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Chromatographic data for pharmacological classification of imidazol(in)e drugs

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ABSTRACT

A set of eighteen imidazol(in)e derivative drugs of various pharmacological activity were analysed under different high-performance liquid chromatographic (HPLC) conditions. Capacity factors were determined employing methanol–buffer eluents at seven volume ratios and at pH 10.9, 7.0 and 2.9. The use of an alkaline buffer was possible owing to the application of poly(butadiene)-coated alumina (PBCA) as the stationary phase. Two systems employing octadecylsilica (ODS) columns were applied, one operated at pH 7.0 and the other at pH 2.9. Capacity factors of the test solute drugs were determined in 21 chromatographic systems. All the data were subjected to chemometric analysis despite the fact that, except for the PBCA systems, only a limited range of linearity of the logarithm of capacity factor *versus* volume fraction of methanol in mobile phase was observed. The matrix of 21 × 18 capacity factors was statistically analysed by the principal component method. The first two principal components accounted for 80% of the variance in the capacity factors studied. The principal component object scores clearly separated the agents into groups in accordance with their pharmacological classification. It was concluded that diverse retention data can provide more information relevant to the bioactivity of solutes than just a one-dimensional hydrophobicity scale.

INTRODUCTION

Since Boyce and Milborrow [1] related thin-layer chromatographic (TLC) data to the biological activity of a series of solutes, chromatography has been extensively exploited by medicinal chemists for the determination of the physico-chemical properties of drugs [2]. The property of greatest importance for bioactivity is the lipophilicity (hydrophobicity) of an agent. Chromatography [in particular high-performance liquid chromatography (HPLC)] offers a convenient approach to hydrophobicity parameterization. Much effort has been devoted to the application of chromatography for this purpose. The most recent HPLC methods and procedures for hydrophobicity determination are reviewed elsewhere [3].

Certainly hydrophobicity is an important but by no means the exclusive structural property determining the pharmacological activity of chemical substances. Thus, the attempts by most workers to prepare "purely hydrophobic" chromatographic systems (or systems mimicking the 1-octanol–water partitioning processes) can be questioned. Dynamic processes of tissue penetration and drug–receptor interactions in a living system resemble the mass transfer and intermolecular

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interactions during chromatographic separation. One can therefore expect the chromatographic data to comprise more information relevant to bioactivity than just a one-dimensional hydrophobicity scale.

To derive a chromatographic measure of hydrophobicity one usually extrapolates logarithms of the capacity factor, log k', obtained in reversed-phase systems at several compositions of an organic–buffer eluent to 100% buffer as a mobile phase. In this way one assumes a linear relationship between log k' and the volume fraction of an organic modifier in a binary aqueous eluent. Such a relationship was suggested by Soczewiński and Wachtmeister [4]. Observed deviations from linearity were accounted for by the quadratic model proposed by Schoenmakers *et al.* [5]. However, Snyder *et al.* [6] demonstrated that over a limited range of compositions of a binary mobile phase the linear relationship can be used as a good approximation of the general quadratic equation. Although the range of linearity varies depending on the nature of the solute and the properties of the chromatographic system employed, the assumption of linearity generally works. Whatever the physical meaning of the extrapolated capacity factors (often different from those determined experimentally with 100% buffer as eluent), satisfactory correlations are usually reported between such extrapolated log k'_w data and the conventional hydrophobicity parameter, namely the logarithm of the 1-octanol–water partition coefficient, log P.

If chromatography is to serve for hydrophobicity determination, then retention data that do not confirm the linear relationship between log k' and the volume fraction of organic modifier in the binary aqueous eluent, X_{org}, are discarded. Such discarded data are reproducible and vary with changes in the chromatographic systems, however. Hence they must comprise systematic information on properties of the solutes chromatographed. Bearing that in mind, we attempted to extract the systematic information concerning a set of test solutes by chemometric analysis of a complete table of retention data determined in various reversed-phase HPLC systems and not subjected to any preselection.

An interesting and challenging task was to relate the structural information provided by chromatography to the pharmacological properties of solutes. Having collected a representative set of imidazol(in)e drugs of different biological activity, we attempted to compare their established pharmacological classification with that resulting from chromatographic behaviour.

The imidazol(in)e test solutes considered here are circulatory drugs exerting their activity through the so-called adrenergic receptors of the α type. Some of the agents are known to bind preferentially to the α-1 subtype whereas the other have higher affinity to the α-2 subtype of adrenoceptor. After binding, some agents stimulate the receptor whereas the other inactivate it. Based on drug–receptor interactions, the imidazol(in)es considered may be classified as α-1 agonists, α-2 agonists, α-1 antagonists and α-2 antagonists. For the sake of this project we applied basically the classification scheme elaborated by Timmermans and van Zwieten [7]. Leaving aside the details not related to chromatography, one should note that the hydrophobicity of the agents does not allow their pharmacological classification [8,9].

EXPERIMENTAL

Materials

The series of eighteen drug solutes consisted of fifteen imidazoline, two imidazole and one thiazine derivatives. The following agents agents were chromatographed after dissolution in the mobile phase: cirazoline hydrochloride (a gift from Dr. J. Cavero, Synthelabo, Paris, France), detomidine hydrochloride and medetomidine hydrochloride (a gift from Dr. A. Karjalainen, Farmos-Group, Oulu, Finland), tiamenidine hydrochloride (a gift from Hoechst, Frankfurt, Germany), oxymetazoline hydrochloride (a gift from Schering, Bloomfield, IN, USA), lofexidine hydrochloride (a gift from Dr. H. Betzing, Nattermann, Cologne, Germany), phentolamine methanosulphate (Regitine; Ciba-Geigy, Basle, Switzerland), clonidine hydrochloride (Haemiton; Germed, Dresden, Germany), xylazine hydrochloride (Rotar; Spofa Prague, Czechoslovakia), moxonidine (a gift from Dr. B. I. Armah, BDF Research Labs., Hamburg, Germany), UK-14, 304 tartrate (a gift from Pfizer Central Research, Sandwich, UK), tramazoline hydrochloride (Rhinospray; a gift from Karl Thome, Biberach am Riess, Germany) and xylometazoline hydrochloride, tymazoline hydrochloride, naphazoline nitrate, antazoline hydrochloride, tetryzoline hydrochloride and tolazoline hydrochloride (Polfa, Warsaw, Poland).

A poly(butadiene)-coated alumina (PBCA) column was kindly supplied by Professor R. A. Hartwick (Rutgers University, Piscataway, NJ, USA). The column was 150 × 4.6 mm I.D., slurry packed with poly(butadiene)-coated Spherisorb A5Y using isopropanol as the slurry solvent and methanol as the packing solvent. The stationary phase was prepared according to the procedure of Schomburg and co-workers [10,11]. An ODS column (100 × 4.0 mm I.D., 5 μm particle size) was purchased from POCh (Lublin, Poland).

Universal buffer (Britton–Robinson buffer) was prepared at pH 2.9, 7.0 and 10.9. To obtain pH 2.9, 18.0 ml of 0.2 *M* NaOH were added to 100 ml of a solution of 0.04 *M* CH_3COOH, 0.04 *M* H_3PO_4 and 0.04 *M* H_3BO_3. To obtain pH 7.0 the corresponding volume of 0.2 *M* NaOH was 52.5 ml and to obtain pH 10.9 it was 82.5 ml.

Mobile phases were made by mixing the buffers with methanol (analytical-reagent grade, POCh) in the proportions 80:20, 70:3, 60:40, 50:50, 40:60, 30:70 and 20:80 (v/v). Following the recommendations of Minick and co-workers [12,13] for eluents used when working with the ODS column 0.25 ml of 1-octanol and 0.15 g of 1-dodecylamine were added to 100 ml of methanol. With the PBCA column the methanol used contained 0.25% of 1-octanol. Before use the eluents were left to stand overnight and then filtered through 0.45-μm nylon 66 filters.

Deuteromethanol (CH_3O^2H) was purchased from IBJ (Swierk/Otwock, Poland).

Methods

The chromatographic system (Altex Scientific, Berkeley, CA, USA) consisted of a single-piston reciprocating pump and a Model 157 UV detector operating at 254 nm. A Rheodyne (Cotati, CA, USA) Model 7410 injection valve fitted with a 20-μl sample loop was used. The flow-rate was 1 ml/min.

To determine the columns dead volumes, the approach [14] was applied

consisting in measuring the position of the peak of deuterated methanol with pure methanol as the eluent. Determinations of capacity factors, k', were made in duplicate at room temperature. In several systems studied the exclusion of solutes was observed as evidenced by negative values of k'. To accommodate such excluded solutes in our studies, we decided to consider the k' values instead of log k'. The capacity factors determined in the three chromatographic systems applied are given in Tables I–III.

In several instances we were unable to obtain measurable capacity factors. To overcome the problem of incompletenes of the capacity factor matrix, we estimated the mising data by linear extrapolation or interpolation of the log k' *vs.* X_{CH_3OH} relationship.

With the PBCA column and the ODS column operated at pH 7.0, the linear parts of the log k' *vs.* X_{CH_3OH} plot were extrapolated to 0% methanol in the eluent. The data thus obtained for individual solutes were designated log k'_w (PBCA) and log k'_w (ODS), respectively.

Statistical analysis

A matrix of capacity factors determined in $3 \times 7 = 21$ chromatographic systems for 18 drug solutes was subjected to statistical analysis by the principal component method [15]. A standard commercially available statistical package was employed.

The first principal component accounted for 60.5% and the second principal

TABLE I

CAPACITY FACTORS, k', OF IMIDAZOL(IN)E DRUGS DETERMINED ON POLY(BUTADIENE)-COATED ALUMINA WITH METHANOL–BUFFER OF pH 10.9 AS ELUENT WITH VARIOUS VOLUME FRACTIONS OF METHANOL, X_{CH_3OH}

Solute No.[a]	X_{CH_3OH} (v/v)						
	80	70	60	50	40	30	20
1	0.1666	0.6333	2.0416	3.6833	9.7500	9.4166	8.9166
2	0.1083	0.1666	0.6416	1.2500	3.1666	6.6326	7.2000
3	0.0251	0.0398	0.0635[b]	0.1000	0.3000	0.8367	0.7333
4	0.0219	0.0167	0.0917	0.4500	1.2500	3.4285	4.6666
5	0.1416	0.5000	1.1666	2.4833	5.9166	11.6938	10.6666
6	0.1166	0.0033	0.1833	0.6083	1.6583	3.9489	4.1166
7	0.0083	0.0333	0.1250	0.5333	1.7833	5.0204	5.5000
8	0.3250	0.5000	1.4000	2.4583	5.3330	11.6938	13.5833
9	0.5000	0.8000	2.1750	4.8083	11.5583	27.6734	24.5000
10	0.2426	0.3750	0.9750	1.5000	2.5666	4.9795	5.5000
11	0.4667	0.2000	0.5416	0.8500	1.8333	3.3469	3.0833
12	0.2000	0.2750	0.6666	0.8500	1.3666	2.7346	2.3330
13	0.0167	1.4666	4.4166	7.5000	14.8830	35.2200	47.5000
14	0.0333	0.1500	0.8500	2.2500	7.3833	16.2857	25.5000
15	0.0500	0.1333	0.3583	0.8333	2.3916	5.7346	6.6166
16	0.6000	0.8000	2.3589	5.8166	14.3330	37.7750	45.8333
17	−0.1500	−0.0833	−0.1416	−0.1000	0.1250	0.0000	0.0000
18	−0.1250	−0.1080	−0.1666	−0.1333	−0.1000	−0.0500	−0.1166

[a] Solutes are numbered as in Fig. 1.
[b] Interpolated data.

TABLE II

CAPACITY FACTORS, k', OF IMIDAZOL(IN)E DRUGS DETERMINED ON OCTADECYL-SILICA WITH METHANOL–BUFFER OF pH 7.0 AS ELUENT WITH VARIOUS VOLUME FRACTIONS OF METHANOL, X_{CH_3OH}

Solute No.[a]	X_{CH_3OH} (v/v)						
	80	70	60	50	40	30	20
1	3.6652	2.8330	2.2160	4.0830	8.8000	15.2545[b]	27.9660
2	4.3666	3.5000	2.9660	4.9500	6.0660	11.3830[b]	13.000
3	0.3660	0.6660	0.7500	1.0000	1.8500	3.7500	4.3333
4	0.6660	1.5830	2.4330	5.1660	15.0330	45.6600	113.470[b]
5	3.1666	2.0000	1.5000	3.3000	6.5830	19.5660	23.2660
6	1.6333	1.6660	1.6330	2.3000	4.5830	27.9330	2.6666
7	0.7333	1.8660	3.6660	7.4160	16.3900[b]	34.8600	2.7830
8	2.8333	2.7330	2.0830	3.0830	10.7160	31.3300	71.0700[b]
9	6.0000	3.0330	2.5830	4.7500	10.5000	26.8300	14.3500
10	3.6660	2.0000	0.0830	0.1660	3.6000	8.0660	6.0000
11	2.0000	1.3660	1.0830	1.6330	2.3330	6.0000	6.0333
12	3.2493	1.7830	1.3000	2.1660	2.0000	1.7160	3.0500
13	5.0000	2.5000	1.8330	3.2160	13.5000	27.5400[b]	74.1300[b]
14	2.6000	5.4600	4.7500	10.6330	16.9660	39.8100[b]	83.1763[b]
15	1.7498	2.2500	2.9160	5.0000	9.7500	12.4580[b]	15.1660
16	8.6333	5.0000	3.7500	8.0000	18.8330	25.7039	47.8630
17	0.5500	0.4500	0.5830	0.6660	1.1497	6.2488	3.4166
18	0.6000	0.5830	0.5414[b]	0.5000	0.6660	6.2488	1.4167

[a] Solutes are numbered as in Fig. 1.
[b] Extrapolated or interpolated data.

TABLE III

CAPACITY FACTORS, k', OF IMIDAZOL(IN)E DRUGS DETERMINED ON OCTADECYL-SILICA WITH METHANOL–BUFFER OF pH 2.9 AS ELUENT WITH VARIOUS VOLUME FRACTIONS OF METHANOL, X_{CH_3OH}

Solute No.[a]	X_{CH_3OH} (v/v)						
	80	70	60	50	40	30	20
1	2.4490[b]	1.7166	1.0357	0.7857	0.6070	0.0500	0.8750
2	2.0333	1.3333	1.0892	0.6428	0.7321	0.6785	0.5892
3	0.9666	0.6833	0.5714	0.3750	0.1562	0.1250	0.1785
4	2.3000	1.6000	0.8571	0.5535	0.7857	0.1750	0.8214
5	1.3333	1.0350	0.7500	0.3307	0.6071	0.5714	0.5731[b]
6	1.3333	1.1833	0.5000	0.4692	0.4107	0.3750	0.3928
7	2.2333	1.6666	1.1250	0.8750	1.0535	0.9285	1.0892
8	2.1333	1.4000	0.6250	0.6071	0.4821	0.4553[b]	0.4285
9	2.0000	1.6000	1.1250	0.8035	1.0000	1.0964[b]	1.2500
10	1.7000	1.2666	0.6071	0.5357	0.3750	0.2500	0.3928
11	1.0666	0.7000	0.5714	0.3928	0.2500	0.2500	0.2500
12	1.6333	1.1333	0.5714	0.4107	0.4285	0.4107	0.0714
13	1.6333	1.2166	0.7678	0.5714	0.4464	0.3214	0.3928[b]
14	2.6333	1.9666	1.4628	1.3035	1.5000	1.8214	1.8214
15	2.0000	1.1166	0.8214	0.4642	0.4464	0.4642	0.4285
16	2.6500	2.2166	1.5892	1.2321	1.5000	1.7321	2.0000
17	1.6330	1.0266	0.7500	0.4645	0.3393	0.3935	0.3571
18	1.0497	2.7327	0.4286	0.3750	0.3214	0.1607	0.1964

[a] Solutes are numbered as in Fig. 1.
[b] Extrapolated or interpolated data.

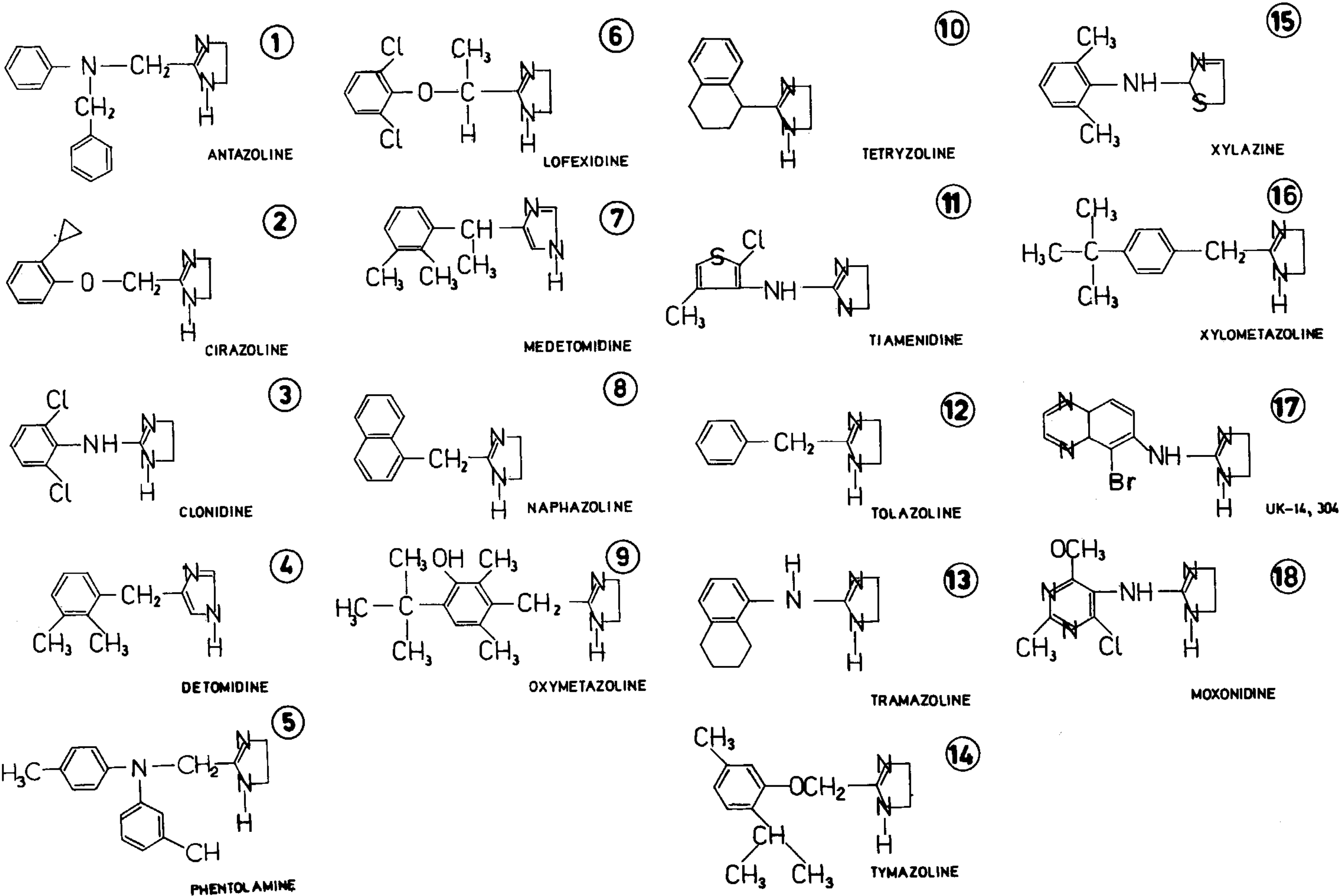

Fig. 1. Structural formulae of the imidazol(in)e derivative drugs studied.

component accounted for 18.9% of the variance in the capacity factors considered. The principal component scores by individual solutes were displayed graphically on the plane spanned by two principal component axes (Fig. 5).

In Fig. 4 the principal component loadings corresponding to the variations in column type and eluent composition are given.

RESULTS AND DISCUSSION

Structural formulae of the drug solutes are given in Fig. 1. There are no distinctive structural features (at least among the imidazoline subgroup) which would justify separation of the agents into classes. However, pharmacologically the drugs are ascribed to two main classes: those binding preferentially to α-1 adrenoceptor and others possessing a higher affinity to the α-2 adrenoceptor [7,16]. As a consequence of these differences, opposite circulatory effects are observed.

The relationship between capacity factors and volume fraction of methanol in the mobile phase can best be illustrated by means of semi-logarithmic plots. Such representative relationships are shown in Fig. 2. Using the poly(butadiene)-coated alumina column and a buffer of pH 10.9 a more or less linear dependence of log k' on

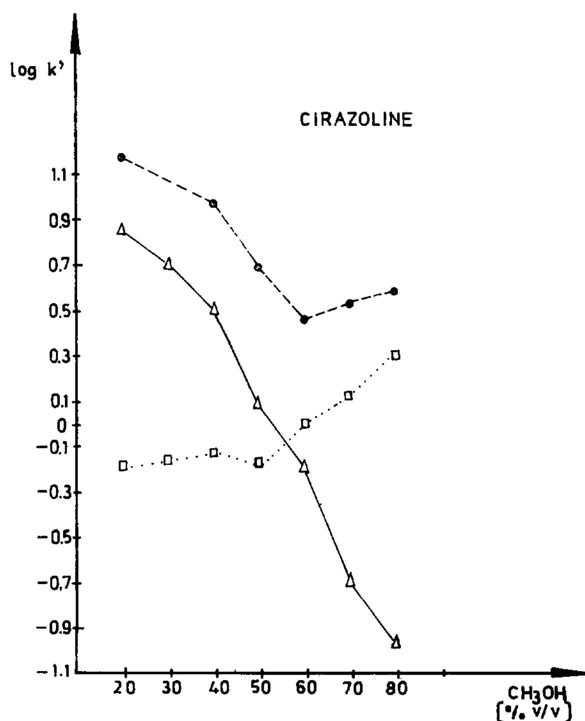

Fig. 2. Relationships between log k' and the volume fraction of methanol in mobile phase for cirazoline. Solid line, retention data determined on poly(butadiene)-coated alumina column operated at pH 10.9; broken line, data obtained on octadecylsilica column at pH 7.0; dotted line, data obtained on octadecylsilica at pH 2.9.

X_{CH_3OH} is obtained over the whole range of composition of the eluent studied. With the ODS column operated at pH 7.0, linearity is at best limited to the range of methanol content in the mobile phase from 20 to 60% (v/v). The results obtained demonstrate the unique advantages of PBCA for the chromatographic determination of the hydrophobicity of organic bases [17]. In the third system studied (ODS, methanol–buffer of pH 2.9), increasing retention of solutes (ionized) was observed with increasing amount of methanol in the mobile phase.

First we attempted to relate pharmacological activity to log k' corresponding to 100% buffer as a hypothetical eluent, log k'_w. Neither log k'_w determined on PBCA nor the corresponding parameter obtained from the system employing ODS at pH 7.0 allowed a reasonable pharmacological classification of the drugs studied (Fig. 3).

Assuming that the set of retention data obtained comprises information suitable for pharmacological classification of the solutes, we turned our attention to modern methods of data analysis. Multivariate methods of data analysis have been applied in chromatography since the early 1970s [18,19]. The reported studies were usually aimed at retention prediction [20,21] and/or explanation of the mechanism of chromatographic separations [22,23]. However, Wold and co-workers [24,25] reported multi-

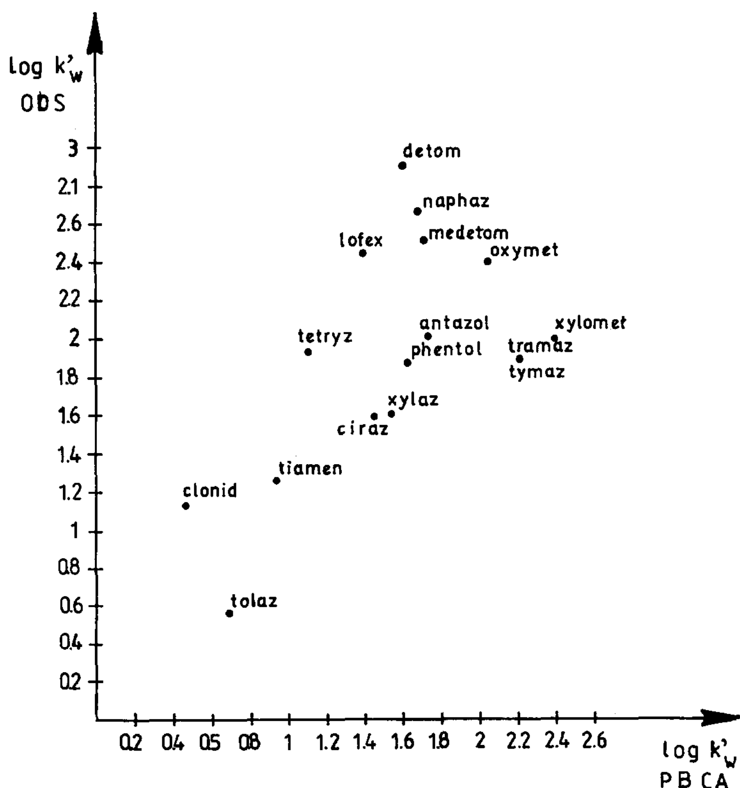

Fig. 3. Distribution of the imidazoline drugs on the plane determined by the logarithms of capacity factors extrapolated to pure buffer as the eluent. The respective retention data were obtained using the ODS (pH 7.0) and the PBCA (pH 10.9) columns.

variate parameterization of amino acid properties based on TLC data. The TLC data considered were R_F values determined in seven isocratic chromatographic systems (normal phase) differing with respect to the type of stationary phase and composition of the eluent. Principal component analysis of the data matrix comprising the R_F parameters, together with Van der Waals volume and molecular mass, resulted in two significant principal components. The principal components were shown to possess predictive capacity and explained about 70% of the variance reported in the literature data on the pharmacological activity of a series of oligopeptides. The differences in pharmacological activities considered by Wold and co-workers [24,25] were quantitative, *i.e.*, all the agents elicited the same effect but of varying magnitude. In selecting the solutes for this project we aimed at a qualitative differentiation of bioactive substances by means of diversified chromatographic data.

The principal component analysis of the retention data given in Tables I–III yielded two main factors accounting for about 80% of the variance in the HPLC capacity factors. Analysing the loadings of the two main principal components by the variables of the chromatographic analysis (Fig. 4), one can note that the first factor (PC1) is loaded mostly by retention data determined under acidic and neutral conditions. However, the input to PC1 by the k' values determined on PBCA with eluents containing higher proportions of water is also significant. Thus, PC1 may reflect a tendency of solutes to undergo hydration and ionizaton. The second principal component, PC2, is evidently loaded by capacity factors determined at pH 10.9 on the PBCA column. One can assume that PC2 reflects the hydrophobicity of non-ionized forms of the solutes. A conspicuous result is the high loading by the capacity factors determined with 70% methanol at pH 2.9 on the ODS column to the fourth principal component and also the marked loadings to the third principal component by the k' values from the 20 and 30% methanol (pH 7.0)–ODS systems and the 80% methanol

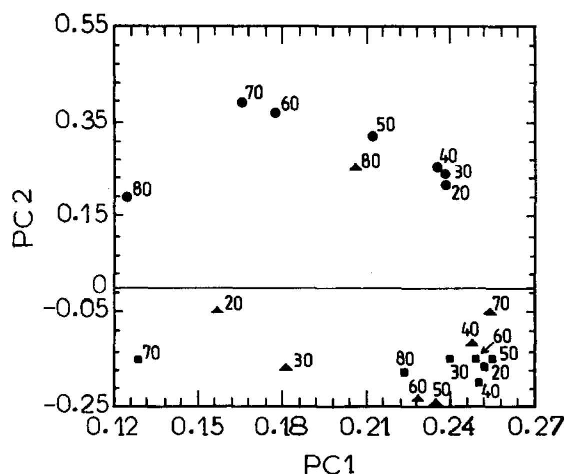

Fig. 4. Two-dimensional scatter plot of the loadings of the two first principal components, PC1 and PC2, by the variables of the chromatographic systems applied. Column: ●, PBCA operated at pH 10.9; ■, ODS operated at pH 2.9; ▲, ODS operated at pH 7.0. Numbers denote the volume percentage of methanol in the eluent.

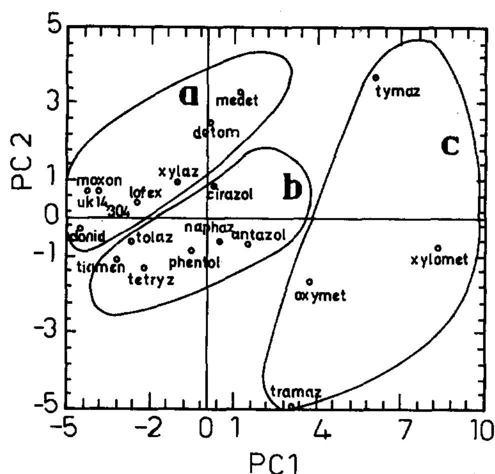

Fig. 5. Two-dimensional scatter plot of the scores by individual imidazol(in)es in the two first principal components, PC1 and PC2.

(pH 10.9)–PBCA system. It is difficult to speculate about the physical meaning of such observations, however.

For individual drug solutes, the principal component object scores were calculated. Thus, positions of the drugs on the plane spanned by two principal component axes could be displayed graphically (Fig. 5). The objects in Fig. 5 can be grouped into three clusters, a, b and c. Such a grouping due to retention behaviour correlates well with the established pharmacological classification of the solutes.

Pharmacology textbooks classify unequivocally the agents belonging to cluster a as selective agonists of α-2 adrenoceptor, whereas those belonging to cluster c are considered to be pure α-1 adrenoceptor agonists. A detailed pharmacological discussion of the results is inappropriate here, but it can be demonstrated that imidazolines belonging to cluster b possess affinity for both subtypes of the α adrenoceptor. For example, tolazoline and phentolamine block both α-1 and α-2 subtypes of adrenoceptor. Tolazoline is asumed to have a higher affinity to α-2 than α-1 adrenoceptor, whereas phentolamine blocks both receptors to the same extent [7,26]. This observation is in accordance with the relative positions of the two α adrenoceptor antagonists in Fig. 5. Tiamenidine is used clinically for to its α-2 adrenoceptor stimulating properties. Nevertheless, adverse effects of the drug can be ascribed to α-1 adrenoceptor stimulation [27]. Naphazoline and tetryzoline are usually classified among agonists of α-1 adrenoceptor. There is evidence that both agents react with α-2 adrenoceptors also, however [7,26,28].

The presence of cirazoline in cluster b appears unexpected as it has been considered to be a strong agonist of α-1 receptors. The positon of cirazoline can be rationalized however, in view of reports [29,30] that although an α-1 adrenoceptor agonist, it is at the same time an α-2 adrenoceptor antagonist. Also, the location of antazoline in cluster b seems reasonable. This agent, being an imidazoline derivative, does not belong to circulatory drugs acting via adrenoceptors but possesses

antihistamine properties instead. In independent experiments [31] we did not observe stimulation of either α-1 or α-2 adrenoceptors by antazoline, but the agent revealed antagonistic properties towards the receptors.

Systematic information extracted by principal component analysis from a set of retention data determined in various HPLC systems suffices for the differentiation of imidazol(in)e drugs in accordance with their pharmacological classification. This result demonstrates the usefulness of diversified chromatographic data for the characterization of solutes and thus for the prediction of their properties. By using multivariate methods of data analysis, the informative capacity of retention data so far neglected in retention–bioactivity relationship studies can be exploited. These neglected data (which reflect generally the ability of a solute to take part in specific intermolecular interactions with a stationary and/or mobile phase) can provide structural information that does not manifest itself within a set of capacity factors changing regularly with changes in chromatographic conditions. Provided that the data are reproducible, more reliable information on solute properties can be extracted the more diverse is the representative set of capacity factors considered. From the point of view of the application of chromatography in studies of quantitative structure–activity relationships (QSAR), it appears more productive to collect a representative set of diverse retention parameters than to concentrate all efforts on the determination of a chromatographic measure of hydrophobicity.

Leaving aside all the uncertainties and ambiguities accompanying hydrophobicity determinations (ionization, pH, ionic strength, reactions with buffer components, etc., in organic–aqueous solvents of varying composition), one cannot expect the one-dimensional hydrophobicity scale to comprise the bioactivity of various drugs. Certainly, the multi-dimensional structural characterization of agents based on their chromatographic retention in various systems appears more realistic.

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