of interaction taking into consideration simultaneously all interacting compounds) was calculated by the spectral mapping technique

[10]. The information content of the spectral and nonlinear maps was compared by correlating the corresponding coordinates.

To elucidate the role of various molecular parameters of amino acids in the interaction, stepwise regression analysis was applied [11]. The software was the product of COMPUDRUG Ltd (Budapest, Hungary). The pK value of α-carboxyl, α-amino group, the pK of the side chain (taken from ref. 12) and the z_1, z_2 and z_3 values related to the hydrophobicity, side chain bulk and electronic properties of amino acids, respectively (taken from ref. 13) were the independent variables. As the linear character of the correlation had not been proved before, the quadratic forms of the independent variables were also included in the calculation. The control Arg, Lys and Tyr were omitted from the calculation, because Arg and Lys showed low mobility under the chromatographic conditions, and the solubility of Tyr did not reach 25 mM in the eluent. Stepwise regression analysis was carried out seven times, the first and second coordinates of both nonlinear maps and the spectral map as well as the potency values being separately the dependent variables.

Results and Discussion

The results of both PCAs are compiled in Table 2. The interaction of the 17 drugs with amino acids can be described by three principal components. In other words, three hypothetical compounds (imaginary, drug-like compounds) are sufficient to obtain the bulk of the information content of the interactions of the

Table 2
Results of principal component analysis carried out on the covariance (A) and on the correlation matrix (B) of the original data set

	Variance explained (%)		Total variance explained (%)	
Number of principal component	A	B	A	B
1	69.21	69.21	74.60	74.60
2	15.43	84.64	10.73	85.33
3	5.27	89.91	5.04	90.37
4	3.32	93.23	2.78	93.15

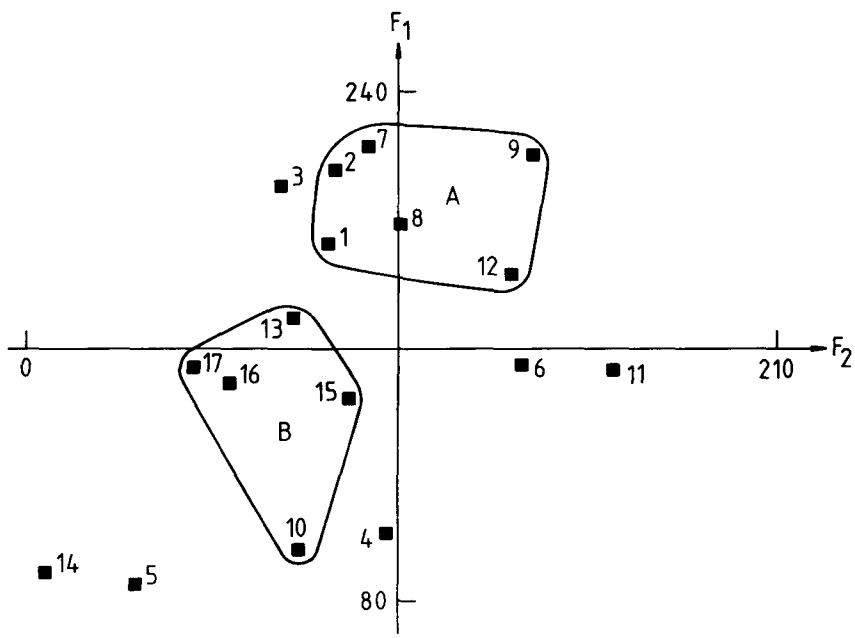


Figure 1
Two-dimensional nonlinear map of PC loadings. Covariance matrix. Number of iterations: 128. Max. error: 1.23×10^{-2}. Numbers refer to monoamine oxidase inhibitory drugs in Table 1. A = substituted benzene derivatives; B = condensed ring structures.

17 drugs with amino acids. Unfortunately, PCA does not define these three compounds, but only indicates their mathematical possibility. As the conclusions drawn from the two-dimensional nonlinear maps of PC loadings calculated with different methods are the same, only the map calculated from the covariance matrix is shown (Fig. 1). The drugs form two distinct clusters according to the substituents of the propargylamine group (cluster A = various benzene substituents; cluster B = various condensed ring substituents). The drugs with heterocyclic rings do not form a cluster. As will be shown later, the interaction of drugs with amino acids is of hydrophilic character and probably involves the polar amino group. The influence of the apolar substituents on the strength of interaction can be explained by the supposition that the electron donor or acceptor capacity of the substituents modifies the polarity of the amino group resulting in changed electrostatic forces involved in the interaction. The two-dimensional nonlinear maps of PC variables calculated with different methods also contain similar information (Fig. 2).

The majority of amino acids form a cluster including the control, i.e. these amino acids probably do not interact with the drugs or the interaction is so weak that it cannot be detected by this charge-transfer chromatographic method. Only amino acids with polar side chains show considerable effect that indicates the polar character of the interaction. The inefficiency of the dibasic amino acids Arg and Lys can be explained by the low mobility of these amino acids in the chromatographic system and by the absence of interaction with the similarly basic drugs. The different behaviour of Gsn and Asn cannot be explained and this phenomenon needs further investigation.

The potency values (reciprocally related to the strength of interaction) of amino acids and drugs calculated with the spectral map technique are compiled in Table 3. The data clearly show that Glu and Asp form the most stable complexes with the propargylamine derivatives. This finding is in good agreement with the results of PCA and suggests that the basic amino group of the drugs and the second carboxyl group of Glu and Asp are involved in the interaction. The differences between the potency values of drugs are higher than those between the amino acids. This result indicates that the strength of interaction of drugs with amino acids is highly different and this may contribute to their different biological activity. Although the spectral maps contain only the selectivity characteristics of drugs and amino acids, their information content (Figs 3 and 4) is similar to those of the nonlinear map of PC loadings (Fig. 1) and variables (Fig. 2). The drugs form clusters according to the character of the substituents and the acidic amino acids differ from the others.

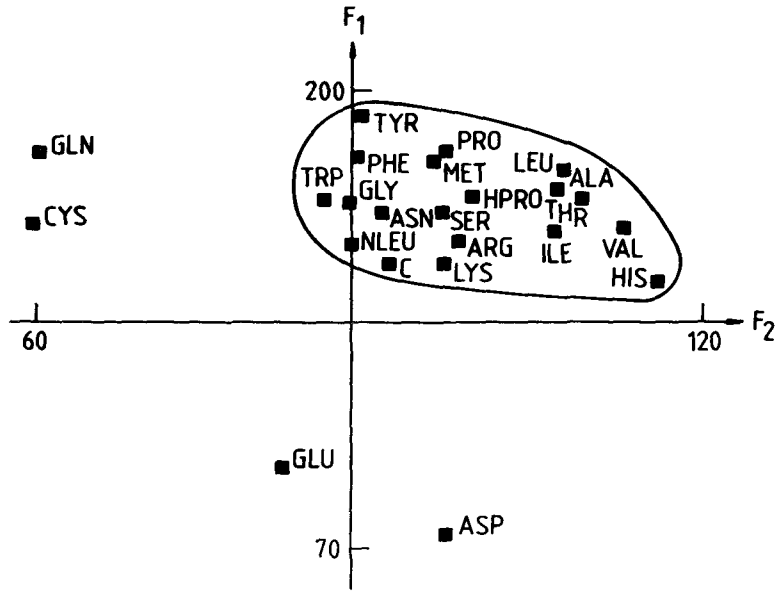


Figure 2
Two-dimensional nonlinear map of PC variables. Covariance matrix. Number of iterations: 52. Max. error: 1.39×10^{-2}. C = amino acid free eluent (control).

Table 3
Potency values (arbitrary units) of amino acids and drugs calculated with the spectral mapping technique. Numbers in the third column refer to monoamine oxidase inhibitory drugs in Table 1

Amino acids		Drugs	
Control	416	1	503
Ala	436	2	459
Arg	421	3	485
Asn	434	4	437
Asp	303	5	405
Cys	451	6	590
Gln	448	7	530
Glu	336	8	549
Gly	438	9	556
His	395	10	492
Hpro	437	11	567
Ile	421	12	592
Leu	443	13	508
Lys	414	14	333
Met	449	15	531
Nleu	421	16	522
Phe	455	17	529
Pro	454		
Ser	433		
Thr	435		
Trp	437		
Tyr	469		
Val	420		

The parameters of correlations between the strength and selectivity of interactions and the physico-chemical parameters of amino acids are compiled in Table 4. Each equation fits the experimental data well, the significance level was in all cases over 99.9% (see F values). In agreement with the results discussed above, only the pK value of the side chain of amino acids has a significant impact on the interaction, it explains about 89–93% of the total variance (see r^2 values). The correlation is markedly nonlinear, the quadratic member having a similar impact on the interaction as the linear one (compare path coefficient values).

The good correlations between the coordinates of two-dimensional nonlinear maps calculated by the different methods (Table 5) indicate that the information loss caused by the normalization is negligible. However, it must be emphasized that the differences between the results of the two methods of calculation may strongly depend on the original data matrix. The correlations between the nonlinear and spectral maps are significant. However, the ratio of variance explained is fairly low. This result indicates that the information content of the techniques are different, they are not interchangeable and the similarity of conclusions drawn from the different maps may be fortuitous.

It can be concluded from our data that the monoamine oxidase inhibitory drugs interact only with dicarboxylic acids and the amino group of the drugs and the second carboxyl group of the amino acids are possibly involved in the interaction. These results make probable

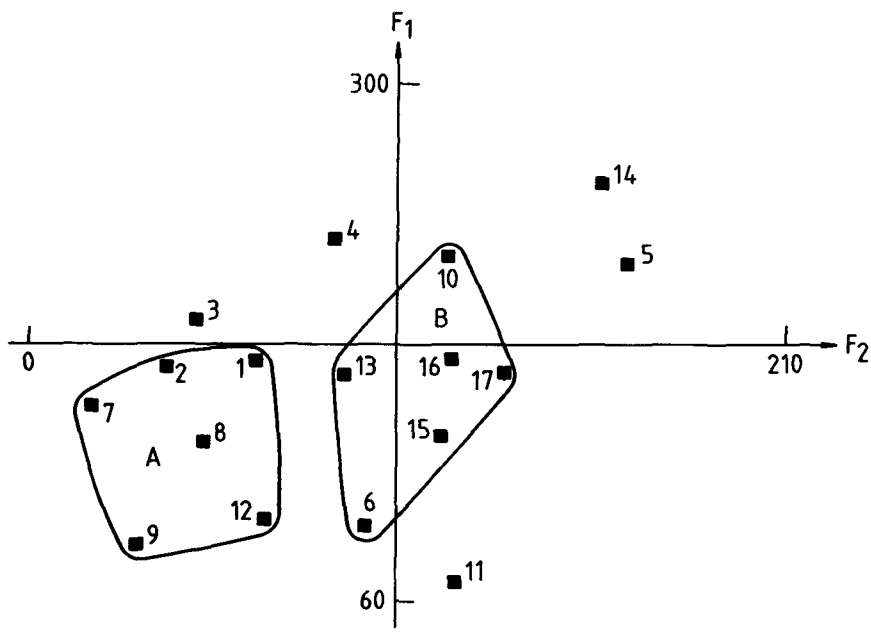


Figure 3
Spectral map of monoamine oxidase inhibitory drugs. Number of iterations: 150. Max. error: 3.49×10^{-2}. Numbers refer to monoamine oxidase inhibitory drugs in Table 1. A = substituted benzene derivatives; B = condensed ring structures.

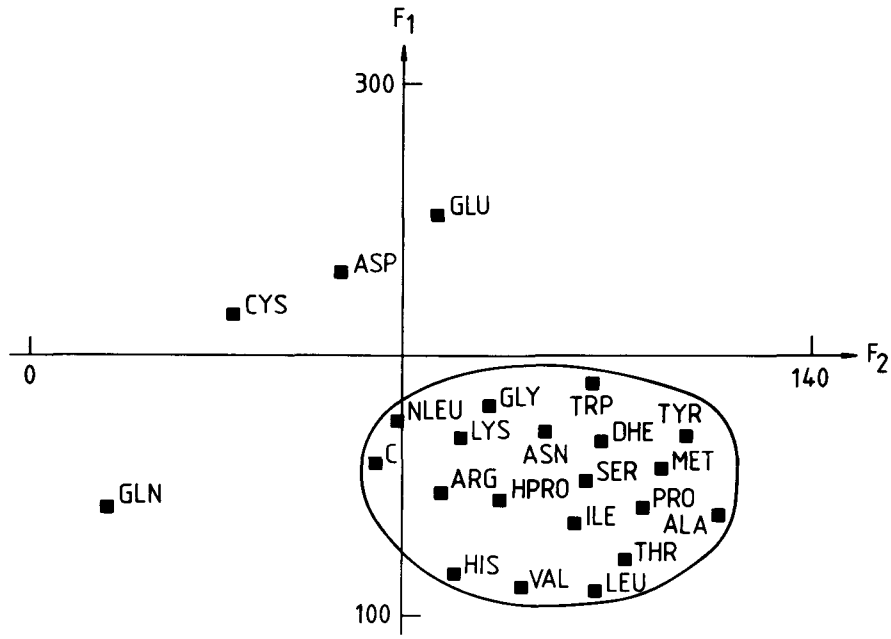


Figure 4
Spectral map of amino acids. Number of iterations: 135. Max. error: 5.03×10^{-2}. C = amino acid free eluent (control).

Table 4
Relationships between the strength and selectivity of interaction and the physicochemical parameters of amino acids

Results of stepwise regression analysis (n = 19)
(I) $Y_1 = a + b_1.x + b_2.(x)^2$
(II) $Y_2 = a + b_1.x + b_2.(x)^2$
(III) $Y_3 = a + b_1.x + b_2.(x)^2$

Parameter	No. of equation		
	(I)	(II)	(III)
a	−160.5	−181.0	23.5
b_1	77.1	84.2	94.8
s_{b1}	8.7	7.0	8.7
Path coefficient (%)	55.7	54.7	56.4
b_2	−4.27	−4.86	−5.11
s_{b1}	0.60	0.49	0.61
Path coefficient (%)	44.3	45.3	43.6
r^2	0.8925	0.9299	0.9319
F	66.45	106.07	109.42

a = intercept; b_{1-2} = slope values; s_{b1-2} = standard deviations of the corresponding slope values; path coefficient (%) = impact of individual independent variables on the change of the dependent variable; r^2 = coefficient of determination (ratio of variance explained by the change of independent variables); F = calculated value of Fisher test, indicator of the fitness of equation to the experimental data; Y_1 = first coordinate of the nonlinear map (correlation matrix); Y_2 = first coordinate of the nonlinear map (covariance matrix); Y_3 = potency; x = pK value of the side chain of amino acids.

the direct interaction of drugs with the target enzyme or enzymes and do not support the hypothesis that the drugs modify the organization of phospholipids surrounding the enzyme resulting in modified enzyme structure and activity. The data indicate that the interaction is of electrostatic character. The calculations proved that the information content of PCA

Table 5
Relationships between the corresponding coordinates of two-dimensional nonlinear and spectral maps. (As the slope and intercept values of the linear correlations are irrelevant only the coefficients of regression are given.)

	$Y = a + b.X$	
Y	X	Coefficient of regression
1	3	0.9873
1	5	0.5150
2	4	0.8258
2	6	0.6898
3	5	0.6214
4	6	0.5090
7	9	0.9220
7	11	0.3839
8	10	0.9483
8	12	0.6821
9	11	0.5641
10	12	0.5260

1 = first coordinate of the two-dimensional nonlinear map calculated from the correlation matrix (amino acids); 2 = second coordinate of the two-dimensional nonlinear map calculated from the correlation matrix (amino acids); 3 = first coordinate of the two-dimensional nonlinear map calculated from the covariance matrix (amino acids); 4 = second coordinate of the two-dimensional nonlinear map calculated from the covariance matrix (amino acids); 5 = first coordinate of the spectral map (amino acids); 6 = second coordinate of the spectral map (amino acids); 7 = first coordinate of the two-dimensional nonlinear map calculated from the correlation matrix (drugs); 8 = second coordinate of the two-dimensional nonlinear map calculated from the correlation matrix (drugs); 9 = first coordinate of the two-dimensional nonlinear map calculated from the covariance matrix (drugs); 10 = second coordinate of the two-dimensional nonlinear map calculated from the covariance matrix (drugs); 11 = first coordinate of the spectral map (drugs); 12 = second coordinate of the spectral map (drugs).

and spectral mapping techniques are different, and the stepwise regression analysis is a useful method to ascribe concrete physicochemical meaning of the PCA and spectral map coordinates.

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