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Note

Inclusion chromatography of alkaloids on cyclodextrin polymer gel beds

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Cyclodextrins form relatively stable inclusion complexes with aromatic compounds in aqueous media (a review of cyclodextrin chemistry was published by Saenger[1]). This effect is also shown by cyclodextrin polymers (CDP), which can be used for the separation of certain water-soluble aromatic compounds by inclusion chromatography on water-swollen CDP gel beds[1,2].

Previously, we reported the separation of natural aromatic amino acids on bead-formed α-, β- and γ-CDP gel beds[3]. This paper reports the inclusion chromatography of some indole alkaloids.

EXPERIMENTAL

Bead-formed β-CDP of medium swelling capacity[3] and indole alkaloids prepared in our laboratory were used.

Column chromatography was performed at atmospheric pressure using automatic equipment consisting of Pharmacia columns (1.6 cm I.D.), an LKB Multi-Perpex pump, an LKB UltroRac fraction collector, an LKB Uvicord III absorptiometer and/or a Thorn NPL automatic polarimeter and an LKB flat-bed recorder.

β-CDP was swollen in mildly acidic citrate buffer, then filled and equilibrated in the columns. The same buffer was used for dissolution of alkaloids and for elution. Alkaloids in the eluates were detected continuously by UV absorption at 280 or 340 nm and/or by the optical rotatory power.

Alkaloids

One of the alkaloids was tabersonine (I), isolated as the main alkaloid from ripe seeds of *Amsonia tabernaemontana*[4]. Further alkaloids, namely (—)-vincadifformine (II), vincamine (III), apovincamine (IV) and ethyl-apovincaminate (V), were genetically and stereochemically related to tabersonine (I) and were prepared from it[4,5]. (+)-Vincadifformine (VI), the optical antipode of (—)-vincadifformine (II), was isolated from green leaves of *Amsonia tabernaemontana*[6] and converted into (—)-

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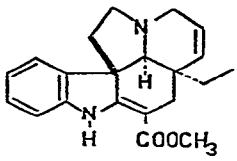
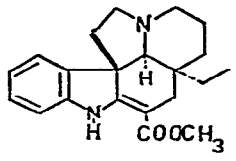
quebrachamine (VII), (—)-N-methylquebrachamine (VIII) and (—)-vincadine (IX)[7]. Some of these alkaloids are of therapeutic values (*e.g.*, vincamine and ethyl apovincaminate).



I



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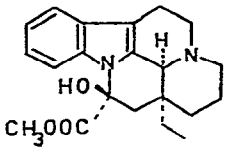
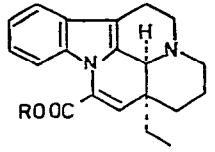
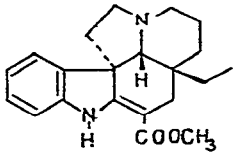


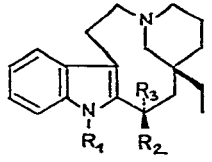
III



IV R = CH_3
V R = C_2H_5



VI



VII R_1 = H, R_2 = H, R_3 = H
VIII R_1 = CH_3, R_2 = H R_3 = H
IX R_1 = H, R_2 = H, R_3 = $COOCH_3$

RESULTS

All nine alkaloids were runned one by one on β-CDP gel beds with mildly acidic citrate buffer solutions under different conditions. Table I summarizes the statistically evaluated relative elution volumes (V_e/V_t) measured on a 28 × 1.6 cm β-CDP gel bed at room temperature and a flow-rate of 30 ml/h using pH 5 citrate buffer for swelling and elution.

All nine alkaloids had unexpectedly high retentions on the β-CDP gel bed under these conditions. The relative elution volumes (V_e/V_t) of the different alkaloids were significantly different in most instances, which made it possible to separate them by chromatography. For example, Fig. 1 shows the chromatography of (—)-vincadine (IX) and (—)-quebrachamine (VII), and Fig. 2 shows the separation of a mixture of vincamine (III) and apovincamine (IV).

It is particularly noteworthy that enantiomers had different retentions on the

TABLE I

RELATIVE ELUTION VOLUMES (V_e/V_t) OF INDOLE ALKALOIDS ON A β-CDP GEL BED AT ROOM TEMPERATURE USING pH 5 CITRATE BUFFER FOR SWELLING AND ELUTION

Alkaloid	*No.*	V_e/V_t
Tabersonine	I	1.8–1.9
(−)-Vincadifformine	II	2.2–2.3
Vincamine	III	1.3–1.4
Apovincamine	IV	2.6–2.7
Ethyl apovincaminate	V	3.0–3.1
(+)-Vincadifformine	VI	1.9–2.0
(−)-Quebrachamine	VII	2.9–3.0
(−)-N-Methyl-quebrachamine	VIII	2.2–2.3
(−)-Vincadine	IX	1.4–1.5

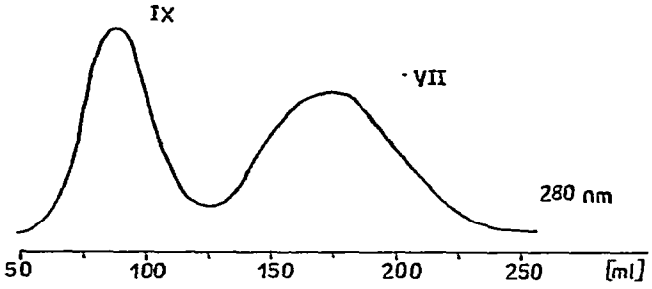


Fig. 1. Chromatogram of a mixture of 2 mg of (−)-vincadine (IX) and 3 mg of (−)-quebrachamine (VII) on a 28 × 1.6 cm β-CDP gel bed. Room temperature; flow-rate 30 ml/h; pH 5 citrate buffer; detection by UV absorption at 280 nm.

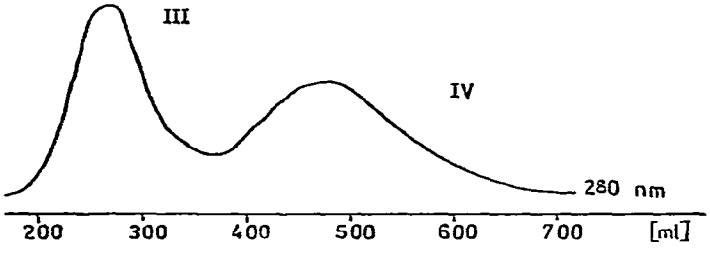


Fig. 2. Chromatogram of a mixture of 2 mg of vincamine (III) and 3 mg of apovincamine (IV) on a 90 × 1.6 cm β-CDP gel bed. Room temperature; flow-rate 80 ml/h; pH 5 citrate buffer; detection by UV absorption at 280 nm.

CDP gel bed containing optically active β-cyclodextrin, and this could provide the possibility of separating optical antipodes by chromatography. For example, Fig. 3 shows the partial resolution of a mixture of (+)-vincadifformine (VI) and (−)-vincadifformine (II).

Both the high and the different retentions of the alkaloids in mildly acidic aqueous media on CDP gel beds can be explained by the reversible formation of inclusion complexes or by a combined effect of complex formation and adsorption

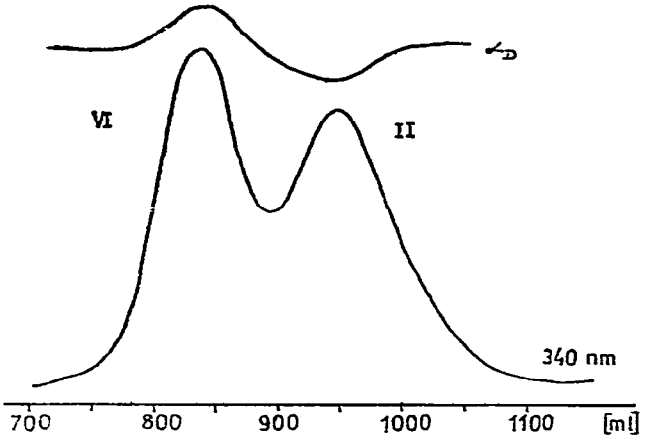


Fig. 3. Chromatogram of a mixture of 4 mg of (+)-vincadifformine (VI) and 4 mg of (−)-vincadifformine (II) on a 225 (= 3 × 75) × 1.6 cm β-CDP gel bed. Room temperature; flow-rate 50 ml/h; pH 4 citrate buffer; detection by UV absorption at 340 nm and by optical rotatory power.

rather than by adsorption. The same high and different effect was not observed on other carbohydrate-type gels under these conditions. For example, values of 1.05, 1.10, 1.10 and 1.20 were measured for the relative elution volumes (V_e/V_t) of vincamine, (−)-vincadifformine, (+)-vincadifformine and ethyl apovincaminate, respectively, on a column filled with Sephadex G-25 gel at room temperature using pH 5 citrate buffer for swelling and elution. On the other hand, no significant changes in retention was observed on CDP gel beds when the salt concentration in the eluates was altered or the temperature was varied between 5 and 35°C. These observations indicated that adsorption played only a minor role in the chromatography of the alkaloids on CDP gel beds.

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