ELSEVIER

Journal of Chromatography A, 967 (2002) 75–84

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Review

Chromatography of the chromone and flavonoid alkaloids

Peter J. Houghton*

Pharmacognosy Research Laboratories, Department of Pharmacy, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 8WA, UK

Abstract

TLC methods have been derived for the qualitative investigation of the small group of compounds known as chromone and flavonoid alkaloids. Quantitation of two of the chromone alkaloids has been achieved by HPLC and a graphite column has been used to separate the unstable stereoisomers of the piperidone chromone alkaloids. A variety of preparative methods, including silica column and droplet counter-current chromatography, has been used to isolate the members of this group of compounds from plant extracts.
© 2002 Elsevier Science B.V. All rights reserved.

Keywords: Reviews; Chromone alkaloids; Flavonoid alkaloids

Contents

1. Introduction 75
2. Qualitative chromatography 76
 2.1. Flavonoidal alkaloids 76
  2.1.1. Thin-layer chromatography (TLC) 76
 2.2. Chromone alkaloids 77
  2.2.1. TLC behaviour—separation systems 77
  2.2.2. TLC behaviour—detection 79
  2.2.3. HPLC 80
 2.3. Quantitative chromatography 82
 2.4. Use of preparative chromatography in the isolation of flavonoid and chromone alkaloids 82
3. Conclusion 84
Acknowledgements 84
References 84

1. Introduction

The chromone and flavonoid alkaloids are of interest because of the biological activities which they have been found to possess. These include antiviral properties [1] and tyrosine kinase inhibition, the latter being the basis of their potential use in treating inflammatory conditions and acting as anti-neoplastic agents [2]. The biological activities of these alkaloids have been reviewed recently [3].

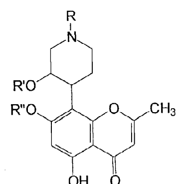
The classification of chromone and flavonoidal

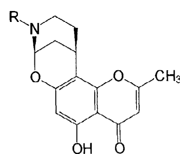
*Tel.: +44-207-8484-775; fax: +44-207-8484-800.
E-mail address: peter.houghton@kcl.ac.uk (P.J. Houghton).

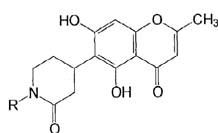
0021-9673/02/$ – see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0021-9673(01)01555-2

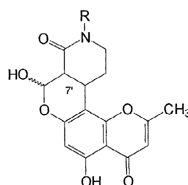
alkaloids is somewhat unusual since most alkaloids are classified chemically according to the nitrogen-containing ring system. However, the class under consideration is typified by the part of the molecule to which the nitrogenous moiety is attached, i.e., a chromone or flavonoid structure. Only one chromone, noreugenin, has so far been found as part of an alkaloid molecule but several different flavonoids have been shown to comprise the non-nitrogenous part of isolated alkaloids. The naturally-occurring chromone alkaloids isolated to date are shown in Figs. 1 and 2 and the flavonoidal alkaloids shown in Fig. 3. All but one of these compounds have been isolated from flowering plants, the exception being tubastraine which was isolated from a coral. The natural sources of the alkaloids so far isolated are shown in Tables 1 and 2.

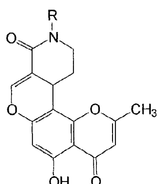
The chromone and flavonoid alkaloids are interesting chemically since they possess phenolic as well as nitrogenous parts of the molecule and this aspect influences their chromatographic behaviour. In some molecules the nitrogen is present as a "typical" alkaloidal tertiary or quaternary amine but in some of the molecules it comprises part of a piperidone lactam which has very little basic character.











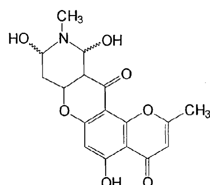
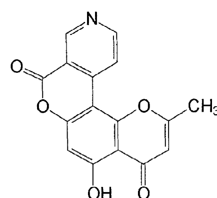
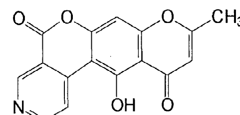
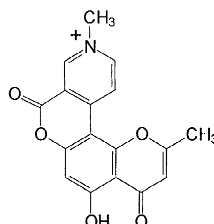
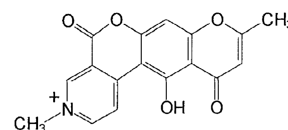


Fig. 1. Noreugenin-derived chromone alkaloids.









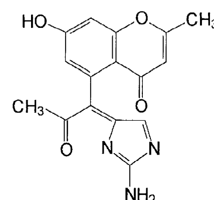


Fig. 2. Noreugenin-derived chromone alkaloids.

Most of the chromatographic work reported has been carried out on the chromone alkaloids, particularly those from *Schumanniophyton* species, and qualitative and quantitative procedures have been described as well as the use of preparative chromatography in isolation of the individual compounds.

2. Qualitative chromatography

2.1. Flavonoidal alkaloids

2.1.1. Thin-layer chromatography (TLC)

Little investigation of the TLC behaviour of the

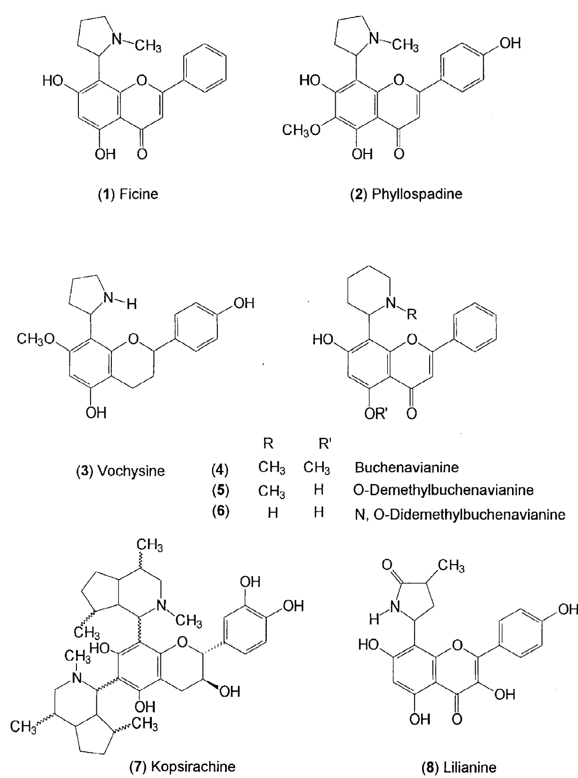


Fig. 3. Flavonoidal alkaloids.

flavonoidal alkaloids has been reported since it is mentioned only as part of the isolation procedure and, in some cases, inadequate information is supplied. In most cases Dragendorff's Reagent has been used as the detecting reagent although the lactam lilialine (**8**), separated by TLC on silica gel/benzene–acetone gave no reaction with this reagent but could be visualised with 5% aq. iron (III) chloride [9]. Phyllospadine (**2**) was separated using cellulose (Avicel) and butan-1-ol–formic acid–carbon tetrachloride–water (4:1:1:1) [5]. The *Buchenavia* flavonoidal alkaloids (**4**, **5**, **6** and others) were analysed by TLC using Kieselgel GF_{254} plates (Merck) and dichloromethane–methanol (95:5) as developing solvent [7].

There are no reports concerning high-performance liquid chromatography (HPLC) analysis of the flavonoidal alkaloids.

2.2. Chromone alkaloids

2.2.1. TLC behaviour—separation systems

In contrast to the flavonoidal compounds, a reasonable amount of data has been published concerning the qualitative analysis of the noreugenin-derived chromone alkaloids since details of TLC behaviour have been provided in most of the publications dealing with their isolation and structural elucidation.

The compounds in question exhibit a wide range of polarities from the comparatively non-polar tertiary pyridine bases schumanniophytine (**21**) and isoschumanniophytine (**22**) to their very polar water-soluble quaternary analogues **23** and **24**. The TLC separation systems which have been used reflect this

Table 1
Flavonoidal chromone alkaloids from natural sources

Compound	Source	Ref.
Ficine (**1**)	*Ficus pantoniana* King. leaves	[4]
Isoficine	*F. pantoniana* King. leaves	[4]
Phyllosadine (**2**)	*Phyllospadix iwatensis* Makino leaves	[5]
Vochysine (**3**)	*Vochysia guianensis* (Aubl.) Poir. fruits	[6]
Buchenavianine (**4**)	*Buchenavia macrophylla* Eichl. leaves, fruits	[7]
N-Demethylbuchenavianine (**5**)	*B. macrophylla* Eichl. leaves	[7]
O-Demethylbuchenavianine	*B. macrophylla* fruits	[7]
O,N-Demethylbuchenavianine (**6**)	*B. macrophylla* fruits	[7]
N-Demethylcapitavine	*B. macrophylla* fruits	[7]
5,7-Dihydroxy-6-(*N*-methyl-2″-piperidinyl)flavanone	*B. macrophylla* fruits	[7]
Capitavine	*Buchenavia capitata* Eichl. seeds	[7]
5,7,4′-Trihydroxy-6-(*N*-methyl-2″-piperidinyl)flavone	*B. capitata* Eichl. seeds	[7]
Kopsirachine (**7**)	*Kopsia dasyrachis* Ridl. leaves	[8]
Liliaine (**8**)	*Lilium candidum* L. flowers	[9]

Table 2
Noereugenin-derived chromone alkaloids from natural sources

Compound	Source	Refs.
Piperidine-type related to rohitukine		
Rohitukine (**9**)	*Amoora rohituka* Wight and Arn. leaves, steam.	[10]
	[Syn. *Aphanamixis polystachya* (Wall.) R.N. Parker]	
	Dysoxylum binectariferum Hook. stembark	[11]
	Schumanniophyton magnificum Harms. rootbark, stembark	[12]
	Schumanniophyton problematicum (A.Chev.) Aubrev. rootbark, stembark	[13]
Tubastraine (**10**)	*Tubastraea micrantha*	[14]
N-Demethylrohitukine-3′-acetate (**11**)	*Schumanniophyton magnificum* Harms. stembark	[15]
Schumagnine (**12**)	*Schumanniophyton magnificum* Harms. rootbark	[12]
N-Methylschumagnine (**13**)	*Schumanniophyton magnificum* Harms. rootbark	[12]
Noreugenin-6-piperidone-type		
Unnamed alkaloid A (**14**)	*Schumanniophyton problematicum*	[16]
	(A. Chev.) Aubrev. rootbark	
	Schumanniophyton magnificum Harms. stembark	[15]
Unnamed alkaloid B (**15**)	*Schumanniophyton problematicum* (A. Chev.) Aubrev. rootbark	[16]
Noreugenin-8-piperidone-type		
Schumannificine (**16**)	*Schumanniophyton magnificum* Harms. Rootbark, stembark	[17–20]
	Schumanniophyton problematicum	[15]
	(A. Chev.) Aubrev. rootbark, stembark	
N-Methylschumannificine (**17**)	*S. magnificum* Harms. rootbark, stembark	[17–20]
	Schumanniophyton problematicum	[15]
	(A. Chev.) Aubrev. rootbark, stembark	
Anhydroschumannificine (**18**)	*S. magnificum* Harms. rootbark	[12,19,20]
N-Methylanhydroschumannificine (**19**)	*S. magnificum* Harms. rootbark	[12,19,20]
Hydroxy-*N*-methylschumannificine (**20**)	*S. magnificum* Harms. stembark	[15]
Noreugenin-8-pyridine type		
Schumanniophytine (**21**)	*S. problematicum* (A.Chem.) Aubrev rootbark	[16]
	S. magnificum Harms. Rootbark, stembark	[18]
Isoschumanniophytine (**22**)	*S. magnificum* Harms. rootbark	[18]
N-Methylschumannuiophytine (**23**)	*S. magnificum* Harms. rootbark	[12]
N-Methylisoschumanniophyytine (**24**)	*S. magnificum* Harms. rootbark	[12]
Miscellaneous		
Cassiadinine (**25**)	*Cassia siamea* Lam. flowers	[21]

and are shown in Table 3 and the behaviour of each alkaloid, expressed in terms of its hR_F value is shown in Table 4.

Conventional separation on silica gel with moderately polar mobile phases consisting of small amounts of methanol with less polar solvents was successfully used for the pyridine bases, the piperidones and related compounds. The R_F values of the different compounds reflects their polarity, e.g., rohitukine (**9**), which has three OH groups, displays much more affinity for the stationary phase than compounds where the only OH group present is attached to C-5 and is strongly H-bonded to the chromone carbonyl.

Some of the more polar compounds were difficult to separate from each other but it was found that making the silica layer alkaline, by preparing it in 0.1 *M* sodium hydroxide, resulted in the separation of **11**, **15** and **20** from *N*-methylschumannificine (**17**) with which they were concurrent in acidic silica systems [15]. A hypothesis for this could be the more phenolic nature of the three molecules com-

Table 3
Analytical TLC systems for noreugenin-type chromone alkaloids

TLC system	Stationary phase	Mobile phase	Refs.
	Non-polar compounds		
A	Silica gel GF_{254}	Chloroform–butanone (4:1)	[18]
B		Chloroform–methanol (12:1)	[18]
C		Butanone–methanol (12:1)	[18]
D	Silica gel GF_{254} plates prepared	Chloroform–methanol (12:1)	[15]
E	with 0.1 *M* NaOH	Dichloromethane–methanol (10:1)	[15]
	Polar compounds		
F	Silica gel GF_{254}	Acetone–methanol–10% NH_4OH (4:1:1)	[12,18]
G		Butan-1-ol–methanol–10% NH_4OH (4:1:1)	[12,18]
H	Silica gel GF_{254} impregnated with	Citrate buffer, pH 4.2–methanol (3:1)	[22]
J	5% (v/v) liquid paraffin in petroleum	Borate buffer, pH 10.0–methanol (3:1)	[22]

Note: In all cases the silica gel used was obtained from Merck. Plates were made by mixing silica gel and water in the laboratory followed by hand spreading. All plates were dried at room temperature for 2 h then at 105 °C for 60 min prior to use.

pared with **17** and hence their increased affinity for an alkaline layer.

The more polar compounds were separated using very polar mobile phases with silica gel but more recent work showed that they could be separated using reversed-phase (RP) systems where the silica gel had been previously impregnated with liquid paraffin [22]. Nothing has been reported concerning behaviour on chemically-bonded RP plates.

2.2.2. TLC behaviour—detection

Detection of the noreugenin chromone alkaloids after TLC separation is not difficult as many of them are fluorescent and all of them quench short range

Table 4
hR_F values of chromone alkaloids in reported TLC systems [12,15,18,22]

Alkaloid	TLC system (see Table 3)								
	A	B	C	D	E	F	G	H	J
Rohitukine type									
Rohitukine (**9**)	–	–	–	–	–	95	55	22	30
N-Demethylrohitukine-3′-acetate (**11**)	–	40	37	45	27	–	–	–	–
Schumagnine (**12**)	42	62	59	–	–	–	–	–	–
N-Methylschumagnine (**13**)	55	72	64	–	–	–	–	–	–
Piperidone type									
Alkaloid (**15**)	–	51	39	46	52	–	–	–	–
Schumannificine (**16**)	5	37	29	–	–	–	–	–	–
N-Methylschumannificine (**17**)	10	72	40	–	–	–	–	–	–
Anhydroschumannificine (**18**)	18	79	47	–	–	–	–	–	–
N-Methylanhydroschumannificine (**19**)	28	86	45	–	–	–	–	–	–
Hydroxy-*N*-methylschumannificine (**20**)	–	51	39	46	43	–	–	–	–
Pyridine type									
Schumanniophytine (**21**)	9	70	6	–	–	–	–	–	–
Isoschumanniophytine (**22**)	9	72	4	–	–	–	–	–	–
N-Methylschumanniophttine (**23**)	–	–	–	–	–	75	22	25	17
N-Methylisoschumanniophytine (**24**)	–	–	–	–	–	24	1	40	32

–=Not reported.

Table 5
Colours observed for chromone alkaloids with various TLC detection methods [12,15,18]

Alkaloid	Appearance of zone			
	UV 254 nm	UV 366 nm	Daylight after Dragendorff's reagent	Daylight after Dragendorff's reagent followed by 5% aq. ion (III) chloride
Rohitukine type				
Rohitukine (**9**)	Quenches	No colour	Pale orange	Blue–black
N-Demethylrohitukine-3′-acetate (**11**)	Quenches	No colour	Pale orange	Blue–black
Schumagnine (**12**)	Quenches	No colour	Pale orange	Grey–brown
N-Methylschumnine (**13**)	Quenches	No colour	Pale orange	Red–brown
Piperidone type				
Schumannificine (**16**)	Quenches	No colour	Pale orange	Blue–black
N-Methylschumannificine (**17**)	Quenches	No colour	Brown	Dark brown
Anhydroschumannificine (**18**)	Quenches	No colour	Blue	Blue–black
Piperidone type				
N-Methylanhydroschumannificine (**19**)	Quenches	No colour	Red–brown	Dark brown
Hydroxy-*N*-methylschumannificine (**20**)	Quenches	No colour	Orange	Dark brown
Alkaloid (**15**)	Quenches	No colour	Pale orange	Dark brown
Pyridine type				
Schumanniophytine (**21**)	Pale yellow	Lemon yellow	Orange	Dark orange
Isochumanniophytine (**22**)	Pale yellow	Golden yellow	Brown	Dark brown
N-Methylschumanniophytine (**23**)	Yellow	Bright yellow	Orange	Dark orange
N-Methylisoschumanniophytine (**24**)	Yellow	Bright yellow	Orange	Dark orange

UV light. The reactions of most of the isolated alkaloids to detection systems reported in the literature are shown in Table 5. It is noteworthy that some of these compounds, especially the piperidone secondary amines, such as schumannificine (**16**) and anhydroschumannificine (**18**) do not give the classical deep orange zone when sprayed with Dragendorff's reagent. This is presumably because this spray reagent depends on a complex being formed between tertiary amine groups, absent in **16** and **18** and the bismuth molecules in the reagent. The compounds can, however, be easily visualised by using 5% (w/v) aqueous iron (III) chloride solution to overspray the plate after it has been treated with Dragendorff's reagent.

It should be noted, however, that other non-nitrogenous phenolic compounds present, such as the noreugenin glycosides, will also give coloured zones after this treatment due to the iron–phenol complex. A further possibly misleading reaction after the initial treatment with Dragendorff's reagent is the strong orange colour given by the coumarin scopoletin and related compounds which have been found to be present in large amounts in the bark of *Schumanniophyton problematicum* [13].

2.2.3. HPLC

The interesting anti-human immunodeficiency virus (HIV) activity displayed by **16** prompted the investigation of HPLC methods for the screening of samples of plant material in which it and related compounds were found. Since quantitation as well as qualitative information was required, it was considered that HPLC would be more suitable than the TLC procedures already in existence which, although they could be adapted for densitometry, were unlikely to give very precise information.

The procedure leading to the HPLC system finally chosen has been described [13]. A reversed-phase system was employed using an Alphasil 5 ODS column (HPLC Technology, Cheshire, UK) of 250× 4.6 mm I.D. with detection at 254 nm using a Perkin-Elmer LC-290 variable-wavelength UV detector. Various proportions of methanol and 20 m*M* potassium dihydrogenphosphate were used to optimise the separation of schumannificine (**16**), *N*-

methylschumannificine (**17**) and morphine, which was chosen as an internal standard since it resembles the chromone alkaloids in possessing both phenolic and tertiary amine groups. Parameters investigated for the best separation were the pH of the buffer and its concentration which were found to be 5.5 and 20 m*M*, respectively. Further experiments designed to find the optimum mixture of these two solvents showed that a 50:50, v/v, mixture was found to give the best range of capacity factors and so this was used as the mobile phase.

All of the alkaloids found in the plant extracts eluted within 12 min using the above system with a flow-rate of 1.0 ml/min. The various types of alkaloids eluted in the expected sequence with the most polar, e.g., rohitukine (**9**) eluting first, followed by schumannificine (**16**), *N*-methylschumannificine (**17**) and lastly the tertiary pyridine compounds (**21**, **22**).

HPLC has also been used to separate the 7′-H isomers of schumannificine (**16**) and *N*-methylschumannicine (**17**). Nuclear magnetic resonance (NMR) spectroscopic studies had revealed that the isolated compounds were a mixture of two compounds which could not be separated by TLC. When the structures of these compounds were finally determined [20], it could be seen that the two compounds were isomers depending on the orientation of the 7′-H of the hemiacetal bridge. Attempts to separate these isomers by a variety of TLC and HPLC procedures proved futile (see Tables 6 and 7) until a graphite HPLC column was used [23]. The use of this column was based on the consideration that a graphite stationary phase presents a planar non-polar surface. The molecules in question (**16** and **17**) have a major planar moiety in the molecule due to the chromone ring and the hypothesis proposed was that the difference in the orientation of the 7′-OH could result in different affinities of the two isomers for the stationary phase and hence their separation. Experiments using the pure compounds did in fact show that two peaks were obtained [23] and in the same ratio as that shown by the NMR spectra [18], thus demonstrating that the two isomers had been resolved. The system used consisted of a a Perkin-Elmer 410 LC pump; a Rheodyne 7125 injection valve (Cotati, Berkeley, CA, USA) fitted with a 10 μl sample loop [24]. A variable-wavelength UV detector (Perkin-Elmer LC-290; Norwalk, CT, USA) coupled with a Perkin-Elmer integrator and printer, was used for recording the chromatograms. A stainless steel column (100×4.6 mm I.D.)

Table 6
HPLC systems employed for the attempted separation of isomers of schumannificine (**16**) and *N*-methylschumannificine (**17**)

Column	Manufacturer	Mobile phase used	t_R (min)	
			1	2
Hypersil 5 μm Si	Phenomenex UK,	1	5.0	4.2
(150×4.6 mm)	Cheshire, UK	2	6.1	5.4
		3	5.5	4.7
Alphasil 5 μm ODS	HPLC Technology,	5	2.8	3.7
(250×4.6 mm)	Cheshire, UK	6	3.1	3.9
Spherisorb 5 μm	Phase Separations,	5	3.3	4.1
(C_6, C_8, C_{18})	Deeside, UK	6	3.2	4.0
(250×4.6 mm)				
Hypersil CN 3 μm	Phenomenex UK,	1	4.3	3.5
(100×4.6 mm)	Cheshire, UK	2	4.6	3.8
		3	3.9	3.3
		5	2.8	3.7
		6	2.5	3.4
AGP	Enantiopac, LKB	4	12.1	13.0
(100×4.6 mm)	Produkter, Bromma, Sweden			
β-Cyclodextrin	Cyclobond I, Astec,	5	8.7	9.8
(250×4.6 mm)	Whippany, NJ, USA	6	8.0	8.9

Mobile phases: (1) hexane–ethanol (98:2); (2) hexane–chloroform–ethanol (15:3:1); (3) chloroform–methanol (10:1); (4) 1–2% isopropanol in KH_2PO_4 buffer (pH 4.5–6.5); (5) methanol–20 m*M* KH_2PO_4 (50:50); (6) acetonitrile–20 m*M* KH_2PO_4 (60:40).

Table 7
TLC systems used in attempts to separate schumannificine (**16**) and *N*-methylschumannificine (**17**) isomers

Stationary phase	Mobile phase	R_F values	
		1	2
Silica gel GF_{254} (Merck)	Chloroform–methanol (12:1)	0.45	0.56
	Ethyl acetate–methanol (15:1)	0.49	0.58
Silica gel RP-18 (Merck)	Methanol–water (40:60)	0.68	0.61
	Methanol–20 m*M* NaH_2PO_4 (40:60)	0.70	0.63
	Acetonitrile–20 m*M* NaH_2PO_4 (50:50)	0.67	0.59
	Acetonitrile–0.6 *M* NaC1 in 0.1 *M* β-cyclodextrin (50:50)	0.58	0.50
Silica gel GF_{254} impregnated with liquid paraffin	Methanol–water (40:60)	0.70	0.64
	Methanol–20 m*M* NaH_2PO_4 (40:60)	0.72	0.66
	Acetonitrile–20 m*M* NaH_2PO_4 (50:50)	0.68	0.60
Silica gel GF_{254} impregnated with (+)-tartaric acid	Chloroform–methanol (12:1)	0.33	0.46
Silica gel GF_{254} impregnated with (−)-quinine	Chloroform–butanone–acetic acid (4:3:0.5)	0.56	0.71
Silica gel GF_{254} impregnated with (−)-brucine	Chloroform–butanone–acetic acid (4:3:0.5)	0.54	0.70

N.B. All layers were 0.25 mm thick.

packed with Hypercarb S (7 μm) (Shandon Scientific, Cheshire, UK) was used. The optimised mobile phase was pumped at 1.5 ml/min and consisted of tetrahydrofuran (THF)–20 m*M* sodium dihydrogenphosphate (50:50, w/v; adjusted to pH 2.5 with trifluoroacetic acid, TFA). Detection was effected at 254 nm. The isomer having 7′-H α was thought to have the longer retention time since studies with models showed that this possessed a more planar lipophilic face than the corresponding 7′-H β isomer. Attempts to use this system for preparative separation of the two isomers proved futile since it was shown that interconversion took place rapidly within a few hours in the presence of water, presumably due to the lability of the hemiacetal junction.

2.3. Quantitative chromatography

The only quantitative work so far executed has concerned only the noreugenin chromone alkaloids schumannificine (**16**) and *N*-methylschumannificine (**17**) and this utilised HPLC [13].

The primary aim of this work was to quantify these compounds in methanolic extracts of bark obtained from species of *Schumanniophyton* in which they occur. Some of these extracts contain large amounts of non-alkaloidal phenols and so passage of the extract through a Bond-Elut Diol cartridge was used as a clean up procedure prior to HPLC analysis. The eluate containing the alkaloids was dried and then dissolved in the internal standard solution consisting of 0.1 mg/ml morphine in methanol. The separation system consisted of a Perkin-Elmer 410 LC pump adjusted to give a flow-rate of 1.0 ml/min. Samples were injected through a Rheodyne 7125 injection valve fitted with a 10 μl sample loop. A stainless steel cartridge column (250×4.6 mm I.D.) packed with Alphasil 5 ODS was used and the mobile phase consisted of methanol–20 m*M* potassium dihydrogenphosphate (50:50) adjusted to pH 5.5. Detection at 254 nm was effected using a variable-wavelength UV detector (Perkin-Elmer LC-290) coupled with a Perkin-Elmer integrator and printer.

Linear calibration graphs, five replicates, for different concentrations of the two compounds and morphine gave correlation coefficients of 0.9998 for **16** and 0.9997 for **17**. These calibration graphs were then used to compare the amounts of the two compounds in fresh and dried samples of both root- and stem-bark of *Schumanniophyton magnificum* and in dried samples of *S. problematicum* stem- and root-bark.

No other work has been reported on the quantitation of these or other chromone alkaloids.

2.4. Use of preparative chromatography in the isolation of flavonoid and chromone alkaloids

In most cases the alkaloids have been isolated from crude extracts of plant material using separation

Table 8
Preparative normal-phase column chromatography using silica gel used to isolate flavonoidal and chromone alkaloids

Alkaloid	Eluting solvent	Ref.
Flavonoidal alkaloids		
Phyllospadine (**2**)	Benzene–ethyl acetate (3:1)	[5]
Vochysine (**3**)	Chloroform–methanol (95:5)	[6]
Buchenavia alkaloids (**4**–**6**)	Chloroform–methanol gradient	[24]
Kopsirachine (**7**)	Chloroform–methanol (95:5)	[8]
Lilianine (**8**)	Benzene–acetone	[9]
Noreugenin-type chromone alkaloids		
Rohitukine (**8**)	Dichloromethane–methanol (2:1)	[10]
N-Demethylrohitukine-3′-acetate (**11**)	Chloroform–methanol gradient	[15]
Alkaloid (**15**)	Chloroform–methanol gradient	[15]
Schumannificine (**16**)	Chloroform–methanol gradient	[18]
N-Methylschumannificine (**17**)	Chloroform–methanol gradient	[18]
Anhydroschumannificine (**18**)	Chloroform–methanol gradient	[18]
N-Methylanhydroschumannificine (**19**)	Chloroform–methanol gradient	[18]
Hydroxy-*N*-methylschumannificine (**20**)	Chloroform–methanol gradient	[15]
Schumanniophytine (**21**)	Chloroform–methanol gradient	[18]
Isoschumanniophytine (**22**)	Chloroform–methanol gradient	[18]

on silica gel columns. A summary of the procedures used for those alkaloids where this information is provided is shown in Table 8.

The more polar alkaloids, *N*-methylisoschumanniophytine (**24**), rohitukine (**9**) and *N*-methylschumanniophytine (**23**), as well as schumagnine (**14**) and isoschumagnine (**15**) were separated in that order of elution by liquid–liquid droplet countercurrent chromatography on a Buchi 670 DCC instrument [12]. Initial fractionation was achieved by using butan-1-ol–methanol–water (5:1:5) in descending mode and further separation of (**9**) and (**23**) by using butan-1-ol–methanol–10% aq. ammonia (5:1:5) in ascending mode where rohitukine was the first alkaloid to be eluted.

Preparative TLC has been used extensively in the final purification and isolation of this group of alkaloids. In most cases silica gel GF_{254} or P_{254} (Merck) has been used as the stationary phase and the mobile phases used are shown in Table 9. Non-destructive detection of the alkaloids can be achieved by examination under UV light 254 nm and the

Table 9
Preparative silica gel thin-layer chromatographic systems used to isolate flavonoidal and chromone alkaloids

Alkaloid	Mobile phase	Ref.
Flavonoidal alkaloids		
Phyllospadine (**2**)	Benzene–ethyl acetate (3:1)	[5]
Noreugenin-type chromone alkaloids		
N-Demethylrohitukine-3′-acetate (**11**)	Chloroform–methanol (12:1)	[15]
Schumagnine (**12**)	Butanone–methanol (12:1)	[12]
N-Methylschumagnine (**13**)	Butanone–methanol (12:1)	[12]
Alkaloid (**15**)	Chloroform–methanol (12:1)	[15]
Schumannificine (**16**)	Chloroform–methanol (12:1)	[18]
N-Methylschumannificine (**17**)	Chloroform–methanol (12:1)	[18]
Anhydroschumannificine (**18**)	Chloroform–butanone (4:1)	[18]
N-Methylanhydroschumannificine (**19**)	Chloroform–butanone (4:1)	[18]
Hydroxy-*N*-methylschumannificine (**20**)	Chloroform–methanol (12:1)	[15]
Schumanniophytine (**21**)	Chloroform–butanone (4:1)	[18]
Isoschumanniophytine (**22**)	Chloroform–butanone (12:1) gradient	[18]

compounds have been eluted from the removed silica with methanol.

Preparative HPLC has not been employed widely in the isolation of these compounds. Kopsirachine (**7**) was isolated from the crude extract of leaves of *Kopsia dasyrachis* using a preparative silica column and eluting with chloroform–methanol–25% ammonia solution (90:10:1) [8]. The separation of the the two 7′-H isomers of schumannificine (**16**) and *N*-methylschumannificine (**17**) has been mentioned above [23]. No preparative column was available to isolate the two isomers so small amounts were repeatedly injected on to the analytical column of graphite used for qualitative analysis. Relevant fractions were collected and the respective fractions combined but it was found that each of the two collected fractions contained both isomers and it was shown that interconversion took place rapidly in the aqueous mobile phase employed [23].

3. Conclusion

A reasonably extensive knowledge of the TLC behaviour of the flavonoid and chromone alkaloids has now been acquired as has also the application of chromatography in their separation and isolation. Although some progress has been made in HPLC separation of this group of compounds, more needs to be carried out should the quantitation of most of them be required at any future stage.

Acknowledgements

Some of the work reported here, involving the chromatography of the noreugenin-type chromone alkaloids, was supported by a grant from the Medical Research Council AIDS programme.

References

[1] P.J. Houghton, T.Z. Woldemariam, A.I. Khan, A. Burke, N. Mahmood, Antiviral Res. 25 (1994) 235.
[2] N.J. De Souza, in: A.D. Kinghorn, M.F. Balandrin (Eds.), Human Medicinal Agents from Plants, ACS Symposium Series, Vol. 534, American Chemical Society, 1993, p. 331.
[3] P.J. Houghton, in: A. ur-Rahman (Ed.), Studies in Natural Product Chemistry, Elsevier, Amsterdam, 1999, in press.
[4] S.R. Johns, J.H. Russell, Tetrahedron Lett. (1965) 1987.
[5] M. Tagaki, S. Funahashi, K. Ohta, T. Nakabayashi, Agric. Biol. Chem. 44 (1980) 3019.
[6] G. Baudouin, F. Tillequin, M. Koch, M. Vuilhorgne, J.-Y. Lallemand, H. Jacquemin, J. Nat. Prod. 46 (1983) 681.
[7] A. Ahond, A. Fournet, C. Moretti, E. Philogene, C. Poupat, O. Thoison, P. Potier, Bull. Soc. Chim. Fr. 2 (1984) 41.
[8] K. Homberger, M. Hesse, Helv. Chim. Acta 67 (1984) 237.
[9] I. Masterova, D. Uhrin, J. Tomko, Phytochemistry 26 (1987) 1844.
[10] A.D. Harmon, U. Weiss, J.V. Silverton, Tetrahedron Lett. 20 (1979) 721.
[11] R.G. Naik, S.L. Kattige, S.V. Bhat, B. Alreja, N.J. de Souza, R.H. Rupp, Tetrahedron 44 (1988) 2081.
[12] P.J. Houghton, Y. Hairong, Planta Med. 47 (1987) 262.
[13] P.J. Houghton, T.Z. Woldemariam, Phytochem. Anal. 4 (1993) 9.
[14] A. Alam, R. Sanduja, G.M. Wellington, Heterocycles 27 (1988) 719.
[15] P.J. Houghton, Planta Med. 48 (1988) 239.
[16] E. Schlittler, U. Spitaler, Tetrahedron Lett. 19 (1978) 2911.
[17] J.I. Okogun, J.O. Adeboye, D.A. Okorie, Planta Med. 49 (1983) 95.
[18] P.J. Houghton, Y. Hairong, Planta Med. 48 (1985) 23.
[19] P.J. Houghton, Planta Med. 47 (1987) 264.
[20] P.J. Houghton, I.M. Osibogun, T.Z. Woldemariam, K. Jones, Planta Med. 61 (1995) 154.
[21] K.M. Biswas, H. Mallik, Phytochemistry 25 (1986) 1727.
[22] P.J. Houghton, I.M. Osibogun, Phytochem. Anal. 1 (1990) 22.
[23] P.J. Houghton, T.Z. Woldemariam, Phytochem. Anal. 6 (1995) 85.
[24] J.A. Beutler, J.H. Cardellina II, J.B. McMahon, M.R. Boyd, G.M. Cragg, J. Nat. Prod. 55 (1992) 207.