Journal of Chromatography, 205 (1981) 57–64
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,157

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF EBURNANE ALKALOIDS

II. SEPARATIONS ON SILICA

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(Received June 30th, 1980)

SUMMARY

Separation of eburnane alkaloids on silica was investigated using various eluent mixtures composed of solvents having different elution strengths. It was found that the efficiency and selectivity of the separation was strongly dependent on the eluent composition and the best resolution was achieved using an appropriate mixture of hexane, chloroform, acetonitrile and methanol. The method can be applied for the separation of closely related eburnane alkaloids such as stereo- and structural isomers. A satisfactory separation of ester derivatives of apovincaminic acid, vincaminic acid and epivincaminic acid was achieved, suggesting that the separation mechanism is based on liquid–liquid partition.

INTRODUCTION

In the previous part[1] of this series the separation possibilities of eburnane alkaloids using reversed-phase chromatography were described. As was mentioned in this paper, only some of the analytical problems could be solved by the systems investigated. For example, the separation of stereoisomers and ester homologues can be achieved, but structural isomers cannot be separated from each other.

This was one reason for the present study of the separation on silica. The other reason was that in some cases the elution order obtained on chemically bonded octadecyl or octyl silica stationary phases was unfavourable for solving practical separation problems. Because the separation on silica results in a radical change of the elution order, this separation mode should eliminate this disadvantage of reversed-phase chromatography.

EXPERIMENTAL

A Varian Model 8500 liquid chromatograph, equipped with a variable-wavelength UV detector (Variscan Model 635) and stop-flow injector, was used.

0021-9673/81/0000–0000/$02.50

The separations were performed on columns of 10 μm Micropak Si 10 (250 × 2 mm I.D.) (Varian Aerograph, Walnut Creek, CA, U.S.A.), 5 μm LiChrosorb Si 60 (250 × 4.6 mm I.D.) (Pierce Eurochemie, Rotterdam, The Netherlands) and 10 μm μBondapak CN (300 × 3.9 mm I.D.) (Waters Assoc., Frankfurt/M, G.F.R.).

The solvents used were of analytical grade (Reanal, Budapest, Hungary) and were freshly distilled 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

Most of the compounds investigated were those described in the first part[1] of this series. They are listed in Table I.

TABLE I

EBURNANE ALKALOIDS INVESTIGATED

II	(+)-*cis*-Vincamine
III	(−)-*cis*-Vincamine
IV	(+)-*cis*-Epivincamine
V	(−)-*cis*-Epivincamine
VI	(+)-*cis*-Vincaminic acid ethyl ester
VII	(+)-*trans*-Vincaminic acid ethyl ester
VIII	(+)-*cis*-Epivincaminic acid ethyl ester
IX	(+)-*trans*-Epivincaminic acid ethyl ester
X	(+)-*cis*-Vincamone
XI	(+)-*cis*-Vincanole
XII	(+)-*cis*-Isovincanole
XIII	(+)-*cis*-10-Bromovincamine
XIV	(+)-*cis*-11-Bromovincamine
XVI	(+)-*cis*-Apovincamine
XVII	(+)-*cis*-Apovincaminic acid ethyl ester
XVIII	(+)-*trans*-Apovincaminic acid ethyl ester
XIX	(+)-*cis*-Apovincaminic acid phenyl ester
XX	(+)-*cis*-Vincamenine
XXI	(+)-*cis*-Dehydrovincamine
XXII	(+)-*cis*-Dehydroepivincamine
XXIII	(+)-*cis*-10-Methoxyvincamine (vincine)

Considering the structures and properties of the compounds, as well as the partly solved and unsolved analytical problems described previously[1], our aims can be summarized as follows: to elaborate optimal separation systems on silica; to compare the separation systems using two-, three- or four-component eluent mixtures according to their efficiency and selectivity; to clarify the separation mechanism by comparing the chromatographic data obtained on silica and chemical bonded nitrile phases and to solve the already mentioned separation problems such as group separation of eburnane alkaloids, separation of stereo- and structural isomers, etc.

First, Micropak Si 10 and LiChrosorb Si 60 stationary phases and chloroform–methanol eluent mixtures were used for the separations, because most of the eburnane alkaloids have polar functional groups. The capacity ratios, k', measured for the compounds are collected in Table II. It is seen that a relatively poor separation is

TABLE II

CAPACITY RATIOS, k', FOR EBURNANE ALKALOIDS ON MICROPAK Si 10 AND LICHROSORB Si 60 COLUMNS USING TWO-COMPONENT ELUENT MIXTURES

Detector: UV, 280 nm. Flow-rate: 20 cm³/h (Micropak Si 10); 60 cm³/h (LiChrosorb Si 60).

No.	*Alkaloid*	*Ratio of chloroform and methanol*			
		95:5	*92:8*	*95:5*	*98:2*
		MicroPak Si 10	*5 μm LiChrosorb Si 60*		
X	(+)-*cis*-Vincamone	1.00	0.19	0.31	0.66
XXI	(+)-*cis*-Dehydrovincamine	—	0.14	0.25	0.45
XXII	(+)-*cis*-Dehydroepivincamine	—	0.35	0.58	1.29
XX	(+)-*cis*-Vincamenine	1.60	0.36	0.60	1.36
XVI	(+)-*cis*-Apovincamine	2.62	0.19	0.50	0.77
XVII	(+)-*cis*-Apovincaminic acid ethyl ester	2.62	0.19	0.50	0.77
II	(+)-*cis*-Vincamine	4.14	0.40	0.63	1.37
VI	(+)-*cis*-Vincaminic acid ethyl ester	4.14	0.40	0.63	1.37
XII	(+)-*cis*-Isovincanole	6.43	1.05	1.62	3.26
IV	(+)-*cis*-Epivincamine	8.23	1.03	1.78	4.22
XI	(+)-*cis*-Vincanole	8.43	1.34	2.34	5.39

TABLE III

CAPACITY RATIOS, k', FOR EBURNANE ALKALOIDS USING THREE-COMPONENT ELUENT MIXTURES

Column: 5 μm LiChrosorb Si 60, 250 × 4.6 mm I.D. Flow-rate: 1 cm³/min. Detector: UV, 280 nm. Eluents: A = hexane–chloroform–methanol (60:30:10); B = 65:25:10; C = 80:10:10; D = 90:5:5.

No.	*Alkaloid*	*Eluent*			
		A	*B*	*C*	*D*
XVIII	(+)-*trans*-Apovincaminic acid ethyl ester	0.11	0.20	0.30	0.35
XX	(+)-*cis*-Vincamenine	0.63	0.64	0.78	1.24
XIX	(+)-*cis*-Apovincaminic acid phenyl ester	0.70	0.75	0.93	1.66
XVII	(+)-*cis*-Apovincaminic acid ethyl ester	0.70	0.75	0.96	1.76
IX	(+)-*trans*-Epivincaminic acid ethyl ester	0.55	0.70	0.78	1.82
X	(+)-*cis*-Vincamone	0.70	0.85	1.07	1.94
XVI	(+)-*cis*-Apovincamine	0.70	0.85	1.15	2.06
VII	(+)-*trans*-Vincaminic acid ethyl ester	0.75	0.90	1.15	2.88
XXI	(+)-*cis*-Dehydrovincamine	0.95	1.11	1.96	4.69
XXII	(+)-*cis*-Dehydroepivincamine	1.25	1.51	2.78	5.59
XIII	(+)-*cis*-10-Bromovincamine	1.41	1.47	2.85	5.90
XIV	(+)-*cis*-11-Bromovincamine	1.63	1.59	3.37	7.51
VI	(+)-*cis*-Vincaminic acid ethyl ester	1.48	1.55	2.59	7.82
II	(+)-*cis*-Vincamine	1.85	1.89	3.30	7.94
III	(−)-*cis*-Vincamine	1.85	1.89	3.30	7.94
XI	(+)-*cis*-Vincanole	3.56	3.60	4.33	10.4
XII	(+)-*cis*-Isovincanole	3.56	3.60	5.00	12.2
VIII	(+)-*cis*-Epivincaminic acid ethyl ester	2.85	3.55	3.96	11.0
XXIII	(+)-*cis*-10-Methoxyvincamine	3.15	3.75	4.30	11.5
IV	(+)-*cis*-Epivincamine	3.15	3.80	4.59	13.6
V	(−)-*cis*-Epivincamine	3.15	3.80	4.59	13.6

TABLE IV

CAPACITY RATIOS, k', FOR EBURNANE ALKALOIDS USING FOUR-COMPONENT ELUENT MIXTURES

Ratios of hexane, chloroform, acetonitrile and methanol in the eluent: 1 = 55:20:25:3; 2 = 55:25:20:3; 3 = 55:22.5:22.5:3; 4 = 60:20:20:3; 5 = 60:25:15:3; 6 = 60:22.5:17.5:3; 7 = 65:17.5:17.5:3; 8 = 65:20:15:3; 9 = 65:15:20:3; 10 = 55:25:20:1; 11 = 55:25:20:5. Other conditions as in Table III.

No.	*Alkaloid*	*Eluent*										
		1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
XVIII	(+)-*trans*-Apovincaminic acid ethyl ester	0.32	0.18	0.22	0.20	0.15	0.20	0.25	0.20	0.35	0.10	0.05
IX	(+)-*trans*-Epivincaminic acid ethyl ester	0.53	0.36	0.40	0.47	0.38	0.45	0.57	0.45	0.65	0.39	0.27
VII	(+)-*trans*-Vincaminic acid ethyl ester	0.68	0.49	0.54	0.56	0.58	0.58	0.74	0.68	0.74	0.61	0.39
X	(+)-*cis*-Vincamone	0.88	0.62	0.68	0.71	0.63	0.68	0.78	0.64	0.78	0.82	0.40
XIX	(+)-*cis*-Apovincaminic acid phenyl ester	0.92	0.68	0.72	0.78	0.68	0.75	0.82	0.62	0.78	0.86	0.40
XVII	(+)-*cis*-Apovincaminic acid ethyl ester	1.00	0.76	0.78	0.82	0.76	0.82	0.85	0.64	0.78	0.96	0.40
XVI	(+)-*cis*-Apovincamine	1.13	0.85	0.85	0.98	0.82	0.88	0.98	0.71	0.88	1.14	0.50
XX	(+)-*cis*-Vincamenine	1.29	0.97	0.92	0.98	0.91	0.96	0.98	0.71	0.77	1.27	0.50
XXI	(+)-*cis*-Dehydrovincamine	1.40	1.05	1.00	1.25	1.15	1.25	1.50	1.23	1.25	1.52	0.60
XXII	(+)-*cis*-Dehydroepivincamine	1.55	1.15	1.10	1.35	1.27	1.35	1.62	1.33	1.35	1.64	0.65
XIII	(+)-*cis*-10-Bromovincamine	1.62	1.26	1.25	1.48	1.37	1.46	1.74	1.43	1.50	1.76	0.80
XIV	(+)-*cis*-11-Bromovincamine	1.77	1.39	1.37	1.62	1.51	1.62	1.89	1.57	1.63	1.93	0.88
VI	(+)-*cis*-Vincaminic acid ethyl ester	2.17	1.65	1.61	1.90	1.74	1.93	2.18	1.71	1.76	2.35	1.04
II	(+)-*cis*-Vincamine	2.35	1.79	1.75	2.15	1.98	2.18	2.49	1.99	2.04	2.60	1.14
III	(−)-*cis*-Vincamine	2.35	1.79	1.75	2.15	1.98	2.18	2.49	1.99	2.04	2.60	1.14
VIII	(+)-*cis*-Epivincaminic acid ethyl ester	3'09	2.56	2.36	2.86	2.97	3.06	3.39	·.94	2.63	4.64	1.59
XXIII	(+)-*cis*-10-Methoxyvincamine	3.09	2.35	2.32	2.86	2.62	2.84	3.39	2.66	2.63	3.60	1.47
IV	(+)-*cis*-Epivincamine	3.43	2.79	2.61	3.24	3.26	3.35	3.92	3.24	3.08	5.34	1.73
XI	(+)-*cis*-Vincanole	3.43	2.79	2.61	3.09	3.26	3.35	3.54	2.94	2.63	4.28	1.78
XII	(+)-*cis*-Isovincanole	3.96	3.13	2.97	3.58	3.54	3.85	4.04	3.24	3.08	4.88	1.88

achieved using this eluent. To increase the efficiency of the separation a mixture of hexane, chloroform and methanol was used as eluent and the effect of the ratio of hexane and chloroform on the separation was investigated. The dependence of the capacity ratios on the eluent composition is shown in Table III. It can be concluded that on decreasing the polarity of the eluent the separation of bromovincamine derivatives is improved, but only a slight improvement can be achieved for other eburnane alkaloids. On the other hand, increasing the hexane concentration in the eluent results in an increase in the retention of the compounds investigated, which is undesirable for practical applications of the method.

Four-component eluent mixtures were also examined, replacing part of the chloroform and methanol by acetonitrile. (Recently we used[2] a similar eluent mixture for the separation of ergot alkaloids.) Table IV shows the capacity ratios measured for the compounds using different ratios of hexane, chloroform, acetonitrile and methanol. A hexane–chloroform–acetonitrile–methanol (55:25:20:3) eluent was found to be optimal. The effect of the methanol concentration in the eluent on the efficiency and selectivity of the separation is illustrated in Fig. 1.

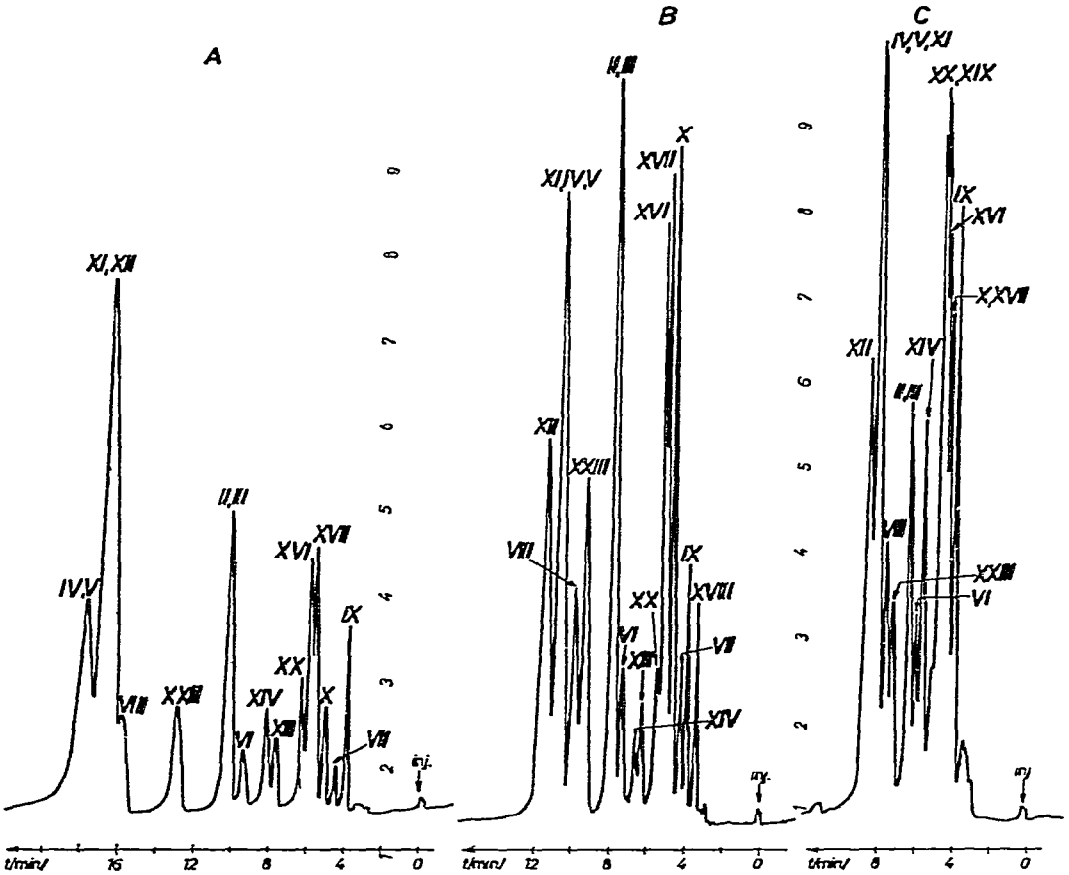

Fig. 1. Separation of eburnane alkaloids on silica using four-component eluent mixtures. Ratios of hexane–chloroform–acetonitrile–methanol in eluent: A = 55:25:20:1; B = 55:25:20:3; C = 55:25:20:5. Other conditions as in Table III.

Comparing the chromatographic data collected in Tables II–IV, it can be concluded that the four-component eluent mixture can be applied for the separation of eburnane alkaloids. Thus group separation of eburnane alkaloids, as in the reversed-phase chromatographic systems, can be achieved, the elution order of compounds having similar structures being as follows: *cis*-apovincamine (XVI), *cis*-dehydrovincamine (XXI), *cis*-dehydroepivincamine (XXII), *cis*-vincamine (II), and *cis*-epivincamine (IV).

Regarding the separation of stereo- and structural isomers, both the three-component (hexane–chloroform–methanol) and the four-component (hexane–chloroform–acetonitrile–methanol) eluent mixtures are suitable. Fig. 2 shows the separation of vincaminic acid ethyl ester isomers on silica using the above mentioned eluent mixtures.

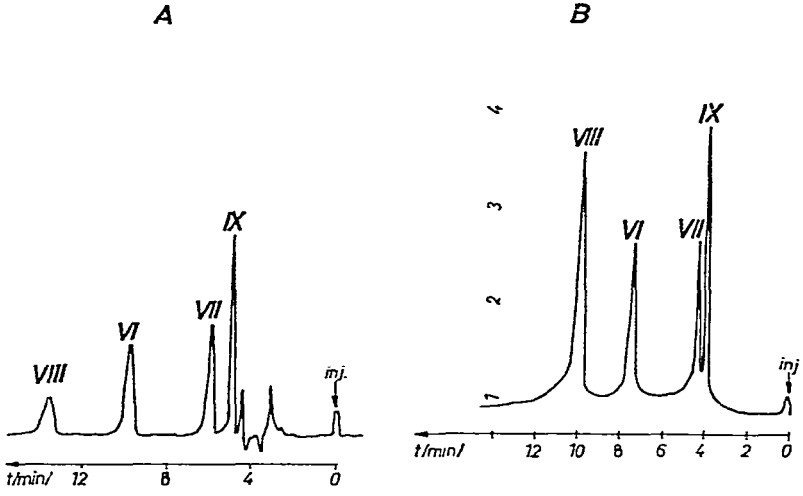

Fig. 2. Separation of vincaminic acid ethyl ester isomers. Eluents: A = hexane–chloroform–methanol (90:5:5); B = hexane–chloroform–acetonitrile–methanol (55:25:20:3). Other conditions as in Fig. 1.

Using a four-component eluent mixture, further selectivity of the separation can be achieved for *cis* and *trans* isomers, namely the *trans* isomers of apovincaminic acid ethyl ester (XVIII), epivincaminic acid ethyl ester (IX) and vincaminic acid ethyl ester (VII) are eluted before the corresponding *cis* isomers (XVII, VIII and VI, respectively). While the elution order of vincaminic acid ethyl ester isomers is independent of the eluent composition, in the case of the separation of two vincanole isomers (XI, XII) the elution order can be reversed by changing the eluent composition, as illustrated by Fig. 3. Thus, using chloroform–methanol (95:5) the isovincanole is first eluted, while in a four-component eluent mixture the isovincanole is retained longer than vincanole.

Fig. 1 also shows that the structural isomers of vincamine derivatives such as 10-bromovincamine (XIII), 11-bromovincamine (XIV) and 10-methoxyvincamine (XXIII) can be separated both from each other and from vincamine (II), and similar results were obtained for other structural isomers of eburnane alkaloids substituted

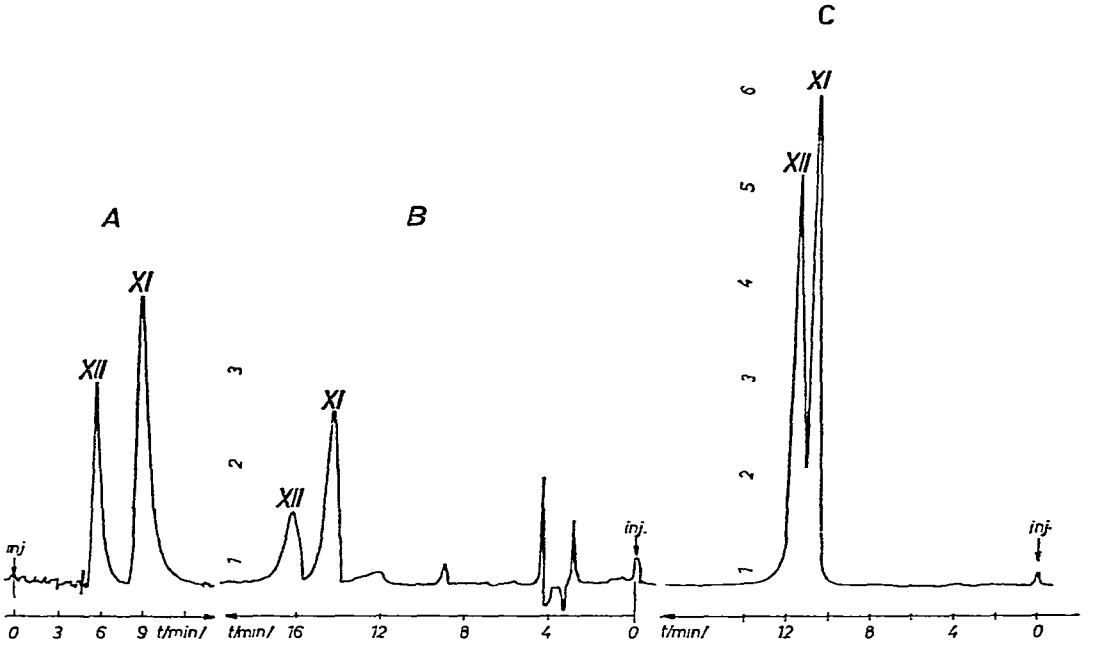

Fig. 3. Separation of vincanole isomers. Eluents: A = chloroform–methanol (95:5), on Micropak Si 10 (250 × 2 mm I.D.), flow-rate 20 cm³/h, B = hexane–chloroform–methanol (8:1:1) on 5 μm LiChrosorb Si 60 (250 × 4.6 mm I.D.), flow-rate 1 cm³/min; C = hexane–chloroform–acetonitrile–methanol (55:25:20:3).

TABLE V

CAPACITY RATIOS, k', FOR EBURNANE ALKALOIDS ON μBONDAPAK CN WITH DIFFERENT ELUENTS

Column: 10 μm μBondapak CN, 300 × 3.9 mm I.D. Flow-rate: 1 cm³/min. Detector: UV, 280 nm. Eluents: A = hexane–chloroform–acetonitrile (65:20:15); B = 70:20:10; C = 75:20:5.

No.	*Alkaloid*	*Eluent*		
		A	*B*	*C*
X	(+)-*cis*-vincamone	0.11	0.27	0.38
XVII	(+)-*cis*-Apovincaminic acid ethyl ester	0.23	0.32	0.57
XVI	(+)-*cis*-Apovincamine	0.25	0.40	0.64
XX	(+)-*cis*-Vincamenine	0.28	0.43	0.64
VI	(+)-*cis*-Vincaminic acid ethyl ester	0.72	0.86	1.36
II	(+)-*cis*-Vincamine	1.00	1.07	1.57
III	(−)-*cis*-Vincamine	1.00	1.07	1.57
VIII	(+)-*cis*-Epivincaminic acid ethyl ester	1.15	1.20	1.80
XXIII	(+)-*cis*-10-Methoxyvincamine	1.28	1.61	1.95
IV	(+)-*cis*-Epivincamine	1.34	2.06	3.79
V	(−)-*cis*-Epivincamine	1.34	2.06	3.79
XI	(+)-*cis*-Vincanole	1.57	2.14	3.86
XII	(+)-*cis*-Isovincanole	2.62	3.00	4.57

in the aromatic ring. This is a significant improvement compared to the reversed-phase systems previously investigated.

Although adsorption chromatography has been considered to be only slightly effective for the separation of ester homologues of carboxylic acids, the chromatographic data in Table IV indicate that the separation of different ester homologues of apovincaminic acid (XVI, XVII, XIX), vincaminic acid (II, VI) and epivincaminic acid (IV, VIII), respectively, can be achieved and the elution order of these ester derivatives is reversed compared to reversed-phase high-performance liquid chromatography. Considering this observation, as well as the altered elution order of vincanole and isovincanole caused by change in eluent composition, it seems that the separation proceeds according to a liquid–liquid partition mechanism. (In the separation of vincanole and isovincanole we assume that adsorption is the dominant mechanism in a two-component eluent mixture, while in three- and four-component eluent mixtures liquid–liquid partition predominates.

The results obtained by using chemically bonded "nitrile" stationary phases and hexane–chloroform–acetonitrile eluent mixtures seem to support the liquid–liquid partition mechanism, because they are similar to the data obtained on silica. The chromatographic data obtained on the "nitrile" phase are collected in Table V.

CONCLUSIONS

The separation of eburnane alkaloids on silica was investigated. From the comparison of the different separation methods it can be concluded that, by using an appropriate mixture of hexane, chloroform, acetonitrile and methanol as eluent, difficult problems such as the separation of closely related eburnane alkaloids, of stereo- and structural isomers, as well as that of ester homologues, can be achieved on silica. No separation can be achieved for optical isomers. Because most of the separation problems can be solved both on silica and reversed-phase packings, the separation mode used should be selected on the basis of the practical aspects of the given analytical problem.

ACKNOWLEDGEMENTS

The authors thank Miss R. Iváncsics and Mrs. G. Reif for their technical assistance.

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