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

II. SEPARATION OF OPTICAL ISOMERS

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SUMMARY

A chiral ion exchanger, (+)- and (−)-antipodes of 10-camphorsulphonic acid, was applied for the normal-phase solvent-generated molecular complexation chromatography of optical isomers of alkaloids. It was dissolved in the less polar phase of a three-component eluent mixture consisting of solvents having different polarities. Separations were performed on a polar bonded stationary phase (cyanopropylsilica). Remarkably high relative retentions were achieved, permitting rapid chiral separation of different eburnane alkaloids selected as models for the investigations. The influence of mobile phase composition and optical antipode selection of the ion exchanger on the selectivity and efficiency of the separation is discussed. Some applications of the proposed method, including the separation of eight optical isomers of vincamine, are discussed.

INTRODUCTION

In recent years, extensive efforts have been made to separate enantiomeric organic compounds. Based on the separation principles used, high performance liquid chromatographic (HPLC) methods developed for this purpose can be divided into the following groups:

(a) the enantiomers can be converted into diastereomeric derivatives and analysed as diastereomers[1,2].

(b) enantiomers can be separated on chemically bonded chiral stationary phases[3-9];

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(c) enantiomeric forms of dansylated amino acids and peptides can be resolved in reversed-phase chromatographic systems using metal chelate additives[10–12];

(d) using zwitterion-pair chromatography, introduced by Knox and co-workers[13,14], enantiomeric forms of nucleotides can be separated;

(e) enantiomers can be separated in the form of their diastereomeric ion pairs by ion-exchange chromatography[15] and HPLC[16] using an optically active eluent.

These separation methods provide excellent possibilities for the solution of special analytical problems; however, they do not seem to be generally applicable to the separation of ionizable organic enantiomers.

In Part I[17], a separation system was introduced in which a polar bonded stationary phase (cyanopropylsilica) and a less polar eluent mixture containing small amounts of anionic ion exchangers such as di(2-ethylhexyl)phosphoric acid or (+)-10-camphorsulphonic acid were used for the separation of ergot and eburnane alkaloids[17]. In this system the increased retention of the free, uncharged alkaloid base can be explained by a molecular complexation mechanism; the complex is formed in the mobile phase.

This paper is a continuation of our recent study using chiral complexing reagents in the eluent for the selective separation of optical isomers. As we mentioned in Part I[17], this system appears very promising for the analysis of optical isomers of ionizable organic substances.

In the meantime, a successful separation of enantiomeric amines has been reported by Petterson and Schill[16] using silica and silica-DIOL stationary phases and (+)-camphorsulphonic acid derivatives dissolved in the organic mobile phase as counter ions. According to their conclusions, the enantiomeric separation proceeds according to a liquid–solid adsorption mechanism of the ion pairs formed.

Our system differs fundamentally from the recently published systems, especially as regards the phase composition, stability, applicability and flexibility of the method, and also in the separation mechanism.

Our investigations have been focused on the investigation of the effects of phase composition, concentration of the chiral complexing reagent and selection of the antipode type of the chiral agent on the selectivity and efficiency of the separation.

EXPERIMENTAL

A Varian 8500 high-performance liquid chromatograph (Varian Aerograph, Walnut Creek, CA, U.S.A.) consisting of a Rheodyne 7010 loop injector (Rheodyne, Berkeley, CA, U.S.A.), a Variscan Model 635 variable-wavelength UV spectrophotometer and a Varian A-25 recorder (Varian Aerograph) and a Liquochrom Model 2010 high-performance liquid chromatograph (Labor MIM, Esztergom-Budapest, Hungary) consisting of a loop injector and a variable-wavelength UV detector (Model OE-312) and a Type 185 recorder (Kutesz, Budapest, Hungary) were used.

Separations were performed on pre-packed Nucleosil 5 CN (150 × 4.6 mm I.D.) and Nucleosil 10 CN (250 × 4.6 mm I.D.) columns (Chrompack, Middelburg, The Netherlands).

All solvents used for eluent preparation were of analytical-reagent grade (Reanal, Budapest, Hungary) and were distilled before use. (+)-10-

Camphorsulphonic acid was obtained from Reanal and (−)-10-camphorsulphonic acid was obtained by courtesy of I. Jelinek (Chinoin Pharmaceutical Works, Budapest, Hungary).

The compounds investigated were produced at the Chemical Works of Gedeon Richter, Ltd. (Budapest, Hungary) and were considered to be of the highest available quality.

RESULTS AND DISCUSSION

To investigate the possibility of chiral separation of ionizable organic amines, eburnane alkaloids were selected as models, because these molecules contain two or three optical centres in positions 3 and 16 (apovincamine-type alkaloids) and 3, 14 and 16 (vincamine-type alkaloids), respectively. Therefore, several optical isomers of

TABLE I

STRUCTURES OF EBURNANE ALKALOIDS INVESTIGATED
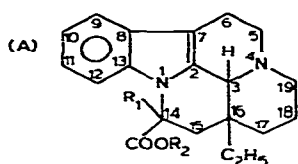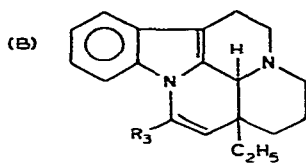
Vincamine-type alkaloids   Apovincamine-type alkaloids

No.	Compound	Type	Substituents			Stereo-positions		
			R_1	R_2	R_3	3-H	14-OH	16-C_2H_5
I	(+)-cis-Vincaminic acid ethyl ester	A	OH	C_2H_5	—	α	β	α
II	(−)-cis-Vincaminic acid ethyl ester	A	OH	C_2H_5	—	β	α	β
III	(+)-cis-Epivincamine	A	OH	CH_3	—	α	α	α
IV	(−)-cis-Epivincamine	A	OH	CH_3	—	β	β	β
V	(+)-cis-Vincamine	A	OH	CH_3	—	α	β	α
VI	(−)-cis-Vincamine	A	OH	CH_3	—	β	α	β
VII	(+)-trans-Epivincamine	A	OH	CH_3	—	β	α	α
VIII	(−)-trans-Epivincamine	A	OH	CH_3	—	α	β	β
IX	(+)-trans-Vincamine	A	OH	CH_3	—	β	β	α
X	(−)-trans-Vincamine	A	OH	CH_3	—	α	α	β
XI	(+)-cis-Vincamone	B	—	—	OH	α	—	α
XII	(−)-cis-Vincamone	B	—	—	OH	β	—	β
XIII	(+)-trans-Vincamone	B	—	—	OH	β	—	α
XIV	(−)-trans-Vincamone	B	—	—	OH	α	—	β
XV	(+)-cis-Apovincaminic acid ethyl ester	B	—	—	$COOC_2H_5$	α	—	α
XVI	(−)-cis-Apovincaminic acid ethyl ester	B	—	—	$COOC_2H_5$	β	—	β
XVII	(+)-trans-Apovincaminic acid ethyl ester	B	—	—	$COOC_2H_5$	β	—	α
XVIII	(−)-trans-Apovincaminic acid ethyl ester	B	—	—	$COOC_2H_5$	α	—	β

the same molecule exist, as illustrated in Table I, where the structures of the compouri.. investigated are shown.

Hydrocortisone and prednisolone were used to control the polarity of the eluents.

Influence of phase composition

To optimize the separation system, first the influence of phase composition on the selectivity and efficiency of chiral separation was investigated. All experiments were performed on a cyanopropylsilica stationary phase, because we found that this

TABLE II

DEPENDENCE OF CAPACITY FACTORS (k') AND SEPARATION FACTORS (r_{ji}) ON THE CONCENTRATION OF CHLOROFORM AND ALCOHOLS IN THE ELUENT

Conditions: instruments, Varian 8500 high-performance liquid chromatograph; column, Nucleosil 10 CN (250 × 4.6 mm I.D.); flow-rate: 1 ml/min; detection at 280 nm. A 1-l volume of eluent contains $2 \cdot 10^{-3}$ mole of (+)-10-camphorsulphonic acid and 10^{-3} mole of DEA.

No.	Compound	Eluent mixture					
		Hexane–chloroform–methanol					
		80:18:2		70:27:3		60:36:4	
		k'	r_{ji}	k'	r_{ji}	k'	r_{ji}
I	(+)-cis-Vincaminic acid ethyl ester	5.24		1.55		0.65	
			1.05		1.06		1.00
II	(−)-cis-Vincaminic acid ethyl ester	5.50		1.65		0.65	
III	(+)-cis-Epivincamine	5.50		1.70		0.72	
IV	(−)-cis-Epivincamine	5.80	1.05	1.79	1.05	0.75	1.04
V	(+)-cis-Vincamine	7.29		2.10		0.82	
VI	(−)-cis-Vincamine	7.81	1.07	2.25	1.05	0.86	1.05
VII	(+)-trans-Epivincamine	17.9		5.15		1.64	
VIII	(−)-trans-Epivincamine	18.4	1.03	5.30	1.03	1.64	1.00
IX	(+)-trans-Vincamine	>20		5.50		1.82	
X	(−)-trans-Vincamine	>20		5.50	1.00	1.82	1.00
XI	(+)-cis-Vincamone	6.50		1.90		0.45	
XII	(−)-cis-Vincamone	6.50	1.00	1.90	1.00	0.45	1.00
XIII	(+)-trans-Vincamone	15.5		3.95		1.36	
XIV	(−)-trans-Vincamone	16.4	1.06	4.10	1.04	1.36	1.00
XV	(+)-cis-Apovincaminic acid ethyl ester	3.86		1.20		0.45	
			1.00		1.00		1.00
XVI	(−)-cis-Apovincaminic acid ethyl ester	3.86		1.20		0.45	
XVII	(+)-trans-Apovincaminic acid ethyl ester	11.0		2.60		0.91	
			1.03		1.00		1.00
XVIII	(−)-trans-Apovincaminic acid ethyl ester	11.4		2.60		0.91	
XIX	Hydrocortisone	10.3		3.40		1.36	
XX	Prednisolone	14.2		4.60		2.09	

moderately hydrophobic phase can be advantageously applied. The retention of the compounds can easily be controlled through the polarity of the eluent.

The mobile phase consisted of three solvents having different polarities: a hydrophobic, a polar and a so-called "moderator" solvent of medium polarity. In Part I[17], the separation of four optical isomers of vincamine using hexane–isopropanol as the eluent was demonstrated. However, we noted that this example illustrated only the possibility of application of the method and served as a starting point for the optimization.

Table II shows the retention data obtained for eburnane alkaloids when certain proportions of hexane and isopropanol are replaced with chloroform and other al-

| *Hexane–chloroform–ethanol* | | | | | | *Hexane–chloroform–isopropanol* | | | | | |
| *80:18:2* | | *70:27:3* | | *60:36:4* | | *80:18:2* | | *70:27:3* | | *60:36:4* | |
k'	r_{ji}	k'	r_{ji}	k'	r_{ji}	k'	r_{ji}	k'	r_{ji}	k'	r_{ji}
6.55		1.90		0.75		10.6		3.48		1.70	
	1.10		1.11		1.10		1.03		1.14		1.10
7.20		2.10		0.83		10.9		3.95		1.87	
7.16		2.10		0.90		11.3		3.48		1.90	
7.88	1.10	2.30	1.10	1.00	1.11	12.6	1.12	3.81	1.09	2.01	1.06
8.90		2.60		1.04		14.8		4.24		2.70	
9.62	1.08	2.80	1.08	1.14	1.09	16.0	1.14	4.81	1.13	3.00	1.10
>20		8.00		2.57		>20		>20		6.33	
>20		8.45	1.06	2.67	1.04	>20		>20		6.90	1.09
>20		10.3		3.38		>20		>20		10.2	
>20		10.8	1.05	3.57	1.06	>20		>20		11.1	1.08
6.14		2.40		1.00		9.50		3.67		1.80	
6.14	1.00	2.40	1.00	1.00	1.00	9.50	1.00	3.67	1.00	1.80	1.00
17.9		7.00		2.81		>20		>20		7.48	
19.2	1.07	7.50	1.07	3.00	1.07	>20		>20		8.14	1.09
5.38		1.60		0.55		12.5		3.90		3.00	
	1.04		1.00		1.00		1.05		1.04		1.05
5.57		1.60		0.55		13.2		4.10		3.19	
18.7		4.85		1.95		>20		>20		19.7	
	1.04		1.03		1.00						1.06
19.5		5.00		1.95		>20		>20		20.9	
12.5		3.70		1.76		20.0		6.14		2.52	
17.2		5.10		2.38		>20		9.00		3.85	

TABLE III

INFLUENCE OF MODERATOR SOLVENTS IN THE MOBILE PHASE

Conditions as in Table II. Eluent: hexane–X–isopropanol (70:25:5).

X	cis-Apovincaminic acid ethyl ester				cis-Vincamine			
	k' (+)	k' (−)	r_{ji}	H (μm)	k' (+)	k' (−)	r_{ji}	H (μm)
Chloroform	3.67	3.67	1.00	—	5.13	5.60	1.09	79
Dioxane	10.0	11.6	1.15	95	12.5	14.6	1.16	88
Dichloroethane	6.03	6.03	1.00	—	9.52	9.97	1.05	172
Dichloromethane	3.74	3.74	1.00	—	5.90	6.40	1.09	170
Acetonitrile	4.75	4.75	1.00	—	8.57	8.57	1.00	—
Tetrahydrofuran	3.53	3.81	1.08	120	4.57	4.83	1.05	81

TABLE IV

INFLUENCE OF HYDROPHOBIC COMPONENTS IN THE MOBILE PHASE

Conditions as in Table II. Eluent: X–dioxane–1-butanol (75:20:5).

X	cis-Apovincaminic acid ethyl ester			
	k' (+)	k' (−)	r_{ji}	H (mm)
Hexane	8.70	10.2	1.18	0.080
Isooctane	5.49	6.59	1.20	0.083
Heptane	5.37	6.36	1.18	0.085
Cyclohexane	4.95	5.69	1.15	0.087

TABLE V

INFLUENCE OF POLAR COMPONENTS IN THE MOBILE PHASE

Conditions: flow-rate, 1.5 ml/min; other conditions as in Table II. Eluent: hexane–dioxane–X (57.5:37.5:5.0).

X	cis-Apovincaminic acid ethyl ester				cis-Vincamine			
	k' (+)	k' (−)	r_{ji}	H (mm)	k' (+)	k' (−)	r_{ji}	H (mm)
Methanol	1.55	1.55	1.00	—	2.12	2.30	1.09	0.130
Ethanol	2.48	2.82	1.14	0.085	2.02	2.46	1.14	0.115
1-Propanol	3.36	3.91	1.16	0.079	4.39	5.08	1.16	0.115
2-Propanol	3.91	4.54	1.16	0.100	4.88	5.69	1.19	0.160
1-Butanol	3.82	4.50	1.18	0.080	4.96	5.53	1.12	0.081
2-Butanol	4.85	5.63	1.16	0.119	6.63	7.41	1.12	0.100
tert.-Butanol	10.0	12.1	1.16	0.246	10.7	11.6	1.18	0.100
Methoxyethanol	2.03	2.49	1.23	0.127	4.41	4.94	1.12	0.089
Ethoxyethanol	2.85	3.21	1.13	0.076	3.66	4.03	1.10	0.095

cohols. It can be seen that a significant increase in selectivity and efficiency can be achieved by using chloroform in the eluent. To increase further the selectivity of the chiral separation, other medium-polarity solvents ("moderator" solvents) were tried.

Table III shows the retention data obtained when different moderator solvents in the same ratio were used. It can be concluded that, when enantiomers are separated by molecular complexation, the correct choice of moderator solvent has great importance, because it can determine the possibility of the separation. For eburnane alkaloids, dioxane and chloroform proved to be the most suitable moderator solvents.

The effect of the hydrophobic component used in the eluent on the efficiency and selectivity of the separation was also investigated (Table IV). Apparently, the hydrophobic solvent does not have a significant influence on the separation.

The importance of the polar solvent is shown in Table V. 1-Butanol showed the best properties in the hexane–dioxane–alcohol system in terms of the chromatographic characteristics.

The dependence of the retention behaviour of apovincaminic acid ethyl ester on the ratio of hydrophobic and moderator solvents is illustrated in Fig. 1. The retention of the compounds can be easily controlled through the ratio of hydrophobic and moderator solvents without significant changes in selectivity and efficiency.

The separation systems found to be optimal for eburnane alkaloids indicated in Table I are collected in Table VI.

From the results obtained, the following general conclusions can be drawn. The selection of the most suitable moderator solvent providing a selective and effective separation of enantiomers is of great importance. For eburnane alkaloids, dioxane and chloroform can be advantageously applied, but in the separation of other enantiomeric amines we also obtained good results with tetrahydrofuran. Easy control of the retention of the compounds can be achieved by changing the ratio of hydrophobic and moderator solvents without a significant loss in selectivity. The most common polar solvents have a favourable effect on the peak shape and separa-

| trans-Vincamine				cis-Vincamone				trans-Vincamone			
k' (+)	k' (−)	r_{ji}	H (mm)	k' (+)	k' (−)	r_{ji}	H (mm)	k' (+)	k' (−)	r_{ji}	H (mm)
6.34	6.61	1.04	0.110	1.23	1.23	1.00	—	4.69	4.84	1.05	0.120
12.7	13.4	1.05	0.134	2.39	2.39	1.00	—	9.64	10.3	1.07	0.077
18.6	19.5	1.05	0.115	2.29	2.29	1.00	—	13.1	14.1	1.08	0.100
25.8	27.9	1.05	0.147	3.65	3.65	1.00	—	15.2	16.3	1.07	0.100
26.0	32.4	1.25	0.044	4.05	4.05	1.00	—	16.9	18.0	1.07	0.086
20.0	21.2	1.06	0.100	4.69	4.69	1.00	—	21.2	22.9	1.08	0.105
>20	>20	—	—	9.85	9.85	1.00	—	>20	>20	—	—
20.0	21.2	1.06	0.140	3.09	3.09	1.00	—	11.2	11.8	1.05	0.170
15.1	16.2	1.06	0.084	2.83	4.10	1.45	0.084	10.2	10.8	1.06	0.112
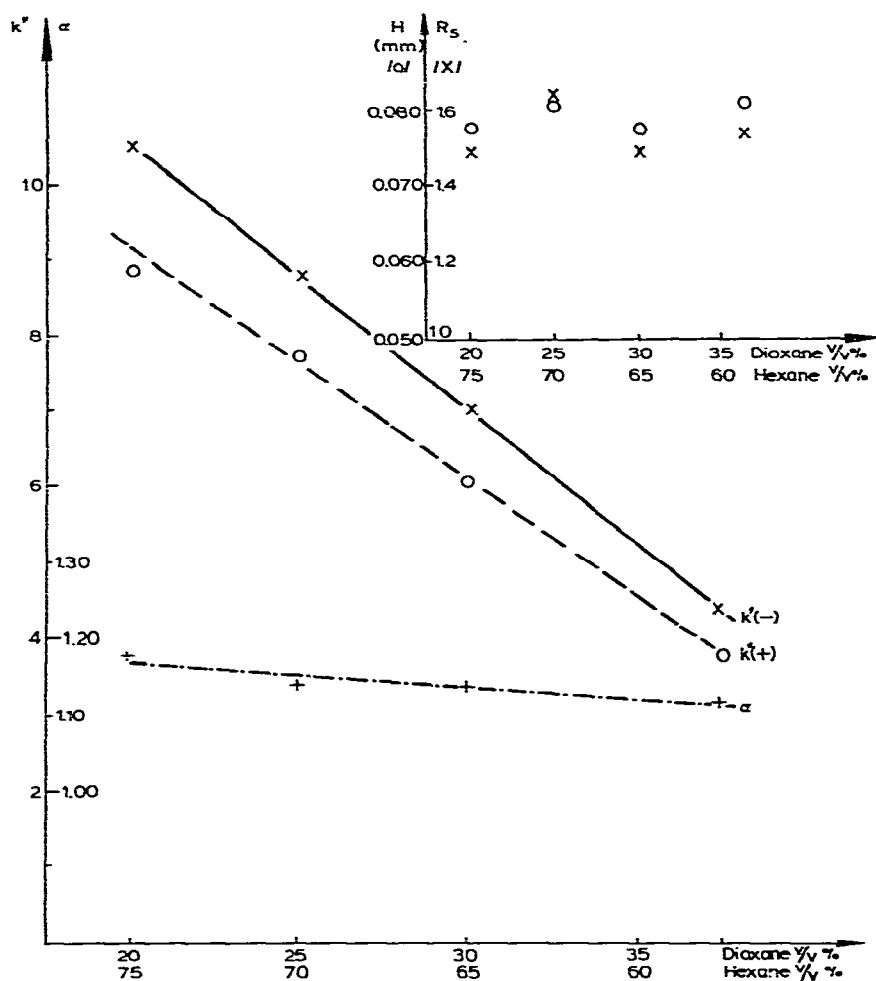
Fig. 1. Dependence of k', r_a, R_s and H measured for racemic apovincaminic acid ethyl ester on the ratio of hydrophobic and moderator solvents in the mobile phase. Instrument, Varian 8500; column, Nucleosil 10 CN (250 × 4.6 mm I.D.); eluent, (hexane–dioxane)–1-butanol (95:5) containing $2 \cdot 10^{-3}$ mole/l of CSA and 10^{-3} mole/l of DEA; flow-rate, 1.5 ml/min; detection at 280 nm.

tion efficiency; special polar solvents (e.g., ethoxyethanol) should improve the selectivity of the separation. We have not experienced detrimental effects of the water content of the solvents used for eluent preparation. The proposed method does not require the elimination of water from the solvents prior to eluent preparation.

Influence of concentration of (+)-10-camphorsulphonic acid (CSA) and diethylamine (DEA) on the separation

In accordance with our earlier experiences, discussed in detail in Part I[17], DEA has a favourable effect on the peak symmetry, probably by excluding the possibility of retention by an adsorption mechanism. On investigating the influences of CSA and DEA on the separation, the same results were obtained. Therefore, a 2:1 ratio of CSA

TABLE VI

OPTIMAL SEPARATION SYSTEMS FOR EBURNANE ALKALOIDS INVESTIGATED

Instrument: system A, Liquochrom Model 2010 high-performance liquid chromatograph; systems B and C, Varian 8500 high-performance liquid chromatograph. Column and mobile phase composition as indicated in the table; detection at 280 nm.

Compound	System*	Nucleosil CN column		k' (+)	k' (−)	r_{ji}	H (mm)	R_s	Asf**
		5 μm	10 μm						
(±)-cis-Epivincamine	A		+	7.95	8.70	1.07	0.060	1.35	0.98
	B	+		1.78	1.95	1.10	0.023	1.26	1.15
(±)-cis-Vincamine	A		+	8.90	10.05	1.13	0.064	1.60	1.27
	B	+		2.21	2.37	1.07	0.026	1.16	1.14
(±)-trans-Epivincamine	B	+		7.04	7.42	1.05	0.041	1.14	1.25
(±)-trans-Vincamine	B	+		9.04	9.51	1.05	0.054	1.20	2.08
(±)-cis-Apovincaminic acid ethyl ester	A		+	7.78	8.83	1.14	0.061	1.77	0.92
(±)-trans-Apovincaminic acid ethyl ester	B	+		4.83	4.97	1.03	0.097	1.22	1.44
(±)-cis-Vincamone	C		+	2.83	4.10	1.45	0.084	3.11	1.20
(±)-trans-Vincamone	C		+	10.2	10.8	1.06	0.112	1.15	2.26

* Systems: (A) hexane–dioxane–1-butanol (70:25:5), flow-rate 1.5 ml/min; (B) hexane–chloroform–ethanol (70:27:3), flow-rate 1.5 ml/min; (C) hexane–dioxane–ethoxyethanol (57.5:37.5:5), flow-rate 1.5 ml/min.
** Asf = back part of the peak/front part of the peak.

and DEA was used in as low concentration as possible ($2 \cdot 10^{-3}$ mole of CSA and 10^{-3} mole of DEA in 1 l of eluent).

Stability and reproducibility of the phase system

One of the main disadvantages of the method proposed by Petterson and Schill[16] is that stable operating conditions can be reached only after about 2 days, having recirculated the water-free eluent. Owing to the different retention mechanism and lower water sensitivity of the system in our work, stable operating conditions are obtained after only 20 min of washing with the eluent. The day-to-day reproducibility of the method is illustrated in Fig. 2 by the analysis of racemic *cis*-apovincaminic acid ethyl ester. Four months passed between the two investigations. Good reproducibility of the chromatographic separation on the same column was obtained.

Influence of optical antipode selection of chiral complexing reagent on the selectivity of the separation

When enantiomers are investigated, the selectivity of the separation can be improved if the optical antipode of the chiral complexing reagent can be changed. For instance, when the (+)-antipode of CSA is used in the eluent, the (+)-isomers of alkaloids are eluted before the (−)-isomers. In contrast, when the (−)-antipode of CSA is dissolved in the eluent, the (+)-isomer has a higher retention than the (−)-isomer. This is illustrated in Fig. 3, where the separation of racemic *cis*-apovincaminic acid ethyl ester using (+)-CSA, (−)-CSA and (±)-CSA as complexing reagents is shown.
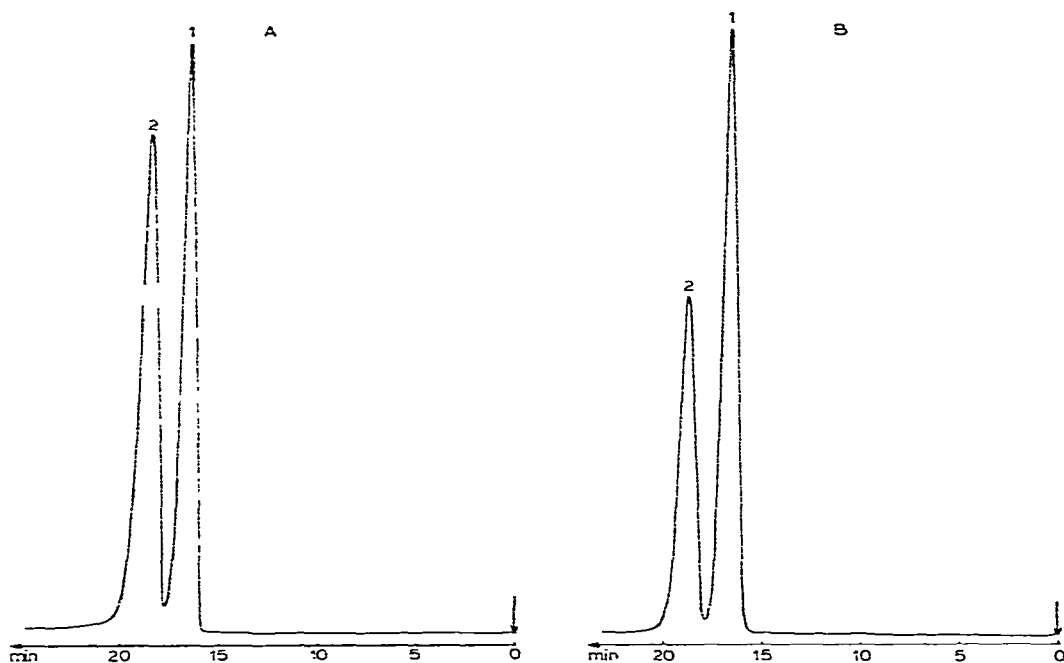
Fig. 2. Illustration of phase system stability. (A) Chromatogram obtained at the start; (B) chromatogram obtained after 4 months using the same column. Conditions: eluent, hexane–dioxane–1-butanol (57.5:37.5:5) containing $2 \cdot 10^{-3}$ mole/l of (+)-CSA and 10^{-3} mole/l of DEA; flow-rate, 1 ml/min; other conditions as in Fig. 1. Compounds: 1 = (+)-cis-apovincaminic acid ethyl ester; 2 = (−)-cis-apovincaminic acid ethyl ester.

As was expected, the opposite elution order for the two components was obtained when (+)-CSA was replaced with its (−)-antipode in the eluent and no separation of the two enantiomers was found when racemic CSA was used in the eluent. The retention data are collected in Table VII.

This possibility of altering the retention behaviour should have great importance, especially when the optical purity of the substance is investigated or when the absolute configuration of the molecule has to be clarified.

Retention principles

We assume, as with the other examples mentioned in Part I[17], that molecular complexation between the uncharged alkaloid base and CSA is responsible for the increase in retention forming a more polar complex in the mobile phase than the free uncharged alkaloid base. When chiral separation is performed, Dalgliesh's three-points rule[18] is generally accepted, assuming the interactions at three points in the vicinity of the chiral carbon atom. According to our experience, this three-point theory does not give an acceptable model in every case, because enantiomers in which the molecule does not contain a hydrogen donor functional group interacting with the oxo-group of CSA or the hydroxyl group is too distant from the amino group can also be well separated. A similar conclusion has been reported by Cardaci *et al.*[19]
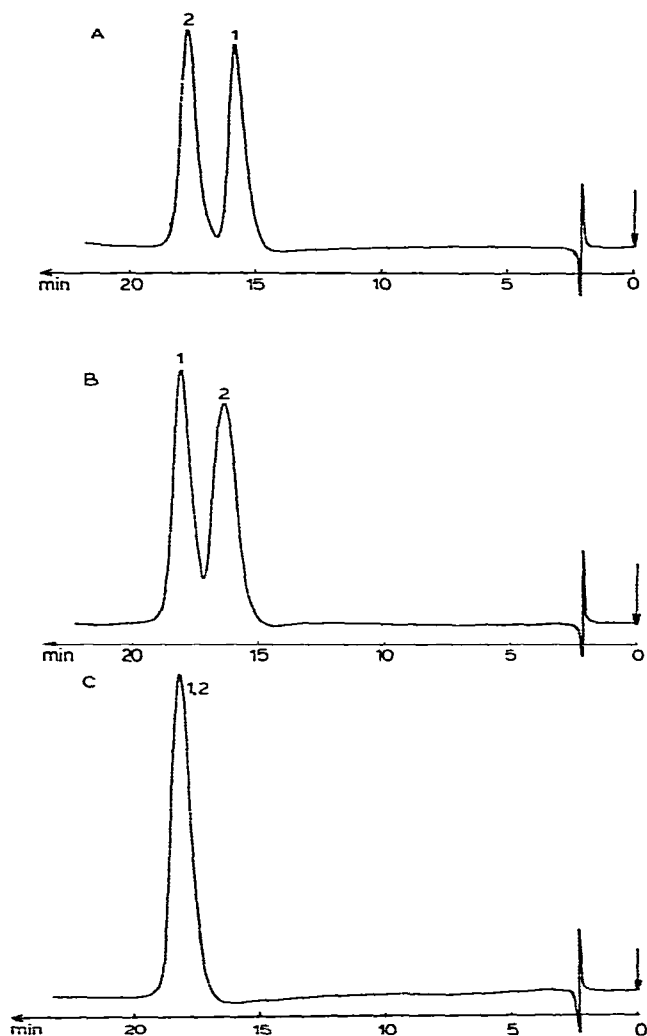
Fig. 3. Dependence of elution order on the antipode type of CSA. Instrument, Liquochrom Model 2010; eluent, hexane–dioxane–1-butanol (70:25:5) containing $2 \cdot 10^{-3}$ mole/l of (A) (+)-CSA, (B) (−)-CSA and (C) (±)-CSA and 10^{-3} mole/l of DEA in all instances; flow-rate, 1.5 ml/min. Other conditions and compounds as in Fig. 2.

when cobalt(III), nickel(II) and iron(III) trisdipyridyl complexes were separated by paper electrophoresis using optically active electrolytes.

In our opinion, in addition to molecular complexation between the alkaloid and CSA, a hydrophobic interaction between the two different ring systems resulting in a fixed, bounded structure of the complexes is responsible for the stereoselectivity. This assumption seems to be supported by the considerable dependence of enantiomeric separation on the selection of the moderator solvent, *i.e.*, these solvents can have an important role in mobile phase solvation, influencing the hydrophobic interactions between the molecules.

TABLE VII

DEPENDENCE OF ENANTIOMERIC SEPARATION AND ELUTION ORDER ON THE ANTIPODE SE-
LECTION OF 10-CAMPHORSULPHONIC ACID

Conditions: instrument, Liquochrom Model 2010 high-performance liquid chromatograph; column, Nucleosil 10 CN
(250 × 4.6 mm I.D.); eluent, hexane–dioxane–1-butanol (70:25:5) containing CSA ($2 \cdot 10^{-3}$ mole/l) and DEA (10^{-3}
mole/l); flow-rate, 1.5 ml/min; detection at 280 nm.

Complexing reagent	cis-Apovincaminic acid ethyl ester					cis-Vincamine				
	k' (+)	k' (−)	r_{ji}	H (μm)	R_s	k' (+)	k' (−)	r_{ji}	H (μm)	R_s
(+)-10-Camphor-sulphonic acid	7.78	8.83	1.14	61	1.77	8.90	10.1	1.13	64	1.60
(−)-10-Camphor-sulphonic acid	9.00	8.00	1.13	56	1.73	10.3	9.30	1.10	57	1.37
(±)-10-Camphor-sulphonic acid	8.60	8.60	1.00	−	−	9.80	9.80	1.00	−	−

Application of the method

　　One of the main aims of our work was to find suitable chromatographic sys-
tems that permit the investigation of the optical purity of eburnane alkaloids of
biomedical interest. Vincamine has been used in medical practice for a long time and
its optical purity is tested by measuring its optical rotation. The chromatograms of a
(+)-*cis*-vincamine sample without and with addition of 1.0 and 0.5% of (−)-*cis*-
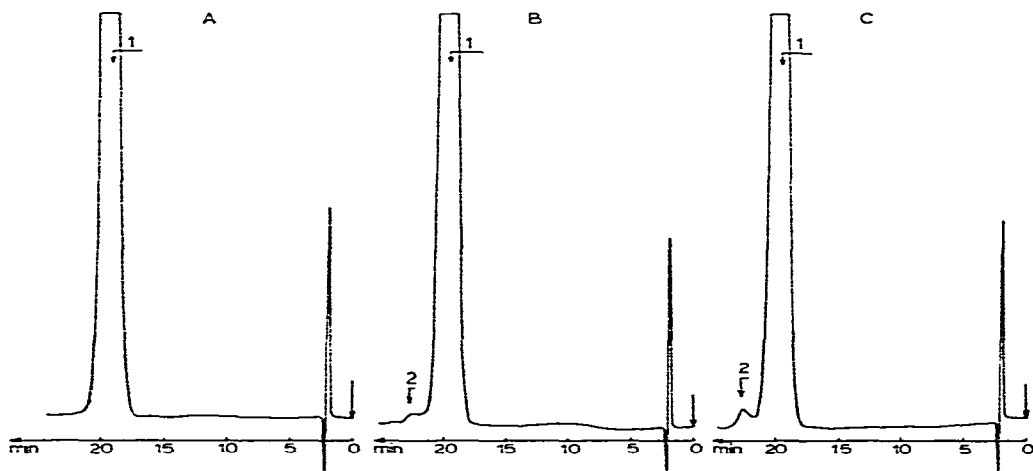vincamine using hexane–dioxane–1-butanol as the eluent are shown in Fig. 4.

Fig. 4. Separation of isomer impurities of vincamine. Chromatograms: (A) original sample; (B) sample
solution with 0.5% of (−)-*cis*-vincamine added to the original sample; (C) sample solution with 1.0% of
(−)-*cis*-vincamine added to the original sample. Instrument and conditions as in Fig. 3A. Compounds:
1 = (+)-*cis*-vincamine; 2 = (−)-*cis*-vincamine.

Fig. 5 shows the separation of isomer impurities present in a (−)-*cis*-vincamine sample. Hexane–chloroform–ethanol was used as the eluent and the separation was performed on a 5-μm Nucleosil CN column.

Apovincaminic acid ethyl ester (vinpocetine). produced by Chemical Works of Gedeon Richter, was introduced into medical practice a few years ago. The proposed method is also suitable for the investigation of the optical purity of this substance, as is illustrated in Fig. 6 by adding 0.1, 0.2 and 0.5 % of (−)-*cis*-apovincaminic acid ethyl ester to the original sample. The same separation conditions were as used for vincamine.

From the results in Figs. 4–6, it can be concluded that optical isomer impurities of the compounds can be detected in low concentration ranges, providing a good possibility for their accurate determination.

The example of the separation of eight optical isomers of vincamine (four diastereomers and four enantiomers) illustrates well the versatility of the proposed
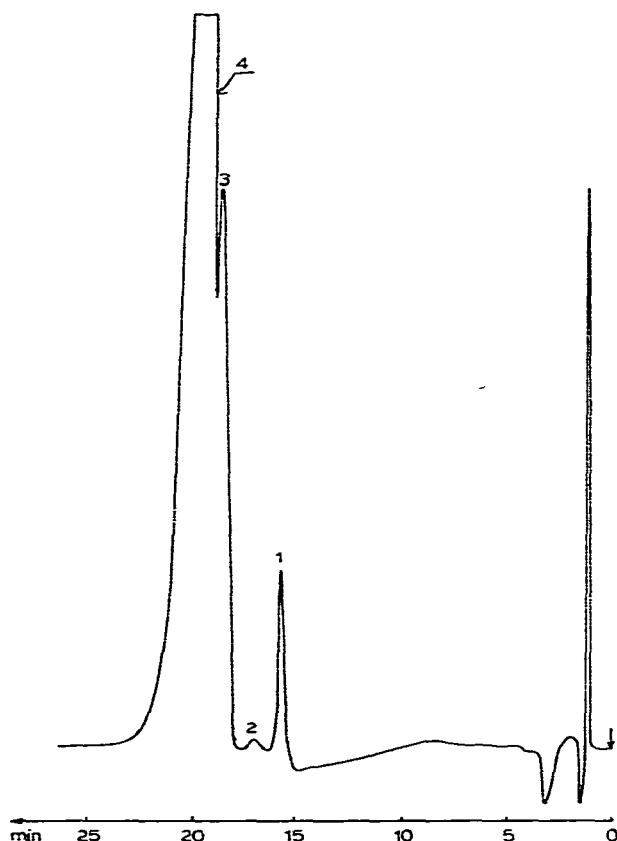
Fig. 5. Separation of optical isomer impurities present in (−)-*cis*-vincamine sample. Instrument, Varian 8500; column, Nucleosil 5 CN (150 × 4.6 mm I.D.); eluent, hexane–chloroform–ethanol (80:18:2) containing $2 \cdot 10^{-3}$ mole/l of (+)-CSA and 10^{-3} mole/l of DEA; flow-rate, 2 ml/min; detection at 280 nm. Compounds: 1 = (+)-*cis*-epivincamine; 2 = (−)-*cis*-epivincamine; 3 = (+)-*cis*-vincamine; 4 = (−)-*cis*-vincamine.
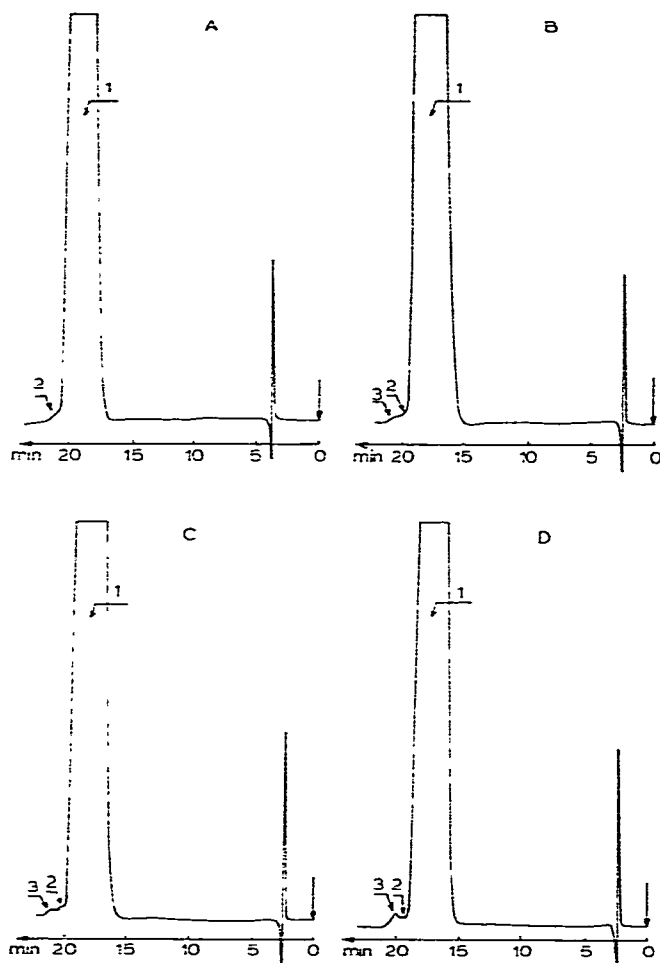
Fig. 6. Separation of (−)-*cis*-apovincaminic acid ethyl ester impurity in vinpocetine. Instrument and operating conditions as in Fig. 4. Chromatograms: (A) vinpocetine sample; (B) vinpocetine sample spiked with 0.1 % of (−)-*cis*-isomer; (C) vinpocetine sample spiked with 0.2 % of (−)-*cis*-isomer; (D) vinpocetine sample spiked with 0.5 % of (−)-*cis*-isomer. Compounds: 1 = vinpocetine; 2 = (+)-*cis*-apovincamine; 3 = (−)-*cis*-apovincaminic acid ethyl ester.

method. Fig. 7 shows the structure of the optical isomers and the chromatogram obtained by using the optimal eluent composition for the separation (see Table VI) can be seen in Fig. 8.

Fig. 8 shows that the method gives a satisfactory separation of the compounds investigated and provides good selectivity for the different types of optical isomers, namely the *trans*-isomers (VII, VIII. IX and X) are more retarded on the column than the *cis*-isomers (III, IV, V and VI), the 14-*epi*-isomers (III, IV and VII, VIII, respectively) are eluted before the corresponding normal isomers (V, VI and IX, X, respectively), and the (−)-isomers. as was mentioned, have higher retentions than the corresponding (+)-isomers.
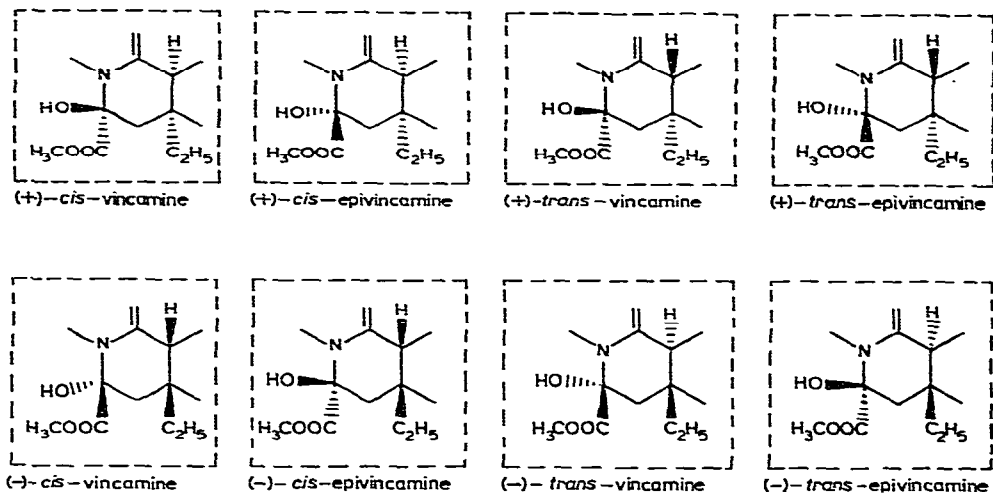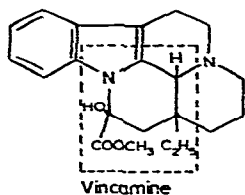
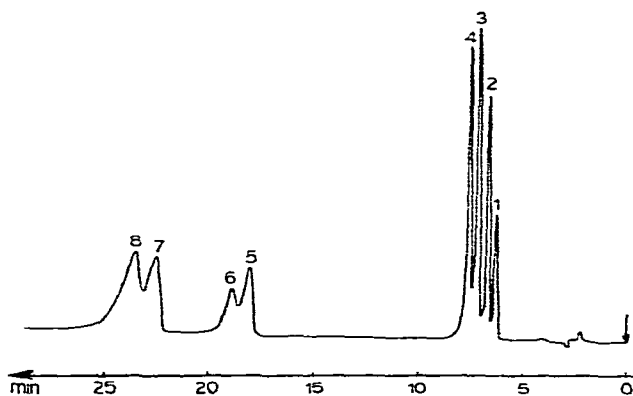Fig. 7. Structure of vincamine optical isomers.

Fig. 8. Separation of eight optical isomers of vincamine. Eluent, hexane–chloroform–ethanol (70:27:3) containing $2 \cdot 10^{-3}$ mole/l of (+)-CSA and 10^{-3} mole/l of DEA; flow-rate, 1 ml/min. Instrument and other conditions as in Fig. 5. Compounds: 1 = (+)-*cis*-epivincamine; 2 = (−)-*cis*-epivincamine; 3 = (+)-*cis*-vincamine; 4 = (−)-*cis*-vincamine; 5 = (+)-*trans*-epivincamine; 6 = (−)-*trans*-epivincamine; 7 = (+)-*trans*-vincamine; 8 = (−)-*trans*-vincamine.

CONCLUSIONS

It can be concluded that normal-phase dynamic (solvent-generated) molecular complexation chromatography using a chiral anionic ion exchanger can be advantageously applied for the enantiomeric separation of alkaloids. This system provides good selectivity and efficiency for the separation, is highly stable and is not sensitive to the water content of the solvents used for eluent preparation.

In our opinion, with flexible changes in the experimental conditions such as the suitable selection of the moderator solvent and solvent composition according to the particular analytical problem and trying other chiral complexing reagents, etc., the method can be generally used for the separation of optical isomers of ionizable organic substances. Therefore, chiral cationic ion exchangers can be utilized for the separation of enantiomeric organic acids. Work on this aspect is in progress.

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