Journal of Chromatography A, 659 (1994) 307–315
Elsevier Science B.V., Amsterdam

CHROM. 25 638

Structure–retention relationships of diastereomeric mixtures of lipidic amino acid conjugates on reversed-phase stationary phases

D. Korakas [☆], K. Valkó *, I. Wood, W.A. Gibbons and I. Toth [☆☆]

Department of Pharmaceutical Chemistry, School of Pharmacy, University of London, 29–39 Brunswick Square,
London WC1N 1AX (UK)

(First received September 22nd, 1993; revised manuscript received October 11th, 1993)

ABSTRACT

The retention parameters of diastereomeric mixtures of 19 lipidic amino acid conjugates have been determined on Spherisorb ODS and Supelcosil LC-ABZ stationary phases by changing the acetonitrile concentration in acetonitrile–0.1% trifluoroacetic acid–water mobile phases. In general, better resolution of the diastereomeric mixtures was obtained on the Supelcosil LC-ABZ stationary phase but in some cases the Spherisorb ODS column showed better separation. The presence of silanol groups improved the separation of the diastereomers in those cases. The log k' values were plotted against the acetonitrile concentrations. In the case of basic derivatives parabolic relationships could be observed on the Spherisorb ODS stationary phase which contains free silanol groups, while always straight lines were obtained on the Supelcosil LC-ABZ column. The slope and the intercept values were calculated for the straight lines obtained. The octanol–water partition coefficients (log P) and the dissociation constants (pK_a) of the compounds were also calculated and were related to the chromatographic parameters. It was found that the chromatographic parameters obtained on the Spherisorb ODS stationary phase showed slightly better correlations with the calculated hydrophobicity and pK_a parameters than those obtained on the Supelcosil LC-ABZ column, which shows that different retention mechanism can be expected on the two stationary phases.

INTRODUCTION

The lipidic amino acids are possible conjugates for biologically active molecules through their bifunctional nature as it was described by Gibbons *et al.* [1]. They have a membrane-like character and are able to facilitate drug absorption to the receptor site. The quantitative structure–activity correlation study [2] revealed the role of the lipidic side chain in the *in vivo* activity of the lipidic amino acid conjugates of β-lactam antibiotics. As the racemic forms of lipidic amino acids are used for conjugation the synthesis always results in diastereomeric mixtures. The separation of diastereomers does not require chiral separation conditions and therefore is easier and cheaper. However, the lipidic amino acid conjugates have amphophilic and surface-active character, so even their separation is not straightforward, and the structure–retention relationship study can reveal their retention mechanism. As the Supelcosil LC-ABZ column was designed specially for anionic, basic and zwitterionic compounds without silanophilic effects it seemed worth trying for the separation of the diastereomeric mixtures.

---

* Corresponding author. On leave from Central Research Institute for Chemistry, Hungarian Academy of Sciences, P.O. Box 17, H-1025 Budapest, Hungary.

[☆] On leave from Department of Chemistry, Section of Organic Chemistry and Biochemistry, University of Ioannina, 451 10 Ioannina, Greece.

[☆☆] On leave from Central Research Institute for Chemistry, Hungarian Academy of Sciences, P.O. Box 17, H-1025 Budapest, Hungary

0021-9673/94/$07.00
SSDI 0021-9673(93)E1075-B

In this study the retention parameters of 19 lipidic amino acid conjugate mixtures were measured and the structure–retention relationships were studied. The chemical structure of the compounds was characterized by calculated log P values (the logarithmic values of the octanol–water partition coefficients) and the dissociation constants (pK_a).

EXPERIMENTAL

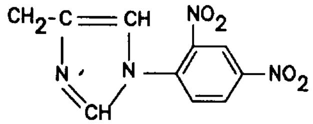
The lipidic dipeptides **1–19** were synthesized by coupling the appropriately protected amino acids to methyl amino tetradecanoate employing the hydroxybenzotriazole assisted carbodiimide coupling method as described by Gibbons *et al.* [1]. Infrared spectra were recorded with a Perkin-Elmer 841 spectrophotometer. ^{1}H NMR spectra were obtained with Varian XL-300 and Bruker AM500 instruments operating at fields of 300 and 500 MHz, respectively; chemical shifts were checked in ppm downfield from internal tetramethylsilane. Mass spectra were run with a VG analytical ZAB-SE instrument, using the fast-atom bombardment (FAB) techniques; 20 kV Cs^+ ion bombardment, with 2 μl appropriate matrix either 3-nitrobenzyl alcohol or thioglycerol with NaI (MeOH solution) added when necessary to produce nitrated species where no protonated molecule ions were observed. The reaction process was monitored by thin-layer chromatography on Kieselgel PF_{254} using CH_2Cl_2–MeOH (10:1), (10:0.5) or (10:0.25) solvent as required. Purification was achieved by TLC using Kieselgel $PF_{254+366}$ (Merck) on 20 × 20 cm plates 1.5 mm thick with the before-mentioned solvent systems. Solvents were evaporated under reduced pressure with a rotary evaporator. The chemical structure and their identification parameters can be seen in Table I.

The compounds were dissolved in 1 mg/ml concentration in acetonitrile–water–trifluoro-

TABLE I

CHEMICAL STRUCTURES OF THE INVESTIGATED DIASTEREOMERIC LIPIDIC AMINO ACIDS

$(CH_3)C{-}O{-}CO{-}N^*H{-}C(R)H{-}CO{-}NH{-}CH(C_{12}H_{25}){-}COOCH_3$

Compound	R	Yield (%)	MS (*m/z*)
1	H	78.6	438
2	$CH(CH_3)_2$	72.8	479
3	CH_3	76.8	453
4	$CH_2{-}CH(CH_3)_2$	72.8	494
5	$CH(CH_3)C_2H_5$	67.4	495
6	$-CH_2{-}CH_2{-}CH_2{-}N^*$ [a]	68.6	478
7	$CH_2{-}C_6H_5$	51.2	528
10	$CH_2{-}S{-}CH_2{-}NH{-}CO{-}CH_3$	65.5	555
11	$(CH_2)_2{-}S{-}CH_3$	65.6	512
12	$CH(CH_3){-}O{-}CH_2{-}C_6H_5$	76.5	572
13	$CH_2{-}O{-}CH_2{-}C_6H_5$	80.1	558
14	$(CH_2)_4{-}NH{-}CO{-}O{-}CH_2{-}C_6H_5{-}o{-}Cl$	58.9	678
15	$(CH_2)_3{-}NH{-}C(NH){-}NH{-}SO_2{-}C_6H_5{-}p{-}CH_3$	63.9	712
16		77.3	684
17	$CH_2{-}CO{-}O{-}CH_2{-}C_6H_5$	65.2	586
18	$(CH_2)_2{-}CO{-}O{-}CH_2{-}C_6H_5$	74.3	601
19	$CH_2{-}CO{-}NH_2$	64.1	495

[a] * denotes the position for forming a ring in compound **6**.

acetic acid (TFA) (95:5:0.1) and 20 μl of this solution were injected three times on the columns. The solvent peak was regarded as the dead time, and the log *k'* values were calculated from the average retention time obtained from the three injections.

A Gilson HPLC system (pump Model 303, variable-wavelength UV detector M 115, Rheodyne injector) was obtained from Anachem (Luton, UK). The flow-rate was always 1.00 ml/min. The detection was carried out at 210 nm with 1 AUFS. Spherisorb ODS 5 μm (50 × 4.6 mm) (Phase Sep, Deeside, UK) and Supelcosil LC-ABZ (150 × 4.6 mm) (Supelco, Bellefonte, PA, USA) columns were used for the separations. The mobile phases were various concentrations of acetonitrile ranging from 95 to 65% (v/v), water and 0.1% TFA.

The calculation of the log *P* and pK_a values were carried out by ELUEX 3.0 expert system developed by CompuDrug Chemistry (Budapest, Hungary) for predicting the optimum mobile phase composition for HPLC separations. The correlation study was carried out by Drugidea software developed by Chemicro (Budapest, Hungary).

RESULTS AND DISCUSSION

The log *k'* values were determined for each compound on both stationary phases using various concentrations of acetonitrile in the mobile phase. The log *k'* values were plotted against the acetonitrile concentration. In most cases straight lines were obtained with correlation coefficients higher than 0.99. Only for compounds **16a** and **16b** a parabolic relationship was found as can be seen in Fig. 1 for the data obtained on the Spherisorb ODS column.

As can be seen from Table I, compound **16** has basic nitrogens which can interact with the free silanol groups of the Spherisorb ODS stationary phase, and that causes the parabolic relationship between the log *k'* values and the acetonitrile concentrations. Fig. 2 shows the straight lines obtained for compounds **16a** and **16b** on the Supelcosil LC-ABZ column which does not have free silanol groups. The letters **a** and **b** denote the separated diastereomers. Three diastereomer pairs could not be separated on the two columns at all, compounds **11**, **18** and **19**; no satisfactory explanations can be given. Only their hydrophobicities do not differ sufficiently to be able to differentiate between them under the studied chromatographic conditions. Compound **1** is an enantiomeric mixture; therefore no separation can be expected in non-chiral chromatographic conditions.

In general, higher resolution of the diastereomers could be observed on Supelcosil LC-ABZ

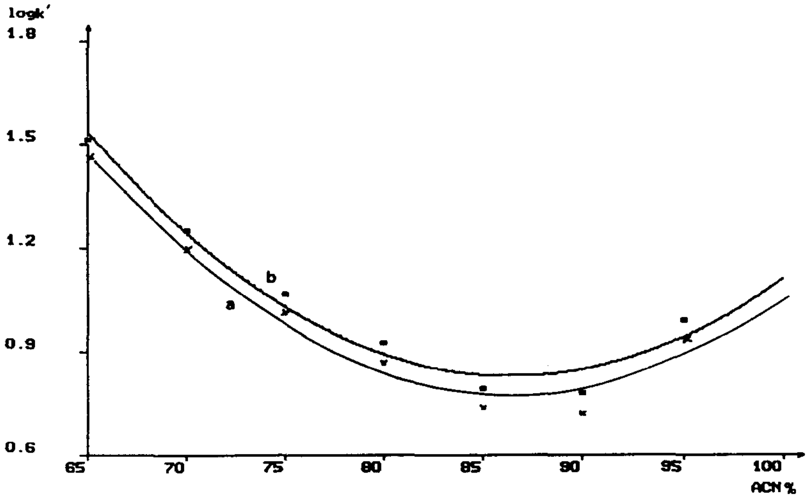


Fig. 1. The plot of log *k'* *vs.* acetonitrile concentration in the mobile phase for compounds **16a** and **16b** obtained on a Spherisorb ODS column.

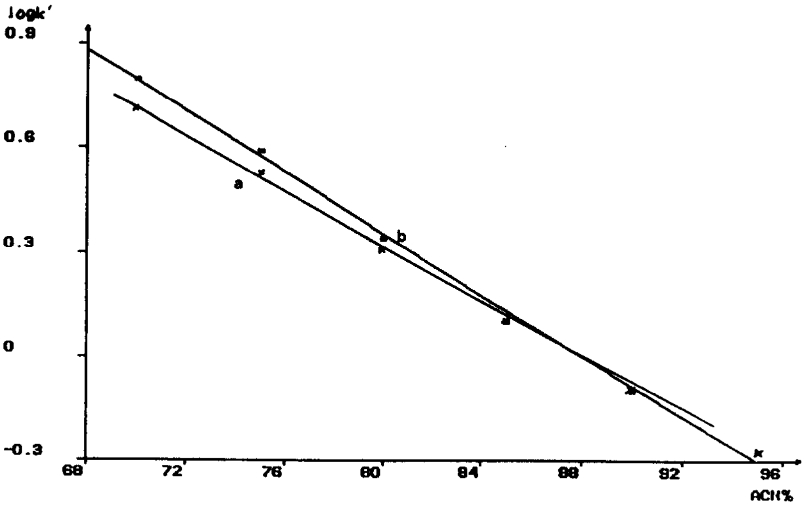


Fig. 2. The plot of log k' *vs.* acetonitrile concentration in the mobile phase for compounds **16a** and **16b** obtained on a Supelcosil LC-ABZ column.

column as can be seen in Figs. 3–5. For compounds **12** and **13** better separation was obtained on the Spherisorb ODS column. These two compounds were the only aromatic ether type in the series (see Figs. 6 and 7).

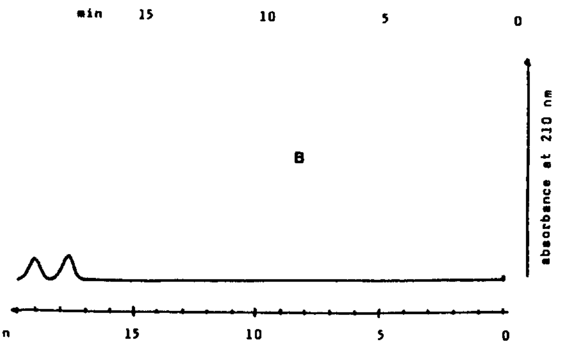


Fig. 3. Separation of the diastereomeric mixture of compound **2** by using acetonitrile–TFA–water (70:0.1:30, v/v/v) as the mobile phase. Flow-rate: 1.00 ml/min. Detection at 210 nm. (A) Spherisorb ODS 5 μm, 50 × 4.6 mm column; (B) Supelcosil LC-ABZ 150 × 4.6 mm column.

Tables II and III present the calculated slope (S) and intercepts (log k'_0) values of the straight lines obtained by plotting log k' values against the acetonitrile concentration (ϕ) for each compound on the Spherisorb ODS and Supelcosil LC-ABZ columns, respectively. When parabolic relationships were observed only the linear portion of the relationship was considered in the calculation of the values. Table III also shows the calculated hydrophobicity values (log P) and the dissociation constants (pK_a). In order to reveal the structure–retention relationships, correlation analysis was carried out on the data shown in Tables II and III. The correlation coefficients summarized in a correlation matrix can be seen in Table IV. On both columns the slope (S) and intercept (log k'_0) values showed high correlation coefficients (−0.95 and −0.98) which means that the set of compounds behaved as structurally related [3]. As expected the dissociation constants (pK_b) alone did not show good correlations to the chromatographic parameters on the reversed-phase columns where hydrophobicity governs the retention. The log P values showed better correlation to the chromatographic parameters obtained on the Spherisorb ODS column when the data obtained at higher organic concentrations, where the silanol effect could be observed, were not included in the calculations. This suggests that the

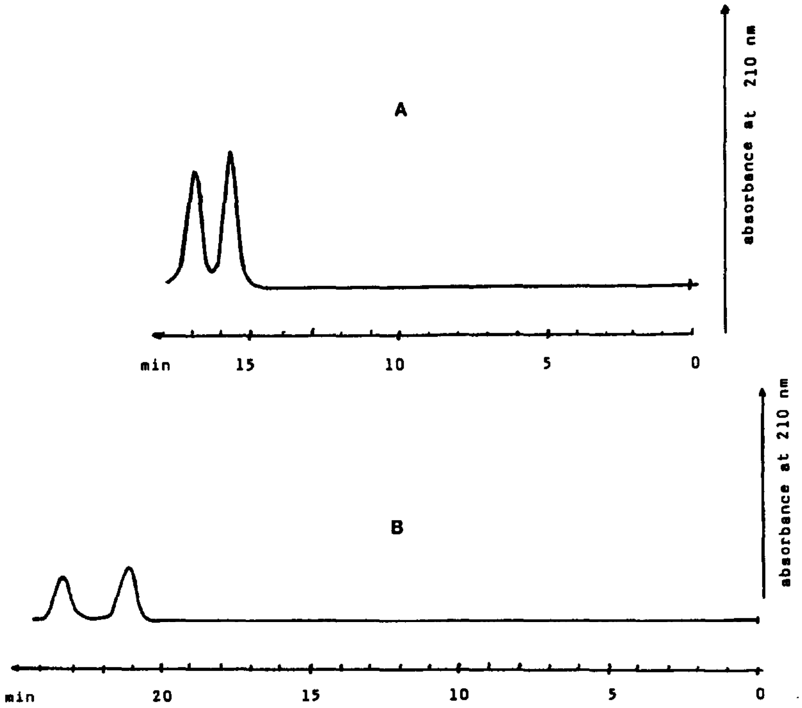


Fig. 4. Separation of the diastereomeric mixture of compound 4. Conditions and columns as in Fig. 3.

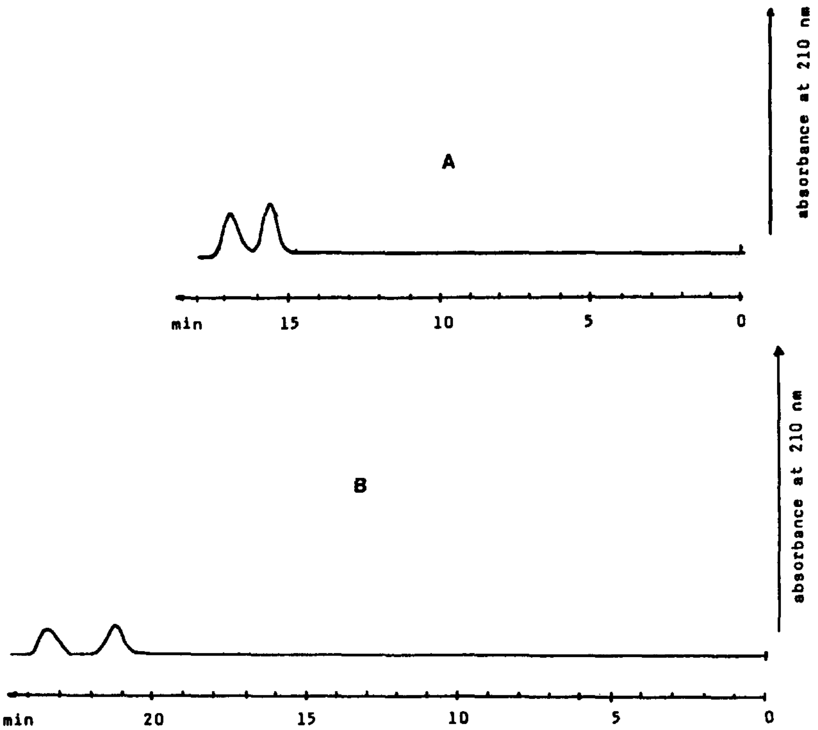


Fig. 5. Separation of the diastereomeric mixture of compound 5. Conditions and columns as in Fig. 3.

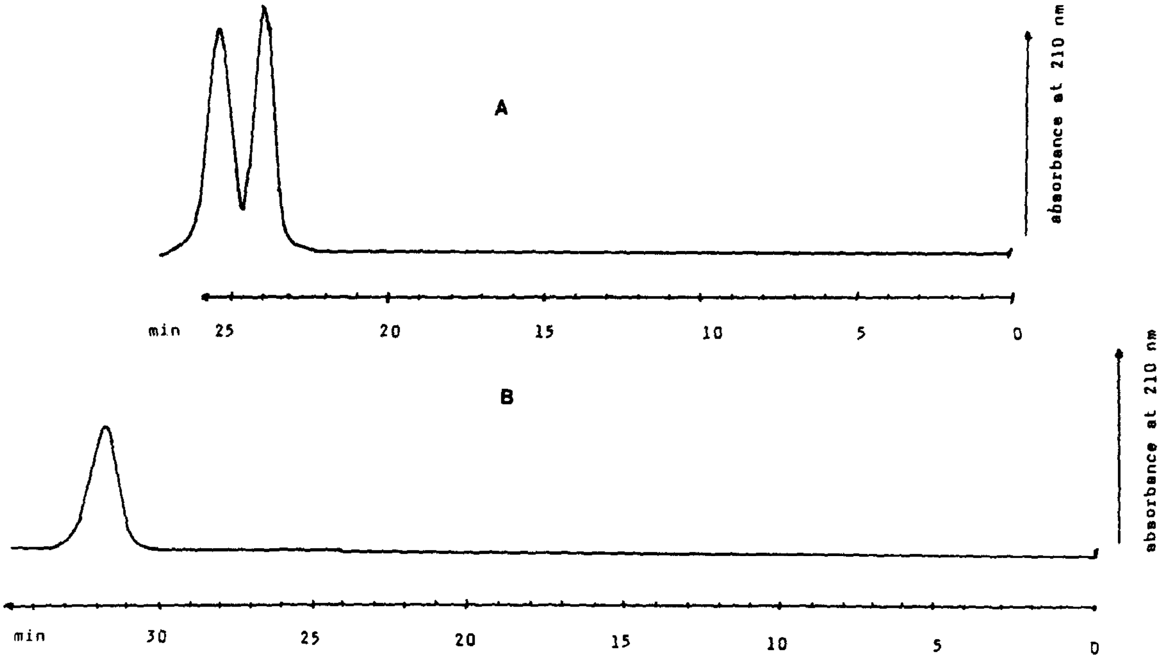


Fig. 6. Separation of the diastereomeric mixture of compound 12. Conditions and columns as in Fig. 3.

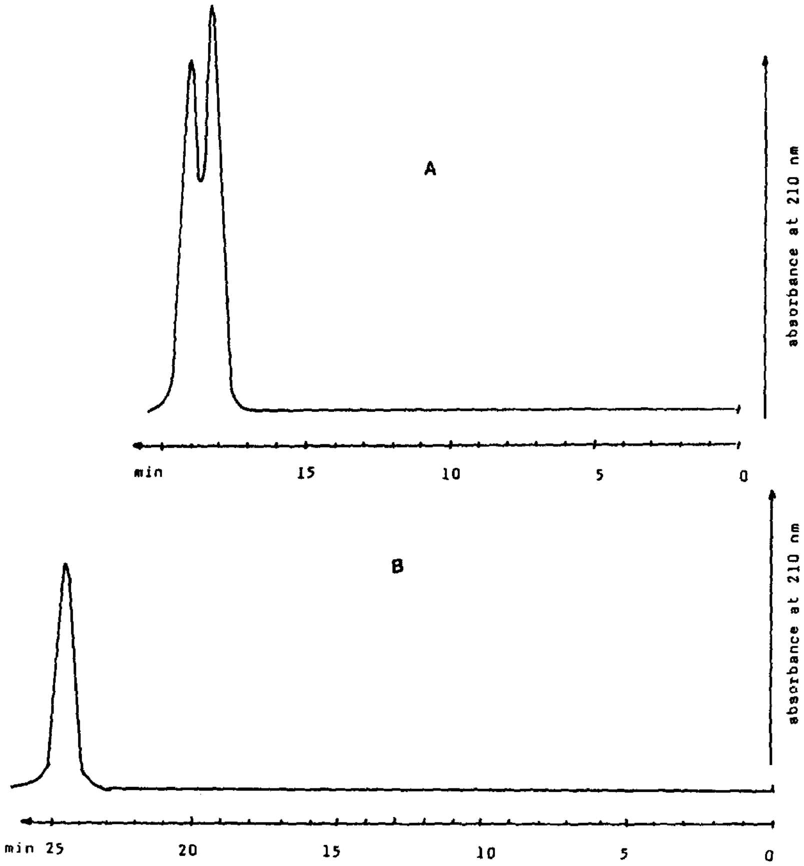


Fig. 7. Separation of the diastereomeric mixture of compound 13. Conditions and columns as in Fig. 3.

TABLE II

SLOPE (S), INTERCEPT (LOG k'_0) AND CORRELATION COEFFICIENT (R) VALUES OF THE COMPOUNDS OBTAINED ON SPHERISORB ODS (Sph) COLUMN ACCORDING TO THE EQUATION LOG $k' = \text{LOG } k'_0 - S\phi$ (WHERE ϕ IS THE VOLUME FRACTION OF ACETONITRILE)

Compound	S_{Sph}	log $k'_{0\text{Sph}}$	R
1	36.3	3.366	0.999
2a	40.6	3.828	0.999
2b	40.3	3.830	0.999
3a	35.5	3.357	0.998
3b	35.5	3.357	0.998
4a	40.4	3.853	0.998
4b	41.8	4.011	0.998
5a	42.9	4.056	0.999
5b	42.4	4.059	0.999
6a	31.7	3.254	0.998
6b	31.7	3.254	0.998
7a	43.0	4.048	0.995
7b	43.2	4.089	0.998
10a	34.8	3.154	0.996
10b	32.9	3.062	0.994
11	41.1	3.829	0.998
12a	46.5	4.497	0.999
12b	47.0	5.565	0.999
13a	42.6	4.065	0.996
13b	42.8	4.134	0.997
14a	43.5	4.075	0.997
14b	44.4	4.158	0.996
15a	38.9	3.455	0.996
15b	40.5	3.610	0.998
16a	30.4	3.308	0.999[a]
16b	30.5	3.377	0.999[a]
17a	43.5	4.080	0.990
17b	44.4	4.158	0.993
18	43.1	4.051	0.993
19	28.5	2.570	0.998

[a] In these cases parabolic relationships were found by plotting the log k' values as a function of the organic phase concentration. For the calculation of the S, log k'_0 and R values, only the linear section of the curve was considered.

TABLE III

SLOPE (S), INTERCEPT (LOG k'_0) AND CORRELATION COEFFICIENT VALUES OF THE COMPOUNDS OBTAINED ON SUPELCOSIL LC-ABZ (Sup) COLUMN ACCORDING TO THE EQUATION LOG $k' = \text{LOG } k'_0 - S\phi$ AND THE CALCULATED HYDROPHOBICITY (LOG P) AND DISSOCIATION CONSTANTS (pK_b) OF THE COMPOUNDS INVESTIGATED

Compound	S_{Sup}	log $k'_{0\text{Sup}}$	R	log P	pK_b
1	40.4	3.459	0.999	7.25	3.3
2a	43.9	3.918	0.998	8.80	3.2
2b	45.0	4.042	0.995	8.80	3.2
3a	42.0	3.643	0.998	7.76	3.3
3b	42.9	3.723	0.998	7.76	3.3
4a	45.5	4.124	0.999	9.32	3.3
4b	44.8	4.117	0.999	9.32	3.3
5a	45.6	4.133	0.998	9.32	3.3
5b	43.6	4.032	0.999	9.32	3.3
6a	38.4	3.433	0.999	6.52	2.2
6b	38.8	3.484	0.999	6.52	2.2
7a	47.0	4.234	0.999	8.90	3.7
7b	47.4	4.288	0.998	8.90	3.7
10a	39.6	3.275	0.999	7.29	3.6
10b	38.5	3.252	0.999	7.29	3.6
11	44.4	3.946	0.999	8.29	3.4
12a	47.9	4.483	0.999	9.46	3.6
12b	47.9	4.483	0.999	9.46	3.6
13a	51.1	4.597	0.990	8.94	3.6
13b	51.1	4.597	0.990	8.94	3.6
14a	48.5	4.281	0.998	10.75	3.3
14b	49.2	4.343	0.998	10.75	3.3
15a	43.9	3.716	0.996	9.76	8.1
15b	45.4	3.856	0.997	9.76	8.1
16a	40.0	3.511	0.999	7.27	3.3
16b	43.8	3.855	0.997	7.27	3.3
17a	49.4	4.466	0.998	9.23	3.8
17b	49.4	4.466	0.998	9.23	3.8
18	51.7	4.607	0.998	9.17	3.5
19	33.1	2.662	0.998	6.19	3.6

hydrophobicity governed the retention. On the contrary in the retention data obtained on the Supelcosil LC-ABZ column other types of interactions are possibly involved as well, as the stationary phase contains charged moieties among the long hydrophobic alkyl chains.

When step-wise regression analyses were carried out on the data it was found that the calculated hydrophobicity values showed good correlations to the S and the pK_b values as described by eqns. 1 and 2 for the data obtained on the Spherisorb ODS and the Supelcosil LC-ABZ columns, respectively.

$$\log P = 1.936(\pm 0.186)S_{\text{Sph}} + 0.23(\pm 0.08)\text{p}K_b + 0.12$$

$$n = 30 \quad r = 0.910 \quad s = 0.517 \quad F = 64.7 \qquad (1)$$

$$\log P = 2.086(\pm 0.279)S_{\text{Sup}} + 0.26(\pm 0.10)\text{p}K_b - 1.86$$

$$n = 30 \quad r = 0.848 \quad s = 0.660 \quad F = 34.5 \qquad (2)$$

TABLE IV

CORRELATION COEFFICIENTS BETWEEN THE CHROMATOGRAPHIC AND PHYSICO-CHEMICAL PARAMETERS

	S_{Sph}	$\log k'_{\mathrm{Sph}}$	S_{Sup}	$\log k'_{\mathrm{Sup}}$	$\log P$	$\mathrm{p}K_b$
S_{Sph}	1.00	−0.95	0.86	−0.88	−0.88	−0.15
$\log k'_{\mathrm{Sph}}$	−0.95	1.00	−0.88	0.94	0.81	−0.02
S_{Sup}	0.86	−0.88	1.00	−0.98	−0.80	−0.13
$\log k'_{\mathrm{Sup}}$	−0.88	0.94	−0.98	1.00	0.78	0.01
$\log P$	−0.88	0.81	−0.80	0.78	1.00	0.37
$\mathrm{p}K_b$	−0.15	−0.02	−0.13	0.01	0.37	1.00

where n is the number of compounds, r is the multiple regression coefficient, s is the standard error of the estimate, F is the Fisher-test value (significant at 0.01% level). Usually the $\log k'_0$ values are expected to show good correlation with the partition coefficients of compounds. Similar, significant correlations can be found in our case as well (see eqns. 3 and 4), but the mathematical statistical parameters are slightly worse than those of eqns. 1 and 2.

$$\log P = 2.124(\pm 0.22) \log k'_{0\mathrm{Sph}} + 0.368(\pm 0.082)\mathrm{p}K_b - 0.738$$
$$n = 30 \quad r = 0.896 \quad s = 0.552 \qquad (3)$$

$$\log P = 1.940(\pm 0.249) \log k'_{0\mathrm{Sup}} + 0.346(\pm 0.095)\mathrm{p}K_b - 0.386$$
$$n = 30 \quad r = 0.857 \quad s = 0.642 \qquad (4)$$

As the S and the $\log k'_0$ values show high correlations with each other, they are interchangeable. As can be seen, slightly better correlation was obtained on the Spherisorb ODS column (again data, where the silanol effect could be observed were not included in the calculations), which proves that on the Supelcosil LC-ABZ other than only hydrophobic interactions took place. The plots of the log P *vs.* the

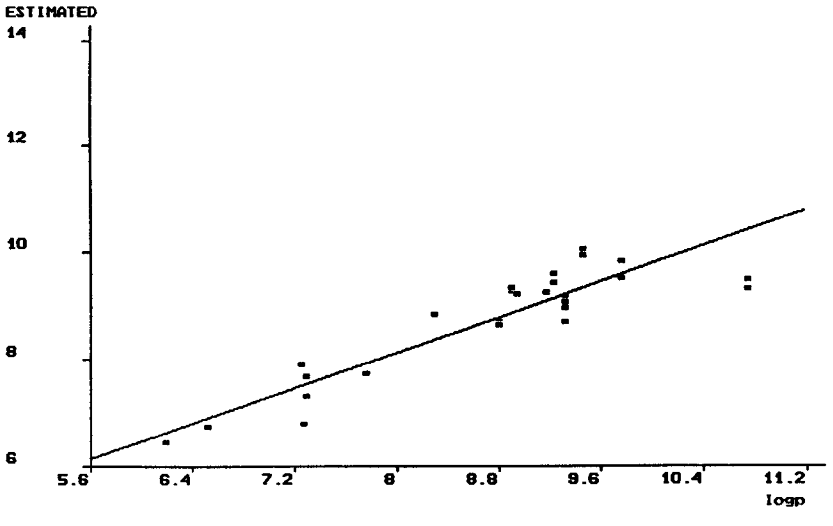


Fig. 8. Plot of the log P values *vs.* the estimated values obtained on the Spherisorb ODS column by eqn. 1.

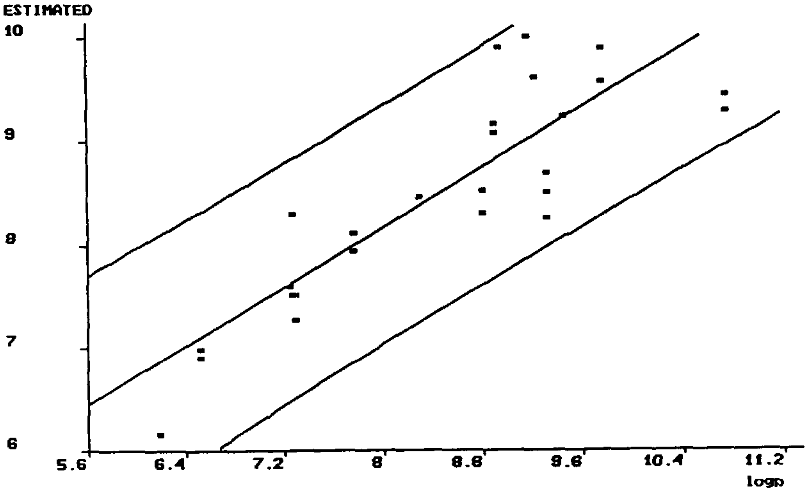


Fig. 9. Plot of the log *P* values *vs.* the estimated values obtained on the Supelcosil LC-ABZ column by eqn. 2.

estimated log *P* values by eqns. 1 and 2 are presented in Figs. 8 and 9, respectively.

In conclusion, most of the diastereomeric lipidic amino acid mixtures could be separated under reversed-phase conditions. The retention of the compounds was governed by hydrophobicity on the Spherisorb ODS column and the effect of the free silanol groups could be observed for basic derivatives. The Supelcosil LC-ABZ column did not show a silanophilic effect, in most cases better separation of the diastereomers could be observed and other than hydrophobic interactions governed the retention as well.

ACKNOWLEDGEMENT

The contribution of K.V. to the work was supported by a Maplethorpe Fellowship from the University of London, which is gratefully acknowledged.

REFERENCES

1 W.A. Gibbons, R. Hughes, M. Charalambous, M. Christodoulu, A. Szeto, A.E. Aulabaugh, P. Mascagni and I. Tóth, *Liebigs Ann. Chem.*, 1990 (1990) 1175.
2 K. Valkó, I. Toth, P. Ward, P. Slegel and W.A. Gibbons, *Int. J. Pharmaceutics*, 87 (1992) 123.
3 K. Valkó, *J. Liq. Chromatogr.*, 10 (1987) 1663.