equipment removed, the number of examples will increase. Essential oils are going that way quickly. For polar substances, the published work shows that each material has to be examined to find the best conditions. It is difficult to assess how many natural products are being routinely extracted in this way, but the steady output of papers indicates continuing interest. As the chlorinated solvents are withdrawn, we can expect to see the use of SFE increase strongly. There is a ready market for some cheaper and less elaborate extraction equipment to meet this growing demand.

See also: **II/Chromatography: Supercritical Fluid:** Theory of Supercritical Fluid Chromatography. **Extraction:** Supercritical Fluid Extraction. **III/Supercritical Fluid Extraction-Supercritical Fluid Chromatography.**

Further Reading

Albert K (1997) Supercritical fluid chromatography-proton nuclear magnetic resonance spectroscopy coupling. *Journal of Chromatography A* 785: 65.

Bevan CD and Marshall PS (1994) The use of supercritical fluids in the isolation of natural products. *Natural Products Reports* 11: 451.

Jarvis AP and Morgan ED (1997) Isolation of plant products by supercritical-fluid extraction. *Phytochemical Analysis* 8: 217. [The whole of *Phytochemical Analysis* 1997; 8(5) is devoted to papers on supercritical fluid extraction.]

Kalampoukas G and Dervakos GA (1996) Process optimization for clean manufacturing: supercritical fluid extraction for β-carotene. *Computers and Chemical Engineering* 20: S1383.

McHugh MA and Krukonis VJ (1986) *Supercritical Fluid Extraction*. London: Butterworth.

Modey WK, Mulholland DA and Raynor MW (1996) Analytical supercritical fluid extraction of natural products. *Phytochemical Analysis* 7: 1.

Reverchon E (1997) Supercritical fluid extraction and fractionation of essential oils and related products. *Journal of Supercritical Fluids* 10: 1.

Smith RM and Hawthorne SB (1997) Supercritical fluids in chromatography and extraction. Special volume of *Journal of Chromatography A* 785.

Thin-Layer (Planar) Chromatography

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Introduction

The use of thin-layer chromatography (TLC) for the analysis of plant extracts began in the 1960s, with the work of Stahl and Randerath. The continuing use, and further development of TLC in plant analysis is justified because of its rapidity and because of the availability of a number of different sorbents. TLC is also useful in plant analysis because it is possible to work on crude extracts, which is not the case with other analytical methods. In plant analysis, derivatization methods serve to increase sensitivity and selectivity in addition to providing evidence concerning the quality of the separation. A selection of the most important derivatization/detection methods are given in **Table 1**. As well as these advantages, TLC is very economical and can be employed for routine use because the consumption of solvent is very low, and it is possible to analyse numerous samples on the same plate. The literature on plant analysis by TLC is very extensive, with the most important contributions being those of Stahl, Randerath, Fried and Sherma, Harbone, and Wagner, who is our main reference on this topic. Most TLC studies are listed in the different pharmacopoeias of the world, as reported by Wagner. The classes of plant compounds separated by TLC covered in this article are as follows:

- Alkaloids
- Glycosides: flavonoids, coumarins, anthocyanins, ginkgolides, anthraquinone glycosides, cardiac glycosides
- Saponins
- Essential oils
- Cannabinoids
- Valepotriates
- Bitter principles

Alkaloids

Most plant alkaloids are tertiary amines; others contain primary, secondary, or quaternary nitrogen (**Figures 1–4**). The basicity of individual alkaloids varies considerably; depending on which of the four types is represented. The pK_b values lie in a range 10–12 for weak bases like purines to 3–7 for stronger bases like opium alkaloids. These factors must be borne in mind for extraction, and also for derivatization. The sample sizes applied to the TLC plate must be calculated, according to the average alkaloid content of the specific extracts. The majority of workers

Table 1 Detection methods and spray reagents

Acetic anhydride	10 mL acetic anhydride, heated at 150°C for about 30 min and then inspected in UV light (365 nm)	Ginkgolides
Anisaldehyde–sulfuric acid	0.5 mL anisaldehyde with 10 mL glacial acetic acid, 85 mL methanol and 5 mL concentrated sulfuric acid, in that order. Spray and heat at 100°C for 5–10 min. Then evaluated in visible light or UV 365 nm; conservation limited, not usable when the reagent is red–violet	Terpenoids, propyl-propanoids, saponins, anthocyanins
Antimony(III) chloride ($SbCl_3$)	20% solution of antimony chloride in chloroform, or ethanol sprayed and then heated for 5–6 min at 110°C	Cardiac glycosides, saponins
Chloramine–trichloroacetic acid	10 mL freshly prepared 3% aqueous chloramine T solution with 40 mL 25% ethanolic trichloroacetic acid, sprayed, then heated at 100°C for 5–10 min then evaluated in UV light (365 nm)	Cardiac glycosides
Dinitrophenylhydrazine	0.1 g, 2,4-dinitrophenylhydrazine in 100 mL methanol, followed by addition of 1 mL 36% hydrochloric acid; evaluation immediately in visible light	Ketones, aldehydes
Dragendorff (Munier–Macheboeuf) reagent	Solution A: 0.85 g basic bismuth nitrate in 10 mL glacial acetic acid and 40 mL water under heating. Solution B: 8 g potassium iodide in 30 mL water. Stock solutions A + B are mixed	Alkaloids
Dragendorff, followed by sodium nitrite	After spraying with Dragendorff, the plate is sprayed with 10% aqueous sodium nitrite. The coloured zones are brown	Alkaloids
Dragendorff (with hydrochloric acid)	5 g bismuth carbonate in 50 mL H_2O, then 10 mL hydrochloric acid and add 25 g potassium iodide, complete with water to 100 mL. The spray reagent is obtained by dilution of 1 mL in 25 mL HCN	Alkaloids, purines (caffeine, theobromine, theophylline)
Fast blue	Fast blue salt B in 100 mL. Spray then look in visible light. A second solution can be sprayed using 10% ethanolic acid, followed by inspection in visible light	Cannabinoids
Iodine	About 10 g solid iodine is spread in a chromatographic tank; the plate is placed into the tank and exposed to iodine vapour, yellow–brown zones are detected in visible light	Compounds with conjugated double bonds
Iodine–chloroform	0.5 g iodine in 100 mL chloroform; after spraying, the plate is warmed at 70°C during 5 min, the plate is evaluated after 20 min in visible or UV light (365 nm)	Ipecacuanha alkaloids
Iodine–hydrochloric acid	Solution A: 1 g potassium iodide and 1 g iodine in 100 mL ethanol. Solution B: 25 mL 25% HCl with 25 mL ethanol. Spray the plate with 5 mL of A followed by 5 mL of B	Purines
Iodoplatinate	0.3 g hydrogen hexachloroplatinate hydrate in 100 mL water with 100 mL 6% potassium iodide solution	Alkaloids (blue–violet)
Iron(III) chloride ($FeCl_3$)	10% in aqueous solution evaluation in visible light	Polyphenols
Kedde reagent	5 mL freshly prepared 3% ethanolic 3,5-dinitrobenzoic acid with 5 mL 2 mol L^{-1} NaOH	Cardenolides
Liebermann reagent	5 mL acetic anhydride and 5 mL concentrated sulfuric acid is added carefully to 50 mL absolute ethanol, while cooling in ice. This agent must be freshly prepared. The plate is warmed at 100°C, 5–10 min and then inspected in UV light (365 nm)	Triterpenes, steroids (saponins)
Marquis reagent	3 mL formaldehyde in 100 mL concentrated sulfuric acid; evaluation in visible light	Morphine, codeine, thebaine
Neu (NP/PEG)	1% methanolic diphenylboric acid β-ethylaminoester (diphenylboryloxyethylamine, NP) followed by 5% ethanolic polyethylene glycol-4000 (PEG)	Flavonoids, anthocyanins

Table 1 *Continued*

Nitric acid (HNO_3 concentrate) for alkaloids	After spraying the plate is heated 15 min at 120°C	Ajmaline, brucine
Nitric acid (HNO_3) + KOH	After spraying, the plate is heated 15 min at 120°C. Then sprayed with 10% ethanolic KOH reagent. Red brown in visible light yellow–brown, brown fluorescence with UV light (365 nm)	Anthracenosides, sennosides
Phosphomolybdic acid reagent	20% ethanolic solution of phosphomolybdic acid spraying then heating at 100°C for 5 min	Essential oils
Potassium hydroxide (KOH) (Borntraeger reagent)	5% or 10% ethanolic potassium hydroxide. In visible light, anthraquinones coloured red; anthrones yellow in UV light (365 nm). Coumarins blue in UV light (365 nm)	Anthracenosides, coumarins
Vanillin–hydrochloric acid	1% in ethanol followed by 3 mL concentrated HCl. In visible light, heating 5 min at 100°C intensifies colours	Essential oils
Vanillin–phosphoric acid	1 g vanillin in 100 mL of 50% phosphoric acid. Heat 10–20 min at 120°C	Essential oils
Vanillin–sulfuric acid	1 g vanillin in 100 mL ethanol then add 2 mL of concentrated H_2SO_4. The plate is sprayed and heated at 100°C (10 min)	Essential oils
Van Urk reagent	0.2 g 4-dimethylaminobenzaldehyde in a cooled mixture of 35 mL water and 65 mL concentrated sulfuric acid. Then add 0.15 mL of a 10% aqueous iron(III) chloride solution	Indolic alkaloids, ergot alkaloids

use silica gel 60 F254 precoated TLC plates but aluminium oxide is also suitable. Many mobile phase systems contain chloroform, the eluting power of which may be decreased by addition of cyclohexane or increased by acetone, ethanol or methanol. The mobile phase is often made alkaline by the addition of ammonia or diethylamine to the less polar solvents but diethylamine is not easy to remove before spraying. The most commonly employed eluents are chloroform–methanol (90 : 10) and chloroform–diethylamine (90 : 10). It is also possible to use a screening system, suitable for the major alkaloids of most drugs employing a solvent mixture of toluene–ethyl acetate–diethylamine (70 : 20 : 10).

The detection of alkaloids is possible by quenching UV light at 254 nm and at 365 nm and by two main spray reagents Dragendorff and iodoplatinate (see Table 1). The chromatographic systems used for plant alkaloids and also the derivatization techniques used for identification are given in **Table 2**.

Glycosides

Glycosides are compounds that yield one or more sugars upon hydrolysis. Among the products of hydrolysis, the non-sugar components of the glycosides are known as aglycones. The classification of glycosides is a difficult matter and here the therapeutic use has been chosen.

Flavonoids

The flavonol glycosides and their aglycones are generally termed flavonoids. A large number of different flavonoids are known to occur in nature, and these yellow pigments are widely distributed throughout the higher plants. The main constituents of flavonoid drugs are 2-phenyl-γ-benzopyrones. The various structural types of flavonoids differ in the degree of oxidation of the C ring. Most of these compounds are present in drugs as monoglycosides or diglycosides. It is possible to classify flavonoids into flavonols, flavones, flavanons, flavanols and flavanolignans in relation to substituents and the presence of double bonds (**Figure 5**).

There are numerous plants containing flavonoids and there are also numerous flavonoids and so here only the main plants and compounds are cited in **Table 3**.

Before TLC is possible, extracts of the plant must be made, and a general method for the extraction of flavonoids is as follows: 1 g of the powered plant material is extracted with 10 mL methanol for 5 min at 60°C and then filtered. The methanolic extract is then analysed by TLC. When the plant contains lipids it is often necessary to use hexane to defat the powder prior to methanolic extraction and TLC. It is also worth noting that when analysing flavonoids, it is often better to examine the aglycones present in hydrolysed plant extracts. Having obtained a suitable extract, it is then possible to separate the various components using TLC and a variety of chromatographic systems have been devised for this purpose.

It is possible to screen flavonoids on silica gel TLC plates with the following solvent: ethyl acetate–formic acid–glacial acetic acid–water (100 : 11 : 11 : 26).

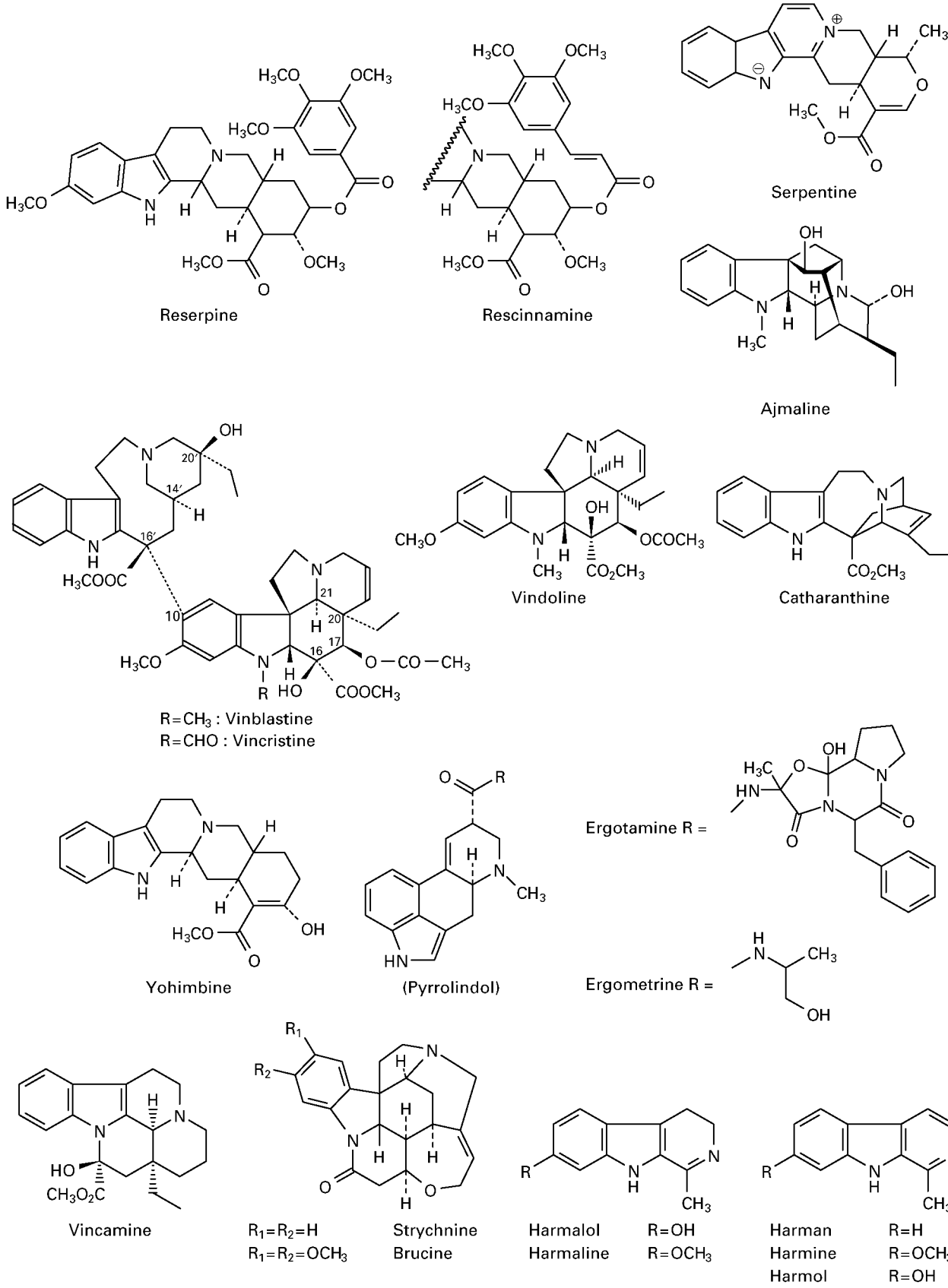


Figure 1 Alkaloids: indoles.

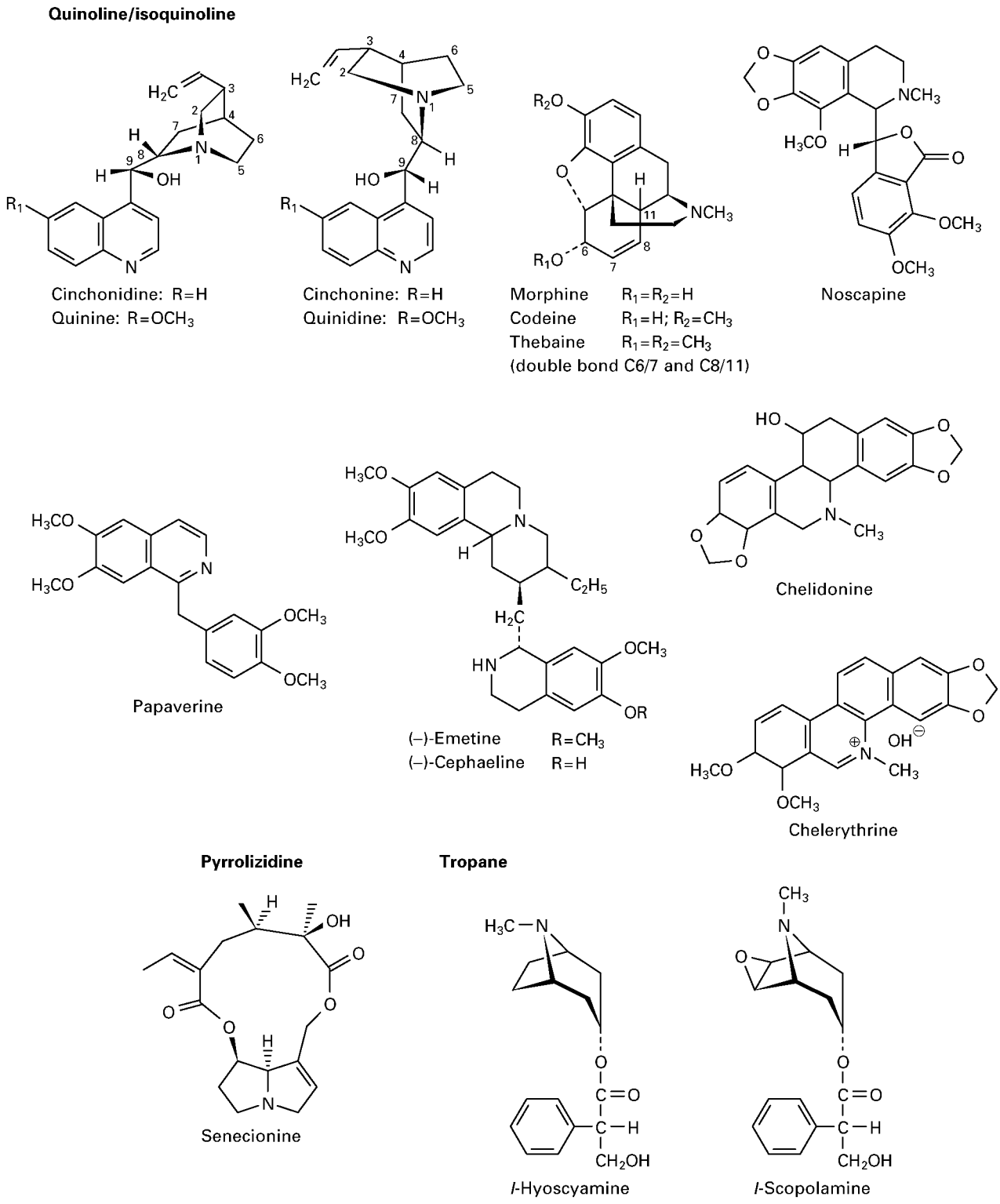
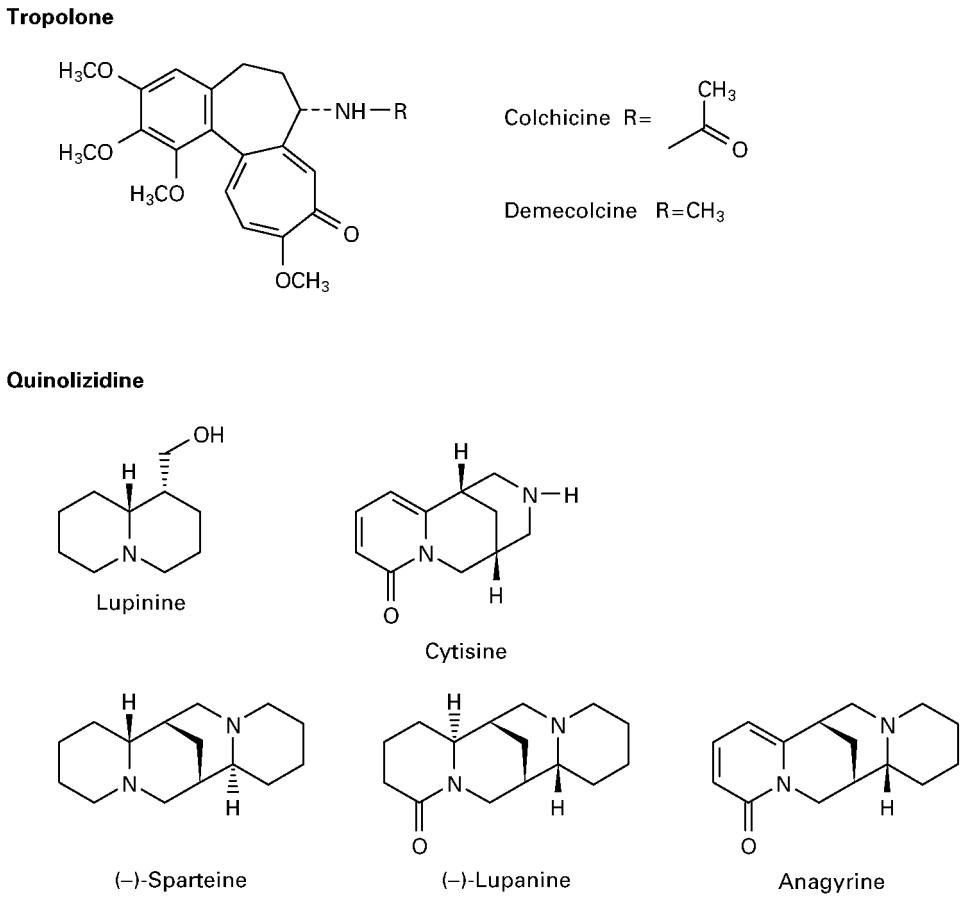


Figure 2 Alkaloids: quinoline/isoquinoline, pyrrolizidine and tropane.

With addition of methyl ethyl ketone (MEK) to give the solvent ethyl acetate–formic acid–glacial acetic acid–MEK–water (50 : 7 : 3 : 30 : 10) it is possible to separate rutin and vitexine-2-O-rhamnoside. Numerous other eluents are also used, such as chloroform–acetone–formic acid (75 : 16.5 : 8.5) for flavanolignans of milk thistle (*Silybum marianum*) and amentoflavone from black haw (*Viburnum*



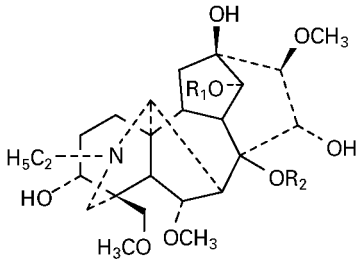
	R_1	R_2
Caffeine	CH_3	CH_3
Theobromine	H	CH_3
Theophylline	CH_3	H

Figure 3 Alkaloids: tropolone, quinolizidine and purines.

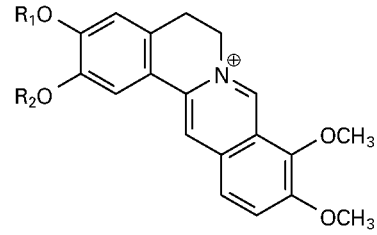
prunifolium). Chloroform–ethyl acetate (60 : 40) has been used for the flavonoid aglycones of orthosiphon (*Orthosiphon aristatus*).

Flavonoid aglycones In addition, the following eluents can be used to separate the aglycones of flavonoids: benzene–pyridine–formic acid (72 : 18 : 10) and toluene–ethyl formate–formic acid (50 : 40 : 10). Toluene–dioxane–glacial acetic acid (90 : 25 : 4) and a further list of suitable sorbents and solvent systems for flavonoids and their aglycones is given in **Table 4**.

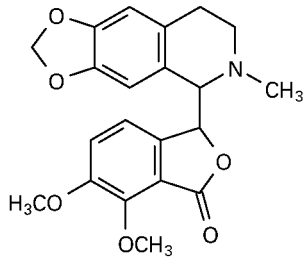
Flavonoids can be detected on TLC plates containing a fluorescent indicator because they cause fluorescence quenching when irradiated with UV light at 254 nm, or 365 nm depending on the structural type. Flavonoids also show dark yellow, green or blue fluorescence, which is intensified and changed by the use of various spray reagents. With the spray reagent diphenylboryloxyethanolamine/polyethylene glycol (NP/PEG), flavonoids and biflavonoids give yellow–orange and green fluorescence when irradiated at 365 nm. Acetic acid reagent gives various blue fluorescent zones after heating.



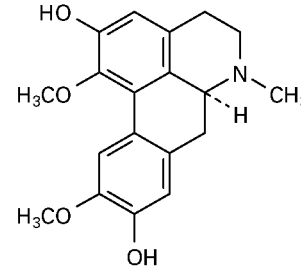
R_1	R_2	
COC_6H_5	$COCH_3$	Aconitine
COC_6H_5	H	Benzoylaconin
H	H	Aconin



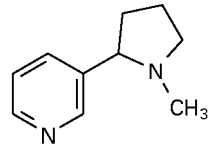
R_1	R_2	
H	CH_3	Jatrorrhizine
CH_3	H	Columbamine
CH_3	CH_3	Palmatine
$-CH_2-$		Berberine



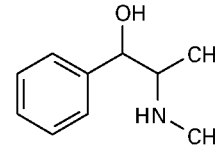
Hydrastine



Boldine



Nicotine



Ephedrine

Figure 4 Miscellaneous alkaloids.

Coumarins

Coumarins are derivatives of benzo-α-pyrones (**Figure 6**).

- *Simple coumarins* consist of coumarin and compounds substituted with OH in umbelliferon, or OCH_3 in scopoletin, and both present in tonka beans (*Coumarouna odorata*), woodruff (*Asperula odorata*) and melilot (*Melilotus officinalis*) or in position C6- and C7-scopoletin which is common in Solanaceae C5 and C8 like fraxin, isofraxidin, fraxetin three compounds of ash bark (*Fraxinus excelsior*).
- *Complex coumarins* belong to two families of plants, the Solanaceae and especially the Apiaceae. C-prenylated coumarins like umbelliprenin are found in angelica root (*Angelica archangelica*). The furanocoumarins possess a furan ring fused at C6 and C7 like psoralen from rue (*Ruta graveolens*), imperatorin, bergapten in ammi (*Ammi majus*), angelica and burnet root (*Pimpinella major*), or a furan ring fused at C7–C8 like angelicin in angelica. Pyranocoumarins have an additional pyran ring at C7–C8, for example visnadin, samidin from ammi (Figure 6).

Table 2 Alkaloids

Group	*Eluents*	*Reagents*	*Compounds*
Quinoline/isoquinoline			
Cinchona (bark): *Cinchona* sp., *Cinchona succirubra*, *Cinchona ledgeriana*	Chloroform–acetone–methanol–ammonia (60 : 20 : 20 : 10)	10% ethanol H_2SO_4, then UV 365 nm	Quinine, quinidine, strong fluorescence
	Chloroform diethylamine (90 : 10)	10% H_2SO_4 or 10% HCOOH, then iodoplatinate	Quinine, quinidine cinchonine, cinchonidine main alkaloids, dihydrocompounds and epiquinine basis give coloration; pink to blue with iodoplatinate
	Toluene–ethyl acetate–diethylamine (70 : 20 : 10)		
Opium (*Opium*)	Toluene–acetone–ethanol–ammonia (45 : 45 : 7 : 3)	Dragendorff and $NaNO_2$	Morphine, codeine, noscapine, papaverine thebaine; all major alkaloids give orange–brown coloration
	Cyclohexane–ethylenediamine (80 : 20)		
	Chloroform–acetone–diethylamine (50 : 40 : 10)	Iodoplatinate	Pink coloration with papaverine, noscapine, thebaine
	Chloroform–methanol (90 : 10)		Blue with morphine and codeine
	Toluene–ethyl acetate–diethylamine (70 : 20 : 10)	Marquis reagent	Violet for codeine and morphine
		NP/PEG, then UV 365 nm	Except codeine, the main alkaloids give a blue fluorescence
Ipeac (*Cephaelis ipecacuanha*)	Toluene–ethyl acetate–diethylamine (70 : 20 : 10)	UV 365 nm	Cepheline, emetine, fluorescence light blue
		Iodine reagent	Cepheline, bright blue emetine yellow–white
		Dragendorff	The major alkaloids give orange–brown coloration
Celandine (*Chelidonium majus*)	Propanol–water–formic acid (90 : 9 : 1)	UV 365 nm	Bright yellow fluorescence of coptisine, sanguinarine; weak yellow–green for chelidonine, chelerytrine
		Dragendorff	Brown. Not stable with main alkaloids
Pyrrolizidine			
Golden senecio (*Senecio vulgaris*)	Chloroform–methanol–ammonia–pentane (82 : 14 : 2.6 : 20)	UV 254 nm	Senecionine, Senecionine *N*-oxide, agmatine
	Acetone–methanol–ammonia (40 : 30 : 20)		
Tropane			
Belladonna (*Atropa belladonna*), Thorn apple (*Datura stramonium*), Henbane (*Hyoscyamus niger*)	Toluene–ethyl acetate–diethylamine (70 : 20 : 10)	Dragendorff	Scopolamine, (−)-hyoscyamine or atropine
	Acetone–water–ammonia (90 : 7 : 3)	Iodoplatinate	
Tropolone			
Meadow saffron (*Colchicum autumnale*)	Chloroform–methanol (95 : 5)	UV light (254 nm) Dragendorff, 10% ethanol Hydrochloric acid gives a yellow coloration	Colchicine, demecolcine, 3-demethylcolchicine
	Benzene–ethyl acetate–diethylamine–methanol–water (15 : 12 : 3 : 6 : 12)		
	Chloroform–acetone–diethylamine (80 : 10 : 10)		

Table 2 *Continued*

Group	*Eluents*	*Reagents*	*Compounds*
Indole alkaloids			
Rauwolfia: *Rauwolfia* sp., *Rauwolfia vomitoria*, *Rauwolfia serpentina*	Toluene–ethyl acetate–diethylamine (70 : 20 : 10)	UV 254 nm	Ajmaline: prominent quenching
	Heptane–methyl ethyl ketone–methanol (53 : 34 : 8)	Dragendorff (orange–brown)	All alkaloids, ajmaline, serpentine, rescinnamine, rauwolcine give orange colours
	Cyclohexane–diethyl ether (60 : 40)	Nitric acid	Ajmaline give red colour
Catharanthus leaves (*Catharanthus* sp.)	Ethyl acetate–ethanol–benzene–ammonia (100 : 5 : 5 : 3) Chloroform–methanol (90 : 10) two dimensional: direction 1, ethyl acetate–methanol (80 : 20); direction 2, dichloromethane–methanol (12 : 1)	Dragendorff	Vinblastine, vincristine, vindoline Catharanthine, and other minor alkaloids give a brown coloration
Yohimbe bark (*Pausinystalia yohimbe*)	Toluene–ethyl acetate–diethylamine (70 : 20 : 10)	UV 365 nm	Yohimbine (blue fluorescence)
		Dragendorff	Yohimbine, pseudoyohimbine, coryantheine (orange zones)
Ergot (*Claviceps purpurea*)	Toluene–ethyl acetate–diethylamine (70 : 20 : 10) Toluene–chloroform–ethanol (28.5 : 57 : 14.5)	Van Urk	Ergocristine, ergotamine, ergometrine give blue zone
Common periwinkle leaves (*Vinca minor*)	Ethyl acetate–methanol (90 : 10)	UV 254 nm	Vincamine, vincaminine, vincamajine, vincine give blue–green fluorescence
		Dragendorff	Weak brown, with major alkaloids
Nux vomica (*Strychnos nux vomica*) Ignatius beans (*Strychnos ignatii*)	Toluene–ethyl acetate–diethylamine (70 : 20 : 10)	UV 254 nm	Strychnine, brucine give strong blue fluorescence
		Dragendorff	Brown for brucine, strychnine and minor orange–brown zones for pseudostrychnine, and α,β-colubrines for nux vomica
		Iodoplatinate	Blue zones with brucine and strychnine
		Nitric acid	Brucine give a red colour
Syrian rue (*Peganum harmana*)	Chloroform–acetone–diethylamine (50 : 40 : 10) Chloroform–methanol–ammonia 10% (80 : 40 : 1.5)	UV 365 nm	Harmanol, harmaline, harmine, harmane, harmon, give a strong blue fluorescence
Miscellaneous alkaloids			
Aconite (*Aconitum napellus*)	Ether–chloroform–ammonia (25 : 10 : 1) Cyclohexane–ethyl acetate–ethylenediamine (80 : 10 : 10) Hexane–chloroform (60 : 40) Chloroform–methanol (80 : 20)	UV 254 nm Dragendorff $NaNO_2$	Aconitine, mesaconitine, hypoaconitine, give orange colours

Table 2 *Continued*

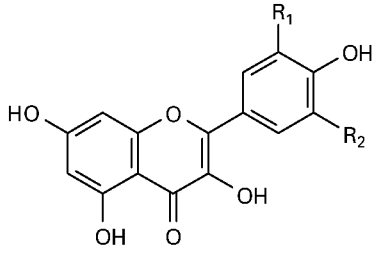
Group	*Eluents*	*Reagents*	*Compounds*
Barberry (*Berberis vulgaris*)	n-Butanol–formic acid–water (90 : 1 : 9)	Dragendorff	Berberine, protoberberine, jateorrhizine, palmitine give orange colours
	n-Butanol–ethyl acetate–formic acid–water (30 : 50 : 10 : 10)	Without treatment	Berberine, yellow in visible light
Hydrastis (*Hydrastis canadensis*)	(See barberry, *Berberis vulgaris*)	UV: hydrastine, blue–white fluorescence with Dragendorff	Berberine, hydrastine
Boldo (*Peumus boldus*)	Toluene–ethyl acetate (93 : 7)	Dragendorff	Aporphinic alkaloids, boldine
Tobacco (*Nicotiana tabacum*)	Toluene–ethyl acetate–diethylamine (70 : 20 : 10)	UV for nicotine, Dragendorff	Nicotine, nornicotine, anabasine, give red–orange colours
Desert tea (ma huang) (*Ephedra* sp.)	Toluene–chloroform–ethanol (28.5 : 47 : 14.5)	Ninhydrin (violet–red for ephedrine)	Ephedrine, norephedrine, pseudoephedrine give orange colours
Quinolizidine			
Lupines (*Lupinus* sp.) Broom tops (*Sarothamnus* sp.)	Chloroform–methanol ammonia (85 : 14 : 7) Chloroform–methanol (80 : 20) Cyclohexane–diethylamine (70 : 30) Toluene–acetone–ethanol–ammonia (30 : 40 : 12 : 4) Cyclohexane–dichloromethane–diethylamine (40 : 40 : 20)	UV and iodine vapours, Dragendorff Iodoplatinate Heating the plate to 100°C, then UV 254 nm Note: quantification by densitometry (565 nm) after derivatization by Dragendorff	Lupanine, sparteine, cytisine, *N*-methylcytisine, hydroxylupanine, matrine Fluorescence blue
Purines			
Coffee (*Coffea* sp.) Thea (*Thea sinensis*) Cocoa (*Theobroma cacao*) Kola nuts (*Kola nitida*) Mate (*Ilex paraguanensis*) Guarana (*Paullinia cupana*)	Ethyl acetate–methanol–water (100 : 13.5 : 10) Ethyl acetate–formic acid–glacial acetic acid–water (100 : 11 : 11 : 26)	UV light (254 nm) Dragendorff, acidic Iodine–hydrochloric acid reagent	Fluorescence quenching for caffeine, theobromine, theophylline range coloration Dark brown coloration

For analysis by TLC, it is necessary to first prepare an extract and for this 1 g is extracted with 10 mL methanol for 30 min under reflux on a water bath. After filtration the solution is evaporated to about 1 mL before application to the plate. For separation on silica gel TLC plates, the following eluents are used: toluene–ether (10 : 10; saturated with 10% acetic acid) – this eluent is used for coumarin/aglycones; and ethyl acetate–formic acid–glacial acetic acid–water (10 : 11 : 11 : 26) for glycosides. Following chromatography, the coumarins can be detected by irradiation with UV light as there is distinct fluorescence quenching for all coumarins at 254 nm and 365 nm. Simple coumarins give blue or blue green fluorescence, and furano and pyranocoumarins yellow, brown, blue, or blue–green fluorescence. The non-substituted chromones show less intense fluorescence: visnagin (pale blue); khellin (yellow brown). They can also be detected by using spray reagents and these include NP/PEG and KOH.

Anthocyanins

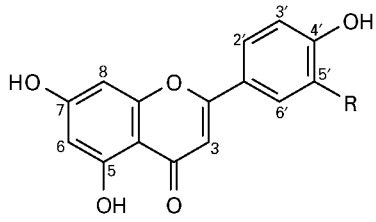
Anthocyanins are the most significant group of coloured substances in plants; they are responsible for the pink, mauve, red, violet, and blue colours of flowers and other plant parts. They are present in plants as glycosides of flavylium salts in petals and leaves. In the fruits of higher plants, they are mostly present as glycosides of hydroxylated 2-phenylbenzopyrylium (**Figure 7**). Anthocyanins found in numerous plants used therapeutically include the following: hibiscus (*Hibiscus sabdariffa*) (hibiscin); corn flowers (*Centaurea cyanus*) (cyanin; pelargonin); common mallow (*Malva sylvestris*) (malvin

Flavonols



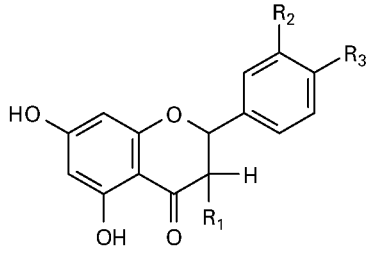
R_1	R_2	Aglycone	Quercetin
OH	H	Quercetin	Q-3-O-glucoside (isoquercitrin)
H	H	Kaempferol	Q-3-O-rhamnoside (quercitrin)
OH	OH	Myricetin	Q-3-O-galactoside (hyperoside)
OCH_3	H	Isorhamnetin	Q-3-O-rutinoside (rutin)
			Q-4′-O-glucoside (spiraeoside)

Flavones

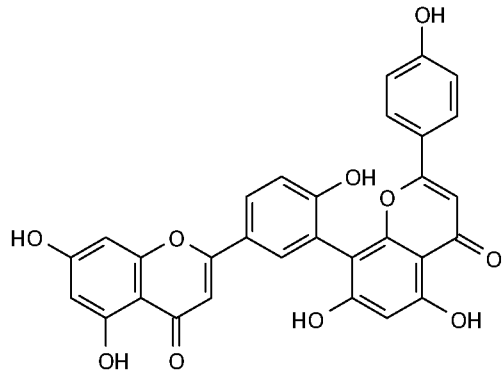


Aglycone	Glycoside
Apigenin	A-8-C-glucoside (vitexin)
R=H	A-6-C-glucoside (isovitexin)
	A-7-O-apiosyl-glucoside (apiin)
	A-6-α-L-arabinopyranoside-8-C-glucoside (schaftoside)
Luteolin	L-5-O-glucoside (galuteolin)
R=OH	L-8-C-glucoside (orientin)
	L-6-C-glucoside (iso-orientin)

Flavanones



R_1	R_2	R_3	
H	H	OH	Naringenin
			Naringin
H	OH	OH	Eriodyctiol
			Eriocitrin
H	OCH_3	OH	Homocriodyctiol
H	OH	OCH_3	Hesperetin
			Neohesperidin
			Hesperidin



Amentoflavone

Figure 5 Flavonoids.

Table 3 Plant flavonoids: eluents

Plant	*Eluents*	*Compounds*
Arnica (*Arnica montana*)	Ethyl acetate–glacial acetic–formic acid–water (100 : 11 : 11 : 26)	Quercetin-3-O-glucoside and 3-O-glucogalacturonide, luteonin-7-O-glucoside, kaempferol-3-O-glucoside
Ginkgo leaves (*Ginkgo biloba*)	Ethyl acetate–glacial acetic–formic acid–water (100 : 11 : 11 : 26) Chloroform–acetone–formic acid (75 : 16.5 : 8.5) Toluene–acetone (70 : 30)	Quercetin, kaempferol and isorhamnetin glycosides: flavonol acylglycosides Biflavonoids: amentoflavone, bilobetin, ginkgogetin, isoginkgogetin Ginkgolides a, b, c, catechin and epicatechin
Acacia flowers (*Robinia pseudoacacia*)	Ethyl acetate–formic acid–glacial acetic acid–water (100 : 11 : 11 : 26)	Kaempferol-3-O-rhamnosylgalactosyl-7-rhamnoside (robinin) Acacetin-7-O-rutinoside, acaciin
Roman camomile (*Chamaemelum nobile*)	Ethyl acetate–formic acid–glacial acetic acid–water (100 : 11 : 11 : 26)	Apigenin-7-O-glycoside, 7-apiosil glucoside (apiin) Quercitrin
Marigold flowers (*Calendula officinalis*)	Ethyl acetate–formic acid–glacial acetic acid–water (100 : 11 : 11 : 26)	Isorhamnetin glycosides Isorhamnetin-3-O-glucoside (narcissin)
Hawthorn flowers, leaves (*Crataegus* sp.)	Ethyl acetate–formic acid–glacial acetic acid–water (100 : 11 : 11 : 26)	Quercetin glycosides: rutin, hyperoside spiraeoside Flavon-C-glycosides: vitexin, isovitexin rhamnoside
Coltsfoot (*Tussilago farfara*)	Ethyl acetate–formic acid–glacial acetic acid–water (100 : 11 : 11 : 26)	Quercetin glucosides, rutin, hyperoside isoquercetin
German chamomile flowers (*Matricaria chamomilla*)	Ethyl acetate–formic acid–glacial acetic acid–water (100 : 11 : 11 : 26)	Flavonoid aglycones, apigenin-7-O-glucoside, luteolin-7-O-glucoside
Meadow-sweet (*Filipendula ulmaria*)	Ethyl acetate–formic acid–glacial acetic acid–water (100 : 11 : 11 : 26)	Quercetin-4′-O-glucoside (spiraeoside) Hyperoside Kaempferol glycosides
Lime flowers (*Tilia* sp.)	Ethyl acetate–formic acid–glacial acetic acid–water (100 : 11 : 11 : 26)	Quercetin glycosides: quercitrin, isoquercitrin Kaempferol glycosides
Mullein flowers (*Verbascum album*)	Ethyl acetate–formic acid–glacial acetic acid–water (100 : 11 : 11 : 26)	Kaempferol, rutine, hesperidin, apigenin
Blackcurrant (*Ribes nigrum*)	Ethyl acetate–formic acid–glacial acetic acid–water (100 : 11 : 11 : 26)	Quercetin, kaempferol, myricetin and isorhamnetin glycosides
Round-headed bush clover (*Lespedeza capitata*)	Ethyl acetate–formic acid–glacial acetic acid–water (100 : 11 : 11 : 26)	Flavon-C-glycosides: orientin, iso-orientin, vitexin, isovitexin
Passion flower (*Passiflora incarnata*)	Ethyl acetate–formic acid–glacial acetic acid–water (100 : 11 : 11 : 26)	Flavon-C-glycosides: isovitexin, vitexin, orientin, iso-orientin Flavon-O-glycosides: rutin, hyperoside, isoquercitrin
Lemon and other Aurantiaceae (*Citrus* sp.)	Ethyl acetate–formic acid–glacial acetic acid–water (100 : 11 : 11 : 26)	Flavanon glycosides, eriocitrin, naringin, hesperidin
Sophora buds (*Sorphora japonica*)	Ethyl acetate–formic acid–glacial acetic acid–water (100 : 11 : 11 : 26)	Flavonol glycosides: rutin

Table 4 Plant flavonoids: sorbents/eluents

Plant/sorbent	*Eluents*[a]	*Compounds*
Silica gel		
Elm (*Ulmus* sp.)	Ethyl acetate–formic acid–acetic acid–water (100 : 11 : 11 : 27)	Quercetin glycoside, kaempferol glycoside
Lipocedrus	Chloroform–methanol–formic acid (90 : 10 : 1)	Flavonol glycoside
Calendula officinalis (flowers)	Benzene–methanol–acetic acid (90 : 16 : 8)	Flavonol glycoside
Henry anisetree (*Illicium henryii*) (root cortex)	Butanol–acetic acid–water–methanol (40 : 20: 10 : 50)	Flavonoids
Sedum sediform	Toluene–acetone–formic acid (60 : 60 : 12)	Phloroglucinol glycoside
Olea europea	Ethyl acetate–formic acid–water (60 : 10 : 10)	Flavonoids
Polyamide (aglycones)		
Alnus glutinosa	Toluene–petroleum ether–MEK–methanol (50 : 25 : 11 : 13)	Flavonoid aglycones
Keckiella	Benzene–MEK–methanol (80 : 13 : 7)	Flavonoid aglycones
Viguiera sp.	Toluene–MEK–methanol (60 : 25 : 15)	Flavonoid aglycones
Polyamide (flavonoids)		
Lastenia californica	Water–butanol–acetone–dioxane (70 : 15 : 10 : 5)	Flavonoids
Rosa cultivars	Methanol–acetic acid–water (90 : 5 : 5)	Flavonoids
Cleome sp.	Methylene chloride–benzene–methanol (75 : 5 : 5) Benzene–petroleum ether–MEK–methanol (60 : 60 : 7 : 7 or 60 : 30 : 7 : 7) Benzene–MEK–methanol (40 : 30 : 30)	Flavonoids
Illicium henryii	Butanol–acetic acid–methanol–water (40 : 10 : 20 : 50)	Flavonoids

[a]MEK = methyl ethyl ketone.

and delphinidin glycosides); hollyhock (*Althea rosea*) (delphinidin-3-glycoside, malvidin-3-glycoside); bilberry (*Vaccinium myrtillus*) (delphinidin-3-glycoside = myrtillin A).

For analysis, anthocyanins must be extracted from plants with solvents containing acetic or hydrochloric acid. Plants are extracted for 15 min with methanol–HCl 25% (9 : 1) and the filtrates are subsequently used for chromatography. For TLC on silica gel, the following eluents are commonly used: ethyl acetate–glacial acetic acid–formic acid–water (100 : 11 : 11 : 26); and n-butanol–glacial acetic acid–water (40 : 10 : 20 or 40 : 10 : 50). Because these compounds are coloured, detection is possible visually without the need for chemical treatment or the developed TLC plates can be sprayed with anisaldehyde–H_2SO_4 reagent.

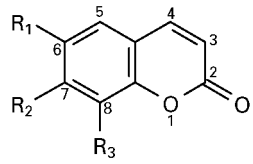
Anthraquinone glycosides

A number of glycosides with aglycones related to anthracene are present in such drugs as cascara (*Cascara sagrada*), aloes (*Aloe* sp.), alder buckhorn (*Rhamnus frangula*), rhubarb (*Rheum officinalis*) and senna (*Cassia senna*). These drugs are employed as cathartics. On hydrolysis, the glycosides yield aglycones which are di-, tri-, or tetrahydroxyanthraquinones or derivatives of these compounds (**Figure 8**). The anthraquinones possess phenolic groups on C1 and C8 and keto groups on C9 and C10; in the anthrones and anthranol, only C9 carries an oxygen function. Most compounds in this group are present in the plant as O-glycosides. In the O- and C-glycosides, the only sugars found are glucose, rhamnose and apiose.

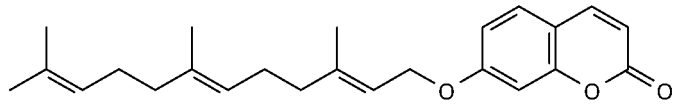
Prior to TLC, the powdered plant material is extracted for 5 min with methanol (1 g of plant in 100 mL) then filtered. It is necessary to hydrolyse the extract to characterize the aglycones and for this 1 g of powder plant is heated under reflux with *X* mL 7.5% hydrochloric acid for 15 min. After cooling, the mixture is extracted by shaking with *X* mL of chloroform or ether. The organic phase is then taken and concentrated to about 1 mL, and then used for TLC. Chromatography is performed on silica gel precoated plates with light petroleum–ethyl acetate–formic acid (75 : 25 : 1) or ethyl acetate–methanol–water (100 : 13.5 : 10) for all anthracene drug extracts except for senna. In this case, n-propanol–ethyl acetate–water–glacial acetic acid (40 : 40 : 29 : 1) is used.

For the non-laxative dehydrodianthrones of St John's wort (*Hypericum perforatum*) (Figure 8), TLC is performed with the eluent toluene–ethyl formate–formic acid (50 : 40 : 10).

Following TLC, all anthracene derivatives can be readily detected because they quench fluorescence



	R_1	R_2	R_3	
	H	H	H	Coumarin
	H	OH	H	Umbelliferone
	OH	OH	H	Aesculetin
	OCH_3	OH	H	Scopoletin
	OCH_3	OH	OH	Fraxetin
	OCH_3	OH	OCH_3	Isofraxidin
	OCH_3	OH	O-gluc	Fraxin



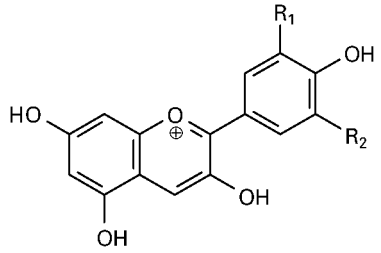
Umbelliprenin

7,6-Furanocoumarins	R_1	R_2	
	H	H	Psoralen
	H	OCH_3	Xanthotoxin
	H	OH	Xanthotoxol
	OCH_3	H	Bergapten

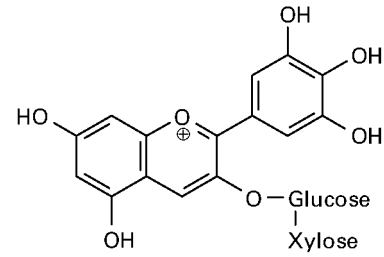
7,8-Furanocoumarins	R_1	R_2	
	H	H	Angelicin
	OCH_3	H	Isobergapten

Pyranocoumarins	R	
	$-CO-CH=C(CH_3)_2$	Samidin
	$-CO-CH_2-CH(CH_3)_2$	Dihydrosamidin
	$-CO-CH(CH_3)-C_2H_5$	Visnadin

Figure 6 Coumarins.



	R_1	R_2
Pelargonidin	H	H
Cyanidin	H	OH
Malvidin	OCH_3	OCH_3
Petunidin	OH	OCH_3
Delphinidin	OH	OH



Hibiscin

Figure 7 Anthocyanins.

when irradiated at UV 254 nm and give yellow or red–brown fluorescence. Different specific reagents are also used for detection (see the Appendix). Thus anthraquinone appears red in the visible after spraying with KOH. With NP/PEG, anthrones and anthranones give intense yellow fluorescence when irradiated at 365 nm. For the characterization of sennosides, the TLC plate is sprayed with HNO_3 and then heated for 10 min at 120°C. Before spraying with ethanolic KOH, these appear brown–red in UV 365 nm and brown in visible light. Hypericin gives red fluorescence when irradiated at 365 nm.

Cardiac glycosides

There are some steroids present in nature, known as the cardiac glycosides, which are characterized by the highly specific and powerful action that they exert upon cardiac muscle. These steroids occur as glycosides with sugars in the 3-position of the steroid nucleus. The steroid aglycones or genins are of two types, either a cardenolide or a bufadienolide.

The steroids are structurally derived from the tetracyclic 10,13-dimethylcyclopentanoperhydrophenanthrene ring system. They possess a γ-lactone ring for the cardenolide or a δ-lactone ring for the bufadienolide attached in the position at C17. The sugar residues are derived from deoxy- and/or C3-O-methylated sugars, and they are linked glycosidically by the C3-OH groups of the steroid aglycones (**Figure 9**). The main plants containing cardenolides are white foxglove (*Digitalis lanata*), red foxglove (*Digitalis purpurea*), oleander (*Nerium oleander*), strophanthus (*Strophantus gratus*; *Strophantus kombe*), adonis (*Adonis vernalis*) and lily of the valley (*Convallaria majalis*). The main plants containing bufadienolides are hellebores (*Helleborus s*p.) and squill (*Urginea maritima*).

For analysis, 1–10 g of powered plant is extracted by heating for 15 min under reflux with 20 mL 50% ethanol, with the addition of 10 mL 10% lead(II) acetate solution. After cooling and filtration, the solution is extracted twice with 15 mL dichloromethane. The combined lower organic phases are then filtered over anhydrous sodium sulfate and evaporated to dryness. The residue to dissolved in 1 mL of dichloromethane–ethanol (1 : 1) and the solution obtained is used for chromatography.

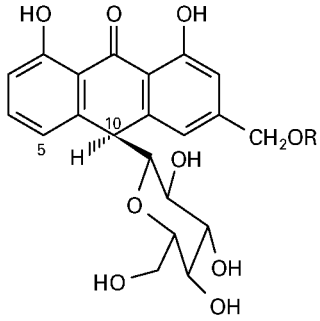
The TLC of the cardiac glycosides is accomplished on silica gel with the following solvents: ethyl acetate–methanol–water (100 : 13.5 : 10) or (81 : 11 : 8) and ethyl acetate–methanol–ethanol–water (81 : 11 : 4 : 8); and the lower phase of chloroform–methanol–water (35 : 25 : 10) for Hellebore bufadienolides.

The separated analytes can be detected under UV light at 254 nm as there is weak fluorescence quenching for cardenolides which is more distinct for bufadienolides. Spray reagents for detection include antimony chloride in chloroform and heating at 100°C, chloramine T, sulfuric acid and Kedde reagent (see the Appendix).

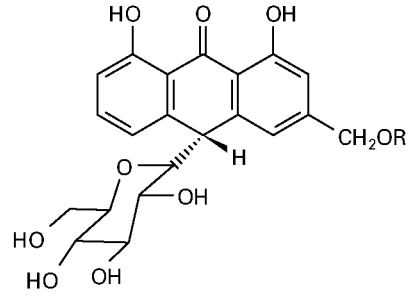
Saponins

The formation of persistent foams during the extraction or concentration of plant extracts indicates the presence of saponins. The saponins are mainly triterpene derivatives, with similar amounts of steroid present. The most important plants are described in **Table 5**. Ginseng roots (*Panax ginseng*) contain triterpene glycosides: the ginsenosides a, b, c, d, e, f,

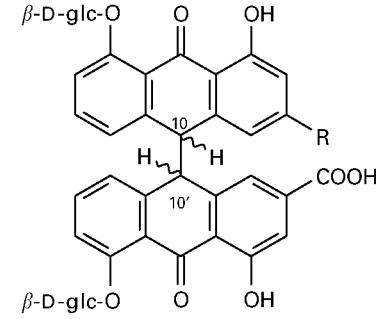
Anthraquinone glycosides



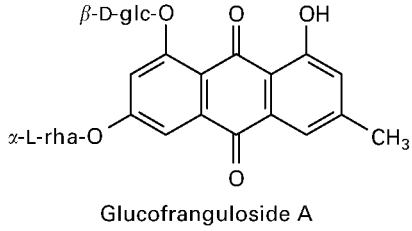
R=H, aloine A
R= α-L-Rha, aloinoside A



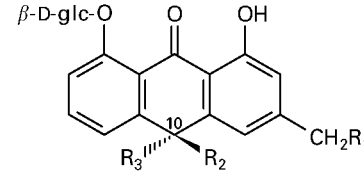
R=H, aloine B
R= α-L-Rha, aloinoside B



R	C-10	C-10′	
COOH	R	R	sennoside A
COOH	R	S	sennoside B
CH_2OH	R	R	sennoside C
CH_2OH	R	S	sennoside D

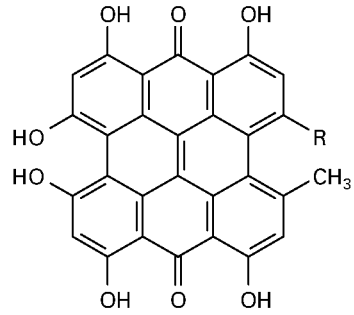


Glucofranguloside A



R_1=OH, R_2=β–D-glc, R_3 =H, cascaroside A
R_1=OH, R_2=H, R_3=β–D-glc, cascaroside B
R_1=H, R_2=β–D-glc, R_3 =H, cascaroside C
R_1=H, R_2=H, R_3=β–D-glc, cascaroside D

Dehydrodianthrones

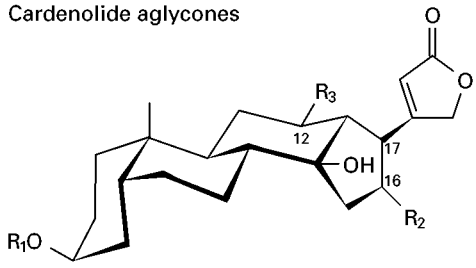


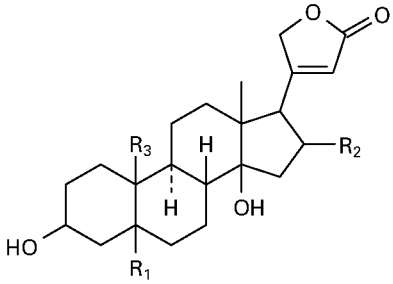
R=CH_3, hypericine
R=CH_2OH, pseudohypericine

Figure 8 Anthraquinones.

g, h. Horse chestnuts (*Aesculus hippocastanum*) contain the pentacyclic triterpenes glycosides aescine and aescinol, liquorice roots (*Glycyrrhiza glabra*) contain saponins aglycone from the glycyrrhetic acid, milkort root (*Polygala senega*) triterpene ester saponins 'senegenines', red soapwood root (*Saponaria officinalis*) triterpene saponins, Indian pennywort (*Centella asiatica*) asiaticoside A, B, 'madecassoside'

Cardenolides

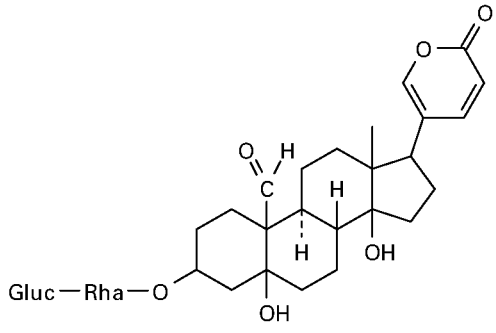
Digitalis lanatae and *Digitalis purpureae*		R_1	R_2	R_3
Cardenolide aglycones 	Digitoxigenin	H	H	H
	Gitoxigenin	H	OH	H
	Digoxigenin	H	H	OH
	Diginatigenin	H	OH	OH
	Gitaloxigenin	H	O—CHO	H



	R_1	R_2	R_3	
Adonis	OH	H	CHO	
	K-Strophanthidin (*S*)			Cymarin (*S*-cymaroside) desglucocheirotoxin (*S*-gulomethyloside) k-Strophanthidin-β, k-strophanthoside
	H	OH	CHO	Adonitoxin (*A*-rhamnoside), *A*-2-O-acetyl-rhamnoside, *A*-3-O-acetylrhamnoside, and glucosides and xylosides
	Adonitoxigenin (*A*)			
	H	OH	CH_2OH	Adonitoxigenol (-rhamnoside)
	OH	OH	CHO	Strophadogenin (-diginoside)
Strophanthus	OH	H	CHO	Cymarin (*S*-cymaroside), helveticoside (*S*-β-D-digitoxide)
	k-Strophanthidin (*S*)			Erysimoside (*S*-digitoxoside-glucoside), k-strophanthin-β, k-strophanthoside

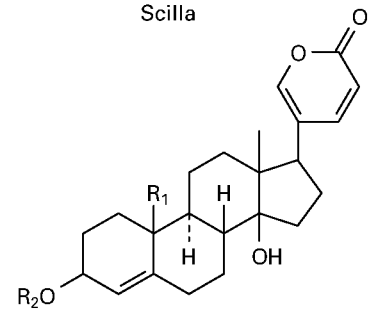
Bufadienolides

Helleborus



Hellebrin

Scilla



	R_1	R_2
Scillarenin	CH_3	H (Aglycon)
Proscillaridin A	CH_3	Rham
Scilliphaeoside	H	Rham
Scillaren A	CH_3	Gluc-Rham
Glucoscillaren A	CH_3	Gluc-Gluc-Rham

Figure 9 Cardiac glycosides.

Table 5 Saponosids

Plants	*Eluents*	*Reagents*	*Compounds*
Ginseng roots (*Panax ginseng*)	Chloroform–methanol–water (70 : 30 : 40) Butanol–ethyl acetate–water (40 : 10 : 10)	Vanillin–phosphoric acid gives red zones in the visible and red; fluorescence in UV 365 nm Sulfuric acid then heated 110°C, 7 min	Triterpene glycosides, ginsenosides R*x* (x = a, b_1, b_2, d, e, f, g_1, h) Derived from dammaranne (protopanaxtriol, panaxadiol)
Eleutherocoque roots (*Eleutherococus senticosus*)	1,2-Dichloroethane–ethanol–methanol–water (65 : 22 : 22 : 7)	Vanillin–sulfuric acid detection at UV 285 nm	Triterpenes: eleutherosides
Liquorice roots (*Glycyrrhiza glabra*)	Chloroform–glacial acetic acid–methanol–water (60 : 32 : 12 : 8) Ethyl acetate–ethanol–water–ammonia (65 : 25 : 9 : 1)	Anisaldehyde–sulfuric acid	Saponosids: glycyrrhizin, glycyrrhizic acid Aglycone: glycyrrhetic acid
Milkwort roots (*Polygala senega*)	Chloroform–glacial acetic acid–methanol–water (60 : 32 : 12 : 8)	Anisaldehyde–sulfuric acid: five red saponin zones	Triterpene ester saponins = senegenins
Red soapwood (*Saponaria officinalis*)	Chloroform–glacial acetic acid–methanol–water (60 : 32 : 12 : 8)	Anisaldehyde–sulfuric acid give six violet zones and one brown band	Triterpene saponins derived from gypsogenin (quillaic acid)
Butcher's broom (*Ruscus aculeatus*)	Chloroform–glacial acetic acid–methanol–water (60 : 32 : 12 : 8)	Anisaldehyde–sulfuric acid: six to eight yellow or green bands	Steroid saponins = neoruscogenin glycosides Aglycones: ruscogenin and neoruscogenin
Sarsapilla (*Smilax* sp.)	Chloroform–glacial acetic acid–methanol–water (60 : 32 : 12 : 8)	Anisaldehyde–sulfuric acid: six yellow–brown saponins	Steroid saponins: smilax saponin, spirostanol-saponin Aglycones: sarsapogenin and its isomer smilagenin
Indian pennywort (*Centella asiatica*)	Chloroform–glacial acetic acid–methanol–water (60 : 32 : 12 : 8)	Anisaldehyde–sulfuric acid: violet–blue in fluorescence. Brown–violet zone	Esters saponins Madecassoside, a mixture of asiaticoside A and B
Soap bark (*Quillaja saponaria*)	Chloroform–glacial acetic acid–methanol–water (60 : 32 : 12 : 8)	Anisaldehyde–sulfuric acid: brown-to-violet zones	Quillaja saponins constitute a mixture of hydroxy gypsogenins
Horse chestnut seeds (*Aesculus hippocastanum*)	Chloroform–glacial acetic acid–methanol–water (60 : 32 : 12 : 8) Propanol–ethyl acetate–water (40 : 30 : 30)	Anisaldehyde–sulfuric acid gives main blue–violet–black of aescins Iron(III) chloride at UV 540 nm	Pentacyclic triterpene glycosides, aescine, aescinol β-Aescine

and soap bark (*Quillaja saponaria*) quillaja saponins (**Figure 10**).

Steroid saponins are present in Butcher's broom (*Ruscus aculeatus*) like ruscogenin and *Sarsaparilla smilax* sp. smilax saponins.

In order to obtain a suitable sample for TLC the plant powder is extracted by heating for 10 min under reflux with 10 mL of 70% ethanol. After filtration and evaporation, this solution is used for TLC. Ginseng radix is extracted with 90% ethanol under the same conditions.

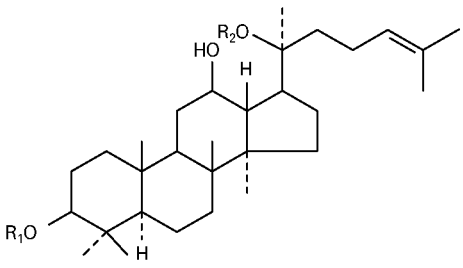
Chromatographic solvents for the separation of these compounds on silica gel include chloroform–glacial acetic acid–methanol–water (64 : 32 : 12 : 8) which is suitable for separation of numerous saponin mixtures. For ginsenosides

chloroform–methanol–water (70 : 34 : 4) is used whilst ethyl acetate–ethanol–water–ammonia (65 : 25 : 9 : 1) is useful for glycyrrhetic acid.

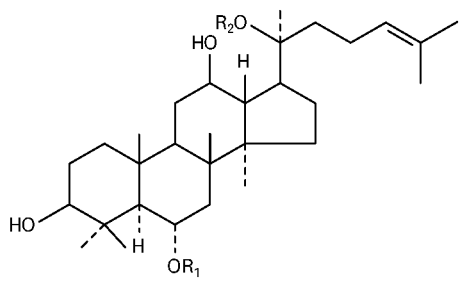
Once separated, the various analytes can be seen by inspection under UV light at 254 or 365 nm for glycyrrhizin and glycyrrhetic acid. Spraying with vanillin–sulfuric acid reagent gives a range of colours for the saponins in the visible spectrum mainly blue, blue–violet and sometimes red and yellow–brown zones. The anisaldehyde–sulfuric acid reagent gives the same colours as vanillin. Vanillin–phosphoric acid reagent with ginsenosides gives red–violet colours in the visible spectrum, and reddish or blue fluorescence when viewed under UV light at 365 nm.

Essential Oils

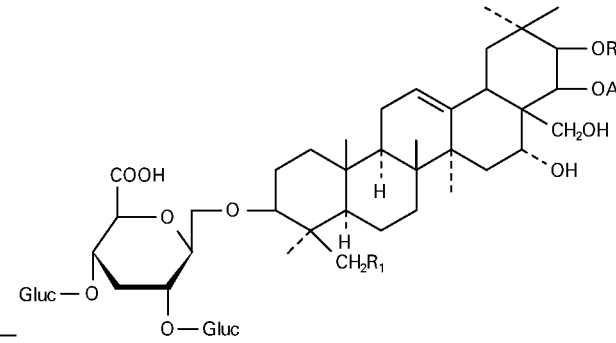
Essential oils are the odorous principles found in various plant parts (**Table 6**) because they evaporate when exposed to the air at room temperature, they are called 'volatile oils', 'ethereal oils' or 'essential oils'; the last term is applied since volatile oils represent the 'essences' or odoriferous constituent of the plants. Odorous principles consist either of (a) terpenes, i.e. alcohols (borneol, geraniol, linalool, menthol), aldehydes (anisaldehyde, citral), ketones (carvone fenchone, menthone, thujone), esters (bornyl acetate, linalyl acetate, menthyl acetate oxides, 1,8-cineole) or (b) phenylpropane derivatives i.e. anethole apiole, eugenol and safrole (**Figure 11**). Es-



R_1				R_2	
(20S-Protopanaxadiol)	H			H	
Ginsenoside Rb_1	β-D-Glu (1→2)	β-D-Glu	β-D-Glu	(1→6)	β-D-Glu
Ginsenoside Rb_2	β-D-Glu (1→2)	β-D-Glu	α-L-Ara*p*	(1→6)	β-D-Glu
Ginsenoside Rc	β-D-Glu (1→2)	β-D-Glu	α-L-Ara*f*	(1→6)	β-D-Glu
Ginsenoside Rd	β-D-Glu (1→2)	β-D-Glu	β-D-Glu		

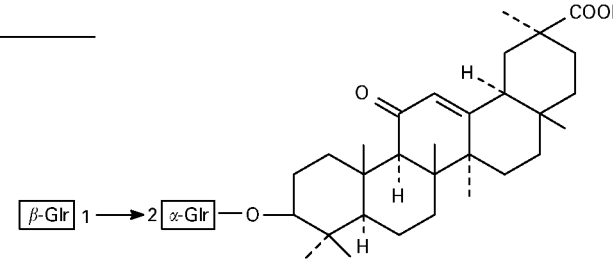


	R_1		R_2
(20S-Protopanaxatriol)	H		H
Ginsenoside Re	α-L-Rha (1→2)	β-D-Glu	β-D-Glu
Ginsenoside Rf	β-D-Glu (1→2)	β-D-Glu	H
Ginsenoside Rg_1	β-D-Glu		β-D-Glu
Ginsenoside Rg_2	α-L-Rha (1→2)	β-D-Glu	H
Ginsenoside Rh_1	β-D-Glu		H

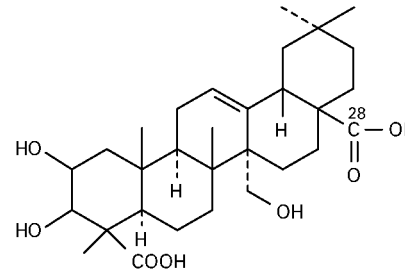


Escin [Aescin]

R_1=OH	Aglycone: Barringtogenol C
R_1=H	Aglycone: Protoaescigenin
R_1=Tigloyl-,	Angelicoyl, 2-Methylbutyryl- or Isobutyryl-

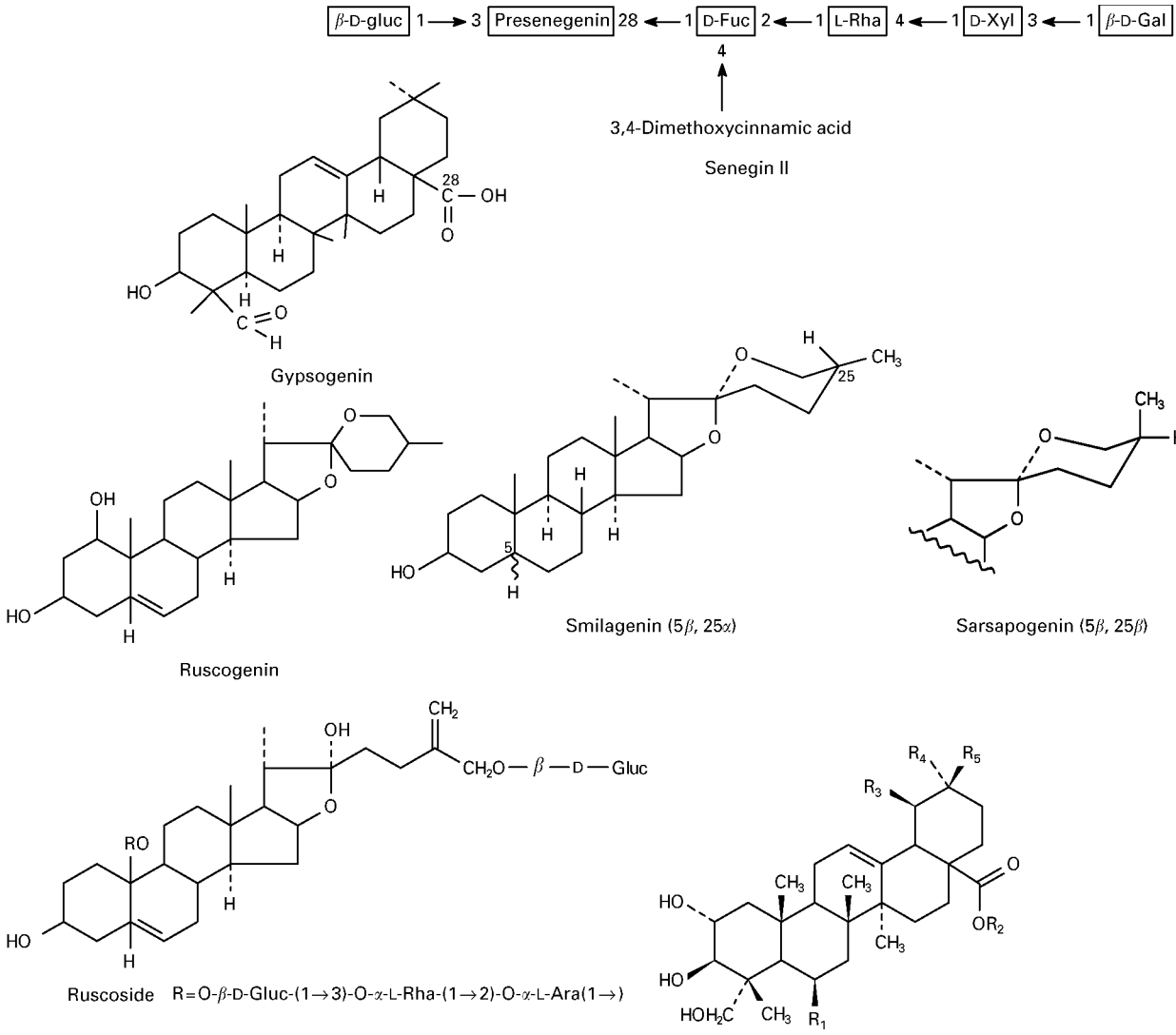


Glycyrrhizic acid, Glycyrrhizin



Presenegin

Figure 10 Saponins.



	R_1	R_2	R_3	R_4	R_5
Asiaticoside	–H	→1)-β-Gluc-(6→1)-β-D-Gluc-(4→1)-α-L-Rha	$-CH_3$	$-CH_3$	–H
Asiaticoside A	–OH	→1)-β-Gluc-(6→1)-β-D-Gluc-(4→1)-α-L-Rha	$-CH_3$	$-CH_3$	–H
(Asiaticoside B	–OH	→1)-β-Gluc-(6→1)-β-D-Gluc-(4→1)-α-L-Rha	–H	$-CH_3$	$-CH_3$)

Figure 10 *Continued.*

sential oils are soluble in ethanol and toluene and are mostly obtained by steam distillation of plant material.

For the preparation of extracts, a micro-steam distillation method is used to obtain the essential oil; a standard method is described in some pharmacopoeias. The essential oil is recovered in toluene or xylene and constitutes the sample to be analysed by TLC, but it is also possible to isolate it with hexane, ether or acetone.

Silica gel is the most widely used sorbent for the essential oils, with solvents such as benzene or toluene, chloroform, methylene chloride ethyl acetate for development. Because of its toxicity, however, benzene can no longer be recommended as a solvent for TLC. The eluent toluene–ethyl acetate (93 : 7) is suitable for the analysis and comparison of all of the important essential oils. Different eluents can be employed in special cases, e.g. toluene (*Pimpinella*

Table 6 Essential oils

Plant family	*Plants*	*Main compounds*	*Colour with vanillin–sulfuric acid*
Apiaceae	Anise (*Pimpinella anisum*)	*Trans*-anethole	Red–brown
	Fennel seed (*Foeniculum vulgare*)	*Trans*-anethole	Red–brown
	Parsley fruits	Apiol	Violet–brown
	(*Petroselinum crispum*)	Myristin	Violet–brown
	Caraway fruits	Carvone	Red–violet
	(*Carvum carvi*)		
	Coriander fruits	Linalool	Blue
	(*Coriandrum sativum*)		
Asteraceae	Camomile flowers	Chamazulene	Red–violet
	(*Chamomilla reticula*)	Bisabolol	Violet
	Roman camomile	Easters of angelicin	Grey–violet
	(*Chamaemelum nobile*)	1,8-cineole, thujone	Blue
	Worm seed (*Artemisia cina*)		
Lamiaceae	Peppermint leaves	Menthol	Blue
	(*Mentha* sp.)	Menthone	
	Rosemary leaves	1,8-cineole	Green
	(*Rosmarinus officinalis*)	Borneol, pinene, camphene	Blue
	Lemon balm	Citronellal	Blue–violet
	(*Melissa officinalis*)	Citral	Black–blue
		Citronellol	Violet–blue
			Black–blue
	Lavander flowers	Linalool	Blue
	(*Lavandula officinalis*)	Nerol	Blue
		Borneol	Blue–violet
	Basil	Methyl chavicol	Red
	(*Ocinum basilicum*)		
	Thyme (*Thymus vulgaris*)	Thymol	Red–violet
		Carvacrol	Red
		Linalool	Blue
	Sage leaves	Thujone	Pink–violet
	(*Salvia officinalis*)	1,8-Cineole	Blue
		Borneol	Blue–violet
	Greek sage (*Salvia triloba*)	1,8-Cineole	Blue
		Thujone	Pink–violet
Lauraceae	Cinnamon bark	Cinnamaldehyde	Grey–blue
	(*Cinnamomum zeylanicum*)		
	Chinese cinnamon	Cinnamaldehyde	Grey–blue
	(*Cinnamomum aromaticum*)		
Myrtaceae	Cloves	Eugenol	Yellow–brown
	(*Syzygium aromaticum*)		
	Blue gum leaves	1,8-Cineole = eucalyptol	Blue
	(*Eucalyptus globulus*)		
Rutaceae	Bitter orange peel	Limonene	Grey–violet
	(*Citrus aurantium* sp.)	Citral	Blue–violet
	Bergamot	Limonene	Grey–violet
	(*Citrus aurantium* var. *amara*)	Citral	Blue–violet
	Orange flowers (*Citrus sinensis*)	Linalyl acetate	Blue
		Linalool	Blue
	Lemon peel (*Citrus limon*)	Limonen	Grey–violet
		Citral	Violet–blue

anisum), chloroform (*Melissa officinalis*), methylene chloride (*Pimpinella*, *Juniperus*, *Lavandula*, *Rosmarinus*, and *Salvia*), toluene–ethyl acetate–(*Eucalyptus*, *Mentha*), chloroform–toluene (75 : 25) for *Thymus vulgaris* and *Chamomilla recutica* (Table 2).

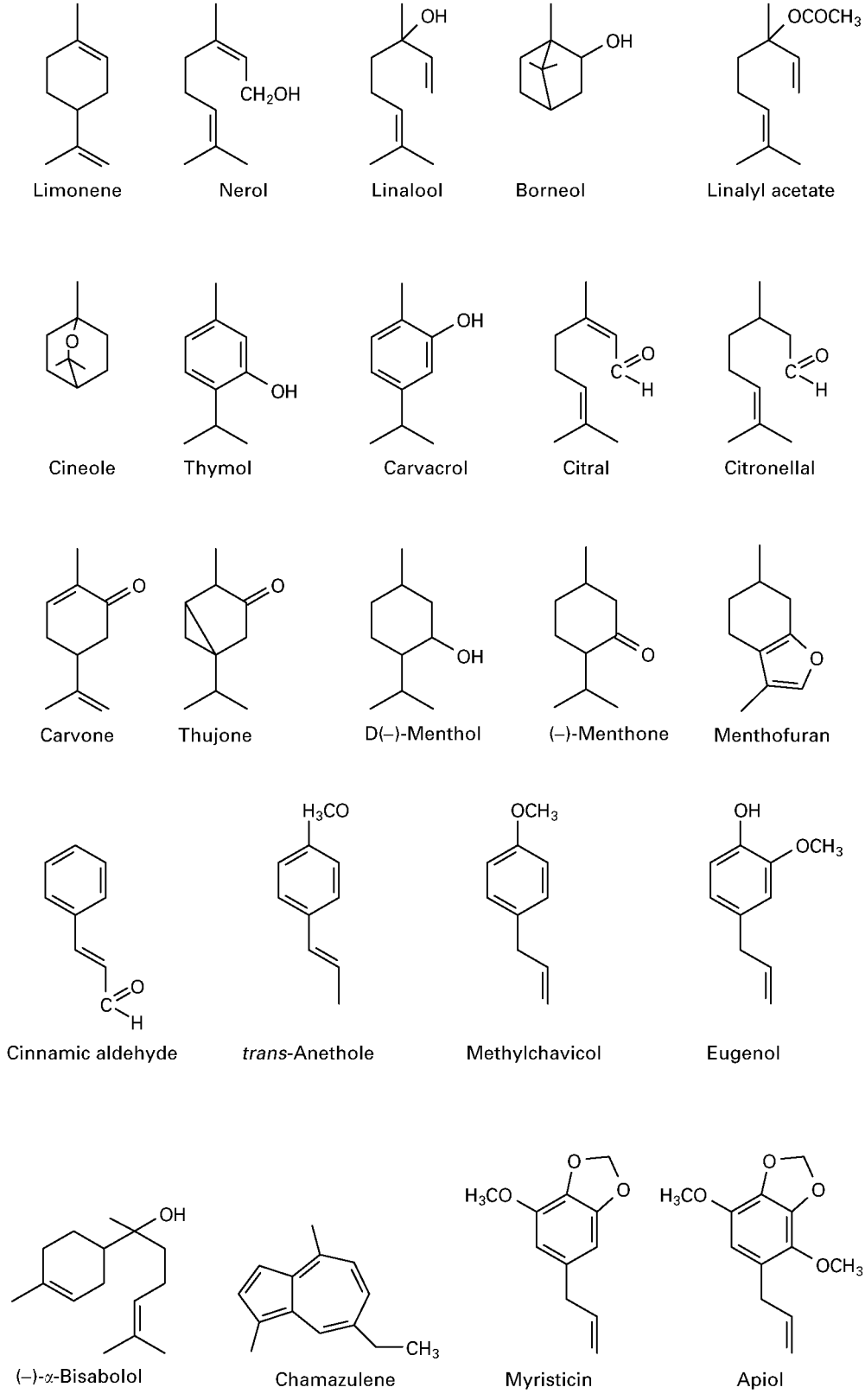


Figure 11 Essential oils.

Under UV light at 254 nm, compounds containing at least two conjugated double bonds quench fluorescence and appear as dark zones against the light-green fluorescent background of the TLC plate. This is the case for the derivatives of phenylpropane (anethole, safrole, apiol, myristicin, eugenol) and compounds such as thymol. The spraying reagents that can be used for the essential oils are (see Appendix) anisaldehyde–sulfuric acid, which gives blue, green, red and brown coloration, and phosphomolybdic acid, which gives uniform blue zones on a yellow background. However, the reagent most widely used for these compounds is vanillin–sulfuric acid which gives a range of different colours (Table 6).

Cannabinoids

The cannabinoids are found in Indian hemp (marihuana; *Cannabis sativa* var. *indica*) (**Figure 12**). The cannabinoids are benzopyran derivatives but only $\Delta^{9,10}$-tetrahydro cannabinol (THC) shows hallucinogenic activity. The type and quantity of the constituents present in the plant depends on the geographical origin and climatic conditions. Marihuana is the flowering or seed-carrying, dried branch tips of the female plant. Hashish is the resin from the leaves and flower of the female plant. The most important cannabinoids are cannabidiol, cannabidiol acid, cannabinol, and Δ^9-THC.

For chromatography, the powdered plant material is extracted with chloroform or hexane and separation can be performed on silica gel TLC plates with hexane–diethyl ether (80 : 20) or hexane–dioxane (90 : 10).

The cannabinoids can be detected by irradiation under UV (254-nm) light as they show fluorescence quenching. With the Fast blue reagent the cannabinoids form violet–red, orange–red or carmine zones; standard thymol gives an orange zone.

Valepotriates

The main active constituents of these drugs, the valepotriates are triesters of a terpenoid, trihydric alcohol. This alcohol has the structure of an iridoid cyclopentanopyran with an attached epoxide ring. Valepotriates are present in valerian rhizome (*Valeriana officinalis*). The drug is extracted with dichloromethane at 60°C then filtered and evaporated to dryness. The chromatographic system is silica gel with toluene–ethyl acetate (75 : 25) or n-hexane–methyl ethyl ketone (80 : 20) as eluents. Under UV light (254 nm) they give a yellow fluorescence and in the visible region with the dinitrophenylhydrazine reagent, after heating, green–grey or blue zones appear. The valepotriates characterized in this way are valtrate, isovaltrate, and acevaltrate.

Bitter Principles

The bitter principles are other compounds in plants which can be characterized by TLC. Plants with bitter principles include gentian, hops, condurango, artichoke and bryony root. Most of them possess a terpenoid structure and can be characterized in TLC with ethyl acetate–methanol–water (77 : 15 : 8) and then derivatization with vanillin-sulfuric reagent. However, these compounds are less important

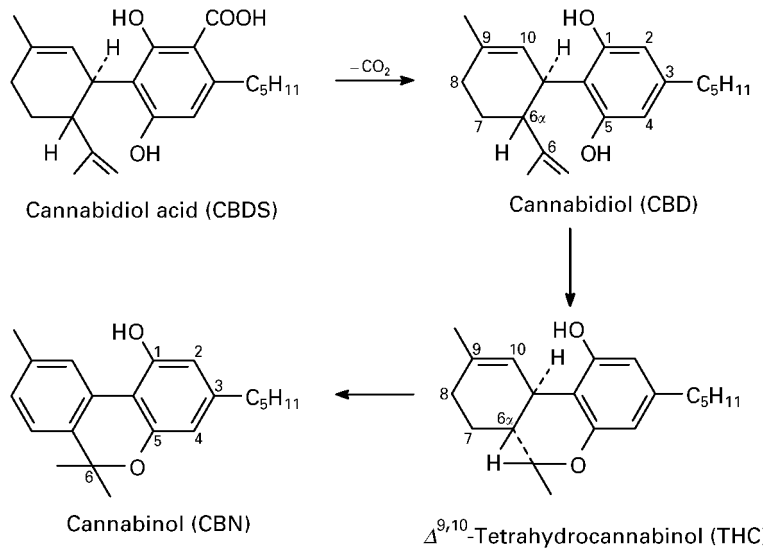


Figure 12 Cannabinoids.

than the compounds mentioned elsewhere in this chapter.

Conclusion

TLC has many advantages for the analysis of herbal products, especially phytopharmaceuticals, for the identification of plants and the quantification of certain marker substances. Planar chromatography has advantages because it allows a parallel evaluation and comparison of multiple samples. In addition, various chromatographic separation systems can be combined with a multitude of specific and non-specific derivatizing agents. Even in samples having complex matrixes such as the pharmaceutical preparations of extracts of plants, sample preparation can be kept simple because of the use of the stationary phase for only one analysis. Unlike column chromatography, contamination of the chromatographic system by carryover cannot occur. In many instances the chemical composition of the herb is not completely known and for many plants, there are often no established methods of analysis available so that a rapid screening technique like TLC is very valuable. Constituents of herbals that belong to very different classes of chemical compounds can often create difficulties in detection, but with this in mind, TLC can offer many advantages.

See Colour Plates 105, 106.

See also: **III/Alkaloids:** High Speed Counter Current Chromatography; Liquid Chromatography; Thin-Layer (Planar) Chromatography. **Citrus Oils: Liquid Chromatography. Essential Oils:** Distillation; Gas Chromatography; Thin-Layer (Planar) Chromatography. **Pigments:** Liquid Chromatography; Thin-Layer (Planar) Chromatography. **Terpenoids: Liquid Chromatography. Appendix 17/Thin-Layer (Planar) Chromatography: Detection.**

Further Reading

Anonymous (1998) Camag bibliography service. CD ROM. Muttenz, Switzerland: Camag.

Baerheim Svendsen A and Verpoorte R (1983) *Chromatography of Alkaloids. Part A: Thin Layer Chromatography*. Elsevier: Amsterdam.

Bruneton J (1999) *Pharmacognosy*, (*Phytochemistry Medicinal Plants*), 2nd edn. Paris: Tec and Doc.

Fried B and Sherma J (1994) *Thin Layer Chromatography: Technique and Applications*. New York: Marcel Dekker.

Fried B and Sherma J (1996) *Practical Thin Layer Chromatography*, pp. 31–47. Boca Raton: CRC Press.

Geiss F (1987) *Fundamentals of Thin Layer Chromatography*. Heidelberg: Hüthig.

Harbone JB (1973) *Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis*. London: Chapman and Hall.

Jork H, Funk W, Fisher W and Wimmer H (1990) *Thin Layer Chromatography Reagents and Detection Methods*, Vol 1a. Weinheim: VCH.

Randerath K (1967) *Dünnschicht Chromatographie*, 2nd edn. Berlin: Springer Verlag.

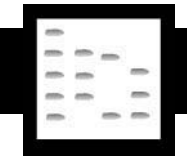
Stahl E (1967) *Dünnschicht Chromatographie*, 2nd edn. Berlin: Springer Verlag.

Touchstone J (1992) *Practical Thin Layer Chromatography*, 3rd edn. London: John Wiley.

Wagner K, Bladt S and Zgainski EM (1966) *Plant Drug Analysis*. Berlin: Springer Verlag.

Wichtl M (1994) *Herbal Drugs and Pharmaceuticals*. Boca Raton: CRC Press.

NEUROTOXINS: CHROMATOGRAPHY



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Chromatography has had a major impact on the discovery and detection of potent, naturally occurring neurotoxins. The neurotoxins discussed in this article were selected because they significantly impact on human health as a result of intoxications from bites and stings or the consumption of contaminated food and water. Many of these toxins target receptors that have implications for the development of potential therapeutic agents. In the neurotoxin topics that have been highlighted here, the role of chromatography in toxin discovery, purification and analysis is emphasized.

Neurotoxins from Marine and Freshwater Algae

It was only in the latter part of the 20th century that scientists appreciated that certain species of microalgae can cause sporadic toxic events that can lead to serious illness, with occasional deaths, in humans as well as farmed and domestic animals. When high populations of toxin-producing microalgae occur