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Determination of hydrophobicity of non-homologous series of anticancer drugs by reversed-phase high-performance liquid chromatography

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Abstract

The hydrophobicity and specific hydrophobic surface area of 21 commercial anticancer drugs were determined by reversed-phase high-performance liquid chromatography on an octadecyl-silica column using methanol–water mixtures as eluents. Linear correlations were calculated between the log k' values and the methanol concentration of the eluent, the intercept and slope were considered as the best estimation of the hydrophobicity and specific hydrophobic surface area. The relationship between retention characteristics and physicochemical parameters of drugs was evaluated by multivariate mathematical statistical methods, such as principal component analysis followed by two-dimensional non-linear mapping, varimax rotation and by cluster analysis. Anticancer drugs can be well separated by reversed-phase HPLC. Various multivariate mathematical statistical calculations indicate that the retention of the investigated drugs is mainly governed by hydrophobic and steric parameters. The results suggest that the use of principal component analysis followed by two-dimensional non-linear mapping is superior to cluster analysis for the evaluation of large retention data matrices.

1. Introduction

Many efforts have been devoted to the elucidation of the mode of action of various anticancer drugs. They can bind to different biomolecules such as model and native membranes [1], DNAs [2,3], and various proteins [4]. The binding of anticancer drugs to proteins may modify protein structure [5], can increase or decrease enzyme activity [6,7] resulting in modified biological efficiency of the drugs [8]. To elucidate the role of individual amino acids in the binding of anticancer drugs to proteins, the interaction of amino acids with anticancer drugs was studied and the involvement of hydrophillic forces in the drug–amino acid interaction has been stressed [9].

Quantitative structure–activity relationship (QSAR) methods play an important role in contemporary drug design [10,11]. Lipophilicity is one of the most important molecular properties applied in QSAR studies [12] because the biological activity of a molecule can generally be correlated with its ability to penetrate the different hydrophobic barriers (membranes) of the target organs or organisms [13]. In addition to

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the classical partition method [14], lipophilicity can be determined by reversed-phase thin-layer chromatography (RPTLC) [15,16], high-performance liquid chromatography (HPLC) [17,18], and gas chromatography (GC) [19].

Multivariate mathematical–statistical methods such as principal component analysis (PCA) [20], cluster analysis [21], etc., have been developed to extract maximal information from large data matrices. Both methods have been successfully used for the evaluation of data structure in HPLC [22,23].

The objectives of the present investigations were to determine the lipophilicity and specific surface area of a non-homologous series of anticancer drugs by reversed-phase HPLC for future QSAR studies, to find the relationship between retention characteristics and physicochemical parameters of anticancer drugs by the use of various multivariate mathematical–statistical methods and to compare the information obtained by various methods.

2. Experimental

2.1. Reversed-phase HPLC

The HPLC system consisted of a Gilson 307 pump (Gilson, Villiers-le-Bel, France), a Cecil CE-212 variable-wavelength UV detector (Cecil Instruments, Cambridge, UK), a Valco injector (Valco, Houston, TX, USA) with a 20-μl sample loop and a Waters 740 integrator (Waters-Millipore, Milford, MA, USA). The reversed-phase column was a Hypersil ODS column (250 × 4 mm I.D., particle diameter 5 μm; Shandon, Cheshire, UK). The flow-rate was 1.0 ml/min and the detection wavelength was 215 nm. Mixtures of 0.025 *M* KH_2PO_4 and methanol were used as eluents. Methanol concentrations ranged from 0 to 90% (v/v). The use of this wide range of methanol concentration was motivated by the highly different lipophilicity of the anticancer drugs. The chemical structure, and the common and IUPAC names of the anticancer drugs are shown in Table 1 and Fig. 1. The anticancer drugs were dissolved in methanol at a concentration of 0.05 mg/ml. The retention time of each compound in each eluent was determined with three consecutive determinations. Linear correlations were calculated between the $\log k'$ value of the drugs and the methanol concentration in the eluent.

$$\log k' = \log k'_0 + b \cdot C \quad (1)$$

where $\log k'$ is the logarithm of the capacity factor, $\log k'_0$ is the logarithm of the capacity factor extrapolated to zero methanol concentration in the eluent, b is the change of the $\log k'$ value caused by a unit change (1 vol.%) in the methanol concentration, and C is the methanol concentration (vol.%). The intercept and slope values were considered as the best estimation of the hydrophobicity and specific surface area [24] of the drugs.

2.2. Multivariate mathematical–statistical methods

The parameters of Eq. (1) (slope = $\log k'_0$ and intercept = b values), the combined hydrophobicity parameter $\log k'_0/b$ [25] and various physicochemical characteristics of drugs (altogether 12 variables) were considered as variables and the anticancer drugs were the observations. The inclusion of the combined hydrophobicity parameter in the calculations was motivated by the recent finding that this parameter seems to be the best descriptor of the hydrophobic character of a solute [26]. The physicochemical parameters included in the calculation were:

– π = Hansch–Fujita's substituent constant characterizing hydrophobicity [27,28]

– H-Do = indicator variable for proton donor properties [29]

– M-RE = molar refractivity [30]

– F, R = Swain–Lupton's electronic parameters characterizing the inductive and resonance effect, respectively [31]

– σ = Hammett's constant, characterizing the electron-withdrawing power of the substituent [32]

Table 1
Chemical structure, common name and IUPAC nomenclature of anticancer drugs

Number	Common name	Chemical composition	Supplier
1	Ftorafur	N-(2-Furanidyl)-5-fluorouracil	Medexport (Russia)
2	Bicnu	N,N-Bis(2-chloroethyl)-N-nitrosourea	Laboratoire BRISTOL (France)
3	Leukeran	4-[Bis(2-chloroethyl)amino]benzenebutanic acid	Wellcome Foundation (UK)
4	Vincristin	22-Oxo-(3α,14β,16α)-14,15-dihydro-14-hydroxy-eburnamenine-14-carbocyclic acid methyl ester	Richter Gedeon (Hungary)
5	Vinblastine	(3α,14β,16α)-14,15-Dihydro-14-hydroxyeburnamenine-14-carbocyclic acid methyl ester	Richter Gedeon (Hungary)
6	Vumon	4′-O-demethyl-1-O(4,6-O-2-thenylidene-β-D-glucopyranosyl)epipodophyllotoxin	Bristol-Arzneimittel (Germany)
7	Provera	17-α-Acetoxy-6-α-(methyl)progesterone	Upjohn Limited (UK)
8	Bleogin	N^1-[3-Dimethyl(sulfonio)propyl]bleomycin amide	Nippon Kayaku (Japan)
9	Paraplatin	9,11,15-Trihydroxy-15-methylprosta-5-13-dienoic acid	Bristol-Arzneimettel (Germany)
10	Farmorubicin	(8S-*cis*)-10-[(3-Amino-2,3,6-trideoxy-α-L-arabino-hexopyranosyl)oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-5,12-naphthacenedione	Farmitalia (Italy)
11	Adriblastine (Doxorubicine)	10-[3-(Amino-2,3,6-trideoxy-α-L-hexapyranosyl)oxy]-7,8,9-tetrahydro-6,8,11-trihydroxy-8-(hydroxyacetyl)-1-methoxy-5-12-naphthacenedione	Farmitalia (Italy)
12	Natulan	N-(1-Methylethyl)4-[(2-methylhydrazino)methyl]-benzamide	Roche (Switzerland)
13	Alexan	4-Amino-1-β-D-arabifuranosyl-2(14)-pyrimidine	Mack (Germany)
14	Mitomycin C Kyowa	[1-aR]-6-Amino-8-[(aminocarbonyl)oxymethyl]-1,1a,2,8,8a,8b-hexahydro-8a-methoxy-5-methyl-azirino-[2′,3′:3,4]pyrrolo[1,1a]indole-4,7-dione	Kyowa (Japan)
15	Estracyt	Estra-1,3,5-(10)-triene-3,17-diol-3-[bis-chloroethyl)carbamate	Aktiebolaget (Sweden)
16	Deticene	5-(3,3-Dimethyl-1-triazenyl)-1*H*-imidazole-4-carboxamide	Rhone-Poulenc (France)
17	Metotrexate	2,4,-Diamino-10-methyl-pteroylglutamic acid	Lachema (Czech Republic)
18	Myelobromol	1,6-Dibrom-1,6-bis(dezoxy)-D-mannitol	Chinoin (Hungary)
19	Zitostop	1,2,5,6-Tetramezil-D-mannitol	EGIS Pharm.Works (Hungary)
20	Elobromol	1,6-Dibrom-1,6-bis(dezoxy)-D-dulcitol	Chinoin (Hungary)
21	Taxol	[2aR-[2aα,4β,4aβ,6β,9α(aR*,βS*),11α,-12α,12aα,12bα]]-β-(Benzoylamino)-α-hydroxybenzenepropanoic acid 6,12b-bis(acetyoxy)-12-(benzoyloxy)-2a,3,4,4a,5,6,9,10,11,12,12a,12b-dodecahydro-4,11-dihydroxy-4a,8,13,-13 tetramethyl-5-oxo-7,11-methano-14-cyclodeca[3,4]benz[1,2-b]oxet-9-yl ester	Sigma Chemie (Germany)

– Es = Taft's constant, characterizing steric effects of the substituent [33]

– B_1, B_4 = Sterimol width parameters [34,35].

The limit of variance explained was set to 99.9%. To facilitate evaluation of the PCA results both two-dimensional non-linear mapping [36] and cluster analysis were carried out on the principal component loadings and variables. Varimax rotation around two axes [37] was carried out only on the principal component loadings. To elucidate the influence of PCA on the data evaluation, cluster analysis was also applied to the original data matrix. The cluster analysis and non-linear mapping technique are theoretically

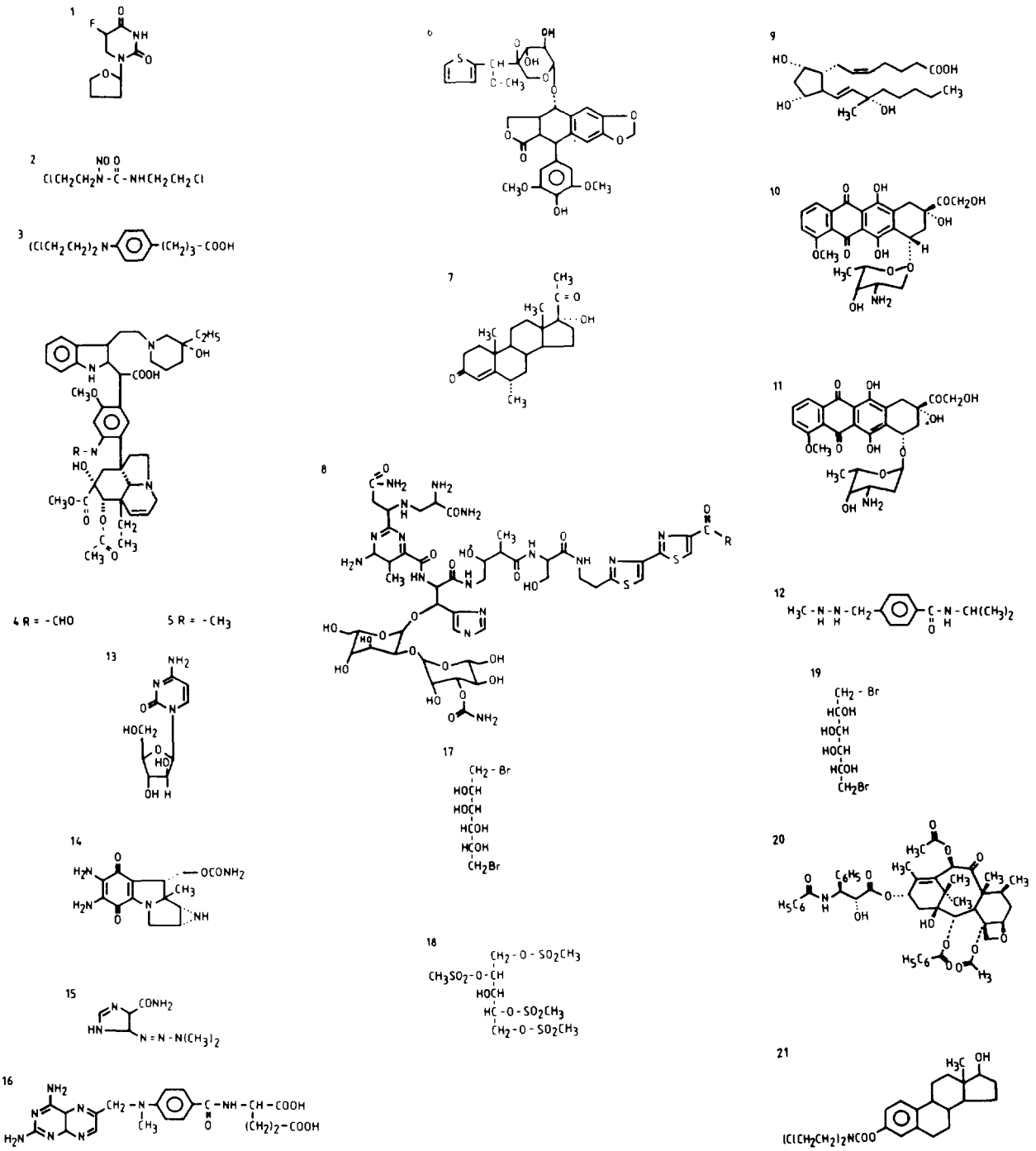

Fig. 1. Structures of the compounds listed in Table 1.

similar: both methods calculate and visualize the relative distances between the members of a data matrix (in our case: the physicochemical and chromatographic parameters of drugs). To find the similarities between the results of both methods, linear correlations were calculated between the corresponding distances on the non-linear map and cluster dendogram:

$$Y = a + b \cdot (X_1 \text{ or } X_2) \quad (2)$$

where Y is the relative distances between the anticancer drugs on the non-linear map, X_1 is the relative distance between anticancer drugs on the cluster dendogram calculated from the original data matrix, and X_2 is the relative distance between anticancer drugs on the cluster dendogram after PCA. To facilitate the calculations only the distances between the nearest neighbour drugs on the maps were included in the equations. The comparison of distances was hampered by the fact that their absolute value depends on the dimensions of the plots. We overcame this difficulty by data normalization: the greatest distances on each map were considered to be 100% and the other distance were calculated accordingly.

To compare the information content of non-linear mapping and varimax rotation techniques linear correlations were calculated between their corresponding coordinates: $Y_1 = a + b \cdot X_1$ and $Y_2 = a + b \cdot X_2$ where Y_1 and Y_2 are the first and second coordinates of the varimax rotation, respectively, and X_1 and X_2 are the first and second coordinates of the non-linear map.

3. Results and discussion

Each drug showed symmetrical peaks in each eluent system (Fig. 2), and thus reversed-phase HPLC can be successfully used for the exact determination of the hydrophobicity parameters of anticancer drugs and these parameters can be applied in future QSAR studies. The parameters of Eq. (1) are compiled in Table 2. The relationships between the logarithm of the capacity factor and the methanol concentration in the eluent was linear in each case.

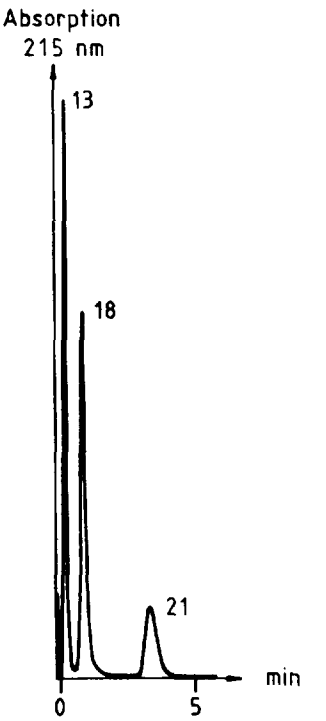

Fig. 2. Separation of anticancer drugs on ODS column. Eluent: methanol–0.025 M K_2HPO_4 (20:80, v/v); flow-rate 1.0 ml/min, detection: 215 nm. Numbers refer to anticancer drugs in Table 1.

The value of the regression coefficient in most cases was over 0.99 proving the applicability of Eq. (1). Both the slope and intercept values show high differences proving that any set of anticancer drugs can be successfully separated by reversed-phase HPLC by using an adequately chosen eluent system.

The PCA results are summarized in Tables 3 and 4. Five principal components explain more than 90% of the total variance. This result indicates that the 12 physicochemical and chromatographic parameters can be substituted by five background variables loosing only 10% of the total information. Unfortunately, PCA does not prove the existence of such background variables as concrete physicochemical entities but only indicates its mathematical possibility. The chromatographic parameters ($\log k'_0$, b,

Table 2
Parameters of linear correlations between the logarithm of capacity factor ($\log k'$) and the methanol concentration (C) in the eluent (s_b = standard deviation of the slope "b")

Compound No	$\log k' = \log k'_0 + b \cdot C$			
	$\log k'_0$	$-b \cdot 10^2$	$s_b \cdot 10^3$	r
1	1.34	5.14	1.6	0.9988
2	0.91	1.30	1.3	0.9922
3	1.40	3.06	2.0	0.9797
4	1.41	2.68	1.9	0.9985
5	1.70	1.45	1.8	0.9854
6	0.98	1.47	0.7	0.9903
7	1.40	1.86	0.5	0.9995
8	0.90	1.62	2.8	0.9865
9	1.03	1.69	1.2	0.9789
10	1.64	2.27	0.6	0.9998
11	1.92	1.43	1.2	0.9987
12	1.55	2.48	1.5	0.9987
13	0.05	2.94	1.3	0.9861
14	1.52	2.48	1.7	0.9935
15	1.20	3.45	1.8	0.9954
16	1.44	3.66	0.5	0.9876
17	0.17	4.86	2.1	0.9902
18	0.14	2.15	1.8	0.9866
19	0.29	2.75	1.1	0.9973
20	3.86	5.10	1.7	0.9978
21	0.15	4.34	1.4	0.9876

$\log k'_0/b$) – together with the calculated molecular hydrophobicity and some electronic parameters of drugs – have high loadings in the second and third PC components. This result indicates that not only the hydrophobicity of drugs but also their electronic parameters influence their retention on ODS column. This finding can be explained by the assumption that the free silanol groups not covered by the hydrophobic ligand

Table 3
Relationship between retention characteristics and physicochemical parameters for anticancer drugs. Results of principal component analysis

Eigen values	Total variance explained (%)
4.61	38.42
2.72	61.13
1.86	76.64
1.04	85.36
0.66	90.90

Table 4
Principal component loadings

Parameter	No of principal components				
	1	2	3	4	5
π	0.21	0.83	0.19	0.35	0.07
H-Do	0.18	−0.05	−0.58	0.63	0.28
M-RE	0.87	0.35	0.13	0.10	0.07
F	0.49	−0.43	0.46	0.32	−0.40
R	−0.72	0.53	0.03	0.23	0.24
σ	0.55	−0.32	0.60	0.32	−0.09
Es	−0.69	0.43	0.38	0.25	0.14
B_1	0.94	−0.09	−0.09	−0.15	0.20
B_4	0.96	0.05	−0.14	0.03	−0.01
$\log k'_0$	0.49	0.81	0.01	−0.17	0.01
b	−0.11	−0.37	−0.67	0.32	−0.16
$\log k'_0/b$	−0.35	−0.55	0.49	0.12	0.49

can interact with the polar substructures of drugs exerting a marked influence on their retention.

The two-dimensional non-linear map of PCA loadings (distribution of HPLC characteristics of drugs and their physicochemical parameters in a plane) is shown in Fig. 3. The $\log k'_0$ and π values of anticancer drugs form a distinct cluster. This suggests that the retention capacity of anticancer drugs on an ODS column is mainly governed by hydrophobicity and by their bulki-

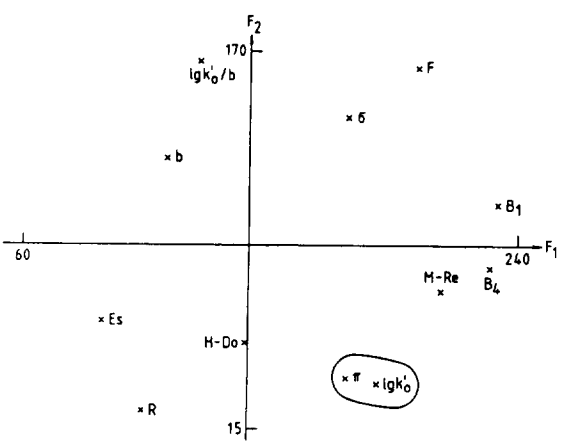

Fig. 3. Similarities and differences between the retention characteristics and physicochemical parameters of anticancer drugs. Two-dimensional non-linear map of PC loadings. Number of iterations: 80, maximum error: $4.7 \cdot 10^{-3}$. For symbols see Experimental.

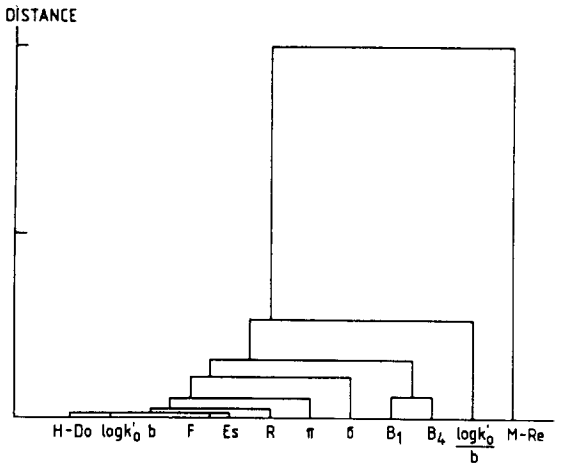

Fig. 4. Similarities and differences between the retention characteristics and physicochemical parameters of anticancer drugs. Cluster dendogram calculated from the original data matrix. For symbols see Experimental.

ness. Comparing the distribution of retention characteristics and physicochemical parameters of anticancer drugs on the two-dimensional non-linear map of PC loadings (Fig. 2) and on the clusters calculated from the original data matrix (Fig. 4) and from PC loadings (Fig. 5) indicates that considerable differences occur. This discrepancy may be due to the different methods of calculation and to the effect of PCA on the structure of the original data matrix. Comparison of the information provided by visualizing techniques resulted only in one significant linear correlation between the relative distances of the two-dimensional non-linear map and those of the cluster analysis carried out on PC loadings: $Y = 0.13 - (1.56 \pm 0.03) \cdot X$, $n = 12$, $r_{calc.} = 0.9974$, $r_{99\%} = 0.9740$.

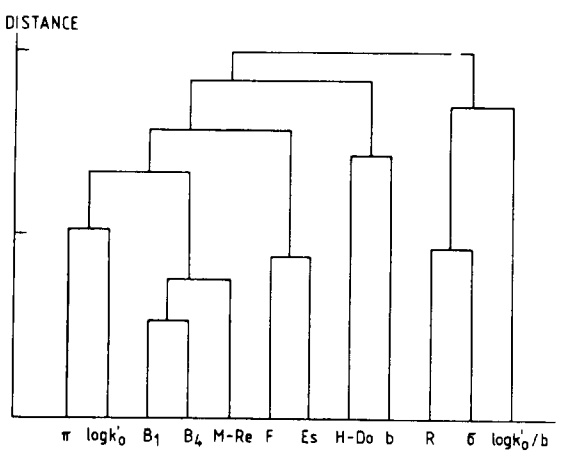

Fig. 5. Similarities and differences between the retention characteristics and physicochemical parameters of anticancer drugs. Cluster dendogram calculated from the PC loadings. For symbols see Experimental.

This finding indicates that the distribution of solutes on a non-linear map and a cluster dendogram may be similar when they are carried out on the same data matrix, but cluster analysis carried out on the original data matrix can result in a different distribution of variables. Although cluster analysis and non-linear mapping give similar results, we strongly advocate the application of the two-dimensional non-linear mapping technique instead of cluster analysis because of its higher dimensionality. We assume that the two-dimensional non-linear map may contain more information than the one-dimensional structure of clusters.

Significant linear correlations were found between the rotated PC loadings and the coordinates of the two-dimensional non-linear map of PC loadings ($n = 12$): $\text{varimax}_1 = -1.03 + (7.80 \pm 0.98) \cdot 10^{-3} \cdot \text{nlmap}_1$, $r_{calc.} = 0.9853$, $r_{99.9\%} = 0.7603$ $\text{varimax}_2 = 0.65 - (4.10 \pm 2.11) \cdot 10^{-3} \cdot \text{nlmap}_2$, $r_{calc.} = 0.8932$, $r_{99.9\%} = 0.7603$.

These data indicate that the results of varimax rotation and two-dimensional non-linear mapping are similar but not identical and both methods can be used to decrease the dimensionality of complicated data matrices.

The distribution of anticancer drugs according to their retention characteristics and physicochemical parameters (two-dimensional non-linear map of PCA variables) is shown in Fig. 6.

Only compounds 8 and 20 are well separated from the other drugs. As these compounds are extremely bulky we assume that this molecular parameter accounts for their separation from the other anticancer drugs. The cluster dendograms of drugs calculated from the original data matrix and from the PC variables are shown in Figs. 6 and 7, respectively. The dendograms calculated from the original data matrix and from the PCA

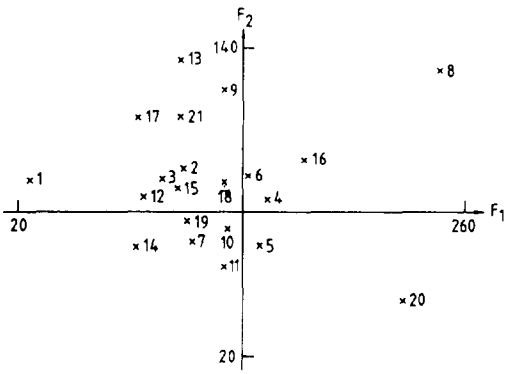

Fig. 6. Distribution of anticancer drugs according to their retention characteristics and physicochemical parameters. Two-dimensional non-linear map of PC variables. Number of iterations: 90, maximum error: $3.2 \cdot 10^{-3}$. Numbers refer to anticancer drugs in Table 1.

variables are different supporting our previous conclusions that PCA can cause data distortion which may influence the distribution of observations and variables in cluster analysis (Fig. 8).

We have to stress that the conclusions discussed above are not the result of theoretical considerations and hence are valid only for this special data set. A generalization of these conclusions can lead to severe misinterpretation.

Summarizing our results it can be concluded that non-homologous series of anticancer drugs

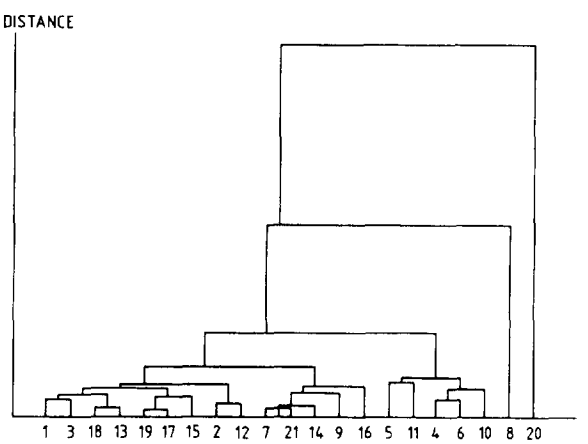

Fig. 7. Distribution of anticancer drugs according to their retention characteristics and physicochemical parameters. Cluster dendogram calculated from PCA variables. Numbers refer to anticancer drugs in Table 1.

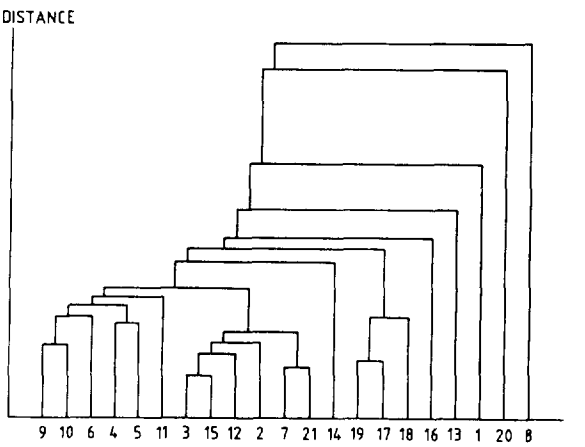

Fig. 8. Distribution of anticancer drugs according to their retention characteristics and physicochemical parameters. Cluster dendogram calculated from the original data matrix. Numbers refer to anticancer drugs in Table 1.

can be well separated on reversed-phase HPLC columns. Various multivariate mathematical statistical calculations indicate that the retention of the investigated drugs is mainly governed by their hydrophobic and steric parameters. The results suggest that the use of principal component analysis followed by two-dimensional non-linear mapping is superior to cluster analysis for the evaluation of large retention data matrices.

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