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Note

High-performance liquid chromatography of iridoid and secoiridoid glucosides*

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Iridoid glucosides are important natural products; more than 100 iridoid and secoiridoid glucosides are known[1,2]. Many are of interest because of their pharmacological activity[3] as well as their importance in chemotaxonomy[4].

Iridoids and secoiridoids are usually separated by thin-layer or column chromatography[1]. Gas chromatography (GC) requires derivatization of the glucosides as trimethylsilyl (TMS) ethers[2]. Small amounts of these glucosides can be separated by reversed-phase high-performance liquid chromatography (HPLC). The method can be used for impurity controls of isolated iridoid glucosides. This note describes the separation of 16 iridoid glucosides, four secoiridoid glucosides and three iridoid glucosides with an acid functional group. A rapid qualitative examination of plant sources is possible with a small quantity of material. Frequently, a methanol extract can be injected directly into the column and known iridoids can be identified with reference samples.

EXPERIMENTAL

Apparatus

A Waters Assoc. (Milford, Mass., U.S.A.) high-performance liquid chromatograph model ALC 201 was used throughout. A Waters Assoc. M-6000 pump was used as the solvent delivery system and a U6K septumless injector. The system was equipped with a Beckmann Model 25 spectrophotometer with a variable-wavelength detector and a Waters LC-25 microcell.

Column

μBondapak C_{18} columns (30 cm × 4 mm, stainless steel) (Waters Assoc.) were used. μBondapak C_{18} has a monomolecular layer of octadecyltrichlorosilane chemically bonded to Porasil beads with an average particle size of 10 μm. The number of theoretical plates based on cinnamic alcohol was 3400 (flow-rate 1 ml/min, solvent

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methanol 48%) for the column used to separate important natural mixtures. Samples (1–10 μl) were injected using a 5 or 25 μl syringe made by Precision Sampling (Baton Rouge, La., U.S.A.).

Materials

Iridoid and secoiridoid glucosides samples were isolated in our laboratory and dissolved in methanol to a concentration of 10 μg/μl (most iridoids) or 1 μg/μl (secoiridoids and harpagoside). For ion-pair chromatography iridoids were dissolved in the eluent (10 μg/μl). Methanol of analytical reagent grade (Fluka, Buchs, Switzerland) and freshly distilled water comprised the solvent system. The aliquot volume of methanol (*e.g.* 400 ml for a 20% solution) was placed in a 2 l volumetric flask and made up with water. The reagent used for ion-pair chromatography, PIC-A (Waters Assoc.) consisted of a solution of tetrabutylammonium phosphate buffered to pH 7.5 for a counter-ion concentration of 0.005 mole/l.

RESULTS AND DISCUSSION

Chromatography

Sixteen iridoid and four secoiridoid glucosides were separated using the reversed-phase system. Retention times depended on solvent composition and glucoside structure. Glucosides with an aromatic group (harpagoside, amarogentin, etc.) eluted only in methanol-rich systems, and the retention time decreased with increasing methanol content. All tested iridoid compounds (except harpagoside) have their main

TABLE I

RELATIVE RETENTION TIMES OF IRIDOID GLUCOSIDES AND THE SECOIRIDOID GLUCOSIDE GENTIOPICROSIDE

Flow-rate, 2.0 ml/min.

Glucoside	*Solvent*				*λ (nm)*
	Water	*10% Methanol*	*20% Methanol*	*50% Methanol*	
Monotropein		0.86	0.85		230
Acetic acid	1.00 (2.28 min)	1.00 (2.12 min)	1.00 (1.87 min)	1.00 (1.73 min)	
Catalpol	2.2	1.11	1.00		210
Aucubin	4.9	1.60	1.15		210
Antirride		1.94	1.28		210
Harpagide		2.41	1.50		210
Galiridoside		3.20	1.62		210
Theviridoside			2.43		230
Asperuloside			2.84		230
Ipolamiide			3.88		230
Gentiopicroside			4.04		230
Verbenalin			4.15		230
Acetylharpagide			5.30		210
Plumieride			5.75		230/210
Loganin			6.23	1.23	230
Antirrinoside				3.10	210
Harpagoside				4.66	210/278

TABLE II

CAPACITY FACTORS OF PHENOLIC SECOIRIDOID GLUCOSIDES IN SOLVENTS WITH DIFFERENT METHANOL CONTENT

Flow-rate, 2.0 ml/min; $t_0 = 60$ sec.

Glucoside	*Solvent*			*λ (nm)*
	40% Methanol	*45% Methanol*	*50% Methanol*	
Amaroswerin	9.3	4.42	2.33	233
Amarogentin	11.9	5.6	2.88	233
Amaropanin	25.5	11.0	5.10	233

absorption maximum in the low UV region. Eluent absorption was minimized, and injections of microgram amounts gave good chromatograms.

Separation of iridoid and secoiridoid glucosides

Table I shows our initial results. Not all the irridoids tested separated completely in the selected systems. However, only a limited number co-occur in natural products. With a correct choice of solvent system every iridoid glucoside tested can be separated from the others. Using a new efficient column we completely separated some important natural iridoid mixtures, such as amaroswerin–amarogentin–amaropanin (*Gentiana* species) (with minimum resolution $R = 1.45$ between amarogentin and amaroswerin) and catalpol–methylcatalpol–aucubin (*e.g.*, *Plantago* species) (Tables II and III). These mixtures cannot be adequately separated using conventional chromatographic procedures. Inouye *et al.*[2] calculated the relative GC retention times with the TMS-aucubin peak as standard. We initially used a similar method, with acetic acid as the basis for relative retention times. Acetic acid (on reversed phase) is not as strong an eluent as water and shows different retention times in relation to the solvent[5]. We determined t_0 by the injection of small amounts of $NaNO_3$ dissolved in water with methanol–water in aliquot parts as eluent[6].

The values of k_{rel} and k' depend on the column (packing, age, etc.) and on the exact solvent composition. Therefore on each column the best conditions for separation must be established. Our studies are intended as guide lines for further work, although the solvent system (methanol–water) and elution sequence are fixed.

Ion-pair chromatography with iridoid acids

Some iridoid glucosides have an acidic functional group, and acetic acid can be

TABLE III

RETENTION TIMES, CAPACITY FACTORS AND RELATIVE RETENTIONS OF IRIDOIDS FROM PLANTAGO SPECIES

Solvent: 20% methanol; flow-rate, 1 ml/min; $t_0 = 115$ sec; 1500 p.s.i.

Glucoside	t_R *(min:sec)*	k'	α	R
Catalpol	4:01	1.10		
			1.40	1.30
Aucubin	4:52	1.54		
			1.87	
Methylcatalpol	7:20	2.83		

used to suppress ionization[7]. However, acetic acid absorbs strongly in the region 210–235 nm, which is within the iridoid glucoside detection range. Furthermore, baseline stability is poor.

Ion-pair formation permits the separation of ionic compounds by reversed-phase chromatography. We used tetrabutylammonium phosphate as counter-ion, buffered to pH 7.5, and added to the mobile phase. The ion-pair formed with the acidic group behaves like a non-ionic species with lipophilic characteristics. A similar system was first developed by Wittmer *et al.*[8]. We used this method in preference to liquid–liquid ion-pair reversed-phase chromatography[9].

Increased tetrabutylammonium ion concentration does not lead to an increase in k'; 0.005 M is the optimum for most analytical separations[10]. Samples should be dissolved in the eluent, otherwise large negative solvent peaks appear in the chromatogram.

Ion-pair chromatography enabled us to detect baseline separation of a mixture of three acidic glucosides, which was not possible using the methanol–water system Table IV).

TABLE IV

CAPACITY FACTORS AND RELATIVE RETENTIONS OF IRIDOID ACIDS IN ION-PAIR CHROMATOGRAPHY

Solvent: 20% methanol with 0.005 M PIC-A reagent; flow-rate, 1 ml/min; $t_0 = 115$ sec; 1500 p.s.i.; $\lambda = 230$ nm.

Glucoside	k'	α
Monotropein	1.31	
		1.35
Geniposidic acid	1.77	
		2.14
Deacetylasperulosidic acid	3.78	
		1.19
Asperuloside (non acidic)	4.48	

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REFERENCES

1 O. Sticher and U. Junod-Busch, *Pharm. Acta Helv.*, 50 (1975) 127, and references therein.
2 H. Inouye, K. Uobe, M. Hirai, Y. Masada and K. Hashimoto, *J. Chromatogr.*, 118 (1976) 201, and references therein.
3 O. Sticher, in H. Wagner and P. Wolff (Editors), *New Natural Products and Plant Drugs with Pharmacological, Biological or Therapeutical Activity. Proceedings in Life Sciences*, Springer, Berlin, Heidelberg, New York, 1977, pp. 137–176.
4 S. R. Jensen, B. J. Nielsen and R. Dahlgren, *Botaniska Notiser*, 128 (1975) 148.

5 K. Karch, I. Sebastian, I. Halász and H. Engelhardt, *J. Chromatogr.*, 122 (1976) 171.
6 C. Horvath, W. Melander, I. Molnar, *J. Chromatogr.*, 125 (1976) 129.
7 L. W. Wulf and C. W. Nagel, *J. Chromatogr.*, 116 (1976) 271.
8 D. P. Wittmer, N. O. Nuessle and W. G. Haney, Jr., *Anal. Chem.*, 47 (1975) 1422.
9 B. Fransson, K.-G. Wahlund, I. M. Johansson and G. Schill, *J. Chromatogr.*, 125 (1976) 327.
10 *Paired-Ion Chromatography*, Waters Assoc., Milford, Mass., May 1976.