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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF *VINCA ROSEA* ALKALOIDS AND THE CORRELATION OF PLATE HEIGHT AND MOLECULAR WEIGHT

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SUMMARY

A gradient elution reversed-phase high-performance liquid chromatographic method for fast analysis of *Vinca rosea* alkaloids is presented. The plate heights of monomeric (e.g. vindoline) and dimeric alkaloids (*e.g.* vinblastine, *etc.*) are distinctly different and have a diagnostic value for identification purposes.

INTRODUCTION

For a programme aimed at the isolation of pure alkaloids from *Vinca rosea*, we needed high-performance liquid chromatographic (HPLC) monitoring methods. Görög *et al.*[1] described an HPLC method using isocratic elution. Retention times for a large number of alkaloids and derivatives are mentioned in their paper, which also gives the relevant chemical structures. The authors also review earlier techniques based on thin-layer chromatography, spectrophotometry, colorimetry and volumetry, and correctly state that these do not fulfil all modern requirements. It is therefore not necessary to repeat earlier references and chemical structures here.

We considered that the wide range of solubilities, molecular weights and polarities of the alkaloids required a gradient elution procedure for fast routine monitoring. Many different stationary phases were tested to this end but, as so often, reversed-phase octadecyl-derivatised silica gel proved to be the best. Our HPLC system is different from that of the Hungarian group[1], and we also mention some different alkaloids.

INSTRUMENTAL DETAILS

All chromatograms were recorded with a Varian LC-5020 instrument. Varichrom detector and Varian CDS-111 integrator. Sample introduction was with a

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Valco 7000-p.s.i. sample loop injector. The LiChroma tubing columns were packed with 10-μm RSiL-C_{18}-HL-D octadecyl-silica gel. The solvents used in the gradient have to be of very high quality or otherwise ghost peaks are generated. The solvents have to contain 0.1 % ethanolamine and, as this dissolves silica gel rather rapidly, a saturating pre-column has to be used. This was 10 cm in length and was packed with 20-μm RSiL, with 20-μm RSiL-C_{18}-HL-D or with a mixture of both. These changes, carried out because of occasional column permeability problems, caused no detectable differences.

RESULTS

The results of our efforts are illustrated in Fig. 1, which shows the chromatogram of the crude alkaloid mixture isolated by acid extraction of a solvent extract of dried *V. rosea* plant material. Peak identification was based on co-chromatography with authentic material isolated in the course of our studies by preparative chromatographic methods. For our structural elucidation efforts, see refs. 2 and 3. Undoubtedly many of the peaks in Fig. 1, although showing the correct retention time, are only partly due to the single alkaloids mentioned. For example, peak 5 (marked vinblastine) integrates for much too large a percentage, considering the actual vinblastine concentration in the plant. This indicates co-elution of other alkaloids at the same retention time. The same is probably true for other peaks in Fig. 1, because

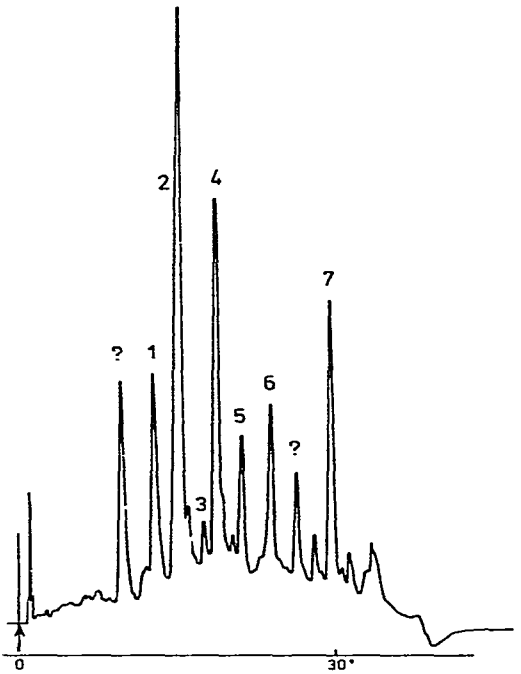

Fig. 1. Chromatogram of crude *V. rosea* extract; details as explained in the text. Column, 25 × 0.46 cm I.D. with pre-column; gradient, from 50 to 85 % methanol in water, both solvents with 0.1 % ethanolamine; flow-rate 2 ml/min; chart speed 20 cm/h; UV detection at 290 nm. Peaks: 1 = vindolinine; 2 = vindoline; 3 = vincristine; 4 = catharantine; 5 = vinblastine; 6 = leurosine; 7 = coronaridine.

more than 70 alkaloids are known to exist in the plant and there are probably many more.

In the large-scale separation scheme developed at this laboratory, the dimeric alkaloids are separated from the above crude extract in two stages. This leads to Fig. 2 and Fig. 3. From the mixture as shown in Fig. 3, pure vinblastine is isolated. The chromatograms shown aptly demonstrate the power of HPLC as a monitoring technique in working out a difficult isolation scheme.

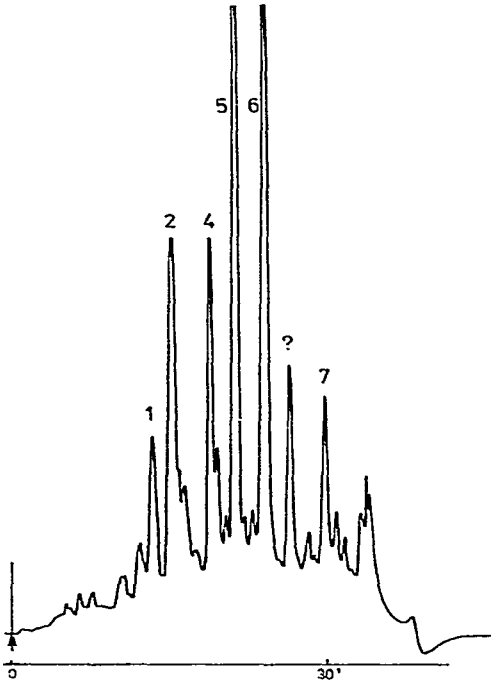

Fig. 2. Chromatogram of first-stage-enriched dimeric alkaloids. Chromatographic conditions and peak identification as in Fig. 1.

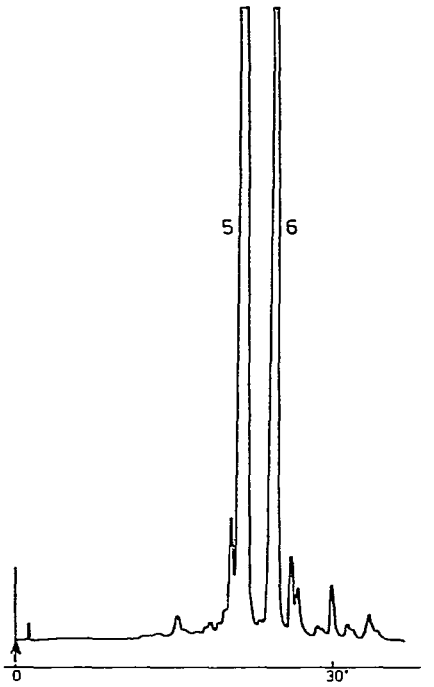

Fig. 3. Chromatogram of second-stage-enriched dimeric alkaloids. Chromatographic conditions and peak identification as in Fig. 1.

With isocratic elution of the alkaloids, and provided the mixtures are not too complex, the plate numbers for the different peaks can be measured. There is a distinct difference between monomeric and dimeric alkaloids. This allowed us to assign the dimeric structure to unknown peaks that were subsequently isolated in larger amounts for structural elucidation[4]. This property is also of interest for identification of the peaks. We attributed the difference to differences in the diffusion rates of the larger (dimeric) and the smaller (monomeric) alkaloids. We expected that a temperature increase would diminish the ratio and *vice versa*. Therefore the plate numbers for a series of pure alkaloids at various temperatures in the range 5–50°C were measured. Changing the temperature (T) not only influences the plate number but also the capacity factor (k'). Going from 5 to 50°C, the k' value is lowered by a factor of 2 or more for monomers and nearly 3 for dimers. A graph of log k' against

$1/T$ is linear, and the slopes of these lines change gradually in the same sense for the group of monomeric and for the group of dimeric alkaloids. The temperature influence on k' is largest for the later eluting peaks. The large changes in retention time emphasize the need for temperature control in HPLC.

The plate numbers more than double on going from 5 to 50°C (Table I).

TABLE I

PLATE NUMBERS (N) AND k' VALUES OF ALKALOIDS AS A FUNCTION OF TEMPERATURE

Alkaloid		*Temperature (°C)*					
		5	*10*	*20*	*30*	*40*	*50*
Vindolinine	N	2061	2495	3013	3580	4362	5076
	k'	11.4	10.7	9.2	8.2	7.1	6.0
Vindoline	N	2526	2769	–	4212	4807	5421
	k'	18.3	15.9	–	11.6	9.7	7.9
Vincristine	N	1603	1890	–	2654	3570	3710
	k'	22.8	21.1	–	16.3	13.9	11.4
Catharantine	N	2866	3433	4155	5247	5741	6371
	k'	31.5	28.1	22.7	19.0	15.5	12.4
Unknown	N	1791	2107	2784	3268	4066	4229
	k'	34.8	31.9	27.5	24.3	20.4	16.6
Vinblastine	N	1798	2069	2623	3339	4018	4312
	k'	40.5	37.3	32.4	28.7	24.0	19.6
Leurosine	N	2277	2484	3416	4096	4444	5281
	k'	84.9	76.0	62.2	52.8	42.4	32.7
Coronaridine	N	4909	6290	–	6907	7819	8226
	k'	131.9	192.3	–	84.0	65.0	49.0

TABLE II

COMPARISON OF PLATE NUMBERS FOR MONOMERIC AND DIMERIC ALKALOIDS AT IDENTICAL k' VALUES AND FOR ROOM TEMPERATURE

	N				
	k' = 20	*k' = 25*	*k' = 30*	*k' = 35*	*k' = 40*
Dimers					
Vincristine	1640	1760	1860	1940	2020
Unknown	1700	1880	2020	2140	2250
Vinblastine	1800	1880	1960	2020	2060
Leurosine	1860	1960	2040	2120	2210
Monomers					
Vindolinine	2320	2380	2440	2470	2500
Vindoline	2820	2890	2920	2920	2920
Catharantine	3100	3360	3540	3620	3600
Coronaridine	3490	3620	3740	3860	3980
Ratio of mean for monomers/dimers	1.68	1.64	1.60	1.57	1.52

At 5°C the counter-pressure of the columns increases considerably, and a flow-rate of 2 ml/min cannot be reached. The data of Table I were therefore all obtained at a flow-rate of 1 ml/min.

The difference in plate number between monomeric and dimeric alkaloids changes only slightly on going from lower to higher temperatures. The ratio of the mean plate number (monomeric/dimeric) is 1.65 at 5°C and 1.43 at 50°C. It is unclear, however, how much the changing k' values influences these results. We therefore wanted to compare the results for similar k' values. This was achieved by changing the solvent composition in numerous chromatographic experiments and by extrapolation where needed (Table II).

The ratio (Table II) is only slightly dependent on k'. The plate number is therefore an indication of molecular size, and this is probably not limited to the present series of alkaloids. It is clear from these data that the unknown mentioned in the tables is a dimeric alkaloid.

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