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Note

High-performance liquid chromatography of Catharanthus alkaloids

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Of the numerous alkaloids of *Catharanthus roseus* (*Vinca Rosea*), vinblastine and vincristine are highly potent antineoplastic drugs and an intense search is being carried out to find even more potent agents among the accompanying alkaloids and their semi-synthetic modifications. Both the quality control of the therapeutically used alkaloids and the analytical control of processing the drug require rapid, sensitive and reliable quantitative methods that will permit the determination of the individual alkaloids in the presence of several structurally closely related derivatives. The existing thin-layer chromatographic[1–7], spectrophotometric[5–8], colorimetric[9,10] and volumetric[11] methods usually do not completely fulfil these requirements. As these compounds have relatively high molecular weights, their gas chromatographic analysis is impossible. This paper describes a high-performance liquid chromatographic (HPLC) method which seems to enable all of the above-mentioned problems to be solved.

EXPERIMENTAL

Chromatographic separations were performed on a Hewlett-Packard 1010B liquid chromatograph equipped with a variable-wavelength UV detector and an HP 3380A integrator. A 25 cm × 4 mm I.D. stainless-steel column packed with LiChrosorb RP-8 (Merck, Darmstadt, G.F.R.) was used at ambient temperature, the chromatograms being monitored at 298 nm. The chromatographic solvent was a 47:53 mixture of acetonitrile (p.a. grade, Reanal, Budapest, Hungary) and 0.01 *M* ammonium carbonate solution. The flow-rate was 1.5 ml/min.

Solutions in acetonitrile of the alkaloids or their sulphates of concentration 0.03% were prepared and 25-μl portions were injected using a Valco loop injector.

All of the alkaloids tested (Fig. 1) were prepared in the research laboratories of Chemical Works of Gedeon Richter. Their identities were proved by comparing their physical constants and spectroscopic data with the literature values.

RESULTS AND DISCUSSION

From tests with several chromatographic systems, the above reversed-phase system proved to be the most suitable for the required separation from the point of view of both the analysis time and resolution power.

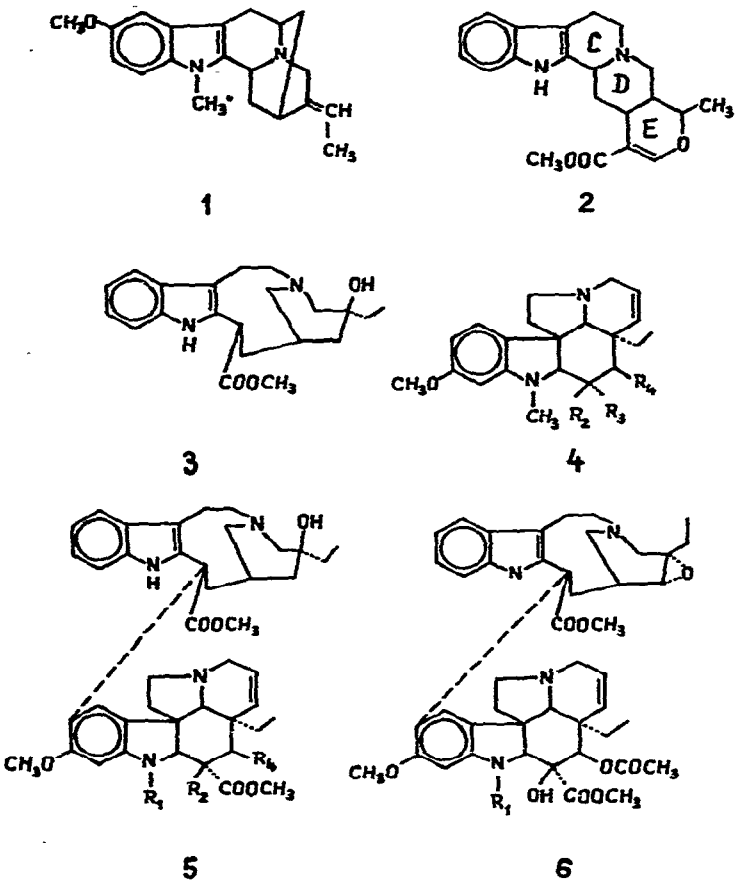

Fig. 1. Formulae of the alkaloids investigated. 1 = Lochnerine; 2 = ajmalicine (β-protons at the C/D/E ring junctions) and tetrahydroalstonine (α-protons at the C/D/E ring junctions); 3 = catharanthine; 4 = vindoline and derivatives; 5 = vinblastine, vincristine and derivatives; 6 = leurosine and derivatives. For details see Table I.

The analytical wavelength used throughout this study was 298 nm, but in the investigation of mixtures with unknown constituents it was found very useful to scan the chromatograms at shorter wavelengths also, as from the ratio of the peak areas at different wavelengths important conclusions can be drawn regarding the structure of the unknown component.

Table I gives the chromatographic data for 26 alkaloids, monomeric and dimeric constituents of *Catharanthus roseus* and some of their semi-synthetic modifications.

A specimen chromatogram of a mixture of some of the compounds listed in Table I is shown in Fig. 2.

It can be seen that the most important constituents of *Catharanthus roseus* (vinblastine, vincristine, vindoline, catharanthine, leurosine, etc.) and many of the structurally closely related pairs of derivatives are well separated, so that the proposed method seems to be suitable for solving a number of practical analytical problems.

TABLE I

RETENTION DATA FOR SOME CATHARANTHUS ALKALOIDS AND SEMI-SYNTHETIC DERIVATIVES

Compound name	*Formula in Fig. 1*				*Retention time (min)*
Monomeric alkaloids					
Lochnerine	1				3.29
Ajmalicine	2				7.90
Tetrahydroalstonine	2				14.65
Catharanthine	3				9.75
Vindoline derivatives	4:	R_2	R_3	R_4	
Vindoline		OH	$COOCH_3$	$OCOCH_3$	6.16
		OH	$COOCH_3$	OH	4.71
		OH	$COOCH_3$	$OCOCH_2Cl$	9.39
		OH	$COOCH_3$	$OCOCH_2N(CH_3)_2$	5.84
		OH	$COOCH_3$	$OCOCH_2NH(CH_3)$	4.30
		$OCOCH_3$	$COOCH_3$	$OCOCH_3$	23.22
Vindolinol		OH	CH_2OH	OH	4.44
Vindorosine (vindoline without aromatic methoxy group)					7.90
Dimeric alkaloids					
Vinblastine and vinoristine derivatives	5:	R_1	R_2	R_4	
Vinblastine		CH_3	OH	$OCOCH_3$	12.37
		CH_3	OH	OH	7.04
		CH_3	OH	$OCOCH_2Cl$	12.52
		CH_3	OH	$OCOCH_2N(CH_3)_2$	10.25
		CH_3	OH	$OCOCH_2NH(CH_3)$	10.09
		CH_3	$OCOCH_3$	$OCOCH_3$	48.10
		H	OH	OH	6.18
		H	OH	$OCOCH_3$	10.04
Desacetoxyvinblastine		CH_3	OH	H	17.15
Vincristine		CHO	OH	$OCOCH_3$	7.22
		CHO	OH	OH	4.87
Leurosine derivatives	6:	R_1			
Leurosine		CH_3			15.65
		H			11.89
Formylleurosine		CHO			9.75

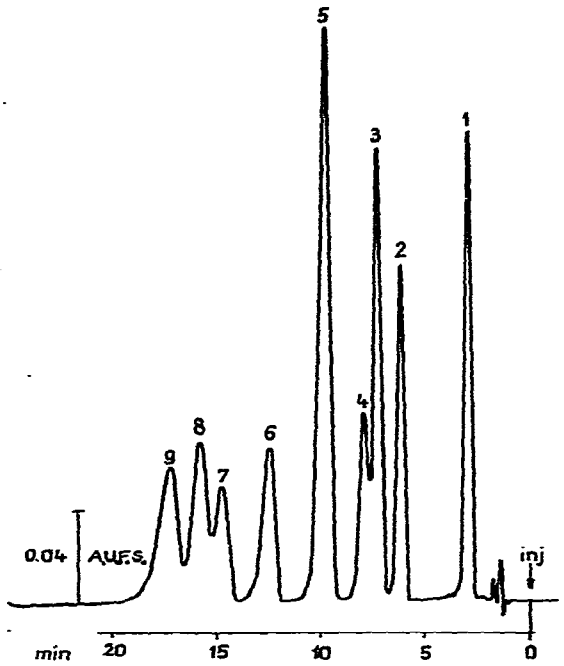

Fig. 2. HPLC separation of some Catharanthus alkaloids. 1 = Lochnerine; 2 = vindoline; 3 = vincristine; 4 = ajmalicine; 5 = catharanthine; 6 = vinblastine; 7 = tetrahydroalstonine; 8 = leurosine; 9 = desacetoxyvinblastine.

Work is in progress to utilize this method for the analysis of *Cantharanthus roseus* drug and the stability assay of the pharmaceutical formulations made from it.

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