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FACTOR ANALYSIS AND EXPERIMENT DESIGN IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

V. SELECTIVITY OF CHALCONE CONFIGURATION ISOMERS ON 23 REVERSED-PHASE PACKINGS

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SUMMARY

The selectivity of 22 pairs of configuration isomers of X,Y-substituted chalcones ($X-C_6H_5-CH=CH-CO-C_6H_5-Y$) on 23 reversed-phase high-performance liquid chromatographic (HPLC) packings has been studied. The main factors influencing the selectivity of these isomers were analysed with principal component analysis (PCA). The chromatographic behaviour of the *E*-s-*cis* and *Z*-s-*cis* chalcones was found to be well related to the substitution pattern on the chalcone core. PCA helps to formulate a general rule for selection of stationary phases suitable for separation of the configuration isomers and enables a classification of the HPLC packings.

INTRODUCTION

The transmission of electronic effects of the X,Y substituents of chalcones through the chain of conjugated double bonds has been intensively studied. The influence of the substituents on the infrared carbonyl stretching frequencies[1–6] and integral intensities[4–6], on the dipole moment[7–11], the ultraviolet spectra [12,13], the half-wave potential [14–17] and the ^{13}C NMR chemical shifts[18–20] have been reported.

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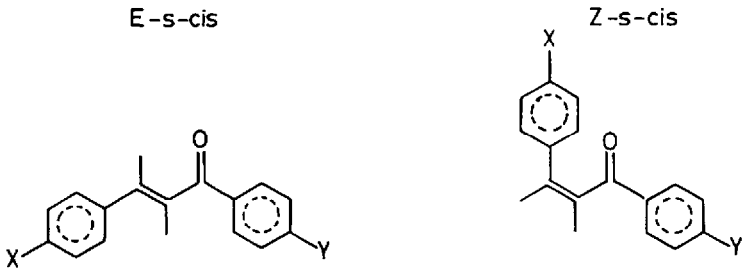

The substituent effects on the chromatographic behaviour of a model series of 53 chalcones have also been analysed by us by reversed-phase high-performance liquid chromatography (RP-HPLC)[21] and by normal-phase HPLC[22–24]. The different contributions of the substituents to the partition coefficient, depending on their position in the 4,4′-substituted chalcones, have been stressed. Additionally, it appears, from our investigations, that these effects depend on the *E*-s-*cis* and *Z*-s-*cis* configuration of the chalcone core, so the separations of these configuration isomers change according to the chemical nature of the substituents.

The separation of the non-substituted *E*-s-*cis* and *Z*-s-*cis* chalcones differs from the separation of the substituted isomers, *e.g.*, methyl, methoxychalcones. Furthermore, and simultaneously, differences are observed in the selectivity of the positional isomers, *e.g.*, CH_3 in position 4 and CH_3O in position 4′ or CH_3O in position 4 and CH_3 in position 4′.

Changes in the configuration of the chalcone core induce changes in molecular volume, contact surface area, polarizability and dipole moment of these isomers. So, it is very difficult to predict the differences in the solubility of the *Z*-s-*cis* and *E*-s-*cis* 4,4′-substituted chalcones. A general rule, which could describe the substituent effects on the separation of the isomers and which could predict their chromatographic behaviour, would be of interest both from the chemistry of chalcones and the chromatographic point of view.

In the present paper, the substituent effects on the selectivity of 22 pairs of the configuration isomers of chalcones are discussed. A study of the ability of 23 RP-HPLC packings to separate the *E*-s-*cis* and *Z*-s-*cis* chalcones was undertaken. The data were analysed with the help of factor analysis methods, which we have used previously to determine trends in the behaviour of various hydrocarbon compounds in gas–solid chromatography[25] or for the model series of 53 chalcones in RP-HPLC[21] or normal-phase HPLC[22–24]. However, instead of using correspondence factor analysis (CFA) to grasp the main trends in selectivity of these configurational chalcone isomers, we will use principal component analysis (PCA) which keeps the information content related to the absolute value of the selectivity parameter analysed.

EXPERIMENTAL

Reagents

The 4- and/or 4′-substituted chalcones considered are listed in Table II. *E*-s-*cis* chalcones were synthesized[26] while the corresponding *Z*-s-*cis* isomers (in our work denoted by asterisks) formed spontaneously in the dichloromethane solution.

The mobile phase consisted of HPLC-grade methanol purchased from Merck (F.R.G.) and of Millipore purified water.

Chromatographic procedure

Prior to the measurements, the columns were washed with the methanol–water (7:3) mobile phase until a constant value was obtained for the retentions of the compounds. Sample solutions (2 mg per 25 ml) were prepared in dichloromethane. All data were collected by averaging three reproducible separations. The same mobile phase and 1 μl of 10^{-3} *M* sodium nitrate, detected at 210 nm, were used to determine the dead time, t_0, for each system. The capacity factor, k', was calculated from the retention time of the solute, t_R, according to the equation $k' = (t_R - t_0)/t_0$. The results are presented in form of selectivity parameters, $\alpha_{X\text{-}Y/X\text{-}Y^*} = k'_{X\text{-}Y}/k'_{X\text{-}Y^*}$, for *E*-s-*cis* (X-Y) and *Z*-s-*cis* (X-Y*) isomer pairs.

Instruments and columns

The HPLC equipment included the following components: a Bruker LC-31 pump, a Rheodyne Model 7125 injection valve, a Schoeffel Model SF 770 spectrophotometer set at 300 nm and a Shimadzu C-RIB recorder.

The commercial columns or columns prepared in our laboratory by slurry-

TABLE I

COLUMN PACKINGS

No.	*Column packing*	*Dimensions (cm × mm)*	*Supplier*	*End-capped*
1	RSIL C_{18} LL*	9 × 4	Alltech	Yes
2	RSIL C_{18} HL*	9 × 4	Alltech	Yes
3	Partisil ODS*	9 × 4	Whatman	No
4	Partisil ODS-2*	9 × 4	Whatman	No
5	Partisil ODS-3*	9 × 4	Whatman	Yes
6	Spherisorb ODS-2*	9 × 4	Phase Separations	Yes
7	μBondapak C_{18}*	9 × 4	Waters	Yes
8	Hypersil C_{18}*	9 × 4	Shandon	Yes
9	Spherosil XOA 600 C_{18}*	9 × 4	Prolabo	No
10	Nucleosil C_{18}*	9 × 4	Macherey-Nagel	Yes
11	Nova-Pak C_{18}	10 × 5	Waters	Yes
12	Resolve C_{18} Radial Pak	10 × 8	Waters	No
13	μBondapak C_{18} Radial Pak	10 × 8	Waters	Yes
14	Zorbax ODS	15 × 4.6	DuPont	No
15	Zorbax C_8*	9 × 4	DuPont	Yes
16	Zorbax TMS	25 × 4.6	DuPont	Yes
17	Zorbax Phenyl	25 × 4.6	DuPont	Yes
18	Zorbax CN	15 × 4.6	DuPont	No
19	Resolve C_8 Radial Pak	10 × 8	Waters	No
20	Resolve CN Radial Pak	10 × 8	Waters	No
21	μBondapak Phenyl	30 × 4.6	Waters	Yes
22	Spherisorb C_6*	9 × 4	Phase Separations	Yes
23	Spherisorb C_8*	9 × 4	Phase Separations	Yes

* Packed in our laboratory.

TABLE II

SELECTIVITY PARAMETERS, $\alpha_{X\text{-}Y/X\text{-}Y^*}$, FOR THE *E*-s-*cis* (X-Y) AND *Z*-s-*cis* (X-Y*) CHALCONES CHROMATOGRAPHED ON 23 RP-HPLC SYSTEMS

No.	*X*	*Y*	*Stationary phase*										
			1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
1	H	CF_3	1.49	1.55	1.36	1.59	1.47	1.56	1.49	1.48	1.66	1.53	1.57
2	H	*tert.*-C_4H_9	1.56	1.62	1.44	1.66	1.52	1.61	1.54	1.52	1.75	1.63	1.60
3	H	iso-C_3H_7	1.55	1.62	1.44	1.65	1.52	1.62	1.54	1.52	1.76	1.63	1.60
4	H	H	1.52	1.55	1.38	1.64	1.44	1.58	1.46	1.46	1.75	1.60	1.54
5	F	H	1.34	1.33	1.25	1.39	1.29	1.33	1.30	1.27	1.45	1.36	1.34
6	H	F	1.48	1.54	1.37	1.60	1.44	1.56	1.47	1.45	1.69	1.54	1.54
7	H	C_2H_5	1.58	1.63	1.45	1.65	1.52	1.65	1.53	1.53	1.76	1.63	1.64
8	H	CH_3	1.60	1.66	1.45	1.76	1.53	1.68	1.54	1.55	1.88	1.68	1.65
9	F	CH_3	1.40	1.41	1.29	1.46	1.36	1.40	1.37	1.33	1.54	1.42	1.43
10	F	F	1.28	1.34	1.20	1.32	1.32	1.34	1.34	1.28	1.38	1.30	1.37
11	CH_3O	CH_3	1.58	1.51	1.43	1.68	1.42	1.52	1.45	1.41	1.78	1.60	1.46
12	CH_3	CH_3O	1.71	1.74	1.51	1.94	1.58	1.78	1.60	1.62	2.09	1.77	1.73
13	F	CH_3O	1.44	1.46	1.31	1.55	1.38	1.44	1.38	1.36	1.63	1.46	1.46
14	H	NO_2	1.44	1.52	1.30	1.55	1.44	1.56	1.45	1.45	1.66	1.47	1.55
15	F	NO_2	1.20	1.31	1.13	1.22	1.31	1.32	1.33	1.28	1.28	1.20	1.38
16	NO_2	CH_3	1.36	1.49	1.22	1.46	1.43	1.51	1.43	1.43	1.57	1.38	1.57
17	NO_2	H	1.31	1.43	1.20	1.41	1.38	1.44	1.35	1.36	1.48	1.34	1.50
18	CH_3O	CH_3O	1.60	1.57	1.44	1.73	1.44	1.54	1.46	1.42	1.84	1.62	1.51
19	NO_2	F	1.24	1.44	1.12	1.32	1.42	1.46	1.41	1.38	1.38	1.26	1.57
20	NO_2	CH_3O	1.40	1.53	1.26	1.54	1.45	1.54	1.44	1.43	1.65	1.42	1.61
21	NO_2	NO_2	1.12	1.46	1.00	1.20	1.44	1.48	1.45	1.44	1.29	1.15	1.61
22	H	OH	1.52	1.61	1.38	1.71	1.48	1.59	1.48	1.48	1.78	1.60	1.58

packing at 6000 p.s.i. with carbon tetrachloride, followed by methanol, are listed in Table I. The information on the packings comes from the supplier's sourcebooks and gives only a rough idea about the percentage of derivatization.

Data processing

The data were processed by PCA[27,28]. The data matrix, [*D*], with i rows and k columns is created with the selectivity parameters $\alpha_{X\text{-}Y/X\text{-}Y^*}$. The elements, α_{ik}, of the data matrix are assumed to have the form

$$\alpha_{ik} = \alpha_i + \sum_{j=1}^{n} r_{ij}c_{jk} + e_{ik}$$

where α_i are the averages of the corresponding variables of the ith column of the data matrix, r_{ij} and c_{jk} are the PCA parameters (cofactors) and e_{ik} is the residual error.

In PCA the eigenvectors are calculated consecutively so as to minimize the residual error, $\sum_i \sum_k e_{ik}$, in each step. Thus each successive eigenvector accounts for a maximum of the variation in the data. The appropriate number of components, j,

12	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*
1.57	1.54	1.59	1.36	1.38	1.33	1.28	1.34	1.20	1.24	1.17	1.28
1.64	1.55	1.62	1.36	1.52	1.31	1.38	1.40	1.25	1.23	1.18	1.31
1.65	1.55	1.65	1.38	1.40	1.31	1.35	1.40	1.24	1.22	1.18	1.32
1.58	1.48	1.60	1.41	1.26	1.31	1.25	1.40	1.18	1.21	1.19	1.33
1.35	1.33	1.36	1.21	1.15	1.19	1.20	1.19	1.13	1.12	1.10	1.17
1.57	1.50	1.58	1.38	1.27	1.28	1.27	1.35	1.18	1.19	1.16	1.28
1.69	1.56	1.68	1.40	1.36	1.31	1.33	1.43	1.23	1.22	1.20	1.34
1.70	1.56	1.70	1.43	1.34	1.30	1.31	1.45	1.21	1.21	1.21	1.36
1.43	1.39	1.43	1.22	1.23	1.19	1.35	1.21	1.16	1.13	1.11	1.18
1.33	1.36	1.37	1.21	1.21	1.18	1.22	1.15	1.14	1.12	1.09	1.16
1.62	1.48	1.55	1.33	1.34	1.21	1.28	1.39	1.20	1.13	1.31	1.28
1.85	1.66	1.81	1.43	1.45	1.27	1.36	1.46	1.24	1.19	1.21	1.36
1.47	1.40	1.50	1.25	1.22	1.18	1.28	1.22	1.18	1.12	1.12	1.21
1.56	1.51	1.58	1.37	1.23	1.29	1.26	1.29	1.11	1.21	1.15	1.25
1.28	1.35	1.35	1.20	1.17	1.20	1.20	1.11	1.11	1.16	1.09	1.14
1.45	1.47	1.54	1.23	1.21	1.17	1.28	1.11	1.18	1.14	1.11	1.17
1.36	1.40	1.48	1.23	1.09	1.17	1.22	1.10	1.14	1.12	1.02	1.16
1.64	1.48	1.60	1.35	1.63	1.20	1.31	1.39	1.21	1.14	1.16	1.29
1.34	1.47	1.50	1.26	1.21	1.19	1.24	1.00	1.13	1.16	1.11	1.17
1.47	1.49	1.61	1.25	1.20	1.17	1.29	1.00	1.17	1.14	1.00	1.18
1.29	1.48	1.51	1.30	1.23	1.23	1.20	1.00	1.09	1.20	1.13	1.18
1.49	1.51	1.62	1.43	1.24	1.28	1.28	1.34	1.15	1.21	1.17	1.33

for a given data set is determined using the imbedded error function (IE)[27]. For the jth factor in the sum, row cofactor r_{ij} is associated with the ith row designee, or identifier of the ith row, of the data matrix and c_{jk} is the corresponding column cofactor associated with the kth column designee (or identifier of the kth column), of the matrix. The row cofactors are called scores and the column cofactors are called loadings.

By factor analysis, we obtain a score matrix which depends solely upon the characteristics of the row designee, and a loading matrix which depends solely upon the column designee.

The teminology used here, designee, score matrix, loading matrix, is in accord with that proposed by Malinowski and Howery[27].

In our study, as all the experimental data are expressed in the same units, the canonic PCA was used, and the matric subjected to analysis was the covariance matrix about the mean. This means that the eigenvectors and eigenvalues, which emerge from the factor analysis of the data matrices $[D]$ and $[D]^{-1}$, are not exactly the same.

If we are interested in the differences between RP-HPLC column packings used for the separation of the configuration isomers of chalcones, the chromatographic systems must be treated as the row designees. If the influence of the X,Y substituents

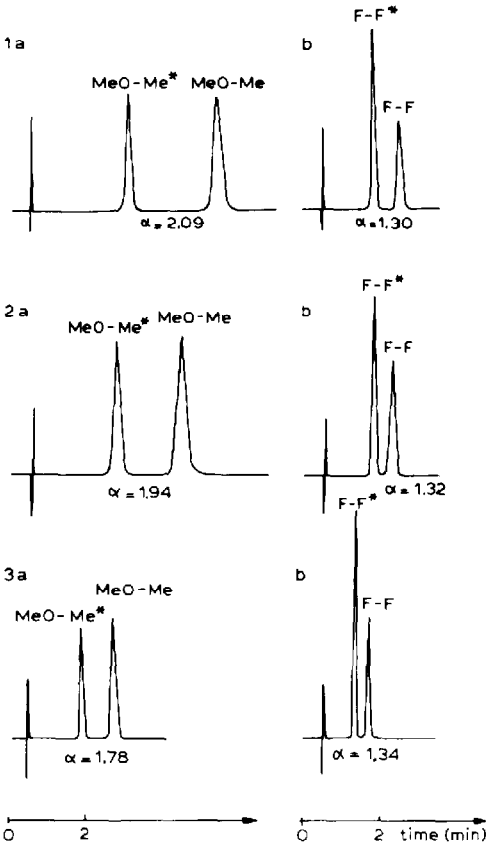

Fig. 1. Separation of (a) CH_3O-CH_3O and CH_3-CH_3O* and (b) F-F and F-F* chalcones on the stationary phases Spherosil XOA C_{18} (1), Partisil ODS-2 (2) and Spherisorb ODS-2 (3). Mobile phase: methanol–water (7:3); flow-rate 1.5 ml/min. Detection: UV at 300 nm. Me = Methyl; MeO = methoxy.

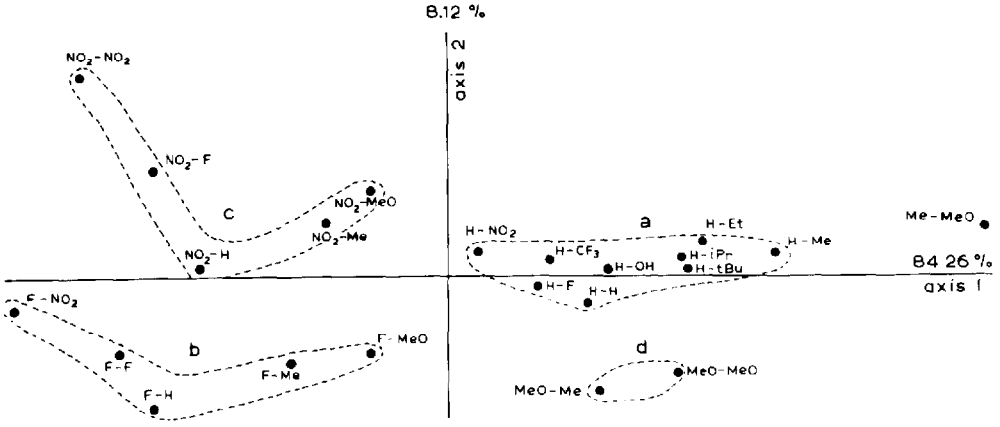

Fig. 2. Projection of 22 pairs of configuration isomers with X,Y substituents onto the plane determined by the two main axes extracted from principal component analysis.

on the separation of the *E*-s-*cis* and *Z*-s-*cis* chalcones is our main object, the pair of X,Y substituents are the row designees. In the first case the score matrix is associated only with the chromatographic systems, in the second, only with the pair of substituents.

RESULTS AND DISCUSSION

The selectivity parameters, $\alpha_{X\text{-}Y/X\text{-}Y^*}$, defined as the ratios of the k' value of the *E*-s-*cis* (X-Y) and *Z*-s-*cis* (X-Y*) chalcones, are listed in Table II. These parameters change from one chromatographic system to another, but in each system they change in a similar manner depending on the substituent pattern of the chalcone core, *e.g.*, in Fig. 1 the separations of the CH_3O-CH_3 and CH_3O-CH_3^* or F-F and F-F* isomers on three different stationary phases are presented.

The average ratio of the capacity factors for *E*-s-*cis* and *Z*-s-*cis* chalcones is 1.39.

To characterize the "true" substituent effect on the isomer separation, independently of the properties of the chromatographic system, and the "true" system ability to separate the configuration isomers, PCA was applied.

Influence of X,Y substituents on the selectivity of E-s-cis and Z-s-cis chalcones

To determine the influence of the X,Y substituents on the selectivity of the *E*-s-*cis* and *Z*-s-*cis* chalcones, the pairs of substituents were treated as the row designees in the data matrix of the $\alpha_{X\text{-}Y/X\text{-}Y^*}$ selectivity parameters. In this case only two main axes create the primary set of factors. The projections of the X,Y substituent pairs onto the plane defined by these axes are presented in Fig. 2.

The X,Y pairs are not grouped in any obvious manner, but some trends in the changes of the $\alpha_{X\text{-}Y/X\text{-}Y^*}$ parameters are seen more easily if one distinguishes the following subclasses of the row designees: a, H,Y; b, F,Y; c, NO_2,Y; d, CH_3O,Y. Within these subclasses only one substituent varies (substituent in position 4'), and the $\alpha_{X\text{-}Y/X\text{-}Y^*}$ parameters increase in the following orders, depending on the nature of this Y substituent:

a : $NO_2 < F < CF_3 < H < OH <$ iso-$C_3H_7 <$ *tert.*-$C_4H_9 < C_2H_5 < CH_3$

b : $NO_2 < F < H < CH_3 < CH_3O$

c : $NO_2 < F < H < CH_3 < CH_3O$

d : $CH_3 < CH_3O$

The influence of the Y substituent on the $\alpha_{X\text{-}Y/X\text{-}Y^*}$ parameters for the H-Y chalcones (subclass a) can be described with only one "abstract" parameter (factor 1). For X-Y chalcones (subclasses b, c) the second "abstract" parameter is necessary (factor 2). The best description of the effect of the Y substituent on isomer selectivity is given by the Hammett parameters, $\sigma_p^{\pm} - \sigma_p$ (ref. 29).

The influence of the X substituent on the $\alpha_{X\text{-}Y/X\text{-}Y^*}$ parameters seems to be more complicated, and two "abstract" factors are required to describe it. Based on isomers with the same Y substituent but with different X substituents, e.g. F-CH_3O, NO_2-

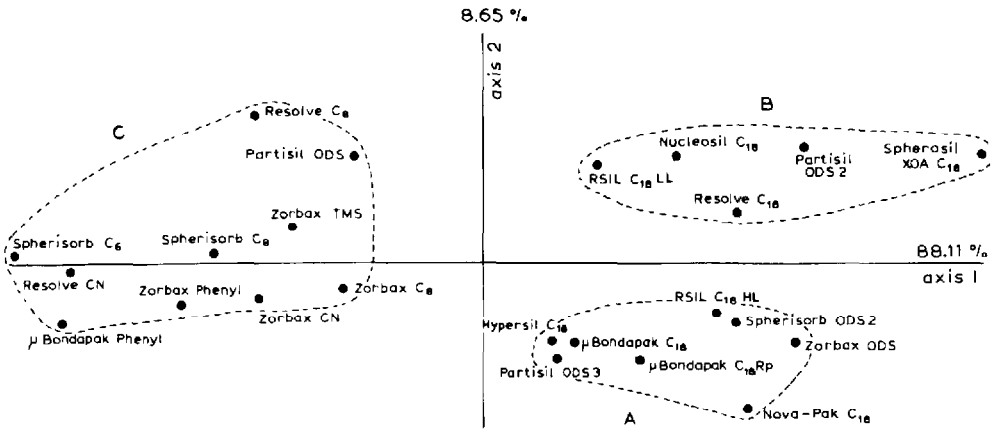

Fig. 3. Projection of 23 chromatographic systems onto the plane determined by the two main axes extracted from principal component analysis.

CH_3O, CH_3O-CH_3O and CH_3-CH_3O, one can order the X substituents as follows:

$$F < NO_2 < CH_3O < CH_3 \text{ (along factor 1)}$$

and

$$CH_3O < F < CH_3 < NO_2 \text{ (along factor 2)}$$

Their effects can be described with the help of Hammett constants σ_I and σ_R^0 (ref. 29).

Hence, the selectivity of *E*-s-*cis* and *Z*-s-*cis* chalcones is well related to their substitution pattern on the chalcone skeleton, and one can generalize the substituents effects in the following way. The selectivity $\alpha_{X\text{-}Y/X\text{-}Y'}$ is highest if both substituents, in positions 4 and 4', are electron-donor groups. The greatest negative influence is observed when electron-acceptor substituents are in position 4.

Influence of the properties of RP-HPLC packings on the selectivity of E-s-cis and Z-s-cis chalcones

To obtain the score matrix, which depends solely upon the characteristics of the stationary phases, these phases were regarded as the row designees in the data matrix. The first four eigenvectors, create a primary set of eigenvectors and the remaining ones account for experimental error only. The factors 3 and 4, associated with smaller eigenvalues, reflect relatively "unique" behaviour associated with only a single designee (Zorbax TMS and Zorbax CN) and will not be taken into consideration below. The results are presented in Fig. 3.

The first eigenvector accounts for the major fraction of the variance in the data and represents a sort of common factor averaged over all the row designees. It defines the best one-factor model for the data. The second principal axis is orthogonal to the first eigenvector. These two factors define a plane passing through the greatest concentration of data points (Fig. 3) and the stationary phases are grouped into the

following subclasses: A, octadecylsilyl endcapped stationary phases; B, octadecylsilyl uncapped or partially capped stationary phases; C, trimethylsilyl, C_6, C_8, phenyl, cyano stationary phases and Partisil ODS which is an uncapped and a low carbon-loaded packing.

The selectivity, $\alpha_{X\text{-}Y/X\text{-}Y'}$, increases along the axis 1 with increasing RP chain length, and with decreasing phase polarity, for the same silica material:

Spherisorb C_6 < Spherisorb C_8 < Spherisorb ODS-2

Resolve CN < Resolve C_8 < Resolve C_{18}

μBondapak Phenyl < μBondapak C_{18}

The additional differences among similarly labelled ODS columns (subclasses A and B) and the differences among the columns within the subclasses A, B and C are also reflected along axis 2.

The selectivity, $\alpha_{X\text{-}Y/X\text{-}Y'}$, seems to increase along the axis 2 with increasing silanol group accessibility: for two phases which have the same coordinates on the axis 1, the selectivity increases in the positive direction of the axis 2, *e.g.*:

Resolve C_{18} > Spherisorb ODS-2

Partisil ODS-2 > Zorbax ODS

Partisil ODS > Zorbax C_8

Resolve C_8 > Zorbax CN

The only common properties of the phases from group A are that they are C_{18} and endcapped. Group B also comprises C_{18} phases and includes uncapped packings: Spherosil XOA C_{18}, Partisil ODS-2 (75% of derivatization) and Resolve C_{18}. The presence of the RSIL C_{18} LL phase in this group can be explained in the following way. This phase is described in the supplier's sourcebook as a capped packing, but as Unger[30,31] and Berendsen and De Galan[32] have shown, a large proportion of the total number of silanol groups, originally present on silica surface, remains underivatized even after an "exhaustive" silanization and can cause a dual retention mechanism. The lower the carbon loading the stronger is the effect of underivatized silanol groups. Probably the same effect causes one of the C_{18} phases to be included in group C. This is the uncapped and low-loaded Partisil ODS. The presence of the Nucleosil C_{18} packing within group B cannot be explained in any obvious way. It is the only polymeric phase in our set of packings.

It is worth stressing that this classification of the stationary phases does not depend on the specific surface area, pore-size distribution, shape of the silica particles or on the character of the organic layer (mono- or multilayer), *e.g.*, subclass A includes Hypersil C_{18} (monolayer) and Partisil ODS-3 (multilayer), RSIL C_{18} HL (irregular shape of silica) and Zorbax ODS (spherical silica) or Nova-Pak C_{18} (surface area 120 m^2/g) and RSIL C_{18} HL (surface area 550 m^2/g).

More generally, the PCA results show that the nature and concentration of the organic "bristle" chemically bonded on the silica surface and the percentage of derivatization of the silica gel play a major rôle in the separation of the *E*-s-*cis* and *Z*-s-*cis* chalcones. (Derivatization is defined as the percentage of the available hydroxyl groups of the silica gel which have reacted with the silane. It is the result both of bonding and sometimes of a final silanization with, *eg.*, methylsilanes in order to minimize unreacted hydroxyl groups.)

CONCLUSIONS

Basing on Rekker's concept[33] of hydrophobic fragmental constants, one cannot predict the difference in solubility of the configuration isomers (*E*-s-*cis* and *Z*-s-*cis*) of chalcones. The present experimental data allow a calculation of the average ratio of the hydrophobicities of the *E*-s-*cis* and *Z*-s-*cis* chalcones, *i.e.*, 1.39.

Due to the transmission of the electronic effects of the substituents, in the 4,4'-substituted chalcones, through the chain of conjugated double bonds, deviations from the additivity of the partition coefficients are observed for the series of *E*-s-*cis* and *Z*-s-*cis* chalcones. These deviations are different for the two series of isomers, so the selectivity of the *E*-s-*cis* and *Z*-s-*cis* isomers changes, and it is difficult to predict their chromatographic behaviour in the RP-HPLC systems. Nevertheless, from the PCA results it is clear that these deviations are well related to the substitution pattern on the chalcone core: the selectivity, $\alpha_{X\text{-}Y/X\text{-}Y'}$, is highest when both substituents in positions 4 and 4' are electron-donor groups. The greatest opposite influence is observed for the electron-acceptor substituents in position 4.

The ability of 23 commercially available RP-HPLC packing materials to separate configuration isomers has also been compared. The nature and the concentration of the organic "bristle" chemically bonded on the surface of silica and the concentration of the polar silanol groups play a major rôle in the separation of *E*-s-*cis* and *Z*-s-*cis* chalcones.

Based on the PCA results (Fig. 3), one can formulate the following general rule for selection of the stationary phase to be used for separation of configurational isomers: the lower the polarity of the organic "bristle", the greater the carbon loading and the lower the percentage of derivatization, the better is the separation of the configurational isomers.

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