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Note

High-performance liquid chromatography of some *Tabernaemontana* alkaloids

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High-performance liquid chromatography (HPLC) has extensively been applied in the analysis of indole alkaloids. Verpoorte and Baerheim Svendsen[1] analyzed *Strychnos* alkaloids on a normal phase, and *Catharanthus* and *Vinca* alkaloids have been analyzed on reversed phases[2–6]. Zsadon *et al.*[7] separated some indole alkaloids by means of inclusion LC. Reversed-phase ion-pair HPLC was used by Szepesi *et al.*[8] for the separation of stereoisomeric vincamine derivatives. Phillipson *et al.*[9] reported the analysis of a series of heteroyohimbine and oxindole alkaloids by means of reversed-phase HPLC and compared the results with previously reported normal-phase thin-layer chromatographic (TLC) data; the previously found correlation between the availability of N_b lone-pair electrons and adsorption on silica could not be confirmed for the retention of the alkaloids in the reversed-phase system. For *Tabernaemontana* alkaloids only a semipreparative separation has been reported by means of HPLC[10], however the chromatograms showed rather poor peak shapes.

In this note a reversed-phase ion-pair and a normal phase HPLC separation are reported for the analysis of a series of *Tabernaemontana* alkaloids. The first method is a modification of a previously reported method for alkaloids[11].

EXPERIMENTAL

Apparatus

An LDC constametric III pump was used to deliver the solvent at a flow-rate of 1 ml/min and an LDC Spectrometer III was used to monitor the eluent at 280 nm. Samples were introduced through a Rheodyne Model 7125 syringe-loading sample injector. All chromatograms were recorded with a W + W 600 Tarkan recorder.

HPLC systems

Reversed-phase ion-pair HPLC was performed on a 300 × 4.6 mm I.D. stainless-steel column packed with LiChrosorb RP-18, 5 μm, subsequently loaded with 0.01 *M* dodecylsulphonic acid in methanol–water (1:1) and 0.02 *M* cetrimide in

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water[11]. Mobile phases: S1, 0.02 *M* methanesulphonic acid in water–dioxane–sulphuric acid (94.5:5:0.5), pH adjusted to 4.0 with sodium hydroxide; S2, 0.02 *M* methanesulphonic acid in water–dioxane–sulphuric acid (98.5:1:0.5), pH adjusted to 3.5 with sodium hydroxide. Normal-phase HPLC was performed on a 300 × 3.9 mm I.D. stainless-steel column packed with μPorasil, with chloroform–methanol–25% ammonia (99:1:0.2) (S3), chloroform–10% ammonia (100:0.2) (S4) or chloroform–methanol–25% ammonia (98 + 2 + 0.4) (S5) as mobile phases.

TLC systems

Thin-layer chromatography was carried out on Merck silica gel F 254 TLC plates (layer thickness 0.25 mm) using the solvent systems toluene–absolute ethanol (saturated with NH_3) (19:1), prior to development the plates were left standing in an atmosphere of ammonia for 20 min, and chloroform–methanol–25% ammonia (95:5:0.2). The spots were visualized by spraying with 1% ceric sulphate in 10% sulphuric acid followed by heating with hot air.

TABLE I

SEPARATION OF SOME *TABERNAEMONTANA* ALKALOIDS BY REVERSED-PHASE ION-PAIR HPLC AND NORMAL-PHASE HPLC AND TLC

S1 = 0.02 *M* methanesulphonic acid in water–dioxane–sulphuric acid (94.5:5:0.5), pH = 4.0; S2 = 0.02 *M* methanesulphonic acid in water–dioxane–sulphuric acid (98.5:1:0.5), pH = 3.5; S3 = chloroform–methanol–25% ammonia (99:1:0.2); S4 = chloroform–10% ammonia (100:0.2); S5 = chloroform–methanol–25% ammonia (98:2:0.4); S6 = toluene–absolute ethanol (saturated with ammonia gas) (19:1), prior to development the plates were placed in an atmosphere of ammonia for 20 min; S7 = chloroform–methanol–25% ammonia (95:5:0.2).

Alkaloid	*No.*	*HPLC k'*					*TLC R_F*	
		S1	*S2*	*S3*	*S4*	*S5*	*S6*	*S7*
Tacamine	V	–	1.67	2.52	–	0.00	0.27	0.33
Tabernaemontanine	IId	–	2.01	0.38	0.67	–	0.36	0.55
Vobasine	IIb	1.15	2.80	0.66	0.68	–	0.32	0.51
Perivine	IIa	1.54	3.99	1.76	2.16	–	0.22	0.32
Dregamine	IIc	1.63	4.87	1.00	1.32	–	0.31	0.45
Stemmadenine	VIII	1.89	5.40	–	–	0.69	0.11	0.28
Vincamine	IV	3.04	8.78	0.93	1.34	–	0.42	0.49
19-*epi*-Iboxygaine	Ib	2.93	10.45	2.05	–	–	0.28	0.27
19-*epi*-Voacristine	If	3.50	14.50	0.17	0.32	–	0.34	0.60
Geissoschizine	XI	3.50	10.25	–	–	0.16	0.23	0.34
Tabersonine	VII	3.75	5.49	0.00	0.00	–	0.62	0.73
Methuenine	IIIa	3.89	2.20	2.72	–	–	0.28	0.32
Ibogamine	Ia	3.96	12.25	0.46	0.55	0.00	0.62	0.48
16-*epi*-Isositsirikine	XII	5.86	23.12	–	–	1.34	0.11	0.13
Apparicine	IX	6.78	22.40	3.48	–	–	0.29	0.21
Voacangine	Id	7.00	10.60	0.00	0.00	–	0.56	0.71
12-Methoxyvoaphylline	VI	8.57	12.80	0.00	0.08	–	0.61	0.72
Isomethuenine	IIIb	11.40	16.40	–	–	0.94	0.08	0.08
Coronaridine	Ic	16.70	31.0	0.00	–	–	0.62	0.71
Isovoacangine	Ie	23.80	14.50	0.00	0.00	–	0.59	0.70
3-Isoreserpiline	X	26.60	–	0.12	0.29	0.00	0.34	0.56

Solvents

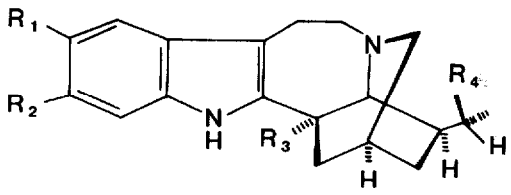
Chloroform and ammonia were of pro analysis quality (E. Merck, Darmstadt, F.R.G.) and methanol was HPLC grade (J. T. Baker).

Alkaloids

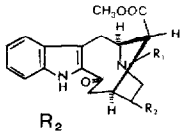
Vobasine, perivine, 19-epi-iboxygaine, 19-epi-voacristine, 12-methoxyvoaphylline, apparicine and isomethuenine were isolated from the leaves of *Tabernaemontana dichotoma*[12]. Stemmadenine, coronaridine, voacangine, ibogamine and tabersonine were isolated from the seeds of *T. dichotoma*[13] and tacamine was isolated from the leaves of *T. eglandulosa*[14]. Tabernaemontanine and dregamine were a gift from Professor A. Cavé, vincamine and 3-isoreserpiline from Dr. N. G. Bisset, geissoschizine from Professor M. Damak, methuenine from Professor P. Potier and isovoacangine from Professor J. F. Cicció. 16-Epi-Isositsirikine was isolated from *T. psorocarpa*[15]. Leaf extracts were prepared according to ref. 12. The chloroform-soluble fraction was used in the analysis.

RESULTS AND DISCUSSION

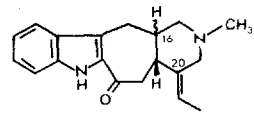
The reversed-phase ion-pair HPLC system was found to be highly selective in the separation of various alkaloids[11]. This is also the case for the separation of *Tabernaemontana* alkaloids. Comparing the six Iboga type alkaloids (Ia–f) rather large differences in capacity factor (k') were observed (Table I) taking into account the rather small structural differences. Apparently the introduction of a 10-methoxy or 19-hydroxy group reduces k', which is readily explained by an increased polarity



	R_1	R_2	R_3	R_4	
1a	H	H	H	H	Ibogamine
1b	OCH_3	H	H	OH	19R-epi-Iboxygaine
1c	H	H	$COOCH_3$	H	Coronaridine
1d	OCH_3	H	$COOCH_3$	H	Voacangine
1e	H	OCH_3	$COOCH_3$	H	Isovoacangine
1f	OCH_3	H	$COOCH_3$	OH	19R-epi-Voacristine

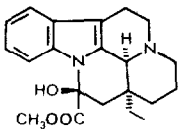


	R_1	R_2	
IIa	H	ethylidene	Perivine
IIb	CH_3	ethylidene	Vobasine
IIc	CH_3	ethyl α	Dregamine
IId	CH_3	ethyl β	Tabernaemontanine

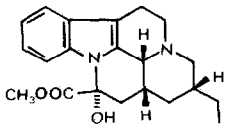


IIIa H(16) β Methuenine

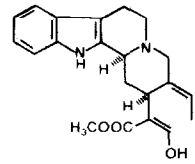
IIIb H(16) α Isomethuenine



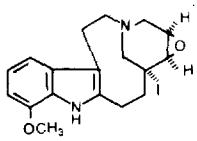
IV Vincamine



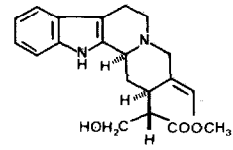
V Tacamine



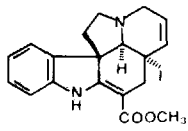
XI Geissoschizine



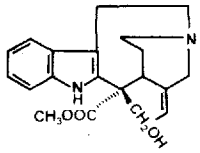
VI 12- Methoxyvoaphylline



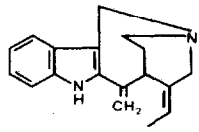
XII 16-epi-Isositsirikine



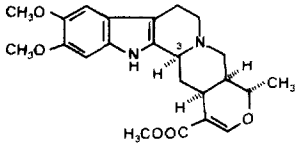
VII Tabersonine



VIII Stemmadenine



IX (-) Apparicine



X 3- Isoreserpiline

of the alkaloid upon substitution (compare Ia–Ib, Ic–Id and If). The increased polarity due to the 19-hydroxy group is also observed in the normal phase HPLC systems and TLC systems. However, the high k' values in the systems S1 and S2 for compounds Ic and Ie are difficult to account for. If Ib and If are compared only a small contribution of the $COOCH_3$ group to the retention of the alkaloid is evident, however comparison of Ia and Ic leads to the opposite conclusion; in both cases the introduction of the $COOCH_3$ group results in an increase of k'. Similarly, in the normal phase HPLC systems a decrease of k' is observed upon introduction of the $COOCH_3$ group. The N_a-acyl indole alkaloids of the vobasine type (IIa–d) are only weakly retained in the reversed-phase system. The sequence of elution is the same as in the normal phase system if perivine is not taken into account. Vincamine (IV) and tacamine (V) are weakly retained in the reversed-phase HPLC system, but are fairly strongly retained in the normal-phase systems. The conclusion of Phillipson *et al.*[9] that in reversed-phase HPLC the retention cannot be explained in terms of the influence of single substituents is thus also supported by this study.

The elution sequences in the TLC system S7 and the normal-phase HPLC systems S3 and S4 are more or less similar as expected. The TLC system S6, which due to its high UV absorption is less suitable for HPLC, showed some differences in the elution sequence of the alkaloids when compared with S7, as expected because the main solvents belong to different classes in Snyder's solvent classification[15,17].

To improve the resolution of some rapidly eluted compounds the mobile phase in the reversed-phase ion-pair HPLC was modified, *e.g.*, the percentage of dioxane was decreased to 1%. Also variations in the pH of the mobile phase resulted in changes of k'. The optimum pH was found to be 4.0 for solvent system S1 and 3.5 for S2. In case of the normal-phase HPLC system the concentrations of ammonia

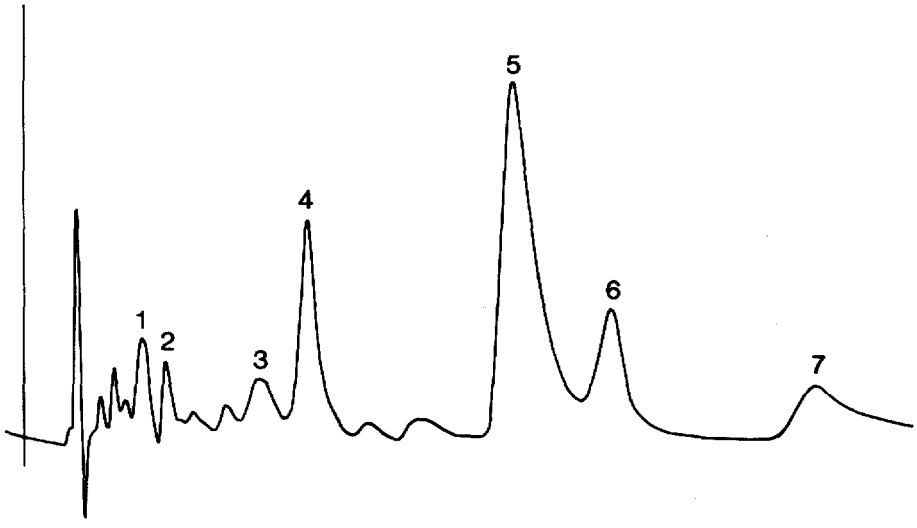


Fig. 1. Separation of alkaloids of *Tabernaemontana dichotoma* leaf extract by reversed-phase ion-pair HPLC. Mobile phase: 0.02 *M* methanesulphonic acid in water–dioxane–sulphuric acid (94.5:5:0.5), pH = 4.0. For other conditions see Experimental. Peaks: 1 = vobasine; 2 = perivine; 3 = 19-*epi*-iboxygaine; 4 = 19-*epi*-voacristine; 5 = (−)appericine; 6 = 12-methoxyvoaphylline; 7 = isomethuenine.

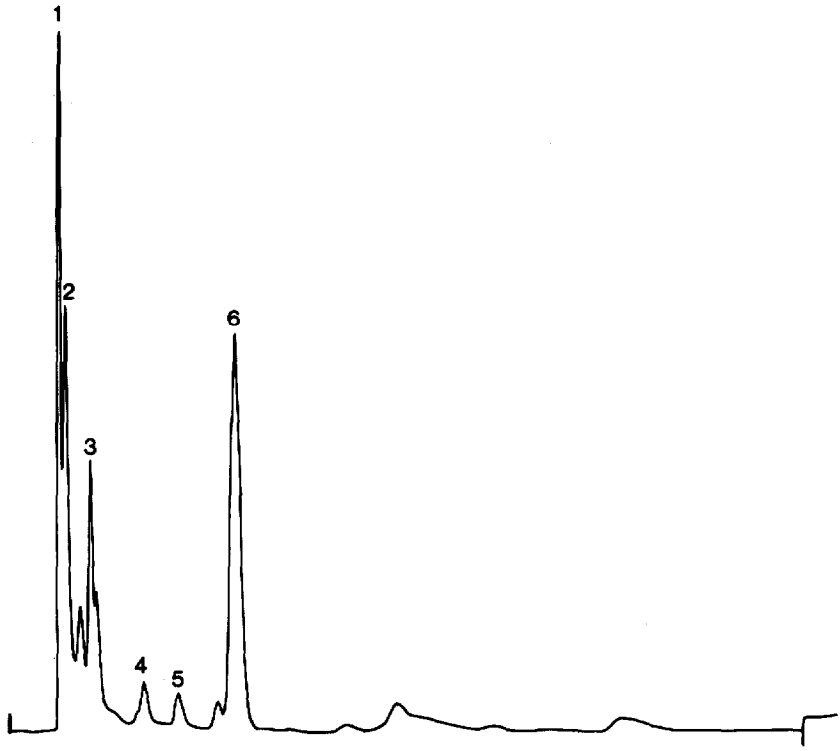


Fig. 2. Separation of alkaloids of *Tabernaemontana dichotoma* leaf extract by normal-phase chromatography. Mobile phase: chloroform–methanol–25% ammonia (99:1:0.2). For other conditions see Experimental. Peaks: 1 = 12-methoxyvoaphylline; 2 = 19-*epi*-voacristine; 3 = vobasine; 4 = perivine; 5 = 19-*epi*-iboxygaine; 6 = (−)appariicine.

and methanol could be decreased to improve the resolution of some rapidly eluted alkaloids, although some iboga type alkaloids were still unretained. Examples of typical separations of alkaloids isolated from plant material are given in Figs. 1 and 2.

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