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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF EBURNANE ALKALOIDS

I. SEPARATION ON REVERSED PHASES

G. SZEPESI* and M. GAZDAG
Chemical Works of Gedeon Richter, Ltd., Budapest (Hungary)

SUMMARY

Separation of eburnane alkaloids using reversed-phase high-performance liquid chromatography was investigated on μBondapak C_{18} and LiChrosorb RP-8 columns with acetonitrile–aqueous 0.01 *M* ammonium carbonate as eluent. The method can be successfully applied for the group separation of eburnane alkaloids as well as for the separation of stereoisomers and of ester homologues.

INTRODUCTION

In the last few years the importance of eburnane alkaloids in pharmacy has considerably increased. Besides vincamine, new representatives of eburnane alkaloids such as apovincaminic acid ethyl ester have been introduced in medical practice.

Only a few methods can be found in the literature for the separation of vincamine and apovincaminic acid ethyl ester. Recently, paper and thin-layer chromatography[1–7] as well as gas chromatography[8,9] have been used for solving special analytical problems. Considering the characteristics and structures of eburnane alkaloids, high-performance liquid chromatography (HPLC) seems to be the most appropriate method for their separation owing to the low selectivity and efficiency of thin-layer chromatography and the thermal instability and low volatility of many eburnane alkaloids.

In the present paper, we report studies using reversed-phase HPLC.

EXPERIMENTAL

The liquid chromatograph consisted of a Model OE-312 Liquopump reciprocating pump (Labor-MIM, Esztergom, Hungary), a Model OE-308 variable-wavelength UV detector (Labor MIM) and a Rheodyne 7120 loop injector (Rheodyne, Berkeley, CA, U.S.A.). The separations were performed on columns of μBondapak C_{18} (300 × 3.9 mm I.D.) (Waters Assoc., Frankfurt/M, G.F.R.) and of 10 μm

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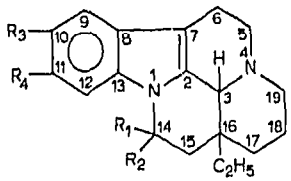
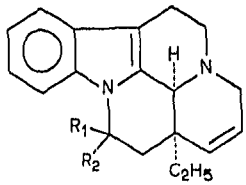
TABLE I

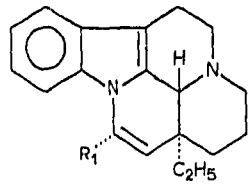
STRUCTURES OF EBURNANE ALKALOIDS INVESTIGATED



Type A
14,15-Dihydroeburnamenine



Type B
Eburnamenine



Type C
14,15-Dihydro-17,18-dehydro-eburnamenine

No.	*Alkaloid*	*Type*	*Substituents*				*Configuration of*			
			R_1	R_2	R_3	R_4	R_1	R_2	*3-H*	*16-*C_2H_5
I	(+)-*cis*-Vincaminic acid	A	OH	COOH	H	H	β	α	α	α
II	(+)-*cis*-Vincamine	A	OH	$COOCH_3$	H	H	β	α	α	α
III	(−)-*cis*-Vincamine	A	OH	$COOCH_3$	H	H	β	α	β	β
IV	(+)-*cis*-Epivincamine	A	OH	$COOCH_3$	H	H	α	β	α	α
V	(−)-*cis*-Epivincamine	A	OH	$COOCH_3$	H	H	α	β	β	β
VI	(+)-*cis*-Vincaminic acid ethyl ester	A	OH	$COOC_2H_5$	H	H	β	α	α	α
VII	(+)-*trans*-Vincaminic acid ethyl ester	A	OH	$COOC_2H_5$	H	H	β	α	β	α
VIII	(+)-*cis*-Epivincaminic acid ethyl ester	A	OH	$COOC_2H_5$	H	H	α	β	α	α
IX	(+)-*trans*-Epivincaminic acid ethyl ester	A	OH	$COOC_2H_5$	H	H	α	β	β	α
X	(+)-*cis*-Vincamone	A	O		H	H	—	—	α	α
XI	(+)-*cis*-Vincanole	A	OH	H	H	H	β	α	α	α
XII	(+)-*cis*-Isovincanole	A	OH	H	H	H	α	β	α	α
XIII	(+)-*cis*-10-Bromovincamine	A	OH	$COOCH_3$	Br	H	β	α	α	α
XIV	(+)-*cis*-11-Bromovincamine	A	OH	$COOCH_3$	H	Br	β	α	α	α
XV	(+)-*cis*-Apovincaminic acid	B	—	COOH	H	H	—	—	α	α
XVI	(+)-*cis*-Apovincamine	B	—	$COOCH_3$	H	H	—	—	α	α
XVII	(+)-*cis*-Apovincaminic acid ethyl ester	B	—	$COOC_2H_5$	H	H	—	—	α	α
XVIII	(+)-*trans*-Apovincaminic acid ethyl ester	B	—	$COOC_2H_5$	H	H	—	—	β	α
XIX	(+)-*cis*-Apovincaminic acid phenyl ester	B	—	$COOC_6H_5$	H	H	—	—	α	α
XX	(+)-*cis*-Vincamenine	B	—	H	H	H	—	—	α	α
XXI	(+)-*cis*-Dehydrovincamine	C	OH	$COOCH_3$	H	H	β	α	β	β
XXII	(+)-*cis*-Dehydroepivincamine	C	OH	$COOCH_3$	H	H	α	β	β	β

LiChrosorb RP-8 (250 × 4.6 mm I.D.) (Pierce Eurochemie, Rotterdam, The Netherlands).

The chemicals and solvents used were of analytical grade (Reanal, Budapest, Hungary). All solvents were freshly distilled and degassed before use. The compounds investigated were prepared in our laboratories and in the Institute for Organic Chemistry, Budapest Technical University, and were considered to be of the highest available quality.

RESULTS AND DISCUSSION

The structures of eburnane alkaloids investigated are summarized in Table I. According to their structures the eburnane alkaloids can be divided into three groups:

(a) vincamine type consisting of a 14,15-dihydroeburnamenine skeleton

(b) apovincamine type consisting of an eburnamenine skeleton

(c) dehydrovincamine type consisting of a 14,15-dihydro-17,18-dehydroeburnamenine skeleton.

Considering the structures and physico-chemical properties of the compounds (Table I), the analytical tasks can be summarized as follows: group separation of the three different types of eburnane alkaloids; separation of stereo- and structural isomers as well as of ester homologues; comparison of the separation possibilities both on octadecyl and octyl silica, and optimization of the separation system. Table II shows the capacity ratios, k', measured for the compounds both on octadecyl and octyl silica stationary phases using different mixtures of acetonitrile and aqueous ammonium carbonate as eluent.

It can be seen from Table II that the group separation of eburnane alkaloids can be achieved on both phases. The elution order of the compounds having similar structure is as follows: *cis*-dehydroepivincamine; *cis*-dehydrovincamine; *cis*-epivincamine; *cis*-vincamine; and *cis*-apovincamine.

As regards the separation of stereoisomers, some interesting conclusions can be drawn. For example, in case of vincaminic acid ethyl ester isomers (their structures are in Fig. 1) eight different stereoisomers should be found depending on the relative positions of the hydrogen atom and ethyl group in positions 3 and 16, and of the ester and hydroxy groups in position 14. However, the (+)-*cis* and (−)-*cis* isomers cannot be separated from each other (see Table II) and we assume the same situation applies for (+)-*trans* and (−)-*trans* isomers [we did have not the (−)-*trans* isomer]; thus only the separation of four isomers can be achieved. In Fig. 1 the dependence of the capacity ratios on the eluent composition using octadecyl and octyl silica stationary phases is shown.

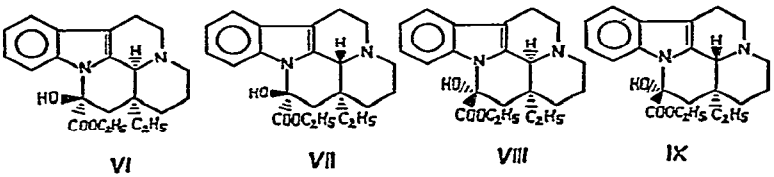
It can be seen from Fig. 1 that a linear relationship between log k' and eluent composition was obtained on both phases. In other respects, however, the results obtained significantly differ from each other. Thus the elution orders are different, and while on octyl silica the elution order is independent of the eluent composition, on octadecyl silica the elution order of *cis*-epivincaminic acid ethyl ester (VIII) and *trans*-epivincaminic acid ethyl ester (IX) can be reversed by changing the eluent composition (Fig. 2).

As has already been mentioned, a limitation of the systems investigated is that the optical isomers of eburnane alkaloids cannot be separated from each other.

TABLE II

CAPACITY RATIOS, k', FOR EBURNANE ALKALOIDS ON OCTADECYL AND OCTYL SILICA PACKINGS WITH DIFFERENT ELUENTS

No.	*Alkaloid*	*Ratio of acetonitrile and 0.01 M $(NH_4)_2CO_3$*								
		4:6	*5:5*	*6:4*	*7:3*	*8:2*	*4:6*	*5:5*	*6:4*	*7:3*
		μBondapak C_{18}					*10 μm LiChrosorb RP-8*			
XI	(+)-*cis*-Apovincaminic acid	0.05	0.05	0.0	0.0	0.0	0.0	0.0	0.0	0.0
I	(+)-*cis*-Vincaminic acid	0.10	0.10	0.07	0.05	0.0	0.0	0.0	0.0	0.0
XXII	(+)-*cis*-Dehydroepivincamine	4.65	2.57	1.57	1.04	0.57	4.61	2.14	1.30	0.66
XXI	(+)-*cis*-Dehydrovincamine	6.03	3.21	1.86	1.31	0.57	5.65	2.60	1.55	0.98
VII	(+)-*trans*-Vincaminic acid ethyl ester	8.93	4.29	2.29	1.32	0.57	10.81	4.43	2.19	1.30
IV	(+)-*cis*-Epivincamine	9.23	4.71	2.71	1.79	0.75	6.02	2.60	1.55	1.04
V	(−)-*cis*-Epivincamine	9.23	4.71	2.71	1.79	0.75	6.02	2.60	1.55	1.04
VIII	(+)-*cis*-Epivincaminic acid ethyl ester	12.4	6.36	3.43	1.97	0.95	8.45	3.60	1.92	1.30
IX	(+)-*trans*-Epivincaminic acid ethyl ester	14.4	6.36	3.43	1.79	0.75	19.8	7.04	3.26	1.83
II	(+)-*cis*-Vincamine	12.4	6.36	3.43	2.14	0.90	10.9	3.53	1.87	1.30
III	(−)-*cis*-Vincamine	12.4	6.36	3.43	2.14	0.90	10.9	3.53	1.87	1.30
X	(+)-*cis*-Vincamone	13.7	7.21	4.04	2.57	1.43	—	5.51	3.19	1.87
XI	(+)-*cis*-Vincanole	15.6	8.07	4.29	3.14	1.71	—	3.60	2.26	1.55
XII	(+)-*cis*-Isovincanole	18.8	9.43	5.43	3.71	1.71	—	4.30	2.83	1.87
VI	(+)-*cis*-Vincaminic acid ethyl ester	16.9	8.47	4.38	2.57	1.43	12.4	5.03	2.57	1.55
XVI	(+)-*cis*-Apovincamine	23.9	14.1	6.78	4.11	2.64	—	9.42	4.79	2.66
XVIII	(+)-*trans*-Apovincaminic acid ethyl ester	30.3	18.5	7.76	3.97	2.03	—	21.8	8.64	4.23
XVII	(+)-*cis*-Apovincaminic acid ethyl ester	32.5	20.2	9.00	6.20	3.27	—	13.6	6.36	3.47
XX	(+)-*cis*-Vincamenine	46.5	31.2	14.0	8.71	5.57	—	19.2	10.4	5.26
XIX	(+)-*cis*-Apovincaminic acid phenyl ester	56.1	36.8	14.0	7.07	4.00	—	26.4	9.80	4.81
XIII	(+)-*cis*-10-Bromovincamine	13.4	9.11	4.86	3.54	1.70	—	7.32	3.57	2.11
XIV	(+)-*cis*-11-Bromovincamine	13.4	9.11	4.86	3.54	1.70	—	7.32	3.57	2.11



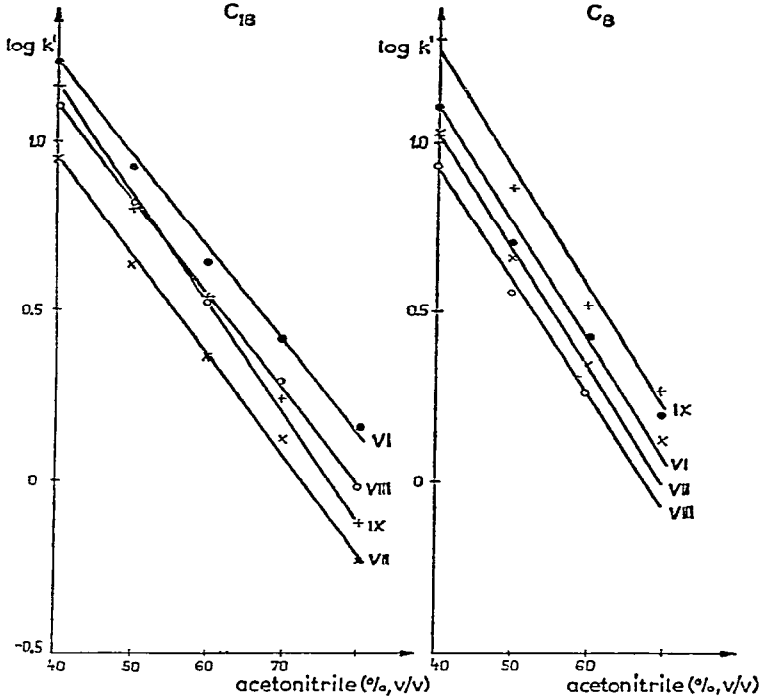


Fig. 1. Dependence of the capacity ratio, k', for vincaminic acid ethyl ester isomers on the eluent composition. Columns: μBondapak C_{18}, 300 × 3.9 mm I.D.; 10 μm LiChrosorb RP-8, 250 × 4.6 mm I.D. Detector: UV, 280 nm. Flow-rate: 1 cm³/min.

Another limitation can be seen in Table II, namely the structural isomers of eburnane alkaloids substituted in the aromatic ring also cannot be separated, being eluted with virtually identical retention in the systems investigated. As shown in Table II and Figs. 3 and 4, the ester homologues of apovincaminic acid and vincaminic acid are well separated both on octadecyl and octyl silica.

To optimize the separation system, a mixture of eburnane alkaloids (Table I) was analyzed. On both octyl silica and octadecyl silica stationary phases, acetonitrile–aqueous 0.01 *M* ammonium carbonate (6:4) was found to be optimal. The separation of these components is shown in Figs. 3 and 4. It thus seems that the best resolution was achieved on octadecyl silica, and by increasing the chain length of the bonded alkyl group on the silica surface, the capacity ratios and to some extent also the selectivity increase for the compounds.

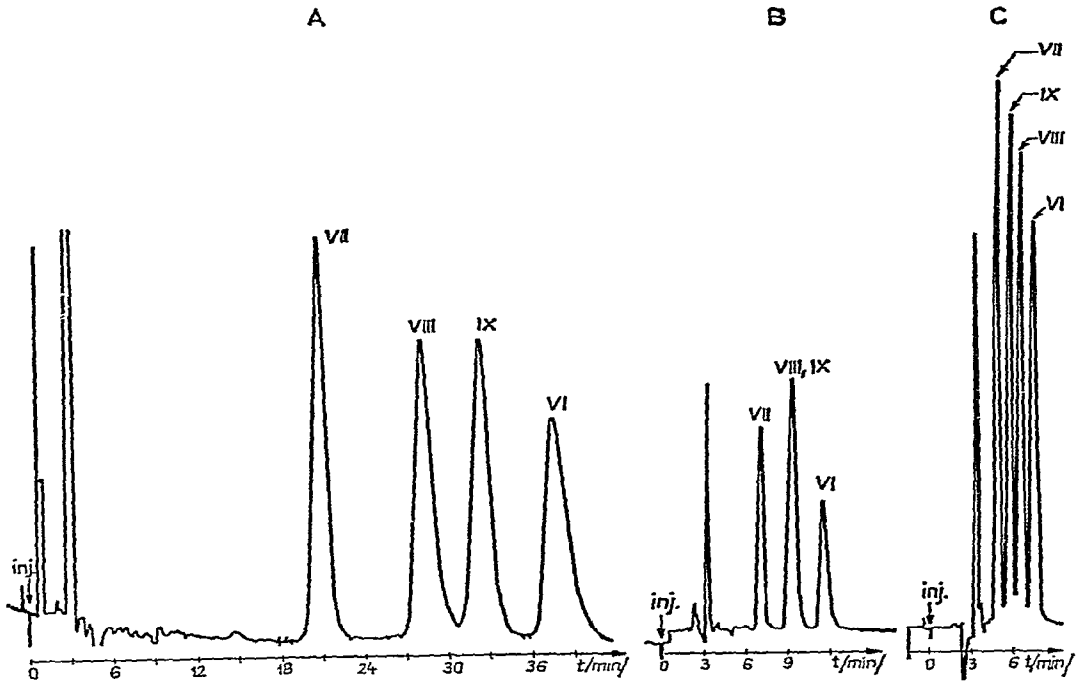


Fig. 2. Separation of vincaminic acid ethyl ester isomers. Column: μBondapak C_{18}, 300 × 3.9 mm I.D. Eluents: (A) acetonitrile–0.01 *M* ammonium carbonate (4:6); (B) 6:4; (C) 7:3. Other conditions as in Fig. 1. For compounds see Table I.

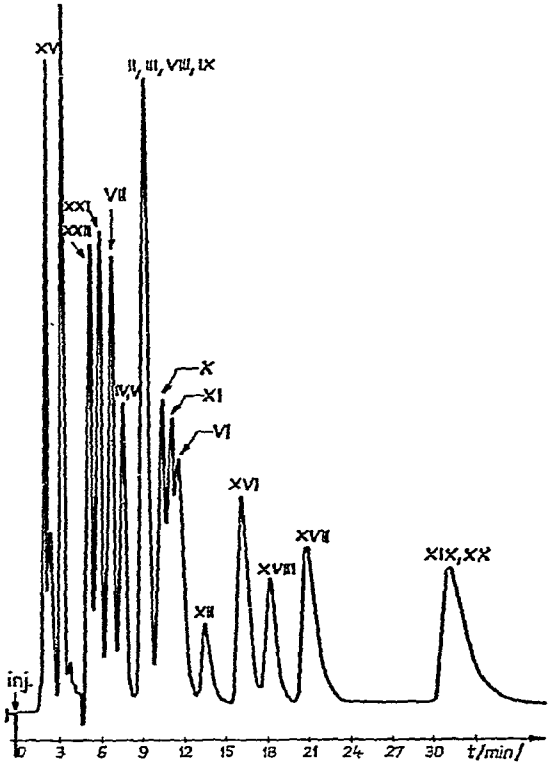


Fig. 3. Separation of eburnane alkaloids on octadecyl silica. Eluent: acetonitrile–0.01 *M* ammonium carbonate (6:4). Other conditions as in Fig. 1. For compounds see Table I.

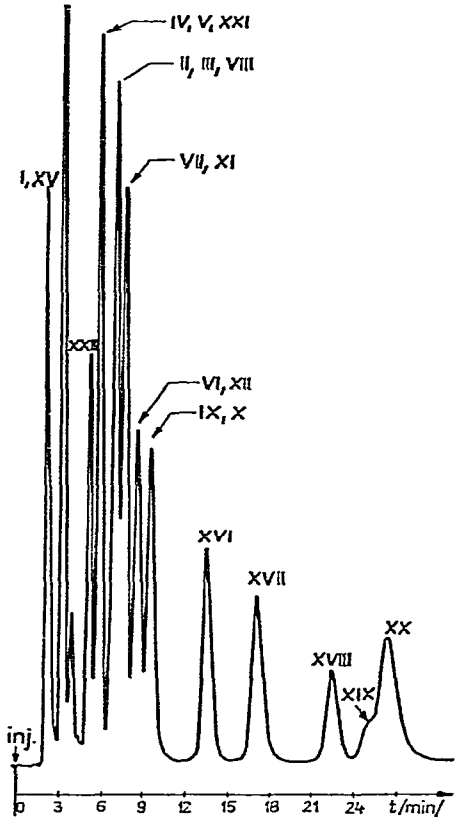


Fig. 4. Separation on octyl silica stationary phase. Conditions as in Fig. 3.

CONCLUSIONS

The separation of eburnane alkaloids by reversed-phase HPLC was studied. It was found that only some of the problems can be solved by the systems investigated. The method is suitable for the separation of closely related eburnane alkaloids as stereoisomers and ester homologues; group separation can also be carried out. However, optical isomers and structural isomers cannot be separated. The investigation of the effect of the chain length on the separation characteristics has revealed a significant difference in selectivity between the octyl and octadecyl silica stationary phases.

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REFERENCES

1 L. Jusiak, *Acta Pol. Pharm.*, 26 (1969) 247.
2 L. Karácsony, I. Gyenes and Cs. Lörincs, *Acta Pharm. Hung.*, 35 (1965) 280.
3 V. Yu. Vachnadze, *Khim. Prir. Soedin.*, (1973) 72.
4 L. A. Sapunova and A. K. Praschurovich, *Chim. Pharm. Zh.*, 11 (1977) 128.
5 V. Vigano, S. Paracchini, G. Piacenza and E. Pesco, *Il Farmaco*, 33 (1978) 583.
6 L. Vereczkey, J. Tamás and H. Vajda-Judit, in A. Frigerio (Editor), *Advances in Mass Spectrometry in Biochemistry and Medicine*, Vol. II, Spectrum Publ., New York, 1976, p. 11.
7 L. Vereczkey and L. Szporny, *Arzneim.-Forsch.*, 26 (1976) 1933.
8 H. Laufen, W. Juhran, W. Fleissig, R. Goetz, F. Scharpf and G. Bartsch, *Arzneim.-Forsch.*, 27 (1977) 1255.
9 M. Gazdag and G. Szepesi, *Z. Anal. Chem.*, in press.