Journal of Chromatography, 241 (1982) 153–167
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,445

NORMAL-PHASE DYNAMIC (SOLVENT-GENERATED) MOLECULAR COMPLEXATION CHROMATOGRAPHY USING ANIONIC ION EXCHANGERS

I. CHARACTERIZATION OF THE SEPARATION SYSTEM

G. SZEPESI*, M. GAZDAG and R. IVÁNCSICS
Chemical Works of Gedeon Richter Ltd., P.O. Box 27, H-1475 Budapest (Hungary)

SUMMARY

A new type of dynamic ion-exchange system has been developed. In this system, polar bonded stationary phases such as cyanopropyl-silica and less polar eluent mixtures such as hexane–chloroform–acetonitrile or hexane–isopropanol containing a small amount of anionic ion exchangers (di-(2-ethylhexyl)phosphoric acid or *d*-camphor-10-sulphonic acid) were used. Some ergot peptide and eburnane alkaloids were selected as models for the investigation of the distribution mechanism. On the basis of the results obtained, we suggest that molecular complexation between the uncharged alkaloid base and the ion exchanger is responsible for the increase in retention, forming a more polar complex in the mobile phase than the free uncharged alkaloid.

The influence of the concentration of ion exchanger and of diethylamine, as well as of eluent composition, on the retention behaviour of the compounds was investigated.

INTRODUCTION

Dynamic (solvent-generated) ion-exchange chromatography is a term which was introduced by Kraak *et al.*[1], and first used by Wittmer *et al.*[2]. It is otherwise known as paired-ion chromatography[3], soap chromatography[4,5] and reversed-phase ion-pair chromatography[6]. In this type of chromatography, column packings with a hydrophobic surface and water–organic solvent mixtures containing a small amount of cationic or anionic ion exchangers have been used for the separation of ionizable organic substances[1,2,4–10]. Based on the models proposed for the retention mechanism in dynamic ion exchange by Kraak *et al.*[1] as well as by Horváth *et al.*[6], a new type of dynamic ion-exchange system has been developed which we call normal-phase dynamic (solvent-generated) molecular complexation chromatography. In this system, polar bonded stationary phases such as cyanopropyl-silica and less polar eluent mixtures containing a small amount of anionic ion exchanger were used. A similar concept was extensively used prior to the introduction of high-performance

0021-9673/82/0000–0000/$02.75

liquid chromatography (HPLC) in thin-layer and paper chromatographic separation of metal ions with inorganic complexants and hydroorganic mobile phases (*e.g.* ref. 16).

In the first part of our paper we introduce the separation system and propose a possible model for the chromatographic retention mechanism. In the second part the application of the proposed method will be introduced for solving difficult analytical problems, including the separation of optical isomers[11].

EXPERIMENTAL

All the experiments were carried out on a Varian 8500 high-pressure liquid chromatograph (Varian Aerograph, Walnut Creek, CA, U.S.A.) consisting of a Rheodyne 7010 loop injector (Rheodyne, Berkeley, CA, U.S.A.), Variscan Model 635 variable-wavelength UV-spectrophotometer and Varian A-25 recorder (Varian Aerograph). For the breakthrough curves a differential refractometer (R 401, Waters Assoc., Milford, MA, U.S.A.) was used. The separations were performed on μBondapak CN column, 300 × 3.9 mm I.D. (Waters Assoc.).

All solvents used for the eluent preparation were of HPLC grade and were obtained from E. Merck (Darmstadt, G.F.R.). Di-(2-ethylhexyl)phosphoric acid (DHP) was from BDH (Poole), (+)-camphor-10-sulphonic acid was obtained from Reanal (Budapest, Hungary) and was purified by recrystallization.

The compounds investigated were produced at the Chemical Works of Gedeon Richter Ltd. and the Institute of Organic Chemistry of Budapest Technical University, and were considered to be of the highest available quality.

RESULTS AND DISCUSSION

To investigate the mechanism of retention of organic amines in the presence of anionic ion exchangers, chemically bonded cyanopropyl-silica as polar bonded stationary phase and mixtures of hexane, chloroform and acetonitrile containing small amount of DHP as the eluent were chosen. Some of ergot peptide and eburnane alkaloids previously investigated by HPLC both in reversed-phase and normal-phase systems by the authors[12–15] were selected as models for the investigation.

The eluents were shown to be less polar than the stationary phase by the elution of prednisolone and hydrocortisone.

The compounds investigated are listed in Table I, and the capacity ratios measured for the compounds in the presence and absence of DHP are given in Table II.

On the basis of the retention data in Table II, from the possible variations of interactions between the organic base and ion exchanger, the ion-pair mechanism can be excluded, because the retention of the alkaloids investigated is considerably increased when ion exchanger is added to the eluent, whereas owing to the increased non-polar nature of the ion pair formed a decreased retention should have been observed. This enhanced retention can be explained in terms of a molecular complexation mechanism, which can be expressed with the following equations:

$$(A)_m \overset{K_1}{\rightleftharpoons} (A)_s \quad K_1 = \frac{(A)_s}{(A)_m} \tag{1}$$

TABLE I

LIST OF COMPOUNDS INVESTIGATED

Eburnane alkaloids			
I	(+)-*cis*-epivincamine	IX	(+)-*cis*-vincamenine
II	(−)-*cis*-epivincamine	X	(+)-*cis*-apovincamine
III	(+)-*cis*-vincamine	XI	(+)-*cis*-vincamone
IV	(−)-*cis*-vincamine	XII	(−)-*cis*-vincamone
V	(+)-*cis*-apovincaminic acid ethyl ester	XIII	(+)-*cis*-vincanol
		XIV	(−)-*cis*-vincanol
VI	(−)-*cis*-apovincaminic acid ethyl ester	XV	(+)-*cis*-vincaminic acid ethyl ester
VII	(+)-*trans*-apovincaminic acid ethyl ester	XVI	(−)-*cis*-vincaminic acid ethyl ester
VIII	(−)-*trans*-apovincaminic acid ethyl ester	XVII	(+)-*cis*-isovincanol
Native and hydrogenated ergot peptide alkaloids			
XVIII	β-ergocriptinine	XXVII	ergotaminine
XIX	α-ergocriptinine	XXVIII	ergometrine
XX	ergocorninine	XXIX	dihydro-β-ergocriptine
XXI	ergocristinine		
XXII	β-ergocriptine	XXX	dihydro-α-ergocriptine
XXIII	α-ergocriptine		
XXIV	ergocornine	XXXI	dihydroergocornine
XXV	ergocristine	XXXII	dihydroergocristine
XXVI	ergotamine	XXXIII	dihydroergotamine
Reference compounds			
XXXIV	hydrocortisone		
XXXV	prednisolone		

$$(A)_m + (DHP)_m \overset{K_2}{\rightleftharpoons} (ADHP)_m \quad K_2 = \frac{(ADHP)_m}{(A)_m\,(DHP)_m} \tag{2}$$

$$(DHP)_m \overset{K_3}{\rightleftharpoons} (DHP)_s \quad K_3 = \frac{(DHP)_s}{(DHP)_m} \tag{3}$$

$$(ADHP)_m \overset{K_4}{\rightleftharpoons} (ADHP)_s \quad K_4 = \frac{(ADHP)_s}{(ADHP)_m} \tag{4}$$

$$(A)_m + (DHP)_s \overset{K_5}{\rightleftharpoons} (ADHP)_s \quad K_5 = \frac{(ADHP)_s}{(A)_m\,(DHP)_s} \tag{5}$$

$$(A)_s + (DHP)_m \overset{K_6}{\rightleftharpoons} (ADHP)_s \quad K_6 = \frac{(ADHP)_s}{(A)_s\,(DHP)_m} \tag{6}$$

where K_1 and K_3 are the partition coefficients of free alkaloid base and DHP between the stationary and mobile phase, respectively; K_2 is the formation constant of the

TABLE II

DEPENDENCE OF CAPACITY RATIOS MEASURED FOR THE COMPOUNDS INVESTIGATED ON DHP CONCENTRATION

Conditions: μBondapak CN column (300 × 3.9 mm I.D.); eluent flow-rate, 1 cm^3/min; detection at 280 nm. See Table I for the numbered compounds.

No.	*Compound*	*Eluent composition (%)*							
		Hexane	65	65	65	65	65	60	70
		Chloroform	20	20	20	20	20	23	17
		Acetonitrile	15	15	15	15	15	17	13
		DHP (mol/dm^3)	–	0.0005	0.001	0.005	0.01	0.025	0.005	0.005
XXXIV	Hydrocortisone		1.89	1.89	1.97	1.92	1.93	1.94	1.48	3.00
XXXV	Prednisolne		2.52	2.50	2.52	2.58	2.55	2.59	1.89	3.90
I–II	(+)-*cis*-Epivincamine		2.93	2.36	4.07	2.36	2.31	3.44	1.96	3.00
III–IV	(+)-*cis*-Vincamine		1.93	1.75	5.34	6.57	7.46	9.04	5.78	10.1
V–VI	(+)-*cis*-Apovincaminic acid ethyl ester		0.45	0.43	2.24	3.36	4.25	4.78	2.78	4.26
IX	(+)-*cis*-Vincamenine		0.93	0.86	3.00	3.64	3.92	4.78	3.30	4.56
X	(+)-*cis*-Apovincamine		0.55	0.54	2.72	4.14	4.42	5.30	3.56	5.44
XI–XII	(+)-*cis*-Vincamone		0.28	0.32	1.62	3.50	4.12	4.63	3.19	4.70
XIII	(+)-*cis*-Vincanol		3.34	2.75	5.83	4.21	3.58	6.33	3.67	5.07
XIV	(−)-*cis*-Vincanol		3.34	2.75	5.83	4.21	3.58	6.33	3.67	5.07
XV–XVI	(+)-*cis*-Vincaminic acid ethyl ester		1.52	1.54	4.14	6.50	5.43	6.93	4.78	7.19
XVII	(+)-*cis*-Isovincanol		5.72	4.18	8.69	10.0	9.08	10.4	7.52	12.7
XVIII	β-Ergocriptinine		0.90	0.89	1.17	4.71	10.4	3.70	4.15	9.67
XIX	α-Ergocriptinine		0.90	0.89	1.17	4.71	10.4	3.70	4.15	9.67
XX	Ergocorninine		0.90	0.89	1.41	6.46	12.9	4.63	4.48	12.7
XXI	Ergocristinine		1.07	1.07	1.41	7.21	14.3	5.22	5.52	14.6
XXII	β-Ergocriptine		1.07	1.18	1.69	1.96	2.38	2.41	1.48	3.37
XXIII	α-Ergocriptine		1.07	1.18	1.69	1.96	2.38	2.41	1.48	3.37
XXIV	Ergocornine		1.28	1.36	1.93	2.21	2.77	2.78	1.67	3.89
XXV	Ergocristine		1.41	1.50	2.10	2.21	2.77	2.78	1.67	4.15
XXVI	Ergotamine		2.72	2.36	3.62	2.93	3.54	4.11	2.37	5.35
XXVII	Ergotaminine		1.48	1.39	5.55	15.9	25.9	16.8	12.0	28.3
XXVIII	Ergometrine		12.0	12.0	15.2					
XXIX	Dihydro-β-ergocriptine		2.79	1.75	1.59	1.41	1.69	2.00	1.15	2.70
XXX	Dihydro-α-ergocriptine		2.79	1.75	1.59	1.41	1.69	2.00	1.15	2.70
XXXI	Dihydroergocornine		3.28	2.00	1.85	1.71	2.00	2.26	1.30	3.10
XXXII	Dihydroergocristine		3.55	2.21	1.97	1.71	2.00	2.26	1.30	3.10
XXXIII	Dihydroergotamine		4.17	3.71	3.28	2.64	3.00	3.37	1.96	4.63

molecular complex in the mobile phase; K_4 is the partition coefficient of the molecular complex; K_5 and K_6 are the formation constants of the molecular complex in the stationary phase when DHP or alkaloid base are strongly adsorbed; A is the uncharged alkaloid base; the subscripts "m" and "s" refer to the mobile and stationary phase, respectively.

We assume that a molecular complexation between the uncharged alkaloid base and the ion exchanger is responsible for the increase in retention that occurs in both the mobile and the stationary phase, and the complexes formed are more polar than the free uncharged alkaloid base.

To elucidate further the retention mechanism, the possible adsorption of DHP on the stationary phase by means of breakthrough curves, as well as the effect of the presence and absence of DHP in the eluent on the capacity ratios, were also studied by using a DHP-loaded column.

Table III shows the retention behaviour of some eburnane alkaloids in the presence and absence of DHP in the eluent when the stationary phase is loaded and unloaded with DHP.

TABLE III

EFFECT OF THE PRESENCE OF DHP IN THE ELUENT ON THE CAPACITY RATIO

No.	*Compound*	*Capacity ratios**		
		A	*B*	*C*
V	Apovincaminic acid ethyl ester	0.14	2.43	0.18
XXVII	Ergotaminine	1.19	9.76	1.16
XXXIV	Hydrocortisone	1.38	1.38	1.38
XXXV	Prednisolone	1.66	1.68	1.69

* A, unloaded column; eluent, hexane–chloroform–acetonitrile (65:20:15); flow-rate, 100 cm^3/h. B, loaded column; eluent, hexane–chloroform–acetonitrile (65:20:15) + 0.005 mol/dm^3 DHP; flow-rate, 100 cm^3/h. C, loaded column; eluent, hexane–chloroform–acetonitrile (65:20:15); flow-rate, 100 cm^3/h.

On the basis of the results obtained it seems reasonable to assume that, under the experimental conditions, liquid–liquid partition of the molecular complex formed in the mobile phase should be an acceptable model for the alkaloid investigated, because a significant decrease of the measured capacity ratios (k') can be observed when the DHP-loaded column and an eluent mixture without DHP were used.

The dependence of k' on the DHP concentration when the complex is formed in the mobile phase can be expressed by the following formula[6]:

$$k' = \frac{k'_0 + K_2K_4\,(\mathrm{DHP})}{[1 + K_2\,(\mathrm{DHP})]\,[1 + K_3\,(\mathrm{DHP})]} \tag{7}$$

where k'_0 is the capacity ratio of the alkaloid base in the absence of DHP.

The influence of the DHP concentration in the eluent on the capacity ratios of ergot peptide and eburnane alkaloids is shown in Fig. 1. A paraboloic dependence of capacity ratios was obtained for eburnane alkaloids whereas a nearly linear relationship can be observed for native ergot peptide alkaloids.

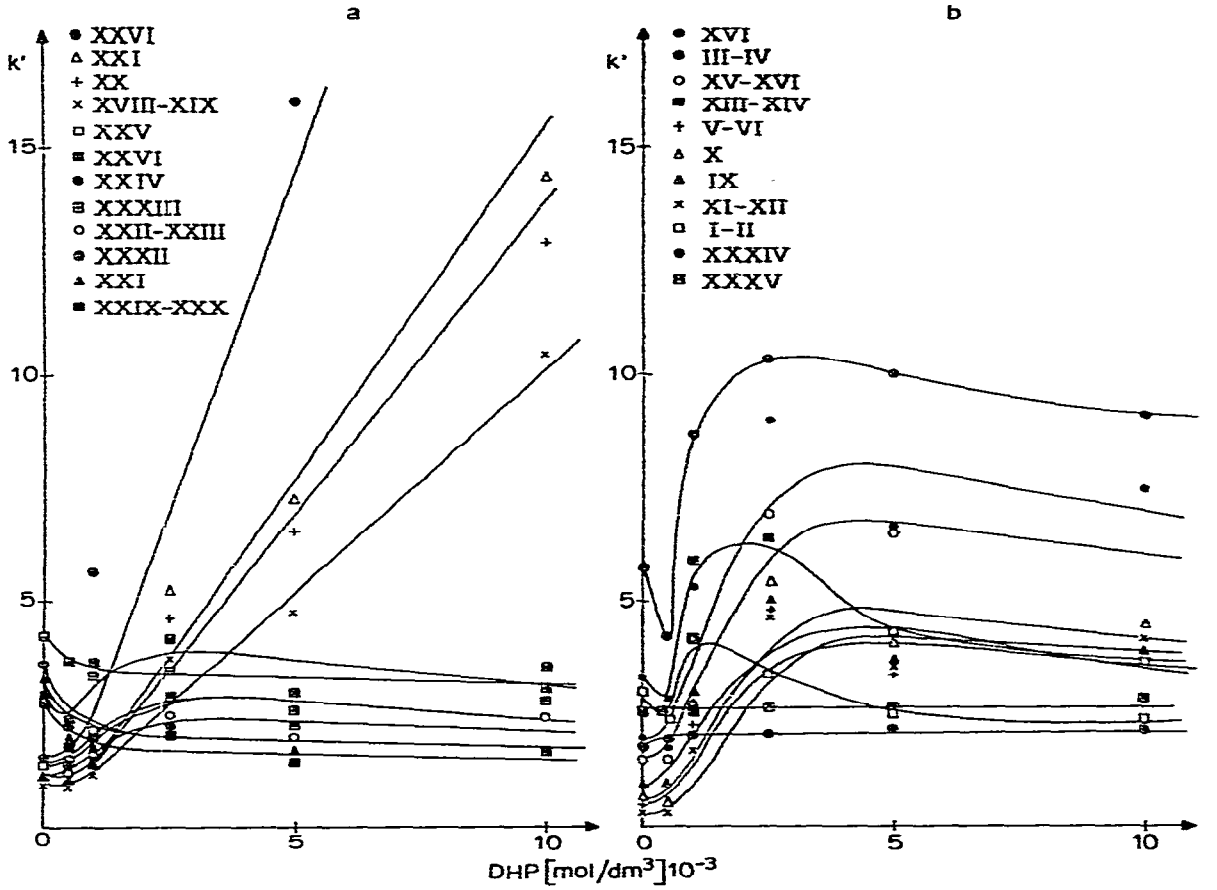

Fig. 1. The influence of DHP concentration in the eluent on the capacity ratios of (a) ergot peptide and (b) eburnane alkaloids. Conditions: μBondapack CN column (300 × 3.9 mm I.D.); eluents, hexane–chloroform–acetonitrile (65:20:15) containing DHP; flow-rates, 1 cm^3/min; detection at 280 nm (for compounds XXXIV and XXXV at 240 nm).

When camphor-10-sulphonic acid (CSA) was used as anionic ion exchanger in the eluent, because of the relatively bad solubility of the reagent in less polar eluent mixtures, diethylamine (DEA) was added to the mobile phase. Because in the presence of DEA, the possible variations of interactions are increased, the influence of the DEA concentration on the retention behaviour of the alkaloids investigated in the absence of ion exchanger in hexane–isopropanol eluent mixture was studied.

It is illustrated in Fig. 2, where the dependence of the capacity ratios on the DEA concentration is shown. In the presence of a small amount of DEA in the eluent, the capacity ratios of the alkaloids investigated are considerably diminished, whereas the retentions of prednisolone and hydrocortisone are unchanged. This change of retention behaviour of alkaloids could be explained by the suppression of ionization of the compounds, as well as by avoiding the adsorption mechanism in the retention of the compounds. A further increase in the DEA concentration led to only a small change in retention.

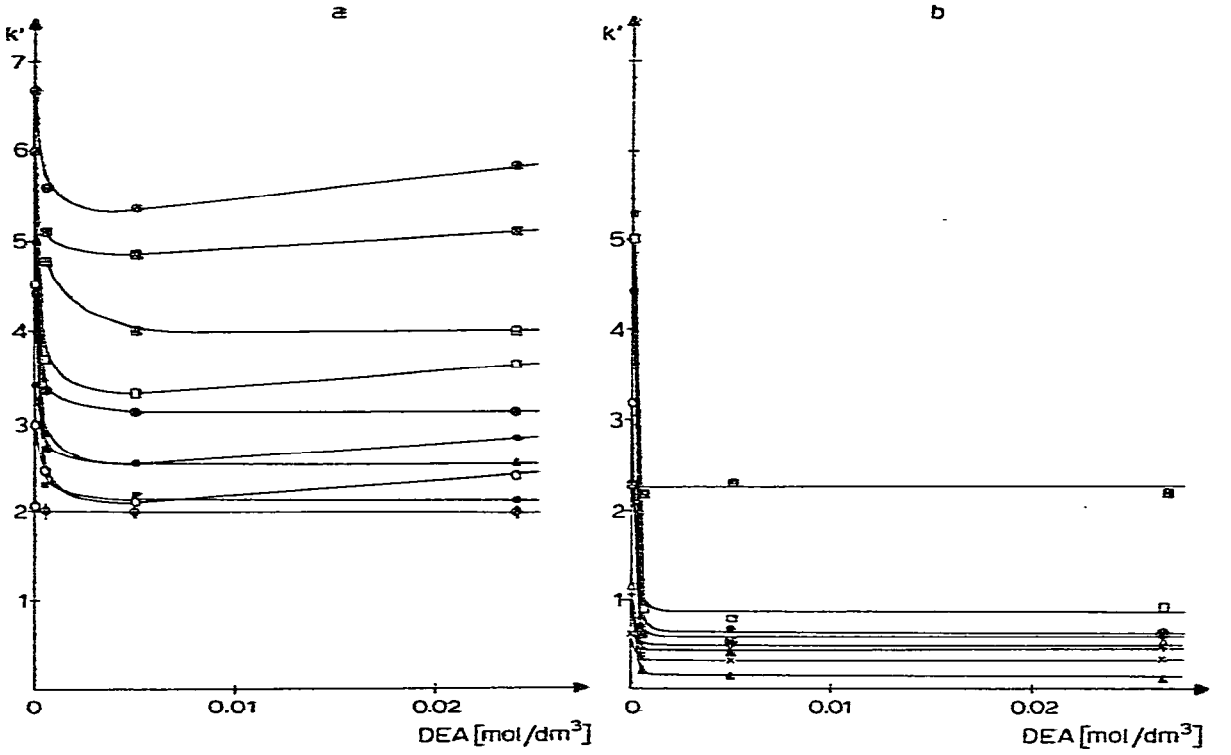

Fig. 2. Dependence of capacity ratios on the DEA concentration in the eluent: (a) native and hydrogenated ergot peptide alkaloids; (b) eburnane alkaloids. Other conditions as in Fig. 1.

The influence of varying the DHP concentration at constant DEA concentration in the eluent on the capacity ratios and selectivity factors of some native and hydrogenated ergot peptide alkaloids is shown in Table IV. When the ratio of DHP and DEA in the eluent is *ca.* 1:1, molecular complex formation between the alkaloid base and DHP can occur and at higher ratio it predominates.

The following equations illustrate the most important interactions involved in the chromatographic process with which eqns. 1–6 can be extended:

$$(DEA)_m \overset{K_7}{\rightleftharpoons} (DEA)_s \quad K_7 = \frac{(DEA)_s}{(DEA)_m} \tag{8}$$

$$(DEA)_m + (DHP)_m \overset{K_8}{\rightleftharpoons} (DEA\text{–}DHP)_m \quad K_8 = \frac{(DEA\text{–}DHP)_m}{(DEA)_m\,(DHP)_m} \tag{9}$$

$$(DEA)_s + (DHP)_m \overset{K_9}{\rightleftharpoons} (DEA\text{–}DHP)_s \quad K_9 = \frac{(DEA\text{–}DHP)_s}{(DEA)_s\,(DHP)_m} \tag{10}$$

$$(DEA\text{–}DHP)_m \overset{K_{10}}{\rightleftharpoons} (DEA\text{–}DHP)_s \; K_{10} = \frac{(DEA\text{–}DHP)_s}{(DEA\text{–}DHP)_m} \tag{11}$$

TABLE IV

INFLUENCE OF DHP CONCENTRATION IN THE PRESENCE OF DEA IN THE ELUENT ON THE CAPACITY RATIOS (k') AND SELECTIVITY FACTORS (r_{ij}) OBTAINED FOR NATIVE AND HYDROGENATED ERGOT PEPTIDE ALKALOIDS

Conditions as in Table II. See Table I for the numbered compounds. Eluent, hexane–isopropanol (80:20).

No.	*Compound* / *DEA (mol/dm³)* / *DHP (mol/dm³)*	$2 \cdot 10^{-3}$ / 10^{-3}		10^{-3} / 10^{-3}		$7.5 \cdot 10^{-4}$ / 10^{-3}		$7.5 \cdot 10^{-4}$ / $1.5 \cdot 10^{-3}$		$7.5 \cdot 10^{-4}$ / $2 \cdot 10^{-3}$		$7.5 \cdot 10^{-4}$ / $4 \cdot 10^{-3}$	
		k'	r_{ij}	k'	r_{ij}	k'	r_{ij}	k'	r_{ij}	k'	r_{ij}	k'	r_{ij}
XXXIV	Hydrocortisone	1.87		1.85		1.87		1.93		1.87		1.90	
XXXV	Prednisolone	2.10		2.10		2.01		2.10		2.12		2.13	
XVIII	β-Ergocriptinine	2.29	1.00	2.40	1.00	2.63	1.07	3.53	1.10	5.20	1.10	10.4	1.11
XIX	α-Ergocriptinine	2.29	1.28	2.40	1.25	2.81	1.10	3.90	1.15	5.70	1.08	11.5	1.09
XX	Ergocorninine	2.94	1.22	3.00	1.32	3.08	1.43	4.50	1.35	6.15	1.40	12.5	1.35
XXI	Ergocristinine	3.58		3.95		4.41		6.07		8.60		16.9	
XXII	β-Ergocriptine	2.06	1.00	2.74	1.00	3.47	1.10	4.53	1.13	4.40	1.13	4.27	1.14
XXIII	α-Ergocriptine	2.06	1.17	2.74	1.18	3.81	1.05	5.13	1.00	4.95	1.00	4.87	1.00
XXIV	Ergocornine	2.42	1.26	3.23	1.39	4.00	1.47	5.13	1.52	4.95	1.50	4.87	1.48
XXV	Ergocristine	3.06		4.48		5.88		7.80		7.51		7.20	
XXVI	Ergotamine	4.35	1.11	4.90	1.20	5.20	1.62	7.12	1.72	8.35	1.98	8.90	2.77
XXVII	Ergotaminine	4.81		5.94		8.40		12.3		16.6		24.5	
XXIX	Dihydro-β-ergocriptine	2.10	1.00	2.87	1.00	2.94	1.00	3.20	1.00	3.26	1.10	3.07	1.21
XXX	Dihydro-α-ergocriptine	2.10	1.18	2.87	1.11	2.94	1.10	3.20	1.15	3.58	1.08	3.70	1.10
XXXI	Dihydroergocornine	2.48	1.23	3.20	1.32	3.25	1.33	3.67	1.43	3.87	1.47	4.07	1.48
XXXII	Dihydroergocristine	3.06		4.23		4.31		5.23		5.68		6.03	
XXXIII	Dihydroergotamine	4.06		4.52		4.81		–		6.03		7.13	

$$(A')_m \overset{K_{11}}{\rightleftharpoons} (A')_s \quad K_{11} = \frac{(A')_s}{(A')_m} \tag{12}$$

$$(A')_m + (DHP)_m \overset{K_{12}}{\rightleftharpoons} (ADHP)_m \quad K_{12} = \frac{(ADHP)_m}{(A')_m\,(DHP)_m} \tag{13}$$

$$(A')_m + (DEA\text{–}DHP)_m \overset{K_{13}}{\rightleftharpoons} (ADHP)_m + (DEA)_m$$

$$K_{13} = \frac{(ADHP)_m\,(DEA)_m}{(A')_m\,(DEA\text{–}DHP)_m} \tag{14}$$

where K_7 and K_{10} are the partition coefficients of DEA and the DEA–DHP complex between the stationary and mobile phase; K_8 is the formation constant of the DEA–DHP complex in the mobile phase; K_9 is the formation constant of the DEA–DHP complex in the stationary phase when DEA is strongly adsorbed; K_{11} is the partition coefficient of alkaloid base between the stationary and mobile phase in the presence of DEA; K_{12} is the formation constant of the ADHP complex in the mobile phase containing DEA; K_{13} is the constant of complex exchange equilibrium; A′ is the uncharged alkaloid base formed in the presence of DEA; "m" and "s" refer to the mobile and stationary phase, respectively.

From the results shown in Table IV, it can be concluded that when DEA is excess in the eluent the interactions represented by eqns. 9, 11 and 12 are responsible for the decrease in retention. When the ratio of DEA to DHP is *ca.* 1:1, the complex exchange reaction should be involved in the chromatographic process, whereas if DHP is excess eqn. 13 represents the dominant mechanism.

Similar variations should exist when DHP is replaced by CSA in the eluent.

Fig. 3 shows the dependence of the capacity ratios measured for some eburnane alkaloids on the CSA concentration of the eluent containing 10^{-3} mol/dm^3 DEA, and the influence of DEA concentration on the retention of the same alkaloids using constant CSA concentration in the eluent can be seen in Fig. 4.

These figures show that CSA, in accordance with its stronger acidic character, can form molecular complexes with eburnane alkaloids in the presence of excess DEA. This can be explained by the complex exchange mechanism. The results obtained also indicate that separation proceeds according to liquid–liquid partition of molecular complexes formed in the mobile phase.

The capacity ratios of the compounds investigated in CSA–DEA system are collected in Table V. When CSA is used as ion exchanger for molecular complexation, it is possible to increase the selectivity of the separation of eburnane alkaloids. However, owing to the very strong interaction between ergot peptide alkaloids and CSA, this type of eluent system is unsuitable for the investigation of the latter alkaloids; it requires a significant change of eluent polarity.

Before demonstrating the applicability of the separation system, we should like to note that the main aim of the work presented in this part of our paper is to characterize the separation system using model mixtures for the investigations. The results obtained indicate that a significant improvement in selectivity can be achieved

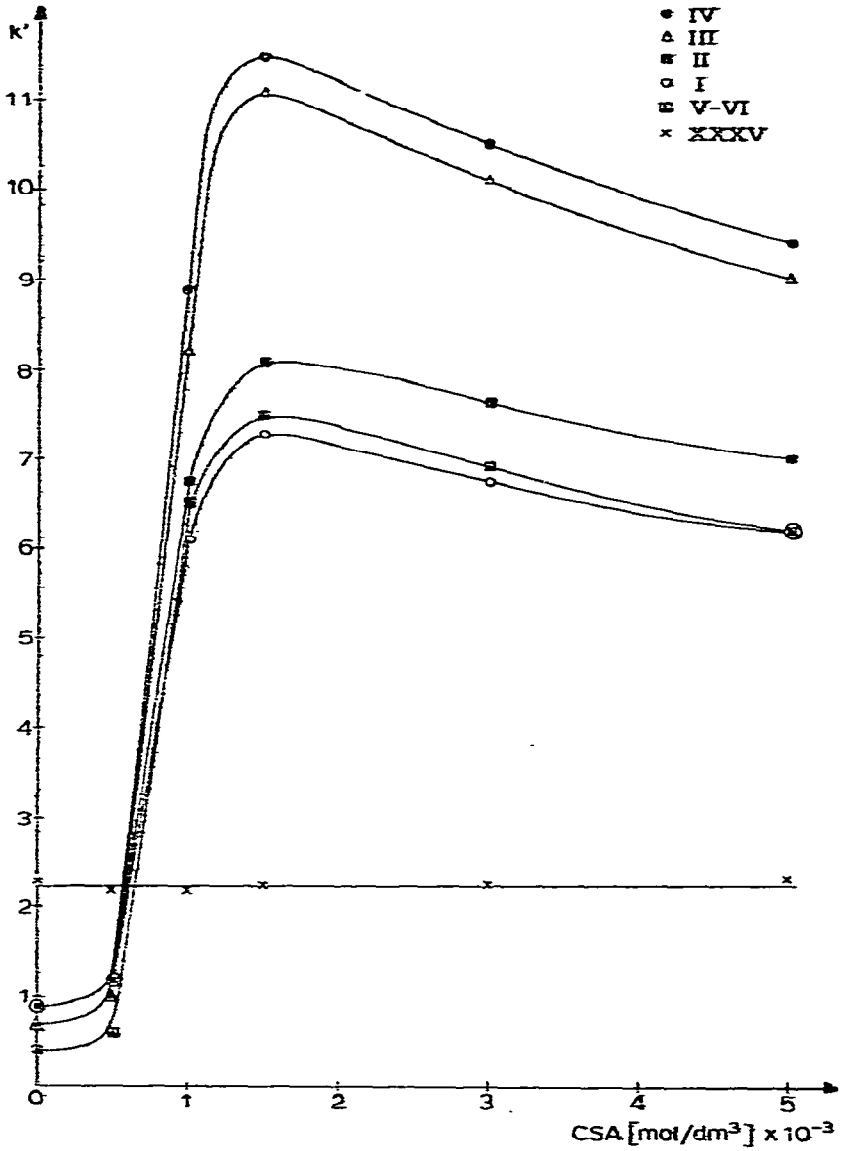

Fig. 3. Influence of CSA concentration on the capacity ratios of eburnane alkaloids using constant DEA concentration in the eluent. Conditions: eluent, hexane–isopropanol (80:20) containing $1 \cdot 10^{-3}$ mol/dm^3 DEA. Other conditions as in Fig. 1.

by molecular complexation, and especially it seems to be very promising for the separation of optical isomers when a chiral ion exchanger is applied. For this reason, the examples shown in this paper served as tests for the optimization of the separation system for solving special analytical problems. It will be discussed in detail in the second part of our paper[11].

The separation of a model mixture of eburnane alkaloids using DHP as ion exchanger is illustrated in Fig. 5.

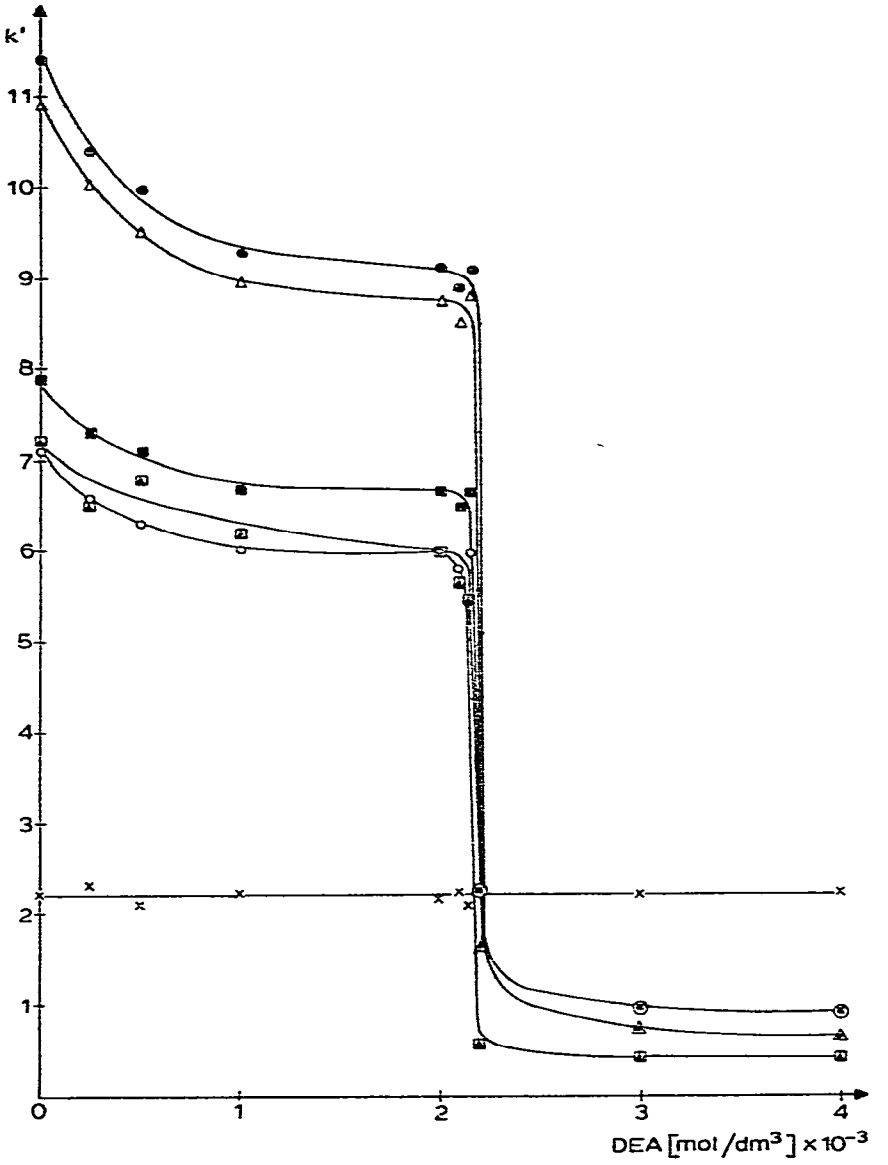

Fig. 4. Influence of DEA concentration on the capacity ratios of eburnane alkaloids using constant CSA concentration in the eluent. Eluent: hexane–isopropanol (80:20) containing $2 \cdot 10^{-3}$ mol/dm^3 CSA. Compounds as in Fig. 3. Other conditions as in Fig. 1.

Fig. 6 shows the separation of "inine" isomers of ergotoxine alkaloids, and the chromatogram of dihydroergotoxine alkaloids can be seen in Fig. 7. In both cases DHP–DEA system was used.

Fig. 8 demonstrates the applicability of the method for the separation of optical isomers. The CSA–DEA system was applied to the separation of four *cis*-vincamine isomers.

TABLE V

DEPENDENCE OF THE CAPACITY RATIOS (k') MEASURED FOR THE COMPOUNDS INVESTIGATED ON THE CSA CONCENTRATION

Conditions as in Table II. See Table I for the numbered compounds.

No.	*Compound*	*Eluent mixtures (hexane–isopropanol, 8:2)*					
	DEA (mol/dm³)	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*
	CSA (mol/dm³)	*–*	*0.0005*	*0.001*	*0.0015*	*0.003*	*0.005*
XXXIV	Hydrocortisone	1.97	1.95	1.95	2.05	2.01	2.00
XXXV	Prednisolone	2.29	2.18	2.19	2.23	2.26	2.30
I	(+)-*cis*-Epivincamine	0.82	1.21	6.08	7.32	6.75	6.23
II	(−)-*cis*-Epivincamine	0.82	1.21	6.75	8.14	7.64	7.00
III	(+)-*cis*-Vincamine	0.65	1.00	8.23	11.1	10.1	8.97
IV	(−)-*cis*-Vincamine	0.65	1.00	8.94	11.5	10.5	9.40
V	(+)-*cis*-Apovincaminic acid ethyl ester	0.41	0.59	6.50	7.46	6.89	6.20
VI	(−)-*cis*-Apovincaminic acid ethyl ester	0.41	0.59	6.50	7.46	6.89	6.20
VII	(+)-*trans*-Apovincaminic acid ethyl ester			13.2	17.0	16.5	14.5
VIII	(−)-*trans*-Apovincaminic acid ethyl ester			13.6	17.6	17.2	15.1
IX	(+)-*cis*-Vincamenine	0.15	0.31	5.52	6.57	5.71	5.20
X	(+)-*cis*-Apovincamine	0.41	0.66	7.80	8.93	7.75	7.10
XI	(+)-*cis*-Vincamone	0.32	0.52	5.71	7.14	6.71	5.97
XII	(−)-*cis*-Vincamone	0.32	0.52	5.71	7.14	6.71	5.97
XIII	(+)-*cis*-Vincanol	0.53	0.62	5.28	7.25	6.75	6.00
XIV	(−)-*cis*-Vincanol	0.53	0.62	5.71	7.50	7.04	6.17
XV	(+)-*cis*-Vincaminic acid ethyl ester	0.47	0.72	8.35	8.71	8.50	7.30
XVI	(−)-*cis*-Vincaminic acid ethyl ester	0.47	0.72	8.72	9.21	9.04	7.70
XXII	β-Ergocriptine	2.12	2.41	⩽10			
XXIII	α-Ergocriptine	2.12	2.41	⩽10			
XXIV	Ergocornine	2.54	2.86	⩽10			
XXV	Ergocristine	3.26	3.62	⩽10			
XXVI	Ergotamine	4.85	6.34	⩽10			
XXVII	Ergotaminine	5.35	6.97	⩽10			
XXI	Dihydro-β-ergocriptine	2.15	2.59	⩽10			
XXX	Dihydro-α-ergocriptine	2.15	2.59	⩽10			
XXXI	Dihydroergocornine	2.50	3.00	⩽10			
XXXII	Dihydroergocristine	3.12	3.62	⩽10			
XXXIII	Dihydroergotamine	4.50	6.24	⩽10			

CONCLUSIONS

A new type of dynamic ion-exchange system has been developed. In this system, a polar bonded stationary phase and a less polar eluent mixture containing DHP or CSA as ion exchanger were used. On the basis of the results obtained it seems reasonable to propose that molecular complexation between the uncharged alkaloid

— 0.002	0.00025 0.002	0.0005 0.002	0.001 0.002	0.002 0.002	0.0021 0.002	0.00215 0.002	0.0022 0.002	0.003 0.002	0.004 0.002
2.20	2.27	2.13	2.23	2.16	2.23	2.11	2.20	2.23	2.23
7.10	6.60	6.33	6.03	6.00	5.81	5.97	2.26	0.94	0.87
7.87	7.30	7.10	6.70	6.67	6.48	6.68	2.26	0.94	0.87
10.9	10.0	9.50	8.93	8.73	8.48	8.77	1.65	0.74	0.65
11.4	10.4	9.93	9.33	9.10	8.87	9.10	1.65	0.74	0.65
7.20	6.53	6.80	6.23	6.00	5.65	5.45	0.55	0.42	0.42
7.20	6.53	6.80	6.23	6.00	5.65	5.45	0.55	0.42	0.42

base and the ion exchanger is responsible for the increase in retention, forming a more polar complex in the mobile phase than the free uncharged alkaloid base. The separation of native and hydrogenated ergot peptide alkaloids can be performed by DHP–DEA system, and the CSA–DEA system provides a good possibility for the analysis of eburnane alkaloids. The CSA–DEA system seems to be very promising in a generalized form for the analysis of optical isomers.

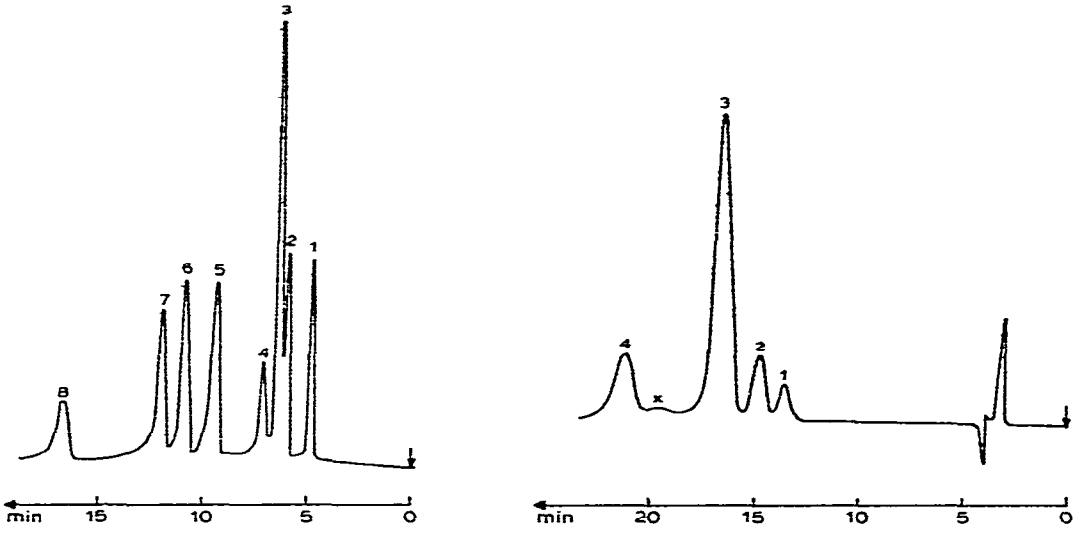

Fig. 5. Separation of a model mixture of eburnane alkaloids. Conditions: column, μ-Bondapak CN (300 × 3.9 mm I.D.); eluent, hexane–chloroform–acetonitrile (65:20:15) containing 10^{-3} mol/dm³ DHP; flow-rate, 1 cm³/min; detection at 280 nm. Compounds: 1 = (+)-*cis*-vincamone (XI); 2 = (+)-*cis*-apovincaminic acid ethyl ester (V); 3 = (+)-*cis*-apovincamine (X); 4 = (+)-*cis*-vincamenine (IX); 5 = (+)-*cis*-epivincamine (I); 6 = (+)-*cis*-vincamine (III); 7 = (+)-*cis*-vincanol (XIII); 8 = (+)-*cis*-isovincanol (XVII).

Fig. 6. Separation of "inine" isomers of ergotoxine alkaloids. Eluent, hexane–isopropanol (80:20) containing $7.5 \cdot 10^{-4}$ mol/dm³ DEA and $1.5 \cdot 10^{-3}$ mol/dm³ DHP. Compounds: 1 = β-ergotriptinine (XVIII); 2 = α-ergocriptinine; 3 = ergocorninine; 4 = ergocristinine; × = unknown. Other conditions as in Fig. 5.

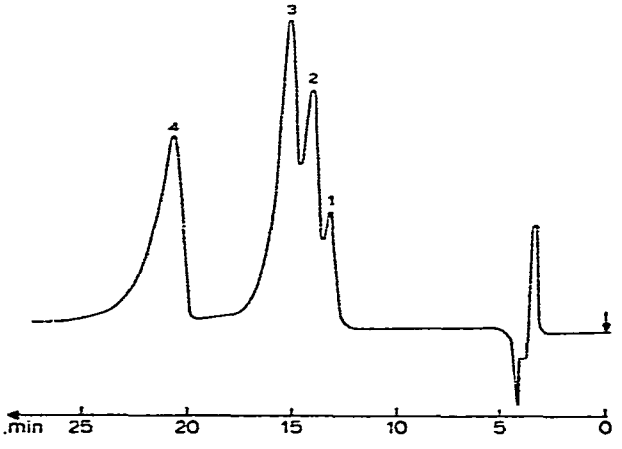

Fig. 7. Separation of dihydroergotoxine alkaloids. Eluent, hexane–isopropanol (80:20) containing $7.5 \cdot 10^{-4}$ mol/dm³ DEA and $2 \cdot 10^{-3}$ mol/dm³ DHP. Compounds: 1 = dihydro-β-ergocriptine (XXIX); 2 = dihydro-α-ergocriptine (XXX); 3 = dihydroergocornine (XXXI); 4 = dihydroergocristine (XXXII). Other conditions as in Fig. 5.

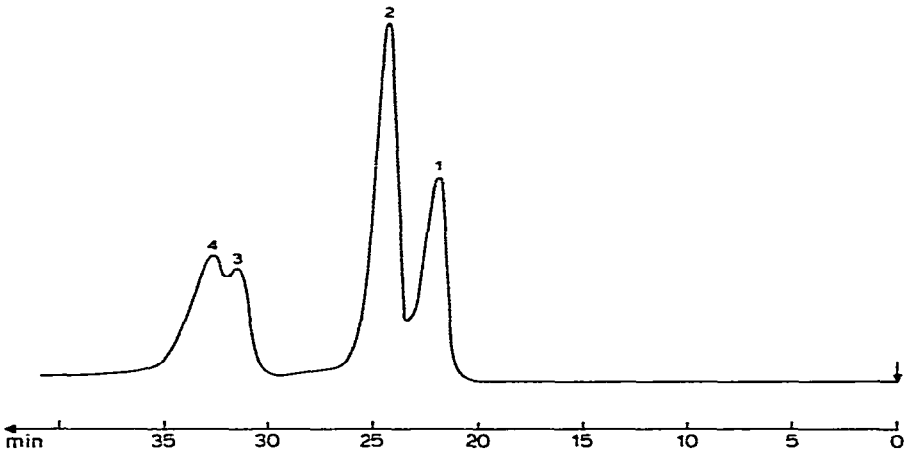

Fig. 8. Separation of four optical isomers of vincamine. Eluent, hexane–isopropanol (80:20) containing $5 \cdot 10^{-4}$ mol/dm^3 DEA and $2 \cdot 10^{-3}$ mol/dm^3 CSA. Compounds: 1 = (+)-*cis*-epivincamine (I); 2 = (−)-*cis*-epivincamine (II); 3 = (+)-*cis*-vincamine (III); 4 = (−)-*cis*-vincamine (IV). Other conditions as in Fig. 5.

ACKNOWLEDGEMENTS

The authors are grateful to L. Dancsi and L. Czibula (Chemical Works of G. Richter, Ltd.) and to Dr. Gy. Kalaus (Budapest Technical University, Institute for Organic Chemistry, Budapest, Hungary) for placing the compounds investigated at our disposal.

REFERENCES

1 J. C. Kraak, K. M. Jonker and J. F. K. Huber, *J. Chromatogr.*, 142 (1977) 671.
2 D. P. Wittmer, N. O. Nuessle and W. G. Hancy, *Anal. Chem.*, 47 (1975) 1422.
3 *Paired Ion Chromatography, an Alternate to Ion Exchange*, Waters Assoc. Milford, MA, 1975.
4 J. H. Knox and G. R. Laird, *J. Chromatogr.*, 122 (1976) 17.
5 J. H. Knox and J. Jurand, *J. Chromatogr.*, 125 (1976) 89.
6 Cs. Horváth, W. Melander, I. Molnar and P. Molnar, *Anal. Chem.*, 49 (1977) 2295.
7 C. P. Terweij-Groen, S. Heemstra and J. C. Kraak, *J. Chromatogr.*, 161 (1978) 69.
8 K.-G. Wahlund, *J. Chromatogr.*, 115 (1975) 411.
9 K.-G. Wahlund and U. Lund, *J. Chromatogr.*, 122 (1976) 269.
10 B. Fransson, K.-G. Wahlund, I. M. Johansson and G. Schill, *J. Chromatogr.*, 125 (1976) 327.
11 G. Szepesi, M. Gazdag and R. Iváncsics, *J. Chromatogr.*, 244 (1982) in press.
12 L. Szepesy, I. Fehér, G. Szepesi and M. Gazdag, *J. Chromatogr.*, 149 (1978) 271.
13 G. Szepesi, M. Gazdag and L. Terdy, *J. Chromatogr.*, 191 (1980) 101.
14 G. Szepesi and M. Gazdag, *J. Chromatogr.*, 204 (1981) 341.
15 G. Szepesi and M. Gazdag, *J. Chromatogr.*, 205 (1981) 57.
16 G. Ghersini and E. Cerrai, in T. Braun and G. Ghersini (Editors), *Extraction Chromatography*, Elsevier, Amsterdam, London, New York, 1975, Ch. 14, p. 366.