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INCLUSION CHROMATOGRAPHY OF ENANTIOMERS OF INDOLE ALKALOIDS ON A CYCLODEXTRIN POLYMER STATIONARY PHASE

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SUMMARY

The chromatographic behaviour (retention and selectivity) of enantioners of a series of indole alkaloids (vincadifformine, aspidospermidine, vincadine, quebrachamine, N-methylquebrachamine, vincamine, apovincamine and eburnamonine) as studied on a stationary phase comprising a polyether-type β-cyclodextrin bead-polymer. Under appropriate conditions, different retention and selectivity could be achieved for enantiomers. Racemic vincadifformine and quebrachamine were resolved in very good yield and optical purity also on a preparative scale, simply by passing their slightly acidic aqueous solutions through a chromatographic column filled with β-cyclodextrin bead-polymer.

INTRODUCTION

The separation of various isomers, particularly enantiomers, represents a potential field of application for cyclodextrins. The separation of enantiomers is based on the differences in physical or chemical properties (*e.g.,* solubility, crystallizability, stability) of the diastereomeric inclusion complex pairs formed from the chiral "host" cyclodextrin (or substituted cyclodextrin) and the "guest" enantiomer pairs. The results achieved previously have been summarized in several reviews[1-4] and papers[5-9].

The traditional batchwise resolution of enantiomer mixtures is laborious even with cyclodextrins, and involves significant losses; at best a moderate degree of optical purity can be obtained with multiple crystallization.

Cyclodextrins maintain their complex-forming ability in polymer structures and in fact more stable complexes are usually formed with these so-called cyclodextrin polymers than with the "monomer" ones. Applying cross-linked cyclodextrin polymers as stationary phases, chromatographic separation can be achieved by means of inclusion chromatography based mostly, although not exclusively, upon the re-

versible complexation of various guest molecules, which is characterized by different stability constants[3,4].

Harada et al.[6] first attempted the separation of enantiomers of mandelic acid on a chromatographic column filled with cyclodextrin polymer. The partial but significant separation efficiencies published show that inclusion chromatography may be a useful method for the resolution of racemates.

In a study of the chromatographic behaviour of alkaloids on a cyclodextrin polymer column packing, we found that some enantiomers showed different retention volumes. The partial separation of (−)-vincadifformine and (+)-vincadifformine was reported[8].

The present paper describes the inclusion chromatography of a series of enantiomers of indole alkaloids on a β-cyclodextrin bead-polymer used as column packing. The aims were: to select the best column packing; to optimize the conditions and to illustrate the utility of the method for preparative purposes.

EXPERIMENTAL

Alkaloids

Eight enantiomer pairs of the natural Vinca alkaloids[10] were studied. These were partly synthesized and partly isolated directly from plants.

Fig. 1 shows the model compounds in one of the two enantiomer series and illustrates their genetic and stereochemical relations. These compounds were synthesized from tabersonine (Iα), an indole alkaloid isolated from the seeds of Amsonia tabernaemontana[11], as described elsewhere[12–15]. In all of these molecules, the ethyl group attached to the chiral centre of key importance (C_5 or C_{16}, respectively) has an α-disposition, hence the index α. On the other hand, the optical antipodes were synthesized from (+)-vincadifformine (IIβ), an indole alkaloid isolated from the leaves of Amsonia tabernaemontana[16] (cf., Fig. 2). The configuration of the chiral centres in these enantiomers is the opposite of those in Fig. 1, and correspondingly the ethyl group attached to the centre of key importance (C_5 or C_{16} respectively) has a β-disposition, hence the index β.

The eight enantiomer pairs studied were as follows:

(−)-vincadifformine (IIα) and (+)-vincadifformine (IIβ)
(−)-aspidospermidine (IIIα) and (+)-aspidospermidine (IIIβ)
(+)-vincadine (IVα) and (−)-vincadine (IVβ)
(+)-quebrachamine (Vα) and (−)-quebrachamine (Vβ)
N-methyl-(+)-quebrachamine (VIα) and N-methyl-(−)-quebrachamine (VIβ)
(+)-vincamine (VIIα) and (−)-vincamine (VIIβ)
(+)-apovincamine (VIIIα) and (−)-apovincamine (VIIIβ)
(−)-eburnamonine (IXα) and (+)-eburnamonine (IXβ)

β-Cyclodextrin bead-polymer

β-Cyclodextrin bead-polymer is a pilot product of Chinoin Pharmaceutical and Chemical Works, Budapest, prepared according to a patented procedure[17,18]; its characteristics were recently reported[19].

The polymer designated as CDP-25 was found to be the most appropriate for the present purposes. It was prepared in the form of small spherules (beads) from
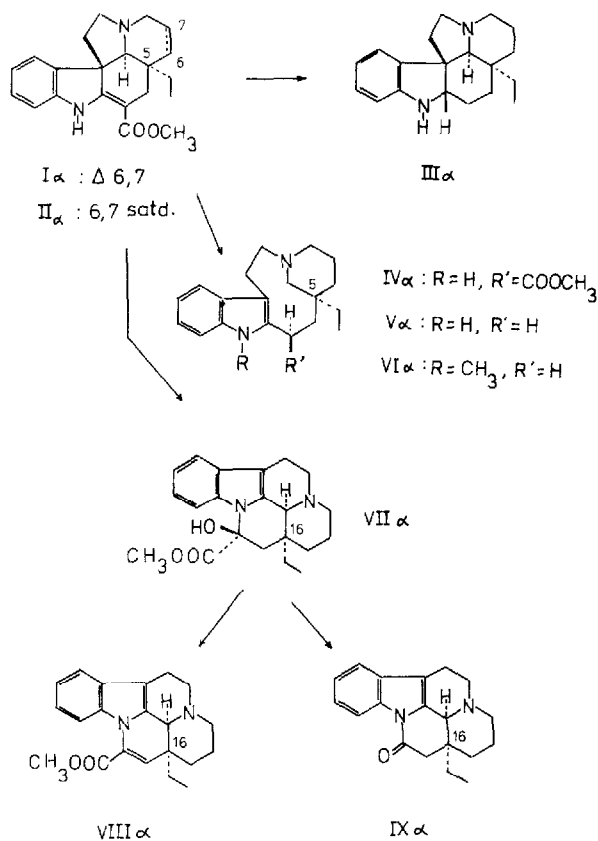
Fig. 1. Genetic and stereochemical relations of the alkaloids synthesized from tabersonine (Iα).

1 M β-cyclodextrin, 4 M epichlorohydrin, 2.6 M ethyleneglycolbis(epoxypropyl ether) and 0.14 M polyvinyl alcohol. The polymer product consisted of 58% β-cyclodextrin and 0.3% polyvinyl alcohol. After separating by sieving, the particle sizes of the two main fractions were 63–90 μm and 90–125 μm; their swelling capacity is illustrated by their gel-bed volumes, 5.0 ml/g and 5.3 ml/g dry polymer, respectively.

Slightly acidic (pH 4–5.5) phosphate and citrate buffers, in which the alkaloids were sufficiently soluble, were used for swelling the cyclodextrin polymer column packing and for elution. An equivalent amount of hydrochloric acid was added to
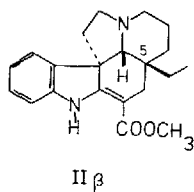

Fig. 2. (+)-Vincadifformine (IIβ), the precursor of the optical antipode series.

the alkaloid bases, and the resulting alkaloid salts were dissolved in the buffers to give stock solutions (2–2.5 mg/ml); aliquots of these were transferred to the column.

For testing small quantities (several mg), a Pharmacia K 16/100 analytical column (100 × 1.6 cm, with thermostatted jacket), and for preparative purposes (over 100 mg) a K 50/100 column (100 × 5 cm) were used.

The alkaloid concentration in the eluate was detected by UV spectrophotometry (LKB Uvicord III absorptiometer). The eluate fractions were collected by an automatic fraction-collector. All chromatographic separations were performed at room temperature.

In the case of preparative separations, the slightly acidic eluate fractions were treated with alkali, the alkaloid bases were extracted with chloroform and after drying with anhydrous Na_2SO_4 and evaporating the organic phase a crude crystalline product was obtained, which was recrystallized from ethanol.

RESULTS AND DISCUSSION

Efficiency of resolution and its dependence on experimental conditions

Preliminary experiments showed our β-cyclodextrin bead-polymer designated as CDP-25 to be suitable as a stationary phase for chromatographic separation of optical antipodes. Aspects of the choice of the most suitable cyclodextrin polymer product for this purpose were as follows: separation of optical antipodes with the highest selectivity; stable and homogeneous column packing with the polymer gel swelled in aqueous buffer; a suitable hydrodynamic behaviour and as high a resolution capacity as possible.

Systematic experiments were performed in aqueous buffer solutions with our CDP-25 β-cyclodextrin bead-polymer and with (−)-vincadifformine (IIα) and (+)-vincadifformine (IIβ) as model enantiomers, in order to determine the most favourable conditions for resolution. It was found that the specific elution volume of the two enantiomers, V_e/V_t, where V_e = elution volume of the chromatographic peak and V_t = total volume of swelled column packing, as well as their ratio (selectivity

TABLE I

SPECIFIC ELUTION VOLUMES, V_e/V_t, AND SELECTIVITY FACTORS OF VINCADIFFORMINE ENANTIOMERS

Stationary phase: CDP-25 β-cyclodextrin bead-polymer (80 × 1.6 cm). Flow-rate: 40–60 ml/h. V_e = Elution volume; V_t = total volume of swelled column packing.

Particle size (μm)	Eluent	V_e/V_t		Selectivity factor
		(−)-Vincadifformine (IIα)	(+)-Vincadifformine (IIβ)	
90–125	Citrate buffer, pH 4.0	2.8	2.5	1.12
	Phosphate buffer, pH 5.0	3.1	2.6	1.19
	Phosphate buffer, pH 5.5	4.6	3.3	1.39
63–90	Phosphate buffer, pH 5.0	3.3	2.5	1.32
	Phosphate buffer, pH 5.5	6.0	4.2	1.43
	Citrate buffer, pH 5.5	4.7	3.5	1.34

factor) depend on the pH and even on the chemical composition of the buffer (Table I). By increasing the pH, the selectivity could also be increased. This can be interpreted by the fact that the non-ionized (non-protonated) bases generally form more stable inclusion complexes than the ionized (and highly hydrated) ones. However, at pH > 5.5 the solubility of the very slightly basic vincadifformine decreases rapidly and its retention increases enormously.

At some pH values a higher selectivity was achieved with phosphate buffer than with citrate buffer. Phosphate buffer at pH 5.5 proved to be optimal, the selectivity factor in this case being 1.40–1.43; this is satisfactory for a very good chromatographic separation.

The resolution efficiency depends on the particle size of the stationary phase and on its distribution. This is partly determined by the height equivalent to a theoretical plate (HETP). For example, under the conditions given in Table I, vincadifformine resulted in the following HETP values at pH 5.5, regarded as optimal: 90–25 μm, phosphate buffer (pH 5.5), HETP = 2.5–3.0 mm; 63–90 μm, phosphate buffer (pH 5.5), HETP = 1.5–2.0 mm; 63–90 μm, citrate buffer (pH 5.5), HETP = 2.5–3.0 mm.

Under or at near optimum conditions, total separation of vincadifformine enantiomers could be achieved. Fig. 3 shows a complete, baseline chromatographic resolution of 8 mg racemic ($\pm$)-vincadifformine.

Comparison of separation effects achieved with different enantiomer pairs

Table II indicates the results of an inclusion chromatographic study on eight different indole alkaloid enantiomer pairs. In every case, 4 mg of the enantiomers were chromatographed under the same experimental conditions on the same column.

The model compounds are divided into three groups in Table II: (1) alkaloids (vincadifformine and aspidospermidine) having aspidospermine structure; (2) alkaloids with quebrachamine structure (quebrachamine, N-methylquebrachamine and vincadine); (3) alkaloids of eburnane structure (apovincamine, eburnamonine and vincamine).

As is seen, great differences were observed in the specific elution volumes and selectivity factors. The best selectivity factor (1.43) was found for vincadifformine.
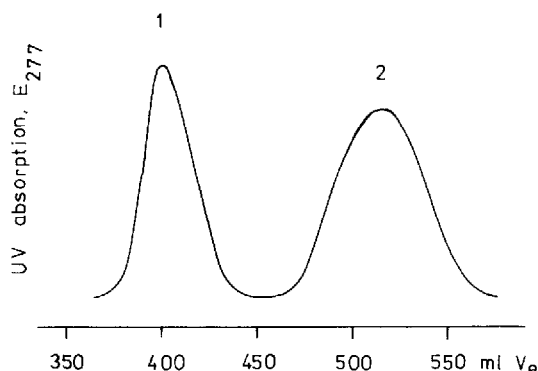

Fig. 3. Complete resolution of 8 mg racemic ($\pm$)-vincadifformine by inclusion chromatography. Stationary phase: CDP-25 β-cyclodextrin bead-polymer (80 $\times$ 1.6 cm), particle size 63–90 μm. Eluent: phosphate buffer, pH 5.0. Peaks: 1 = ($+$)-vincadifformine (IIβ); 2 = ($-$)-vincadifformine (IIα).

TABLE II

COMPARATIVE STUDY OF SPECIFIC ELUTION VOLUMES, V_e/V_t, AND SELECTIVITY FACTORS OF INDOLE ALKALOID ENANTIOMERS

Stationary phase: CDP-25 β-cyclodextrin bead-polymer (80 × 1.6 cm) of particle size 63–90 μm. Eluent: phosphate buffer, pH 5.5. Flow-rate: 40 60 ml/h.

Alkaloid	$[\alpha]_D^{20}$ (in ethanol) (°)	V_e/V_t		Selectivity factor
		α Enantiomer	β Enantiomer	
Vincadifformine (II)	600	6.0	4.2	1.43
Aspidospermidine (III)	17	1.58	1.73	1.09
Quebrachamine (V)	114	4.75	3.98	1.19
N-Methylquebrachamine (VI)	100	3.48	3.63	1.04**
Vincadine (IV)	92	1.82	1.89	1.04**
Apovincamine (VIII)	140	3.85	3.58	1.08
Eburnamonine (IX)	92	5.6	5.3	1.06
Vincamine (VII)	41*	1.69	1.72	1.02**

* In pyridine.
** Values below 1.05 are not significant.

This can be explained only partly by the fact that the experimental conditions were optimal just for the vincadifformine. Within each structural type of alkaloids, the highest selectivity factor was found for the highest specific optical activity, that is for a higher degree of asymmetry. For example, vincadifformine has both an extremely high optical activity and a very large selectivity factor. However, no comparison can be made between compounds belonging to different structural types, since the retention can be influenced by other factors due to the chemical structure.

It is also to be noted that the optimum experimental conditions can be different for different alkaloids. Consequently, under different circumstances, the chromatographic retentions and selectivity factors may have different values.

Preparative chromatographic resolution

The applicability of the cyclodextrin bead-polymer stationary phase for preparative resolution of racemic alkaloid mixtures was tested with vincadifformine and quebrachamine, since maximum selectivity factors were observed just for these two alkaloids.

Fig. 4 shows the resolution of 500 mg racemic (±)-vincadifformine in phosphate buffer (pH 5.5) solution on a preparative column filled with β-cyclodextrin bead-polymer (90–125 μm) gel bed of size 90 × 5 cm. Two fractions of eluate were collected: one from 6.5 to 9 l and the other from 9 to 12 l.

The product isolated from fraction 1 was 230 mg crude (+)-vincadifformine, $[\alpha]_D^{20}$ + 510° (in ethanol); recrystallized from ethanol, $[\alpha]_D^{20}$ + 580° (in ethanol); optically pure (+)-vincadifformine (IIβ), $[\alpha]_D^{20}$ + 600° (in ethanol)[16]. Based on the last value, the crude product had an optical purity of 92.5%, the recrystallized one a purity of 98.2%. These are excellent results, particularly considering the 92% yield of the crude product.

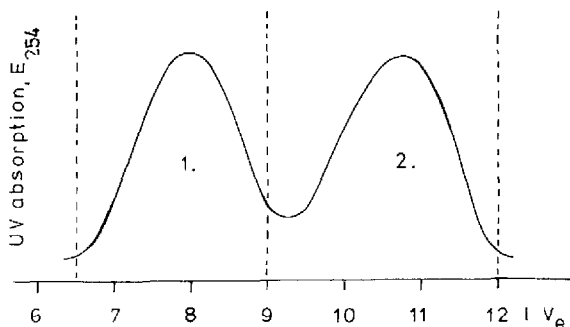The product isolated from fraction 2 was 245 mg (−)-vincadifformine (98%),

Fig. 4. Preparative resolution of 500 mg racemic ($\pm$)-vincadifformine by inclusion chromatography. Stationary phase: CDP-25 β-cyclodextrin bead-polymer (90 $\times$ 5 cm), particle size 90 125 μm. Eluent: phosphate buffer, pH 9.5. Peaks as in Fig. 3.

$[\alpha]_D^{20}$ $-450°$ (in ethanol); after recrystallization, $[\alpha]_D^{20}$ $-510°$ (in ethanol). The optical purity was 87.5% and 92.5%, respectively.

The loading capacity of the preparative column was tested by increasing the amount of the racemic mixture. Separation was achieved at higher loadings, but as expected, the optical purity of both of the enantiomer products somewhat decreased. For example, resolving 800 mg racemic ($\pm$)-vincadifformine under the same circumstances as above gave 350 mg (87.5%) crude ($+$)-vincadifformine from the first eluate fraction, $[\alpha]_D^{20}$ $+490°$ (in ethanol); after recrystallization, $[\alpha]_D^{20}$ $+500°$ (in ethanol). The optical purity was 90.8% and 95%, respectively. Processing the second eluate fraction resulted in 380 mg (95%) crude ($-$)-vincadifformine, $[\alpha]_D^{20}$ $-380°$ (in ethanol); after recrystallization, $[\alpha]_D^{20}$ $-450°$ (in ethanol). In this case the optical purity was 81.6% and 87.5%, respectively.

On the same preparative column, the quebrachamine was also well resolved in a phosphate buffer of pH 5.0, but only in much lower quantities. This is due to the lower selectivity factor of quebrachamine (see Table II).

Fig. 5 shows the resolution of 200 mg racemic ($\pm$)-quebrachamine (V). Processing eluate fraction 1 resulted in 70 mg (70%) crude ($-$)-quebrachamine (Vβ),
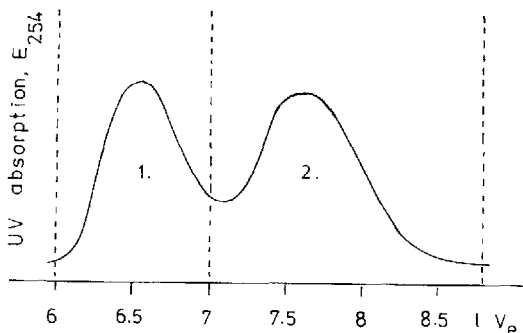

Fig. 5. Preparative resolution of 200 mg racemic ($\pm$)-quebrachamine by inclusion chromatography. Stationary phase as in Fig. 4. Eluent: phosphate buffer, pH 5.0. Peaks: 1 = ($-$)-quebrachamine (Vβ); 2 = ($+$)-quebrachamine (Vα).

$[\alpha]_D^{20}$ $-110°$ (in ethanol); after recrystallization, $[\alpha]_D^{20}$ $-114°$ (in ethanol). The optical purity was 98% and 100% respectively, since for authentic ($-$)-quebrachamine (Vβ), $[\alpha]_D^{20}$ $-114°$ (in ethanol)[14]. The result of processing eluate fraction 2 gave 95 mg (95%) crude ($+$)-quebrachamine (Vα), $[\alpha]_D^{20}$ $+78°$ (in ethanol); after recrystallization, $[\alpha]_D^{20}$ $-106°$ (in ethanol). The optical purity was 84% and 95% respectively.

CONCLUSIONS

Under appropriate conditions, the cyclodextrin polymers used as stationary phase are able to retain enantiomers to different extents and — in the case of adequate selectivity — this enables the separation of mixtures of optical antipodes (enantiomers) by inclusion chromatography. The specific elution volume and the selectivity depend partly on the individual enantiomer pairs, partly on the composition, structure, method of preparation and particle size of the cyclodextrin polymer, as well as on the experimental conditions.

The experimental results, mostly achieved in the analytical and preparative resolution of the enantiomers of the indole alkaloids vincadifformine and quebrachamine, are similar to those achieved with other chiral stationary phases in the field of the chromatographic resolution[20]. The cyclodextrin bead-polymers have the additional advantage in that they can be produced in a relatively simple way from inexpensive and readily available raw materials, and have numerous variations.

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