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Note

Variation of the selectivity in the resolution of alkaloid enantiomers on cross-linked cyclodextrin polymer stationary phases

B. ZSADON*, M. SZILASI and L. DÉCSEI
Department of Chemical Technology, Eötvös Loránd University, Muzeum krt 6-8, Budapest (Hungary)
and
A. UJHÁZY and J. SZEJTLI
Biochemical Research Laboratory, Chinoin Pharmaceutical and Chemical Works, Endrödi S. u. 38, Budapest (Hungary)
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An effective use of cross-linked cyclodextrin polymers as stationary phases both for analytical and preparative separation of optical isomers was demonstrated in our earlier report on the inclusion chromatography of alkaloid enantiomers[1]. These experiments were performed on β-cyclodextrin bead polymer (β-CDP) in mildly acidic phosphate buffer solutions, mostly at pH 5–5.5, with eight non-functionalized enantiomer pairs belonging to different structural types of natural indole alkaloids.

Two of our alkaloids, vincadifformine and quebrachamine, showed excellent enantioselectivity and this enabled the baseline separation and the isolation of their enantiomers in good optical purity on the analytical and preparative scales. However, the other alkaloids showed poorer selectivity (values of less than 1.1 for the selectivity factor, α) and we were not very successful in achieving their resolution under the given experimental conditions. Therefore, further experiments were designed in order to clarify how the selectivity can be influenced through the conditions, namely by altering the pH of the mobile phase and using both α-cyclodextrin polymer (α-CDP) and β-CDP as stationary phases. Our investigations were directed also towards the chromatographic behaviour of different chemical structures under various experimental conditions.

EXPERIMENTAL

Fractions of α-CDP and β-CDP of particle size 80–125 μm and of medium swelling capacity were used, the preparation and characteristics of which have been outlined earlier[1].

Column chromatography was performed in phosphate buffer solutions [dibasic sodium phosphate (0.0667 mol/l) and monobasic potassium phosphate (0.0667 mol/l)] at atmospheric pressure, room temperature and a flow-rate of 40 ml/h, using automatic equipment consisting of Pharmacia columns (1.6 cm I.D.), an LKB MultiPerpex pump, LKB Ultro-Rac fraction collector and LKB flat-bed recorder. The

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alkaloids were injected in amounts of 0.01 mmol (3–4 mg) and detected continuously in the eluates by UV-absorption.

From the indole alkaloids studied previously we chose the enantiomer pairs: (−)-vincadifformine (Iα) and (+)-vincadifformine (Iβ); (+)-quebrachamine (IIα) and (−)-quebrachamine (IIβ); (+)-vincadine (IIIα) and (−)-vincadine (IIIβ); (+)-vincamine (IVα) and (−)-vincamine (IVβ). These belong to three different structural types, *i.e.*, compound I to the aspidospermidine type, II and III to the quebrachamine type and IV to the eburnane type. The indexes α and β indicate the stereochemistry of the ethyl group attached to the chiral centres of importance, C_5 and C_{16}, respectively (*cf.*, Fig. 1).

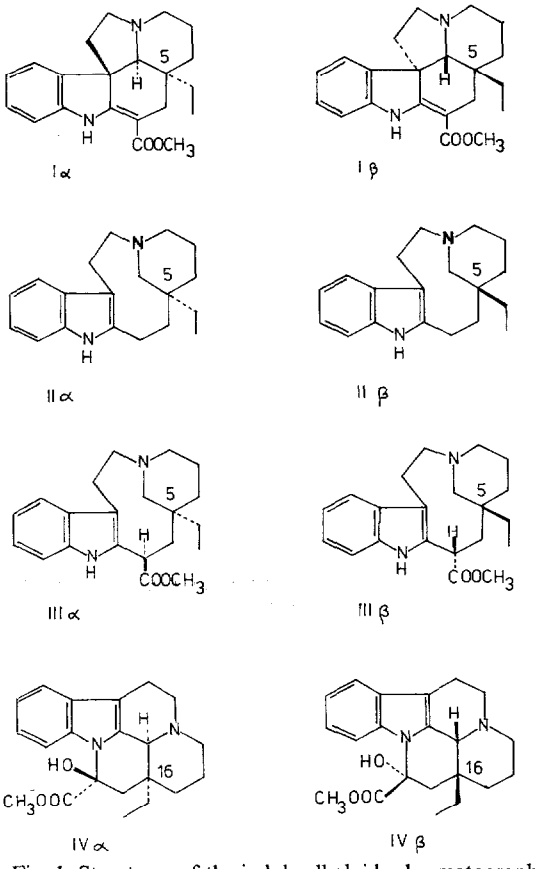


Fig. 1. Structures of the indole alkaloids chromatographed on CDP stationary phases.

RESULTS AND DISCUSSION

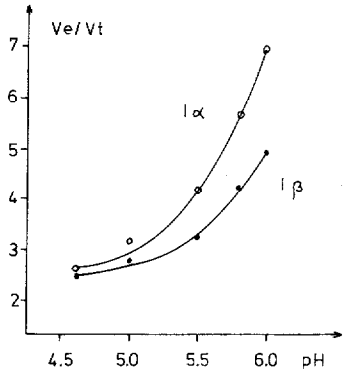
The present observations correspond to the earlier ones, insofar as it was found that the chromatographic retention of the model alkaloids on the CDP stationary phases increased with increasing pH of the mobile phase. However, the magnitude of the pH which could be employed was limited by the decreasing solubility of the

alkaloids. They are insoluble or dissolve only sparingly in water as free bases. Therefore, the limit was pH 6–7.

The strongest dependence on the pH was found in the case of vincadifformine I on β-CDP as stationary phase. Fig. 2 shows that the relative elution volumes, V_e/V_t, for the optical antipodes (−)-vincadifformine (Iα) and (+)-vincadifformine (Iβ) had different values, and not only the absolute values but also their difference and their ratio strongly increased with increasing pH. The chromatographic behaviour of quebrachamine (II) and vincadine (III) was qualitatively similar to that of vincadifformine (I), while the vincamine enantiomers (IVα and IVβ) showed little difference in retention and their retention did not depend significantly on the pH of the mobile phase on β-CDP.

Fig. 3 shows the dependence of the selectivity factor, α (quotient of the greater and smaller relative elution volume), on the pH of the mobile phase. It is seen that a high degree of selectivity exists for vincadifformine (I), quebrachamine (II) and vincadine (III) on β-CDP at pH $\geqslant$ 6.

The chromatographic characteristics of the enantiomers of vincadifformine (I), quebrachamine (II) and vincadine (III) were determined also on α-CDP as stationary phase. In Table I the data obtained on α-CDP and β-CDP at the pH values where the greatest selectivities were found are compared. The results demonstrate that the enantiomers belonging to these structural models can be separated more effectively on β-CDP (*cf.*, Fig. 4). On the other hand, α-CDP proved to be suitable for the separation of the enantiomers (+)-vincamine (IVα) and (−)-vincamine (IVβ). On this stationary phase these enantiomers showed different retentions, and their relative elution volumes, V_e/V_t, and selectivity increased with increasing pH (*cf.*, Fig. 5). The chromatographic characteristics of the vincamine enantiomers on α- and β-CDP at pH 7 are summarized in Table II. As expected, these enantiomers could be separated on α-CDP at pH 7 (Fig. 6).



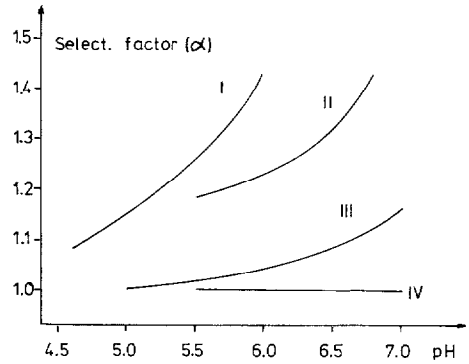


Fig. 2. The relative elution volume, V_e/V_t, of (−)-vincadifformine (Iα) and (+)-vincadifformine (Iβ) as a function of the pH of the phosphate buffer mobile phase, on β-CDP as stationary phase.

Fig. 3. The selectivity factor, α, for the optical antipodes of vincadifformine (I), quebrachamine (II), vincadine (III) and vincamine (IV) plotted against the pH of the phosphate buffer mobile phase, on β-CDP as stationary phase.

TABLE I

CHROMATOGRAPHIC CHARACTERISTICS OF VINCADIFFORMINE, QUEBRACHAMINE AND VINCADINE ENANTIOMERS ON α- AND β-CDP STATIONARY PHASES

Compound	*pH*	*Stationary phases*	V_e/V_t		*Selectivity factor, α*
			α	β	
Vincadifformine (I)	6.0	β-CDP	7.05	4.90	1.44
		α-CDP	4.90	4.10	1.17
Quebrachamine (II)	6.8	β-CDP	6.90	4.80	1.44
		α-CDP	6.85	6.70	1.02*
Vincadine (III)	7.0	β-CDP	4.50	5.25	1.17
		α-CDP	5.90	5.70	1.03*

* Values below 1.05 are not significant.

CONCLUSIONS

The resolution of enantiomers on a chiral CDP stationary phase can be attributed to reversible formation of diastereoisomeric inclusion complexes having different stabilities. In general, the chance of success is determined primarily by the choice of the most suitable "host" CDP, less so by the dimensions and stereochemistry of the "guest" molecules.

At the moment it would be hazardous to describe as universal any correlation between the chance of success and the structure of complicated molecules such as the alkaloids studied. However, analogous chromatographic behaviours were observed and moderate predictions could be made for indole alkaloids of similar structure such as those of the aspidospermidine, quebrachamine or eburnane type.

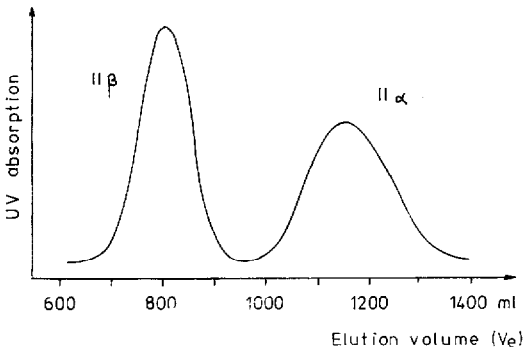


Fig. 4. Baseline resolution of the enantiomers (+)- and (−)-quebrachamine (IIα and IIβ, respectively) on β-CDP as stationary phase. Gel bed: 85 × 1.6 cm. Phosphate buffer: pH 6.8.

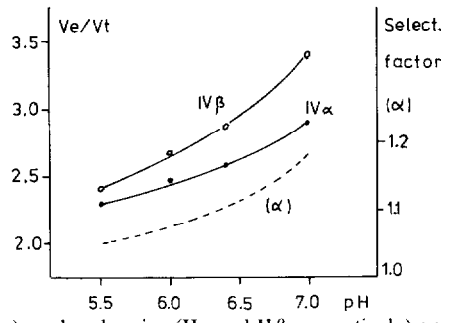


Fig. 5. The relative elution volumes (—○—) of the enantiomers (+)- and (−)-vincamine (IVα and IVβ) and the selectivity factor α (------) plotted against the pH of the phosphate buffer mobile phase, on α-CDP as stationary phase.

TABLE II

CHROMATOGRAPHIC CHARACTERISTICS OF VINCAMINE ENANTIOMERS ON α- AND β-CDP STATIONARY PHASES

Compound	*pH*	*Stationary phases*	V_e/V_t		*Selectivity factor, α*
			α	β	
Vincamine (IV)	7.0	α-CDP	2.90	3.40	1.19
		β-CDP	1.90	1.90	1.00

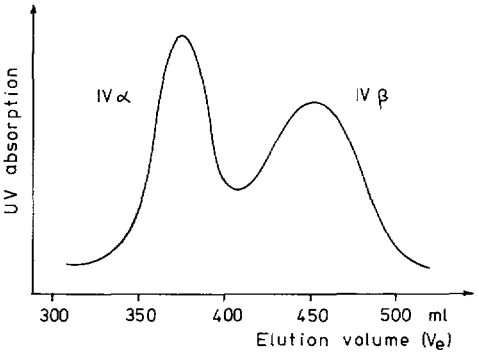


Fig. 6. Chromatography of (+)-vincamine (IVα) and (−)-vincamine (IVβ) on α-CDP as stationary phase. Gel bed: 70 × 1.6 cm. Phosphate buffer: pH 7.

The selectivity for the given enantiomer pairs was found to be sensitive also to the pH of the aqueous mobile phase and could be varied within certain limits. By choosing the most suitable stationary phase (α- or β-CDP) and the optimum pH, good enantioselectivity and effective resolution could be achieved in the case of the model indole alkaloids.

The present observations confirm the conclusion that cross-linked cyclodextrin polymers can serve as stationary phases mainly for specific preparative chromatographic purposes, *e.g.*, for the preparation of enantiomers in good optical purity.

It should be mentioned that very impressive results have been obtained recently by the group of Hinze and Armstrong[2,3] in the fast microanalytical determination of the enantiomeric purity of derivatized amino acids, barbiturates, substituted phenylacetic acids and dioxolanes by high-performance liquid chromatography on a β-CD-bonded stationary phase.

REFERENCES

1 B. Zsadon, L. Décsei, M. Szilasi, F. Tüdős and J. Szejtli, *J. Chromatogr.*, 270 (1983) 127 and references therein.
2 W. L. Hinze, T. E. Riehl, D. W. Armstrong, W. DeMond, Ala Alak and Tim Ward, *Anal. Chem.*, 57 (1985) 237.
3 D. W. Armstrong and W. DeMond, *J. Chromatogr. Sci.*, 22 (1984) 411.