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FACTOR ANALYSIS AND EXPERIMENT DESIGN IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

II. NORMAL-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY ON COLUMNS OF DIFFERENT POLARITIES

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SUMMARY

The separation of 47 chalcones, giving two series of the *E-s-cis*- and *Z-s-cis*-isomers in normal-phase high-performance liquid chromatography (HPLC) on five stationary phases of different polarity (Zorbax C_8, Zorbax ODS, Zorbax NH_2, LiChrospher 100 DIOL and MicroPak CN) is presented. Comparison of the chromatographic behaviour of the chalcones in the investigated HPLC systems was effected with the help of correspondence factor analysis. The specificity of chromatographic systems in relation to the isolated sub-classes of chalcones and the influence of positional and configurational isomerism on the separations are discussed. The results obtained are compared with the data from reversed-phase HPLC systems.

INTRODUCTION

In the framework of a systematic study to evaluate the main factors responsible

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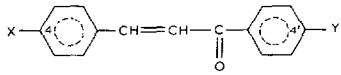
for selectivity in analytical or physico-chemical applications and chiefly with the aim of studying the possibilities to model the activity of series of drugs with chromatographic data[1], we have developed an experiment design based on factor analysis. A series of chalcones were used as model compounds and analysed by reversed-phase high-performance liquid chromatography (RP-HPLC) with non-polar chemically bonded stationary phases[2]. Quantification of the influence of the hydrophobicity of the substituents in the 4- and 4'-positions of the phenyl ring was obtained. The hydrophobic sensitivity to positional isomerism was shown to be dependent on configurational isomerism[2].Although non-specific interactions play a major role in RP-HPLC, specific interactions with nitro-chalcones were observed on a μBondapak-phenyl stationary phase, partly owing to residual silanol groups. In medicinal chemistry, drug transport through the cell membranes can be modelled with Hansch parameters or with RP-HPLC data[1]. Nevertheless, in drug action the specific interactions with receptor sites are also very important. Consequently, more information is required about the electronic and steric properties of drugs. To complement the results obtained in RP-HPLC, a systematic study of potential specific interactions in normal-phase HPLC (NP-HPLC) was undertaken. A similar series of model chalcones:



(denoted as X–Y in the text) were separated on stationary phases of different polarities, in order to establish the relative contributions of the carbonyl group, the phenyl rings and the 4- or 4'-substituents. Owing to their high capacity factors, the *E-s-cis*- and *Z-s-cis*-isomers of the NO_2 NO_2, NH_2 H and H OH compounds were not considered.

EXPERIMENTAL

The chalcones investigated, the chromatographic equipment and the procedures adopted were described in the preceding paper[2].

The following chromatographic columns were used: 7-μm Zorbax ODS (250 × 4.6 mm I.D.), 7-μm Zorbax C_8 (240 × 4.0 mm I.D.), 7-μm Zorbax NH_2 (250 × 4.6 mm I.D.), 10-μm LiChrospher 100 DIOL (250 × 4.6 mm I.D.), and 5-μm MicroPak CN (300 × 4.0 mm I.D.).

The mobile phase consisted of HPLC-grade heptane–tetrahydrofuran (THF) (97:3), purchased from Merck (Darmstadt, F.R.G.).

RESULTS AND DISCUSSION

The aim of this work was to compare the separations of the selected group of chalcones (*E-s-cis* and *Z-s-cis*) in chromatographic systems with stationary phases of different polarity. The capacity factors (k') of the investigated chalcones in the systems employed are summarized in Table I.

From the results obtained, it is evident that the capacity factors generally increase for all columns in the order Zorbax C_8 < Zorbax ODS < LiChrospher 100 DIOL < MicroPak CN < Zorbax NH_2. In addition, differences in selectivity may

TABLE I

CAPACITY FACTORS, k', FOR 47 *E-s-cis-* AND *Z-s-cis*-CHALCONES SEPARATED ON DIFFERENT COLUMNS

Columns: NH_2 = Zorbax NH_2; DIOL = LiChrospher 100 DIOL; CN = MicroPak CN; ODS = Zorbax ODS; C_8 = Zorbax C_8. Mobile phase, heptane–THF (97:3); detection, UV at 280 nm. The *Z-s-cis*-chalcones are denoted by asterisks. Abbreviations: tBu = *tert.*-butyl, iPr = isopropyl, Et = ethyl, Me = methyl, Ø = phenyl.

No.	*Chalcone X–Y*	*NH_2*	*DIOL*	*CN*	*ODS*	*C_8*
1	H–CF_3	3.39	1.14	2.99	0.22	0.29
2	H–tBu	3.49	1.21	3.22	0.37	0.41
3	H–iPr	3.52	1.22	3.31	0.41	0.43
4	H–H	4.04	1.37	3.85	0.67	0.52
5	F–H	4.82	1.67	4.47	0.78	0.55
6	H–F	4.03	1.38	3.7	0.61	0.48
7	H–Et	3.84	1.32	3.56	0.51	0.47
8	H–Me	4.25	1.43	3.88	0.67	0.52
9	F–Me	4.93	1.69	4.45	0.81	0.53
10	F–F	4.6	1.76	4.46	0.71	0.52
11	Me–Ø	6.98	2.25	5.98	0.75	0.5
12	MeO–Me	11.94	3.31	9.29	1.88	1.04
13	Me–MeO	12.07	3.35	9.22	2.03	1.08
14	F–MeO	14.29	4.06	10.74	2.09	1.16
15	H–NO_2	11.29	3.4	9.99	1.17	0.7
16	MeO–Ø	19.84	5.19	14.32	2.23	1.03
17	F–NO_2	15.4	4.57	13.14	1.4	0.75
18	NO_2–Me	16.95	4.69	13.85	1.96	0.88
19	NO_2–H	17.43	4.93	14.99	1.85	0.98
20	MeO–MeO	32.38	7.51	21.59	6.6	2.05
21	MeO–NO_2	22.51	6.82	19.91	2.95	1.26
22	NO_2–F	23.23	5.87	16.94	2	0.96
23	$N(Me)_2$–NO_2	42.83	9.2	29.35	5.33	1.74
24	NO_2–MeO	56.67	11.21	34.02	5.33	2
1*	H–CF_3*	3.23	0.92	2.71	0.36	0.29
2*	H–tBu*	3.49	0.96	3.01	0.22	0.41
3*	H–iPr*	3.52	0.97	3.08	0.18	0.43
4*	H–H*	3.72	1.06	3.34	0.57	0.52
5*	F–H*	3.99	1.12	3.23	0.66	0.55
6*	H–F*	4.03	1.07	3.24	0.61	0.48
7*	H–Et*	3.84	1.04	3.26	0.44	0.47
8*	H–Me*	4.05	1.1	3.53	0.57	0.52
9*	F–Me*	4.13	1.12	3.22	0.66	0.53
10*	F–F*	4.6	1.24	3.43	0.71	0.52
11*	Me–Ø*	6.36	1.63	5.98	0.59	0.5
12*	MeO–Me*	8.46	1.94	6.17	1.09	0.72
13*	Me–MeO*	10.32	2.31	7.31	1.49	0.91
14*	F–MeO*	11.29	2.52	7.38	1.59	1
15*	H–NO_2*	10.69	2.53	8.15	1.29	0.77
16*	MeO–Ø*	14.21	3.03	9.49	1.36	0.74
17*	F–NO_2*	16.65	3.39	10.39	1.94	1.02
18*	NO_2–Me*	16.95	3.4	10.83	2.47	1.17
19*	NO_2–H*	17.43	3.64	11.82	2.26	1.28
20*	MeO–MeO*	22.47	4.29	13.9	3.85	1.46
21*	MeO–NO_2*	30.92	4.05	12.78	2.12	1.03
22*	NO_2–F*	27.08	4.54	14.16	3.32	1.63
24*	NO_2–MeO*	49.67	7.4	24.47	5.86	2.47

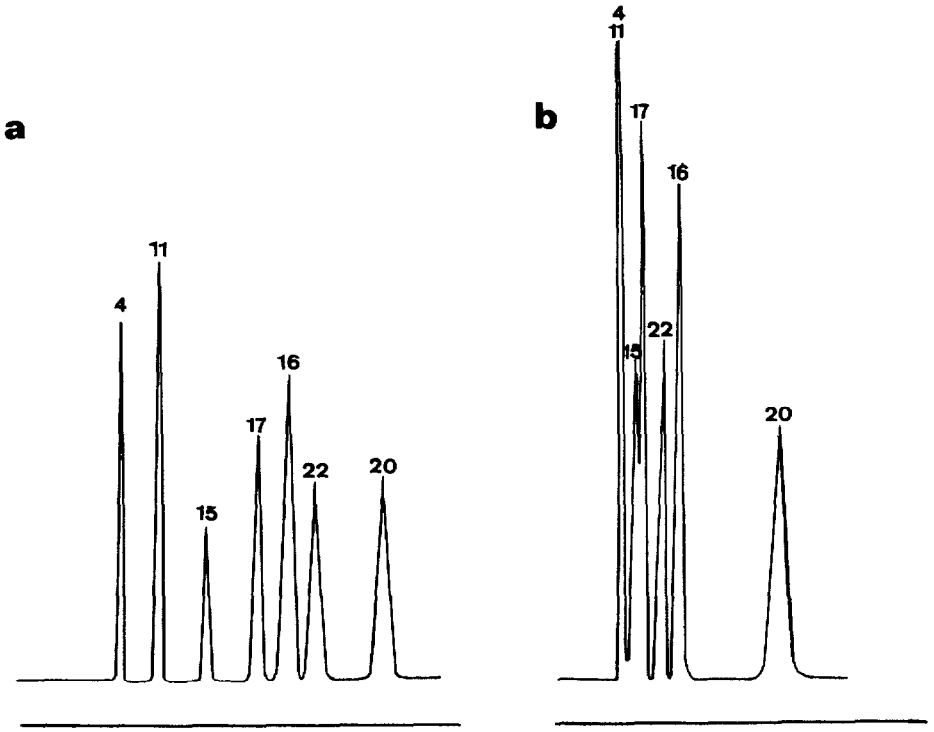


Fig. 1. Separation of selected *E-s-cis*-chalcones on (a) LiChrospher 100 DIOL and (b) Zorbax ODS. Mobile phase, heptane–THF (97:3); flow-rate, 2 ml min^{-1}; detection, 280 nm. Peak numbers correspond to Table I.

be noted that result not only from the retention power of the chromatographic systems investigated, but also from their specificity (Fig. 1 shows the separation of some selected chalcones on LiChrospher 100 DIOL and Zorbax ODS).

It should be stressed that the order of elution of the chalcones in the chromatographic systems with Zorbax ODS and Zorbax C_8 stationary phases and heptane–THF (97:3) as the mobile phase shows that the separation mechanism is a normal-phase process. Thus, weakly polar compounds, such as the unsubstituted chalcone H–H in Table I with $k' = 0.67$ on Zorbax ODS and 0.52 on Zorbax C_8,

TABLE II

CFA OF THE BEHAVIOUR OF 47 CHALCONES: CONTRIBUTION OF THE FIVE CHROMATOGRAPHIC SYSTEMS TO THE MAIN AXES OF INERTIA

Mobile phase: heptane–THF (97:3).

Stationary phase	*Axis 1*	*Axis 2*	*Axis 3*
Zorbax NH_2	43.3	1.2	6.9
LiChrospher 100 DIOL	17.1	2.6	6.6
MicroPak CN	22.8	7.0	2.2
Zorbax ODS	1.3	60.8	31.6
Zorbax C_8	15.6	28.3	52.7

are eluted more readily than polar compounds, such as the nitrochalcone NO_2–H with $k' = 1.85$ on Zorbax ODS and 0.98 on Zorbax C_8. This is surprizing, because these non-polar packing materials are designed to act in the reversed-phase mode. Nevertheless, the phenomenon can be explained by the fact that the coverage of the active surface groups of the silica is incomplete. Unger[3], Roumeliotis and Unger[4] and Berendsen and De Galan[5] have shown that a sizable percentage of the total number of silanol groups originally present on silica surfaces remain underivatized even after "exhaustive" silanization and may cause a mixed retention mechanism. A similar phenomenon was described by Hunter *et al.*[6] for μBondapak C_{18}–Porasil B as the stationary phase and hexane containing 0.5% 2-propanol as the mobile phase.

In order to gain an insight into the specificity of the chromatographic system investigated, correspondence factor analysis (CFA)[2,7,8] was applied to the raw experimental data from Table I. The results of this analysis are presented in Table II and in Fig. 2a and b.

As the three main axes extracted from CFA make contributions of 58.0, 31.2 and 8.9% to the total cluster inertia, we shall discuss the projection of chalcones and chromatographic systems onto the planes defined by axes 1 and 2 (Fig. 2A) and axes 2 and 3 (Fig. 2B).

Axis 1, as determined by the systems containing Zorbax NH_2, MicroPak CN, LiChrospher 100 DIOL and Zorbax C_8 stationary phases (see Table II), shows that the greatest difference is observed for the systems with Zorbax NH_2 and Zorbax C_8 phases. Axis 2 distinguishes alkyl-bonded phases from the polar phases, and axis 3 reflects the differences between systems with Zorbax ODS and Zorbax C_8 stationary phases. The proximity of MicroPak CN- and LiChrospher 100 DIOL-containing systems on both projections testifies to their similarity in the separation of chalcones, *i.e.*, a proportionality of k' between the two systems is observed. This also demonstrates that a longer retention does not necessarily provide better resolution (see Table I).

Whereas in the RP-HPLC systems a certain regularity in the chromatographic behaviour of *E-s-cis*- and *Z-s-cis*-chalcones was observed[2], no such regularity is evident for the NP-HPLC systems considered. The different orientation of the lines joining the isomers in the projection in Fig. 2A illustrates this loss of regularity. Fig. 3 shows these irregularities with the inversion of retention observed among some selected *E-s-cis*-(F–NO_2, MeO–Ø, NO_2–F and MeO–MeO) and *Z-s-cis*-chalcones (MeO–Ø*, F–NO_2*, NO_2–F* and MeO–MeO*) analysed on LiChrospher 100 DIOL stationary phase.

Let us consider first the relative arrangement of *E-s-cis*-chalcones on both projections (Fig. 2a and b). In the space created by the main CFA axes, the investigated chalcones form four sub-classes of substituted *E-s-cis*-chalcones, containing the following groups: (a) NO_2, Ø and CF_3; (b) alkyl and F; (c) MeO; and (d) MeO–MeO. This clustering is created mainly by axes 1 and 2. Axis 3 additionally differentiates the compounds within these groups. The F–F, F–Me and F–H chalcones in group b have positive coordinates on axis 3 and the remaining compounds in this group have negative coordinates. The same is observed for components of group a. Two of them, H–CF_3 and NO_2–F, have reverse coordinates on axis 3 relative to the others.

Based on the relative arrangement of the above-mentioned sub-classes of *E-*

s-cis-chalcones and the chromatographic systems on the discussed projections, one can conclude that this grouping of *E-s-cis*-chalcones arises from interactions between NO_2-, Ø-, and CF_3-substituted chalcones and the polar stationary phases, MeO-substituted chalcones and the Zorbax ODS stationary phase and alkyl- and F-substituted chalcones and the Zorbax C_8 stationary phase.

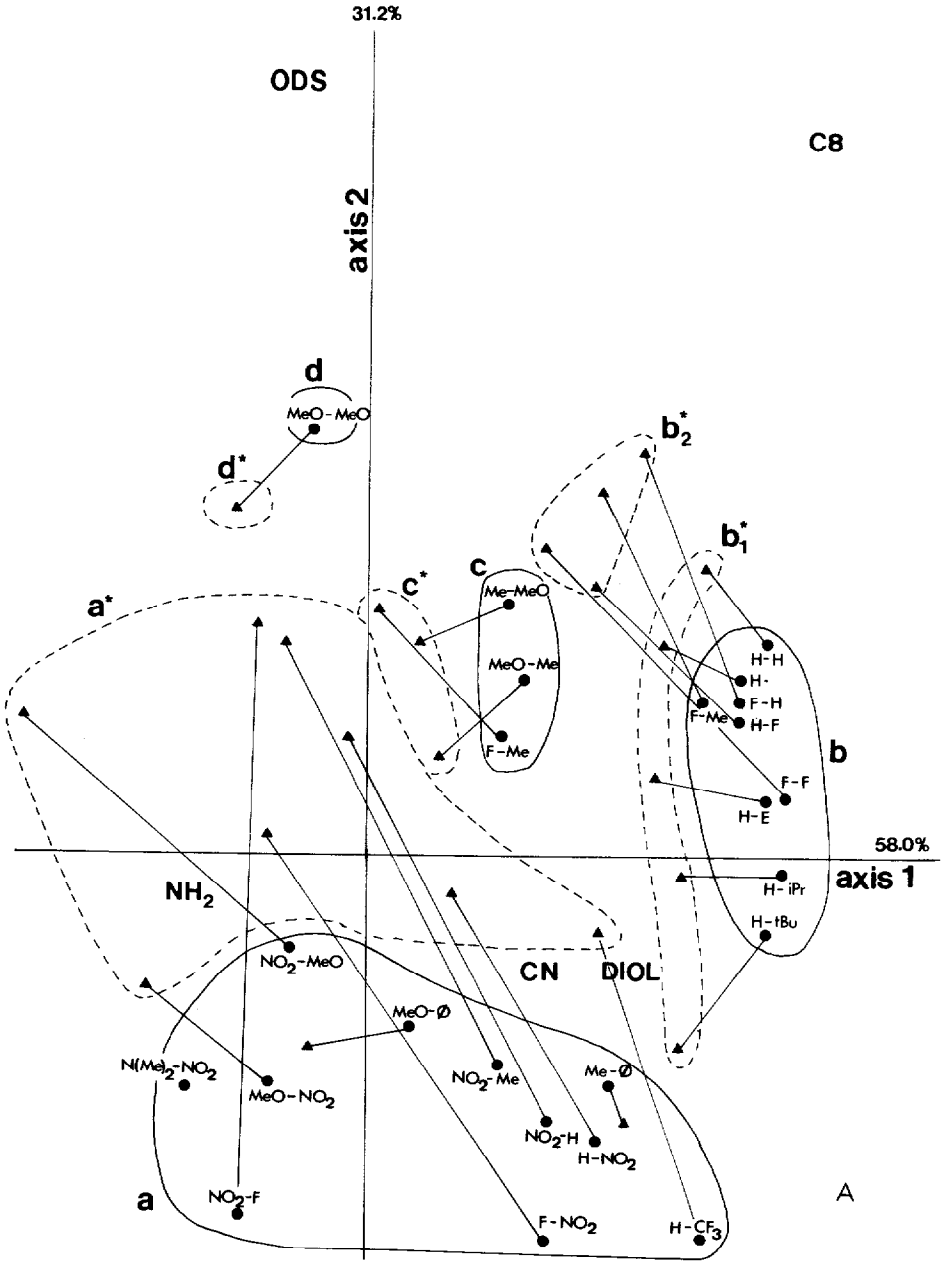


Fig. 2.

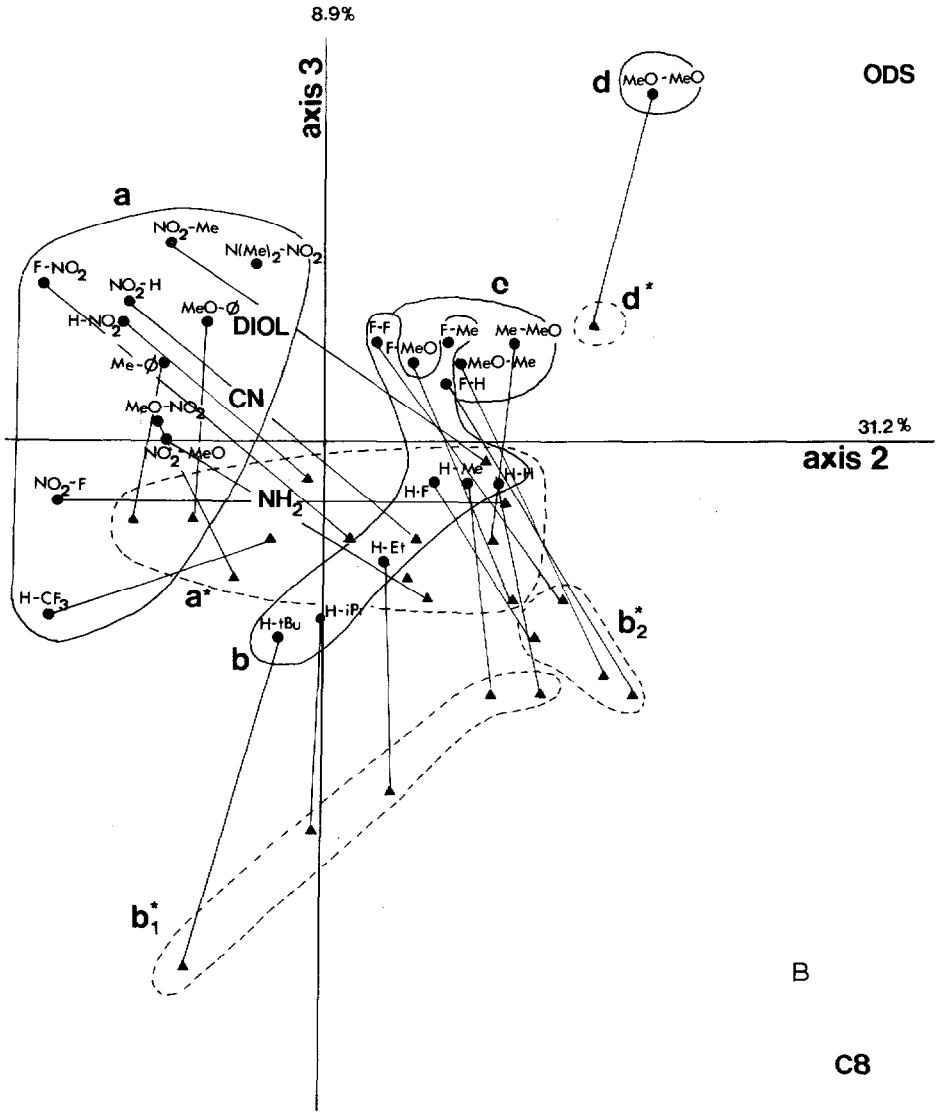


Fig. 2. Simultaneous projection of 47 chalcones (●, *E-s-cis*; ▲, *Z-s-cis*) and five chromatographic systems on to the plane determined by the inertia axes, extracted from CFA. (A) Axes 1 and 2; (B) axes 2 and 3.

The relative distribution of *Z-s-cis*-chalcones on both projections (clusters a*, b_1^*, b_2^*, c* and d* in Fig. 2A and B) is completely different from the arrangement observed for the *E-s-cis*-chalcones (clusters a, b, c and d in Fig. 2A and B). Cluster a, including NO_2-, Ø-, and CF_3-substituted *E-s-cis*-chalcones, is well separated from cluster c, including MeO-substituted *E-s-cis*-chalcones, whereas clusters a* and c*, including the corresponding *Z-s-cis*-chalcones, are grouped together. Simultaneously, F- and alkyl-substituted *Z-s-cis*-chalcones form two separate groups,

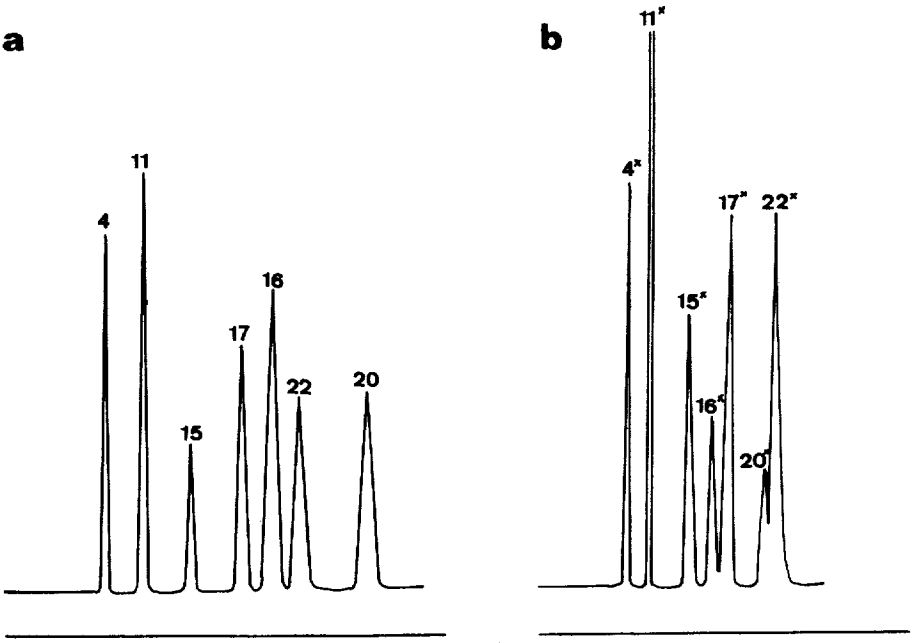


Fig. 3. Separation of some isomeric chalcones: (a) *E-s-cis*; (b) *Z-s-cis*. Stationary phase, LiChrospher 100 DIOL; mobile phase, heptane–THF (97:3); flow-rate, 2 ml min^{-1}; detection, 280 nm. Peak numbers correspond to Table I.

b_1^* and b_2^*. The b_1^* sub-class is additionally set apart from the other groups along axis 3. This axis reflects also the specific behaviour of the MeO–MeO* chalcone (d*) on the Zorbax ODS stationary phase.

These different patterns of *E-s-cis*-chalcones on both projections (Fig. 2a and b) illustrate the great and heterogeneous influence of configurational isomerism on chalcone separations; the heterogeneous influence depends on the chemical nature of the chalcone substituents.

Configurational isomers

The relative retentions of the *E-s-cis*- and *Z-s-cis*-isomer pairs in the chromatographic system considered can be described with help of the following parameter:

$$\alpha_{X-Y/X-Y^*} = k'_{X-Y}/k'_{X-Y^*}$$

(Table III). Careful analysis of the numerical values of $\alpha_{X-Y/X-Y^*}$ leads to the following observations:

(a) The worst selectivity of isomer separation is observed on Zorbax C_8 and the best is obtained on LiChrospher 100 DIOL.

(b) The best correlation between these $\alpha_{X-Y/X-Y^*}$ parameters is observed on LiChrospher 100 DIOL and MicroPak CN; the worst correlation is observed between Zorbax ODS and MicroPak CN.

(c) On the polar stationary phases (*i.e.*, Zorbax NH_2, LiChrospher 100 DIOL and MicroPak CN) the following regularity is observed: the retention of the *E-s-cis*-isomers is stronger than that of their *Z-s-cis* counterparts. The only exception is the F–NO_2 and F–NO_2^* pair, chromatographed on Zorbax NH_2.

(d) On the non-polar stationary phases (*i.e.*, Zorbax ODS and Zorbax C_8) no such regularity is observed: inversion of retention order appears for the pairs of chalcones with the following substituents: (H, CF_3), (H, NO_2), (F, NO_2), (NO_2, Me), (NO_2, H), (NO_2, F) and (NO_2, MeO).

(e) The alkyl-substituted isomers show the best separations LiChrospher 100 DIOL and Zorbax ODS (e.g., $\alpha_{X-Y/X-Y^*}$ for *tert.*-butyl-substituted chalcones on these stationary phases are 1.26 and 1.68, respectively). The separation decreases on MicroPak CN, while on Zorbax C_8 and Zorbax NH_2 no separation is observed.

(f) The best separations are obtained on LiChrospher 100 DIOL and MicroPak CN for fluorine-substituted chalcones, on LiChrospher 100 DIOL and Zorbax ODS for phenyl-substituted chalcones, on LiChrospher 100 DIOL, Zorbax ODS and MicroPak CN for MeO-substituted chalcones and on LiChrospher 100 DIOL and MicroPak CN for NO_2-substituted chalcones.

TABLE III

NUMERICAL VALUES OF THE SELECTIVITY, $\alpha_{X-Y/X-Y^*}$, FOR CHALCONES, SEPARATED ON DIFFERENT COLUMNS

Columns and conditions as in Table I.

No.	*Chalcone X–Y*	$\alpha_{X-Y/X-Y^*}$				
		NH_2	*DIOL*	*CN*	*ODS*	*C_8*
1	H–CF_3	1.05	1.24	1.10	0.61	1.00
2	H–tBu	1.00	1.26	1.07	1.68	1.00
3	H–iPr	1.00	1.26	1.07	1.20	1.00
4	H–H	1.09	1.29	1.15	1.18	1.00
5	F–H	1.21	1.49	1.38	1.18	1.00
6	H–F	1.00	1.29	1.14	1.00	1.00
7	H–Et	1.00	1.27	1.09	1.16	1.00
8	H–Me	1.05	1.30	1.10	1.18	1.00
9	F–Me	1.19	1.51	1.38	1.23	1.00
10	F–F	1.00	1.42	1.30	1.00	1.00
11	Me–Ø	1.10	1.38	1.00	1.27	1.00
12	MeO–Me	1.41	1.71	1.50	1.72	1.44
13	Me–MeO	1.17	1.45	1.26	1.36	1.19
14	F–MeO	1.26	1.61	1.46	1.31	1.16
15	H–NO_2	1.06	1.34	1.22	0.91	0.91
16	MeO–Ø	1.40	1.71	1.51	1.64	1.39
17	F–NO_2	0.92	1.35	1.26	0.72	0.74
18	NO_2–Me	1.00	1.38	1.28	0.79	0.75
19	NO_2–H	1.00	1.35	1.27	0.82	0.76
20	MeO–MeO	1.44	1.75	1.55	1.71	1.40
21	MeO–NO_2	1.37	1.68	1.56	1.39	1.22
22	NO_2–F	1.16	1.29	1.20	0.60	0.59
24	NO_2–MeO	1.14	1.51	1.39	0.91	0.81

Positional isomers

Fig. 4 results from a simplified presentation of Fig. 2 and is limited to ten pairs of position isomers. They differ not only in their retention (Table I) but also, naturally, in their sensitivity to changes in the chromatographic conditions.

A particularly large difference is observed for NO_2–F and F–NO_2 chalcones along axes 1 and 3; it disappears for the NO_2–F* and F–NO_2^* isomer pair. For these

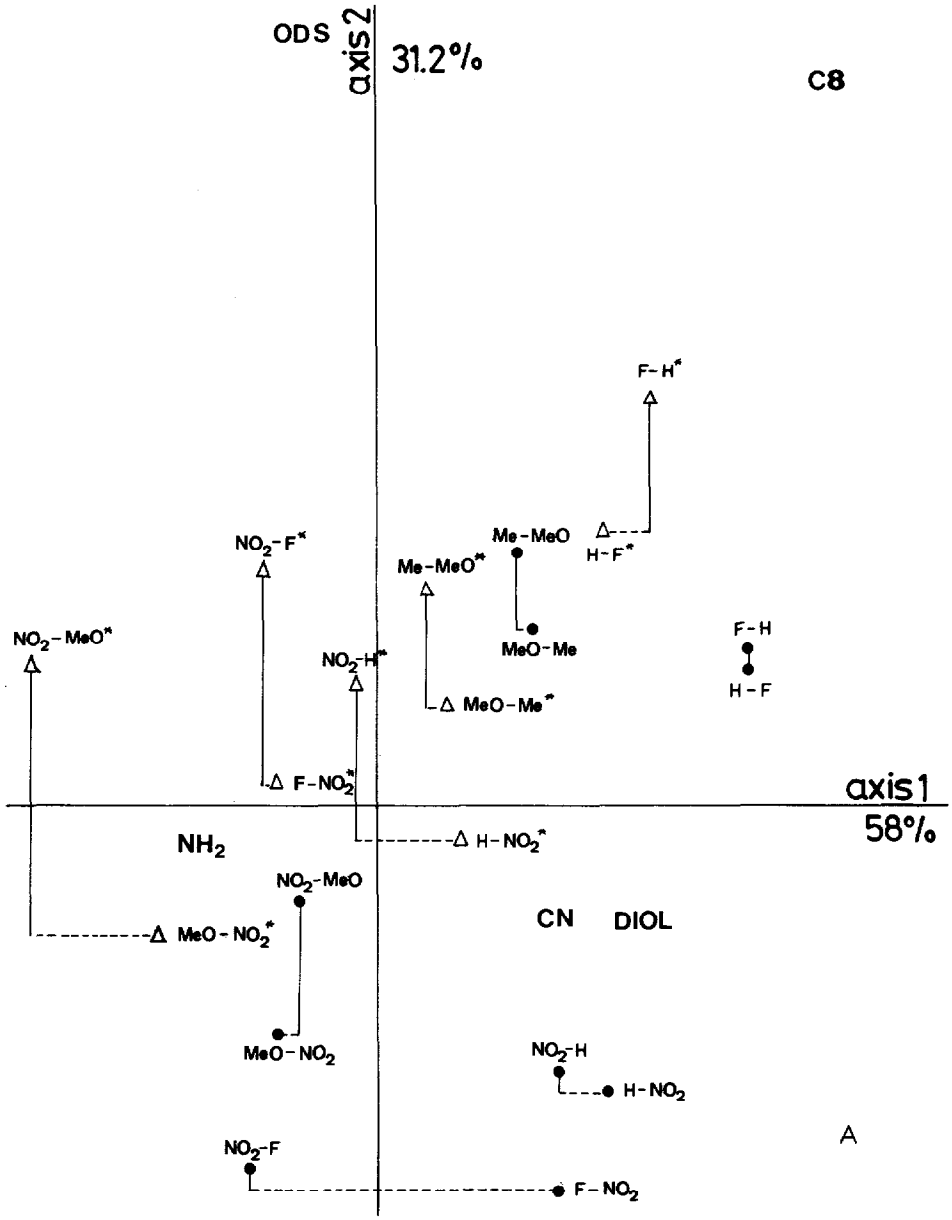


Fig. 4.

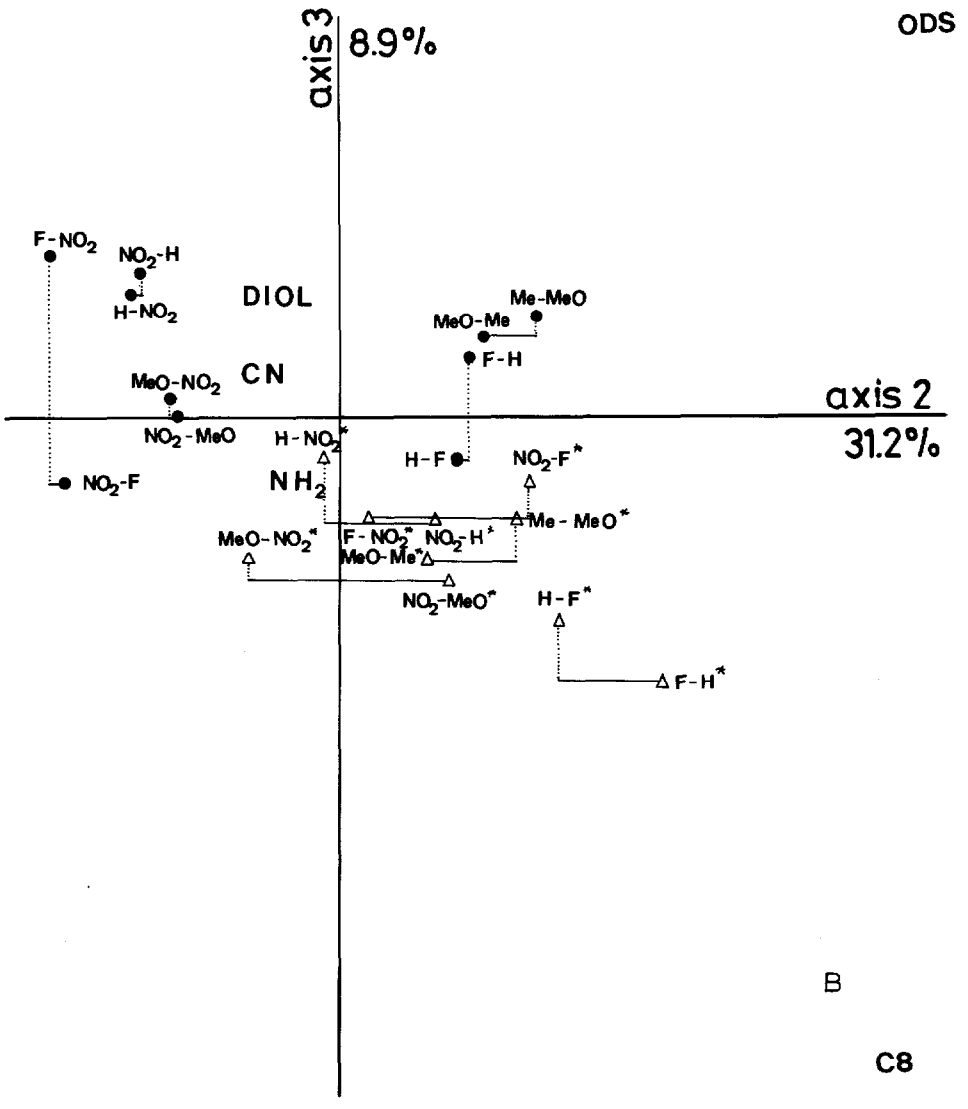


Fig. 4. The same projection as in Fig. 2: an improved presentation, limited to 20 positional isomers. Projection on the plane determined by (A) axes 1 and 2; (B) axes 2 and 3.

the large difference along axis 2 is characteristic. For the remaining substitutional isomers the other regularity is typical: substitutional isomers in the *Z-s-cis* configuration differ to a greater degree than *E-s-cis*-chalcones in position along all the axes. This difference is always greatest along axis 2. Comparison of the displacement direction along a given axis can supply additional information about the changes in the properties of the compounds, *i.e.*, the transition of NO_2 or F groups from the 4'- to the 4-position causes a shift along axis 2 in the opposite direction to the analogous transition of MeO substituent. This means that the electron-acceptor substituents

(NO_2, F) in position 4 decrease the retention on the polar stationary phases in comparison with the alkyl-bonded phases, and the electron-donor (MeO) group causes the opposite effect.

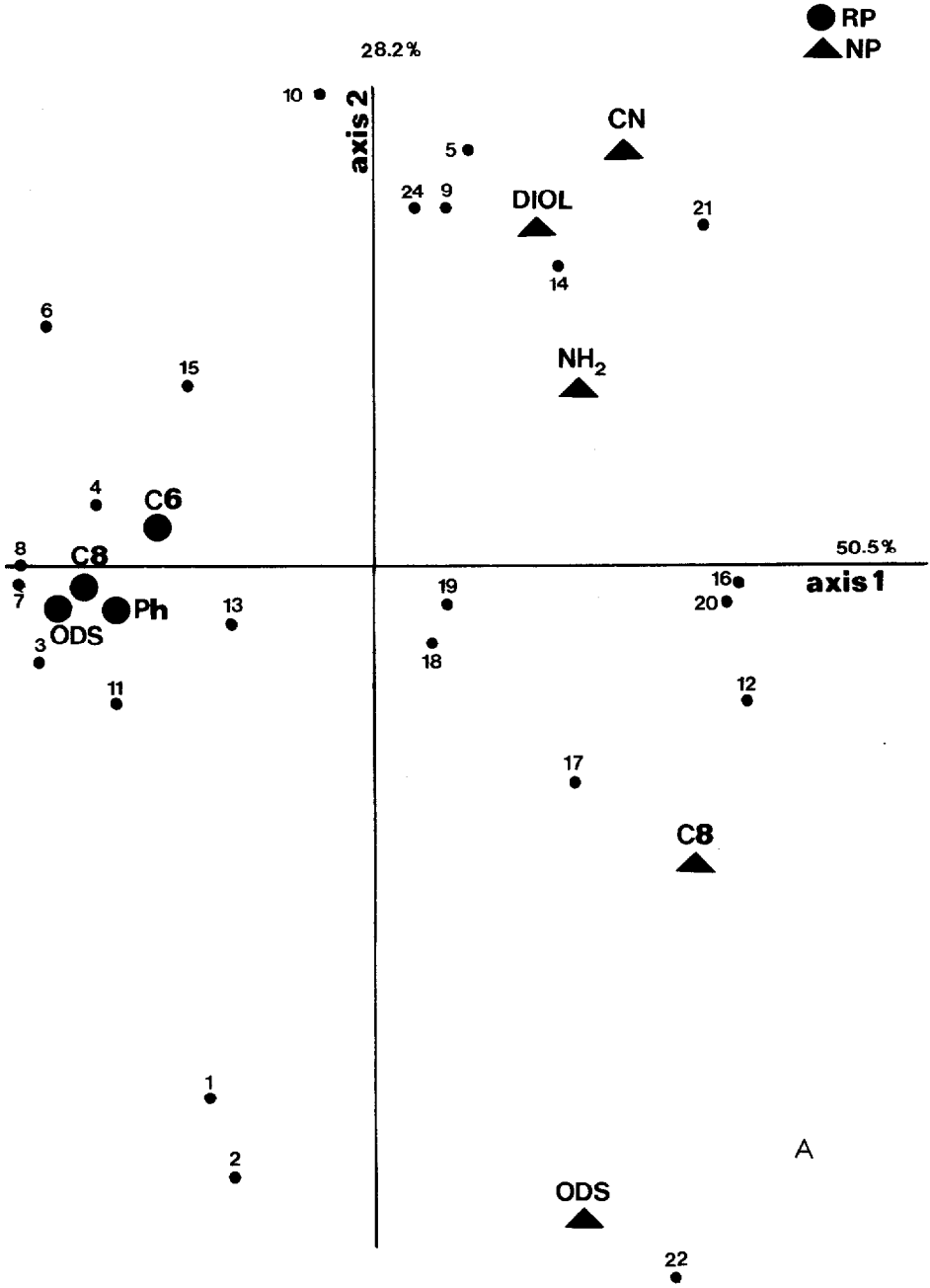


Fig. 5.

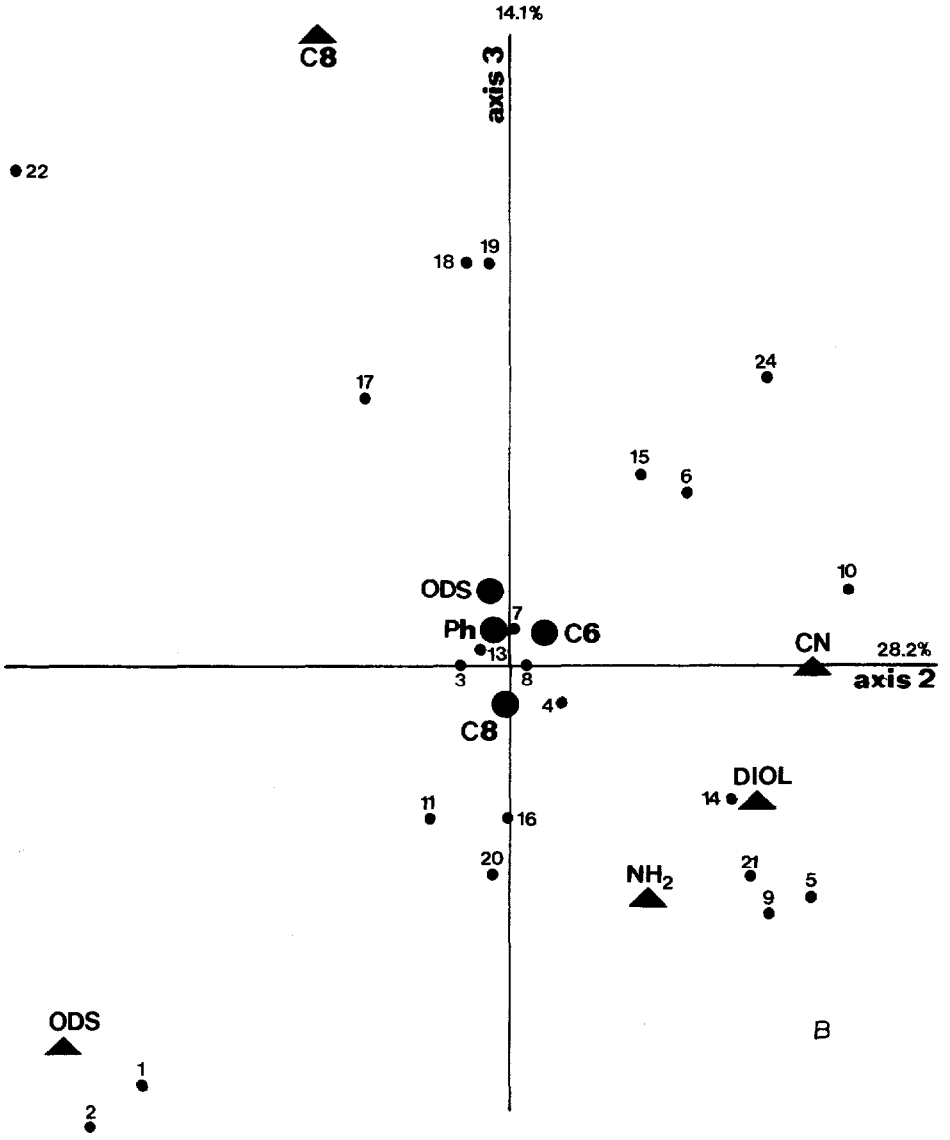


Fig. 5. Simultaneous projection of 23 $\alpha_{X-Y/X-Y}$* parameters and nine chromatographic systems on to the plane determined by the inertia axes extracted from CFA: (A) axes 1 and 2; (B) axes 2 and 3.

Comparison of the chromatographic behaviour of chalcones in RP- and NP-HPLC systems

In all chromatographic systems investigated with stationary phases able to interact specifically (μBondapak-phenyl, Zorbax NH_2, LiChrospher 100 DIOL and MicroPak-CN), NO_2-substituted chalcones behave in the same manner, distinguishing them from the remaining compounds. The specific behaviour of the other groups of compounds is typical only for particular chromatographic systems, *i.e.*, one can

observe the specific behaviour of MeO-substituted chalcones only in normal-phase systems with a Zorbax ODS stationary phase, or Ø-substituted compounds only in reversed-phase system with Zorbax ODS.

The positional isomers behave as expected in normal- and reversed-phase systems. The elution order of positional isomer pairs in the normal-phase systems is opposite to the order in reversed-phase systems. The only exception to this rule is the Me–MeO and MeO–Me isomer pair, which are eluted in the same order in both types of chromatographic systems.

The differences in the relative retentions of compounds of the *E-s-cis* and *Z-s-cis* series in reversed- and normal-phase systems ought to be stressed. Whereas in reversed-phase systems the selectivity of the *E-s-cis*-isomers is very similar to that of the *Z-s-cis*-isomers, in the normal-phase systems, the separation of these two series of compounds is very different. In order to study the differences between configurational isomer separations in both types of chromatographic systems a new CFA study was conducted. A new matrix was formed with the elements $\alpha_{X-Y/X-Y^*}$ if the retention of X–Y is greater than that of X–Y* or $\alpha_{X-Y^*/X-Y}$ if the retention of X–Y* is greater than that of X–Y. The results of this CFA study are presented in Table IV and Fig. 5.

The respective numbers, shown on the CFA projection, refer to the substituent groups of Table III, *i.e.*, point 1 refers to the relative retention of H–CF_3 and H–CF_3^*.

The three main factors gave contributions of 50.5, 28.2 and 14.1% to the cluster inertia. Two first axes divide the chromatographic systems investigated into three groups (Fig. 5a): all reversed-phase chromatographic systems, normal-phase systems with alkyl-bonded stationary phases and normal-phase systems with polar stationary phases. Axis 3 distinguishes additionally systems with Zorbax ODS and Zorbax C_8 with heptane–THF (97:3) as the mobile phase (Fig. 5b).

The above-mentioned axes do not group the α parameters that refer to the

TABLE IV

CONTRIBUTION OF CHROMATOGRAPHIC SYSTEMS TO THE MAIN AXES, EXTRACTED FROM CFA FOR 23 PARAMETERS, $\alpha_{X-Y/X-Y^*}$, DEFINED FOR NINE CHROMATOGRAPHIC SYSTEMS

Chromatographic system		*Axis 1*	*Axis 2*	*Axis 3*
Stationary phase	*Mobile phase*			
Zorbax ODS	Methanol–water (7:3)	6.4	3.8	7.6
Zorbax C_8	Methanol–water (7:3)	5.0	15.8	2.9
Spherisorb C_6	Methanol–water (7:3)	10.5	21.0	0.0
μBondapak-phenyl	Methanol–water (7:3)	5.0	15.8	2.9
Zorbax NH_2	Heptane–THF (97:3)	16.1	8.5	62.3
LiChrospher 100 DIOL	Heptane–THF (97:3)	21.1	0.2	1.5
MicroPak CN	Heptane–THF (97:3)	11.0	0.1	0.2
Zorbax ODS	Heptane–THF (97:3)	14.6	0.0	0.3
Zorbax C_8	Heptane–THF (97:3)	7.6	0.1	0.4

* On leave from Silesian University, Katowice, Poland.

isomer pairs with similar substituents, but what is surprizing and worth stressing is the relative arrangement of the α parameters that refer to positional isomers. It appears that the separation of the MeO–Me and MeO–Me* configurational isomers (No. 12) is better in normal-phase systems, but the separation of the Me–MeO and Me–MeO* isomer pair (No. 13) is better in the reversed-phase systems. Similar differences are observed for the remaining positional isomer pairs (compare the positions of points 5 and 6, F–H and H–F, and 15 and 19, H–NO_2 and NO_2–H).

CONCLUSIONS

With heptane–THF (97:3) as the mobile phase, a normal-phase mechanism is observed for the model series of chalcones, not only on polar LiChrospher 100 DIOL, MicroPak CN and Zorbax NH_2 but also on apolar Zorbax C_8 and Zorbax ODS stationary phases.

The CFA of raw chromatographic data facilitates the comparison of the separation of given groups of compounds in different chromatographic systems. Based on the CFA projection, one can (1) observe which sub-classes of compounds behave specifically in defined chromatographic systems, (2) predict in what manner the relative retention of compounds, grouped in common sub-classes on CFA projection, will change if the chromatographic conditions are changed and (3) choose the best chromatographic conditions for the optimization of the separation of positional or configurational isomers.

A comparison of chalcone behaviour in RP-[2] and NP-HPLC systems shows specific interactions of nitro substituents in all systems investigated and specificity of other different groups in a particular RP- or NP-HPLC system.

Work is under way with LiChrospher 100 DIOL and Zorbax ODS stationary phases in NP-HPLC and different non-aqueous mobile phases to gain an insight into selectivity and the retention mechanism.

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