1 Alkaloid Drugs

Most plant alkaloids are derivatives of tertiary amines, while others contain primary, secondary or quarternary nitrogen. The basicity of individual alkaloids varies considerably, depending on which of the four types is represented. The pK_B values (dissociation constants) lie in the range of 10–12 for very weak bases (e.g. purines), of 7–10 for weak bases (e.g. Cinchona alkaloids) and of 3–7 for medium-strength bases (e.g. Opium alkaloids).

1.1 Preparation of Extracts

Alkaloid drugs with medium to high alkaloid contents (⩾1%)

General method, extraction method A

Powdered drug (1 g) is mixed thoroughly with 1 ml 10% ammonia solution or 10% Na_2CO_3 solution and then extracted for 10 min with 5 ml methanol under reflux. The filtrate is then concentrated according to the total alkaloids of the specific drug, so that 100 µl contains 50–100 µg total alkaloids (see drug list, section 1.4).

Exception

Harmalae semen: Powdered drug (1 g) is extracted with 10 ml methanol for 30 min under reflux. The filtrate is diluted 1:10 with methanol and 20 µl is used for TLC.

Strychni semen: Powdered seeds (1 g) are defatted with 20 ml n-hexane for 30 min under reflux. The defatted seeds are then extracted with 10 ml methanol for 10 min under reflux. A total of 30 µl of the filtrate is used for TLC.

Colchici semen: Powdered seeds (1 g) are defatted with 20 ml n-hexane for 30 min under reflux. The defatted seeds are then extracted for 15 min with 10 ml chloroform. After this, 0.4 ml 10% NH_3 is added to the mixture, shaken vigorously and allowed to stand for about 30 min before filtration. The filtrate is evaporated to dryness and the residue solved in 1 ml ethanol; 20 µl is used for TLC investigation.

Alkaloid drugs with low total alkaloids (<1%)

Enrichment method, extraction method B

Powdered drug (2 g) is ground in a mortar for about 1 min with 2 ml 10% ammonia solution and then thoroughly mixed with 7 g basic aluminium oxide (activity grade I). This mixture is then packed loosely into a glass column (diameter, 1.5 cm; length, 20 cm) and 10 ml $CHCl_3$ is added. Alkaloid bases are eluted with about 5 ml $CHCl_3$ and the eluate is collected, evaporated to 1 ml and used for TLC.

This method is suitable for the Solanaceae drugs, e.g. Belladonnae or Scopoliae radix and Stramonii semen, which should be defatted first by extraction with n-hexane or light petroleum. Leaf extracts contain chlorophylls, which can interfere with the TLC separation. In such cases extraction with sulphuric acid (described below) is recommended.

Sulphuric acid extraction method C

Powdered drug (0.4–2 g) is shaken for 15 min with 15 ml 0.1 N sulphuric acid and then filtered. The filter is washed with 0.1 N sulphuric acid to a volume of 20 ml filtrate; 1 ml concentrated ammonia is then added. The mixture is shaken with two portions of 10 ml diethyl ether. The ether is dried over anhydrous sodium sulphate, filtered and evaporated to dryness and the resulting residue dissolved in 0.5 ml methanol.
This is the preferred method for leaf drugs, e.g. Belladonnae folium (0.6 g), Stramonii folium (0.4 g), Hyoscyami folium (2 g) or Fumariae herba (1 g).

1.2 Thin-Layer Chromatography

Drug extracts

The samples applied to the TLC plate should contain between 50 and 100 µg total alkaloids, which have to be calculated according to the average alkaloid content of the specific drug (see 1.4 Drug List).

Example: Powdered drug (1 g) with a total alkaloid content of 0.3 %, extracted with 5 ml methanol by the general method described above will yield 3 mg in 5 ml methanolic solution, containing approximately 60 µg total alkaloids per 100 µl.

Reference compounds

- Commercially available compounds are usually prepared in 1% alcoholic solution and 10 µl is applied for TLC, e.g. atropine, brucine, strychnine, berberine, codeine.
- Rauvolfia alkaloids are prepared in 0.5% alcoholic solution, and 10 µl is applied for TLC, e.g. reserpine, rescinnamine, rauwolscine, ajmaline, serpentine.
- Colchicine is prepared as a 0.5% solution in 70% ethanol, and 10 µl is applied for TLC.

Alkaloid references can also be obtained from pharmaceutical products by a simple methanol extraction. The sample solution used for TLC should contain between 50 and 100 µg alkaloid.

- Alkaloid content 10–250 mg per tablet or dragée:
  One powdered tablet or dragée is mixed with 1 ml methanol per 10 mg alkaloid and shaken for about 5 min at 60°C. After filtration or centrifugation, the extract is applied directly; 10 µl then corresponds to 100 µg alkaloid.
- Alkaloid content 0.075–1.0 mg per tablet or dragée:
  Ten powdered tablets or dragées are mixed with 10 ml methanol, shaken for about 5 min at 60°C and filtered and the filtrate evaporated to dryness. The residue is dissolved in 1 ml methanol and, if necessary, the solution cleared by centrifugation; 10 µl of this solution contains 100 µg alkaloid (1.0 mg/tablet), or 100 µl contains 75 µg alkaloid (0.075 mg/tablet).

Test mixtures

- Cinchona alkaloids test mixture for Cinchonae (Chinae) cortex (DAB 10)
  A mixture of 17.5 mg quinine, 0.5 mg quinidine, 10 mg cinchonine and 10 mg cinchonidine is dissolved in 5 ml ethanol, and 2 µl of this solution is applied for TLC.
- Test mixture for Solanaceae drugs (DAB 10)
  A total of 50 mg hyoscyamine sulphate is dissolved in 9 ml methanol and 15 mg scopolamine hydrobromide in 10 ml methanol.

  For Belladonnae folium (T1): 1.8 ml scopolamine hydrobromide solution is added to 8 ml hyoscyamine sulphate solution; 20 µl is used for TLC.

For Hyoscyami folium (T2): 4.2 ml scopolamine hydrobromide solution is added to 3.8 ml hyoscyamine sulphate solution; 20 µl is used for TLC.
For Stramonii folium (T3): 4.2 ml scopolamine hydrobromide solution is added to 3.8 ml hyoscyamine sulphate solution; 20 µl is used for TLC.

Adsorbent

Silica gel 60 F_{254}-precoated TLC plates (Merck, Darmstadt, Germany)
▶ The principal alkaloids of the most common alkaloid drugs can be identified.

Aluminium oxide-precoated TLC plates (Merck, Darmstadt, Germany)
▶ More suitable for the separation of berberine, columbamine and jatrorrhizine.

Chromatography solvents

Solvent system	Drug, alkaloids
Toluene–ethyl acetate–diethylamine (70:20:10)	**Screening system**, suitable for the major alkaloids of most drugs
Chloroform–diethylamine (90:10)	Chinae cortex; Cinchona alkaloids
Acetone–light petroleum–diethylamine (20:70:10)	Gelsemii radix
Cyclohexane–ethanol–diethylamine (80:10:10)	Aconiti tuber
Cyclohexane–chloroform–diethylamine (50:40:10)	
Chloroform–acetone–diethylamine (50:40:10)	Harmalae semen
Chloroform–methanol–ammonia 10% (80:40:15)	
Ethyl acetate–isopropanol–ammonia 25% (100:2:1)	Uncariae cortex
Dioxane–ammonia 25% (90:10)	Adhatodae folium
Ethyl acetate–cyclohexane–methanol–ammonia 25% (70:15:10:5)	Ephedrae herba
Ethyl acetate–methanol–water (100:13.5:10)	**Screening system**, suitable e.g. for xanthine derivatives, Colchicum and Rauvolfia alkaloids
Ethyl acetate–methanol (90:10)	Vincae herba
Ethyl acetate–methanol (60:20)	Catharanthi folium
Toluene–chloroform–ethanol (28.5:57:14.5)	Secale alkaloids Ephedrae herba
n-Propanol–formic acid–water (90:1:9)	Berberidis cortex, Hydrastis rhizoma, Colombo radix, Chelidonii herba
n-Butanol–ethyl acetate–formic acid–water (30:50:10:10)	Mahoniae radices cortex

Solvent system	Drug, alkaloids
Ethyl acetate–ethylmethyl ketone–formic acid–water (50:30:10:10)	Fumariae herba, Corydalidis rhizoma
Cyclohexane–chloroform–glacial acetic acid (45:45:10)	Berberine- and protoberberine-type alkaloids
Chloroform–methanol–glacial acetic acid (47.5:47.5:5)	Genistae herba, Sarothamni herba, Spartii scop. flos
n-Butanol–glacial acetic acid–water (40:40:10)	Catharanthus alkaloids

1.3 Detection

- UV-254 nm Pronounced quenching of some alkaloid types such as indoles, quinolines, isoquinolines, purines; weak quenching of e.g. tropine alkaloids
- UV-365 nm Blue, blue–green or violet fluorescence of alkaloids, e.g. Rauvolfiae radix, Chinae cortex, Ipecacuanhae radix, Boldo folium. Yellow fluorescence, e.g. colchicine, sanguinarine, berberine
- Spray reagents (see Appendix A)

– Dragendorff reagent (DRG No.13)
The alkaloids appear as brown or orange–brown (vis.) zones immediately on spraying. The colour is fairly stable. Some types such as purines or ephedrine need special detection. The colour of alkaloid zones can be intensified or stabilized by spraying first with Dragendorff reagent and then with 10% sodium nitrite solution or 10% ethanolic sulphuric acid.

– Iodoplatinate reagent (IP No.21)
Directly after spraying, alkaloids appear as brown, blue or whitish zones (vis.) on the blue–grey background of the TLC plate.

– Special detection

Iodine–potassium iodide–HCl reagent (No.20)	→ purines
Iodine $CHCl_3$ reagent (No.19)	→ emetine, cephaeline
Marquis reagent (No.26)	→ opium alkaloids
van Urk reagent (No.43)	→ secale alkaloids
Ninhydrine reagent (No.29)	→ ephedrine
10% ethanolic H_2SO_4 (No.37)	→ china alkaloids

1.4 Drug List

The chromatograms of the specific alkaloid drugs are reproduced according to their alkaloid types (Fig. 1–30).

Drug/plant source Family/pharmacopoeia	Total alkaloids Major alkaloids (for formulae see 1.5 Formulae)	
Indole Alkaloids		**Fig. 3–10**
Rauvolfiae radix Rauvolfia, snake root Rauvolfia serpentina (L.) BENTH ex KURZ. Rauvolfia vomitoria AFZEL Apocynaceae DAB 10, USP XXII, MD	0.6%–2.4% total alkaloids (R. serpentina) 1.3%–3% total alkaloids (R. vomitoria) >50 alkaloids, yohimbane derivatives: Reserpine (0.14%), rescinnamine (0.01%), epi-rauwolscine (0.08%), serpetine (0.08%), serpentinine (0.13%), ajmaline (0.1%), ajmalicine (=raubasine 0.02%), raupine (0.02%)	**Fig. 3**
Yohimbe cortex Yohimbe bark Pausinystalia johimbe PIERRE Rubiaceae	2.3%–3.9% total alkaloids Yohimbine and ten minor alkaloids, e.g. pseudoyohimbine and coryantheine	**Fig. 4**
Quebracho cortex Aspidosperma bark Aspidosperma quebracho-blanco SCHLECHT Apocynaceae DAC 86	0.3%–1.5% total alkaloids (>30) Yohimbine, pseudoyohimbine, aspidospermine, aspidospermatine, quebrachamine, hypoquebrachamine, quebrachocidine	**Fig. 4**
Catharanthi folium Catharanthus leaves Catharanthus roseus (L.) G. DON. (syn. Vinca rosea L.) Apocynaceae MD	0.15%–0.25% total alkaloids Vinblastine (0.01%), vincristine, vindoline, catharanthine, Root: <0.74% total alkaloids	**Fig. 4**
Vincae herba Common periwinkle Vinca minor L. Apocynaceae MD	0.15%–1% total alkaloids Vincamine (0.05%–0.1%), vincaminine, vincamajine, vincine, minovincine, reserpinine	**Fig. 5**
Strychni semen Poison nuts, Nux vomica seeds Strychnos nux-vomica L. Loganiaceae ÖAB, Helv. VII, MD, Japan	2%–3% total alkaloids Strychnine (>1%) and brucine (>1.5%), α- and β-colubrine, vomicine; psendostrychnine, psendobrucine	**Fig. 6**
Ignatii semen St. Ignaz beans Strychnos ignatii BERG Loganiaceae	2.5%–3% total alkaloids Strychnine (45%–50%), brucine, 12-hydroxy strychnine, α-colubrine, vomicine	**Fig. 6**

	Drug/plant source Family/pharmacopoeia	Total alkaloids Major alkaloids (for formulae see 1.5 Formulae)
Fig. 7	**Secale cornutum** Ergot Claviceps purpurea (FRIES) TULASNE Clavicipitaceae (Ascomycetes) ÖAB, MD	0.2%–1% total alkaloids Ergot alkaloids, lysergic acid alkaloids; amide alkaloids (ergometrine), peptide alkaloids (ergotamine), ergotoxin group (ergocristine)
Fig. 8	**Gelsemii radix** Yellow jasmine, wild woodbine Gelsemium sempervirens (L.) AIT. Loganiaceae MD	0.25%–0.7% total alkaloids Gelsemine, sempervirine, (isogelsemine, gelsemicine)
Fig. 9	**Harmalae semen** Syrian (wild) rue Peganum harmala L. Zygophyllaceae	2.5%–4% total alkaloids Carbolinderivatives: harmaline (>60%), harmine, harmalol, harmidine Quinazoline alkaloids: (−)-vasicine (= (−) peganine), vasicinone
Fig. 10A	**Justiciae-adhatodae-folium** Malabarnut leaves Justicia adhatoda L. (syn. Adhatoda vasica NEES.) Acanthaceae MD	0.5%–2% quinazoline alkaloids Vasicine (45–95%), vasicinine Vasicinone, oxyvasicinine (oxidation products, artefacts)
Fig. 10B	**Uncariae radix** Uncaria ("una de gato") Uncaria tomentosa WILLD. Rubiaceae	>0.9% tetracyclic and pentacyclic oxindoles Rhychnophylline, isorhychnophylline, mitraphylline, isomitraphylline, pteropodine, isopteropodine, uncarine A, F
Fig. 11–16	**Quinoline and isoquinoline alkaloids alkaloids of the morphinane type (phenanthrene type)**	
Fig. 11	**Ipecacuanhae radix** Ipecacuanhae root Cephaelis ipecacuanha (BORT.) RICH. (Rio and Matto-Grosso)	1.8%–6% total alkaloids Emetine and cephaeline (>95%), o-methylpsychotrine and psychotrine (corresponding dehydro compounds) 1:1 → 3:1 ratio of emetine to cephaeline
	Cephaelis acuminata KARSTEN (Cartagena, Panama drugs) Rubiaceae DAB 10, Ph. Eur. I, ÖAB, Helv. VII, BP 88, USP XXII, MD, DAC 86	1.7%–3.5% total alkaloids cephaeline (>50%), emetine; o-methylpsychotrine, psychotrine (0.05%)

Drug/plant source Family/pharmacopoeia	Total alkaloids Major alkaloids (for formulae see 1.5)	
Chinae cortex **Cinchonae cortex** Red Cinchona bark Cinchona pubescens VAHL (syn. C. succirubra PAVON) DAB 10, ÖAB, Helv. VII, MD DAC 86 (tinct.)	4%–12% total alkaloids: approximately 20 alkaloids; diastereomeres Quinine/quinidine and cinchonine/cinchonidine quinine (0.8%–4%), quinidine (0.02%–0.4%), cinchonine (1.5%–3%), cinchonidine (1.5%–5%)	**Fig. 12**
Cinchona calisaya WEDDEL Yellow Cinchona bark Rubiaceae USP XI	Yellow Cinchona bark contains up to 90% quinine	
Opium Opium Papaver somniferum L. subsp. somniferum and varieties Papaveraceae DAB 10, ÖAB, Helv. VII, BP'88, MD, Japan (pulv.), USP XXII (tinct.)	20%–29% total alkaloids raw opium: 30 alkaloids Phenanthrene type: morphine (3%–23%), codeine (0.3%–3%), thebaine (0.1%–3%) Benzylisoquinoline type: papaverine (0.1%–2%), noscapine (narcotine; 2%–12%), narceine (0.1%–2%)	**Fig. 13,14**
Corydalidis rhizoma Hollowroot-birthwort Corydalis cava (L.) SCHWEIGG et KOERTE Papaveraceae, Fumariaceae China, Japan	3–5% total alkaloids Berberine type; corydaline, coptisine tetrahydropalmatine, canadine Aporphine type: bulbocapnine (0.2%–0.3%) (+) corytuberine, corydine Protopine	**Fig. 15**
Fumariae herba Fumitory herb Fumaria officinalis L. Papareraceae (Fumariaceae)	0.5%–1% total alkaloids Protoberberine type (0.2%–0.4%) protopine ► 0.5% flavonoids and phenol carboxylic acids, fumaric acid	**Fig. 16**
Miscellaneous classes of alkaloids		**Fig. 17–26**
Sarothamni (Cytisi) herba Scotch broom tops Cytisus scoparius (L.) LINK (syn. Sarothamnus scoparia (L.)) Fabaceae MD, DAC 86	0.3%–1.5% quinolizidine alkaloids >20 alkaloids. (−)-Sparteine (85%–90%), 17-oxo-α-isosparteine, lupanine, 4- and 13-hydroxylupanine ► 0.2%–0.6% flavonoids: spiraeoside, isoquercitrine, scoparoside, ► coumarins; caffeic acid derivatives	**Fig. 17**

	Drug/plant source Family/pharmacopoeia	Total alkaloids Major alkaloids (for formulae see 1.5)
Fig. 17	**Spartii flos** Spartii juncei flos Broomflowers Spartium junceum L. Fabaceae (Leguminosae)	0.3%–0.4% quinolizidine alkaloids Cytisine (40%) N-methylcytisine (45%) anagyrine ▶ Flavonoids: isoquercitrine, luteolin-4'-O-glucoside
Fig. 18	**Genistae herba** Dyer's weed, Dyer's broom Genista tinctoria L. Fabaceae	0.3%–0.8% quinolizidine alkaloids *N*-methylcytisine, anagyrine, isosparteine, lupanine ▶ 0.5%–3% flavonoids: luteolin glycosides Isoflavones: genistein, genistin
	Note: The trivial name genistein is used for the isoflavone and the alkaloid (α-isosparteine).	
Fig. 19	**Chelidonii herba** Tetterwort, greater celandine Chelidonium majus L. Papaveraceae DAB 10 ▶ Chelidonii radix/rhizoma	0.35%–1.30% total alkaloids (>20) Benzophenanthridine type: chelidonine (>0.07%), chelerythrine (>0.04%) and sanguinarine (>0.01%) Protoberberine type: coptisin (>1.07%), berberine (0.11%). Protopine 2.4%–3.4% total alkaloids: chelidonin (1.2%), and chelerythrine (1%)
Fig. 20	**Colchici semen** Meadow saffron seeds Colchicum autumnale L. Liliaceae DAC 86, MD	0.5%–1% total alkaloids: >20 alkaloids Colchicine (65%), colchicoside (30%), demecolcine, lumialkaloids (artefacts)
Fig. 21	**Berberidis radicis cortex** Barberry root bark Berberis vulgaris L. Berberidaceae MD	>13% total alkaloids Berberine, protoberberine (6%), jateorrhizine (jatrorrhizine), palmatine <5% bisbenzylisoquinolines e.g. oxyacanthine. Magniflorine
Fig. 21	**Hydrastis rhizoma** Golden seal root Hydrastis canadensis L. Ranunculaceae MD	2.5%–6% total alkaloids Berberine (2%–4.5%), tetrahydroberberine (0.5%–1%) (canadine), hydrastine (3.2%–4%; phthalide-isoquinoline alkaloid)
Fig. 21	**Colombo radix** Calumba root Jateorhiza palmata (LAM) MIERS Menispermaceae MD Japan (J. columba MIERS)	2%–3% total alkaloids Palmatine, jatrorrhizine, bisjatrorrhizine, columbamine (protoberberine type) ▶ Furanoditerpenoid bitter principles (palmarin, columbin)

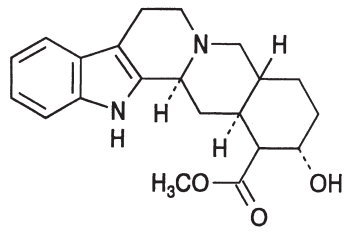
Drug/plant source Family/pharmacopoeia	Total alkaloids Major alkaloids (for formulae see 1.5)	
Mahoniae radicis cortex Mahonia bark, grape root Mahonia aquifolium (PURSH) NUTT (syn. Berberis aquif.) Berberidaceae	1.8%–2.2% total alkaloids Jatrorrhizine, berberine, palmatine, columbamine (protoberberines); magnoflorine, corytuberine (aporphines); oxyacanthine, berbamine, (bisbenzyl-isoquinolines)	Fig. 22
Boldo folium Boldo leaves Peumus boldus J.I.MOLINA Monimiaceae DAC 86, Helv. VII, MD	0.2%–0.5% total alkaloids Aporphine alkaloid boldine ► 2%–3% essential oils: p-cymol, cineole, ascaridole (40%–50%) ► 1% flavonoids	Fig. 23
Nicotianae folium Tobacco leaves Nicotiana tabacum L., N. rustica L. and other varieties Solanaceae	0.06%–10% total alkaloids L-Nicotine, nornicotine, anabasine, nicotyrine	Fig. 24
Aconiti tuber Aconite root Aconitum napellus L. Ranunculaceae MD	0.3%–1.5% total alkaloids: 15 ester alkaloids Aconitine, mesaconitine, hypaconitine (benzoylaconine and aconine: hydrolytic cleavage products)	Fig. 25
Lobeliae herba Lobelia, Indian tobacco Lobelia inflata L. Campanulaceae (Lobeliaceae) ÖAB, BP 88, MD	0.2%–0.6% total alkaloids Lobeline (piperidine ring system) Isolobinine (dehydro, piperidine ring) DL-lobelidine, lobelanine	Fig. 26
Sabadillae semen Caustic barley, Cevadilla seed Schoenocaulon officinale A. GRAY	3%–6% steroid alkaloids (C-nor-C-homo-cholestanes)	Fig. 26
Liliaceae MD	"veratrine" = mixture of cevadine, veratridine, devadilline, sabadine, cevine)	
Ephedrae herba Desert tea (Ma-huang) Ephedra sinica STAPF Ephedra shennungiana TANG E. distachya L. or other species Gnetaceae (Ephedraceae) DAB 10, MD, Japan, China	2.5%–3% total alkaloids L-Ephedrine (0.75%–1%), norephedrine (+)-Pseudoephedrine and norpseudoephedrine	Fig. 26B

	Drug/plant source Family/pharmacopoeia	Total alkaloids Major alkaloids (for formulae see 1.5)
Fig. 27–28	**Tropine alkaloids**	
Fig. 27,28	**Belladonnae folium** Belladonna leaves Solanaceae DAB 10, Ph.Eur.I, ÖAB, Helv. VII, BP 88, USP XXII	0.2%–0.5% total alkaloids (−)-Hyoscyamine/atropine (~87%) scopolamine, apoatropine ► Flavonoids: quercetin glycosides
Fig. 27,28	**Belladonnae radix** Belladonna root Atropa belladonna L. Solanaceae DAC 86, ÖAB, MD, Japan	0.3%–0.8% total alkaloids (−)-Hyoscyamine and scopolamine Minor alkaloids apoatropine, belladonnine, cuskhygrine, ► Coumarins: scopoletin, −7-O-glucoside (see Chap. 5, Fig. 5)
Fig. 27,28	**Scopoliae radix** Scopolia root Scopolia carniolica JACQ. Solanaceae Japan (e.g. Scopolia japonica)	0.4%–0.95% total alkaloids (−)-Hyoscyamine and scopolamine ► Coumarins: scopoletin, −7-O-glucoside (see Chap. 5, Fig. 5)
Fig. 27,28	**Hyoscyami folium** Henbane leaves Hyoscyamus niger L. var. niger Solanaceae DAB 10, PhEur. I, ÖAB, Helv. VII, MD	0.04%–0.17% total alkaloids (−)-Hyoscyamine/atropine (60%) scopolamine, belladonine, apoatropine ► Flavonoid glycosides
Fig. 27,28	**Hyoscyami mutici folium** Hyoscyamus muticus L. Solanaceae MD	0.8%–1.4% total alkaloids (−)-Hyoscyamine/atropine (90%) scopolamine, apoatropine, belladonnine
Fig. 27,28	**Stramonii folium** Thornapple leaves Datura stramonium L. Solanaceae DAB 10, PhEur. I, ÖAB, Helv. VII, MD	0.1%–0.6% total alkaloids (−)-Hyoscyamine/atropine and scopolamine in ratio of approximately 2:1; belladonnine ► Flavonoid glycosides

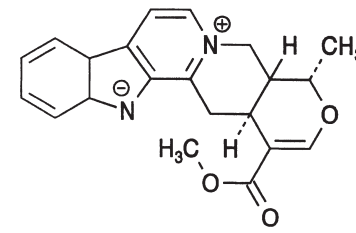
Drug/plant source Family/pharmacopoeia	Total alkaloids Major alkaloids (for formulae see 1.5)	
Purines		**Fig. 29–30**
Cacao semen Cacao beans Theobroma cacao L. Sterculiaceae MD	0.2%–0.5% caffeine 1%–2% theobromine	**Fig. 29,30**
Coffeae semen Coffee beans Coffea arabica L., other species Rubiaceae MD, DAB 10 (caffeine)	0.3%–2.5% caffeine theophylline (traces) ► Chlorogenic acid	**Fig. 29,30**
Mate folium Mate, Jesuit's tea Ilex paraguariensis St.HIL. Aquifoliaceae DAC 86, MD	0.3%–1.7% caffeine 0.03%–0.05% theophylline 0.2%–0.45% theobromine ► 10% chlorogenic-, iso- and neochlorogenic acid, isoquercitrin ► Triterpene saponines: ursolic and oleanolic acid derivatives	**Fig. 29,30**
Theae folium Tea Camellia sinensis (L.) KUNTZE Theaceae MD	2.5%–4.5% caffeine 0.02%–0.05% theophylline 0.05% theobromine ► Polyphenols; tannins: catechin type (10%–20%), dimeric theaflavins, oligomeric procyanidins; flavonoid glycosides	**Fig. 29,30**

Note: Colae semen contains 0.6%–3% caffeine (Cola nidita, C. acuminata SCHOTT et ENDL, Sterculiaceae)

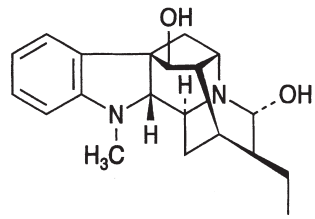
1.5 Formulae



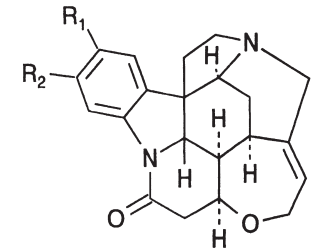
Yohimbine



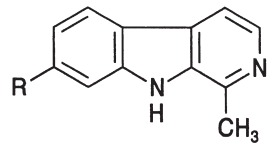
Serpentine



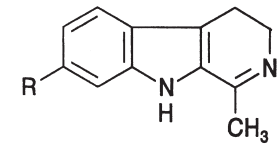
Ajmaline



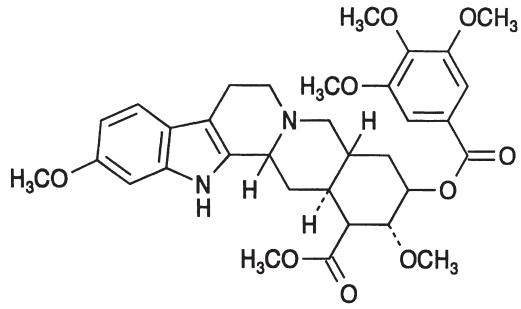
$R_1 = R_2 = H$ Strychnine
$R_1 = R_2 = OCH_3$ Brucine



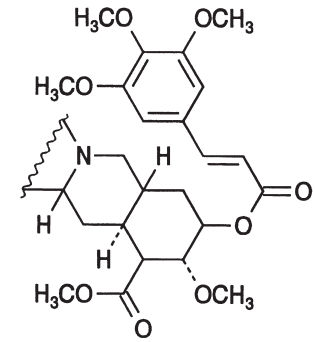
Harman R = H
Harmine R = OCH_3
Harmol R = OH



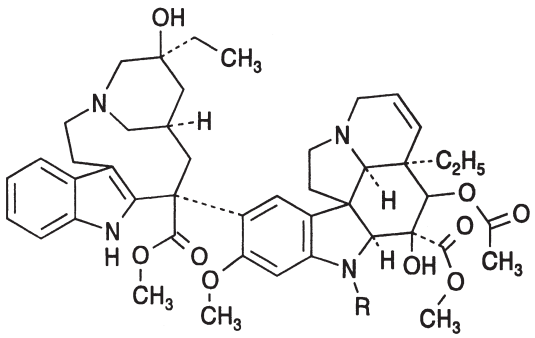
Harmalol R = OH
Harmaline R = OCH_3



Reserpine

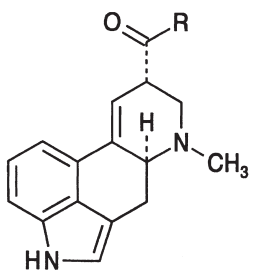


Rescinnamine



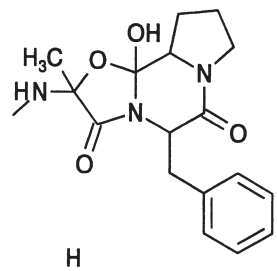
Vincaleucoblastine R = CH_3

Leurocristine R =

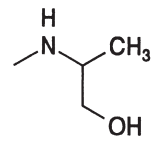


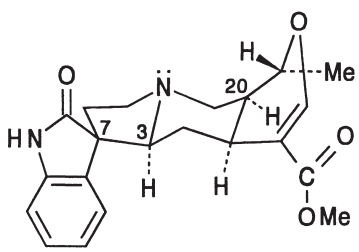
(Pyrrolindol)

Ergotamine R =

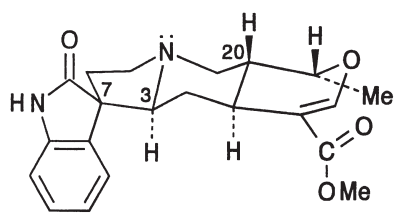


Ergometrine R =

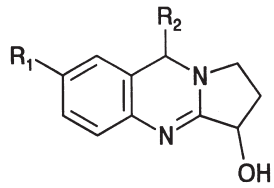




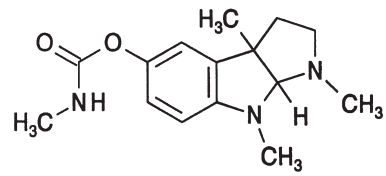
Pteropodine



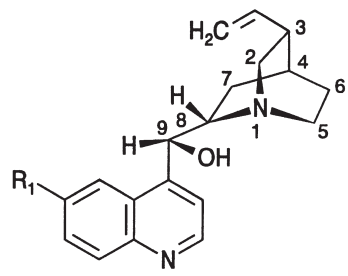
Mitraphylline



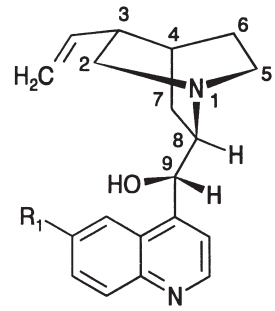
	R_1	R_2
Vasicine	—H	$-H_2$
Vasicinone	—H	=O
Oxyvasicine	—OH	$-H_2$



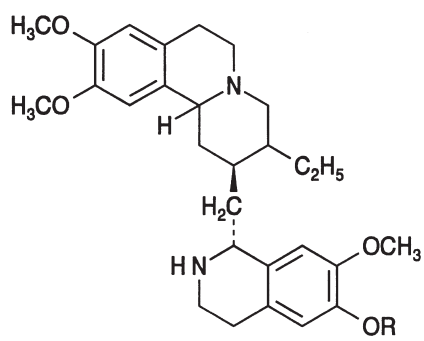
Physostigmine



Cinchonidine: R = H
Quinine: R = OCH_3

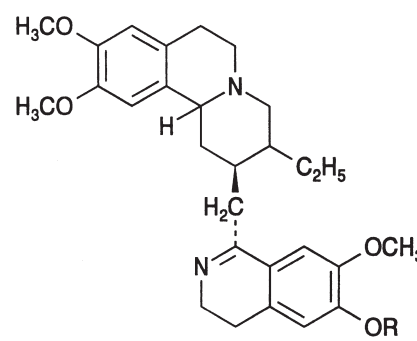


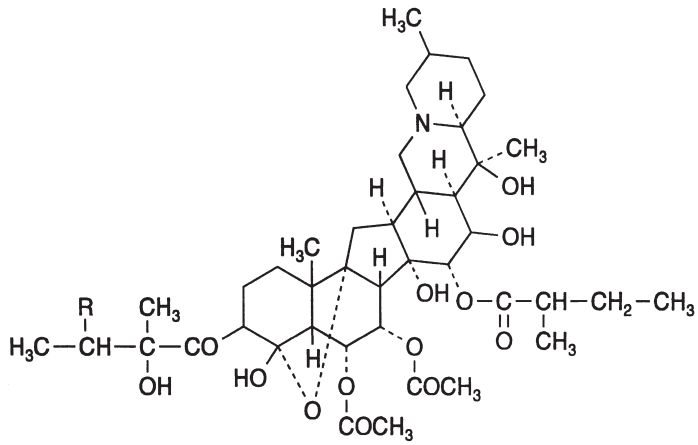
Cinchonine: R = H
Quinidine: R = OCH_3



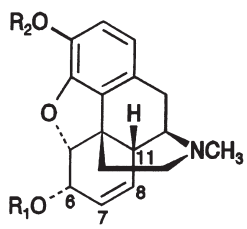
(-) Emetine R = CH_3 — - 2H → O-Methylpsychotrine

(-) Cephaeline R = H → Psychotrine

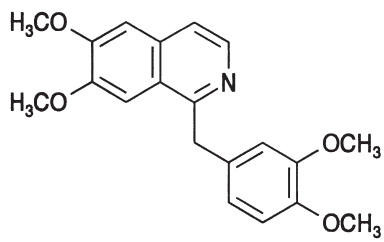




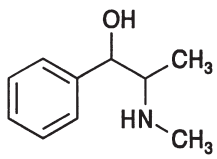
Protoveratrine A : R = H
Protoveratrine B : R = OH



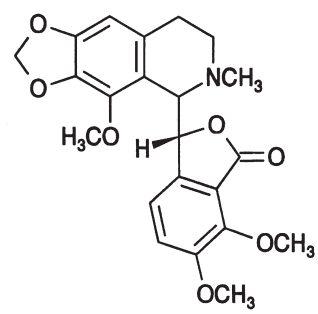
Morphine $R_1 = R_2 = H$
Codeine $R_1 = H;\ R_2 = CH_3$
Thebaine $R_1 = R_2 = CH_3$
(double bond C 6/7 and C 8/11)



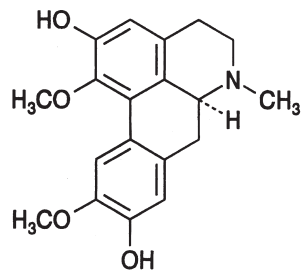
Papaverine



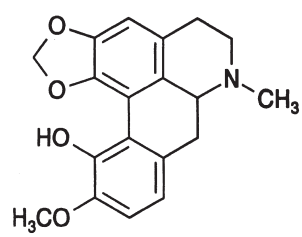
Ephedrine



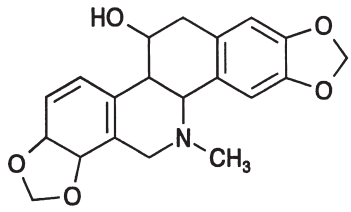
Noscapine



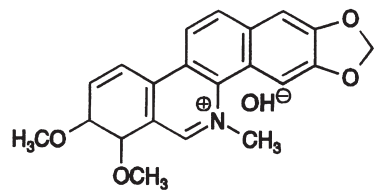
(S)-Boldine



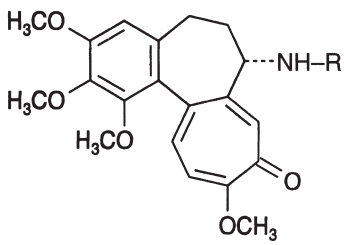
(S)-Bulbocapnine



Chelidonine

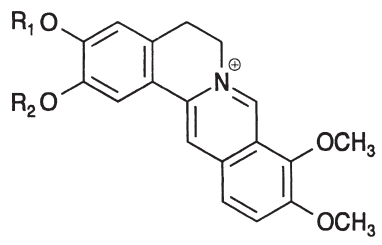


Chelerythrine

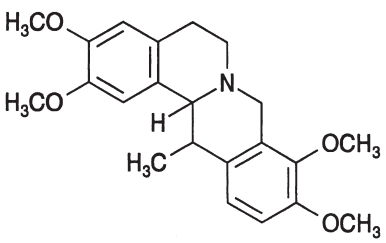


Colchicine R = 

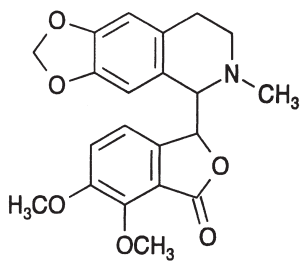
Demecolcine R = CH_3



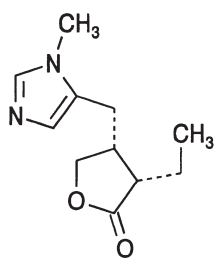
R_1	R_2	
H	CH_3	Jatrorrhizine
CH_3	H	Columbamine
CH_3	CH_3	Palmatine
$-CH_2-$		Berberine



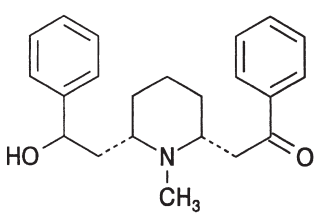
(-)-Corydaline



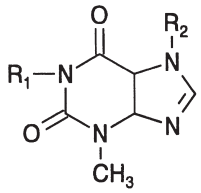
Hydrastine



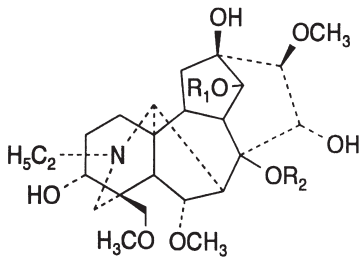
Pilocarpine



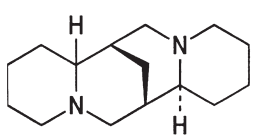
Lobeline



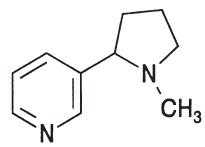
	R_1	R_2
Caffeine	CH_3	CH_3
Theobromine	H	CH_3
Theophylline	CH_3	H



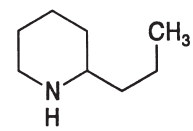
R_1	R_2	
COC_6H_5	$COCH_3$	Aconitine
COC_6H_5	H	Benzoylaconin
H	H	Aconin



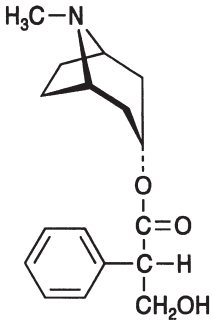
Sparteine



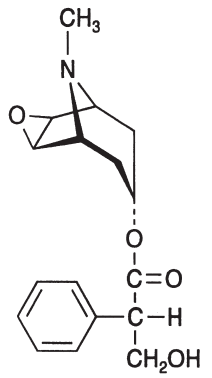
Nicotine



Coniine



L-Hyoscyamine



L-Scopolamine

1.6 TLC Synopsis of Important Alkaloids

Alkaloids I

Reference compounds detected with Dragendorff reagent

1 colchicine	9 atropine	16 nicotine
2 boldine	10 codeine	17 veratrine
3 morphine	11 cinchonine	18 emetine
4 pilocarpine	12 scopolamine	19 papaverine
5 quinine	13 strychnine	20 lobeline
6 brucine	14 yohimbine	21 mesaconitine ▶aconitine
7 cephaeline	15 physostigmine	22 noscapine (=narcotine)
8 quinidine		

Solvent system Fig. 1 toluene–ethyl acetate–diethylamine (70:20:10)

Detection
A Dragendorff reagent (No. 13A) → vis
B Dragendorff reagent followed by sodium nitrite (No. 13B) → vis

Fig. 1 With Dragendorff reagent alkaloids spontaneously give orange–brown, usually stable colours in the visible. With some alkaloids, e.g. boldine (2), morphine (3) and nicotine (16), the colour fades rapidly and can be intensified by additional spraying with sodium nitrite reagent. The zones then appear dark brown (e.g. morphine, 3) or violet–brown (e.g. atropine, 9). The colours of pilocarpine (4) and nicotine (16) are still unstable.

Alkaloids II

Reference compounds that fluoresce in UV-365 nm

23 serpentine	27 cinchonidine	31 noscapine
24 quinine	28 cephaeline	32 hydrastine
25 cinchonine	29 emetine	33 berberine
26 quinidine	30 yohimbine	34 sanguinarine

Solvent system Fig. 2 toluene–ethyl acetate–diethylamine (70:20:10)

Detection
A Dragendorff reagent (No. 13A) → vis
B Sulphuric acid reagent (10%- No. 37A) → UV-365 nm

Fig. 2 The fluorescence of these alkaloids, predominantly light blue, can be intensified by treatment with 10% ethanolic sulphuric acid.
In the case of the quinine alkaloids, the initial light blue fluorescence of quinine and quinidine becomes a radiant blue (this appears white in the photo), while cinchonine and cinchonidine show a deep violet fluorescence (hardly visible in the photo).
Berberine (33) and sanguinarine (34) are exceptions in showing a bright yellow fluorescence.
Colchicine shows a yellow–green fluorescence (see Fig. 20, Alkaloid Drugs).

Remarks: The commercial alkaloid reference compounds (e.g. hydrastine (32)) frequently show additional zones of minor alkaloids or degradation products.

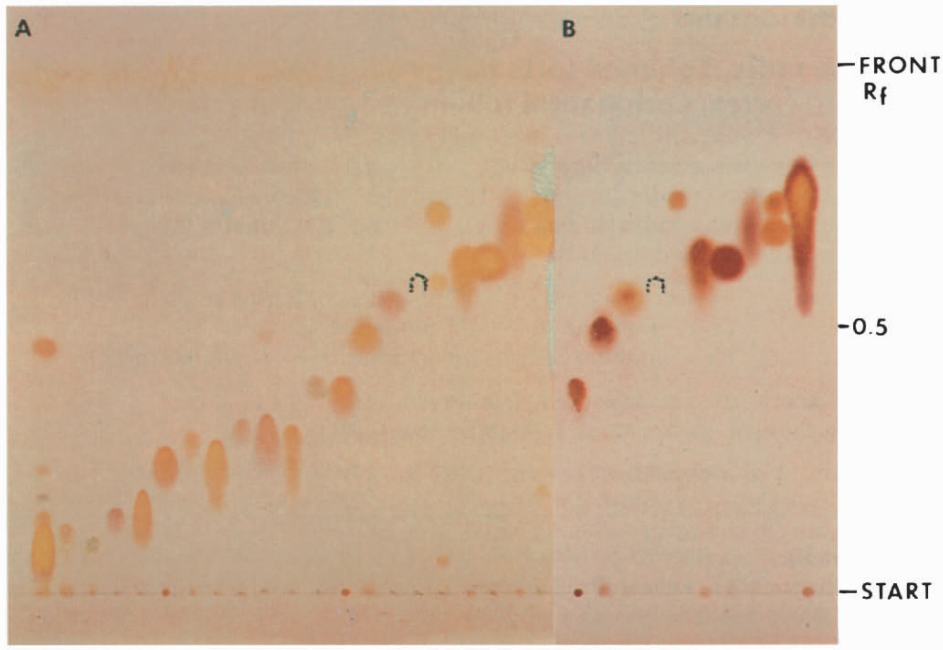

A
B
FRONT
Rf
0.5
START
T- 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 13 14 15 16 17 18 19 20 21 22

Fig. 1

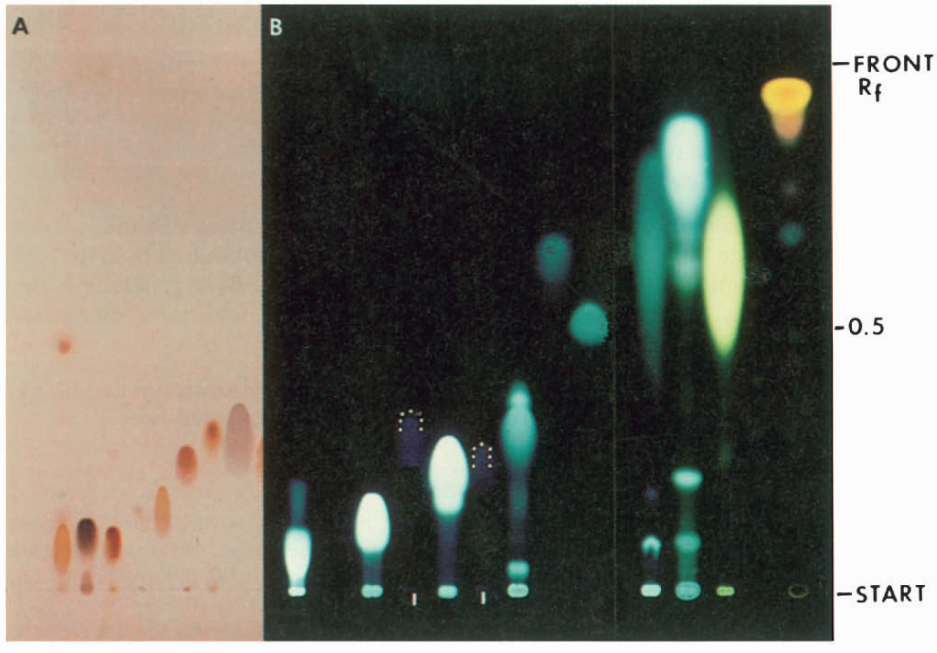

A
B
FRONT
Rf
0.5
START
T- 1 2 3 4 5 6 7 9 23 24 25 26 27 28 29 30 31 32 33 34

Fig. 2

1.7 Chromatograms

Rauvolfiae radix, Yohimbe cortex, Quebracho cortex, Catharanthi folium

Drug sample

1 Rauvolfiae serpentinae radix (Siam drug)
2 Rauvolfiae vomitoriae radix
3 Rauvolfiae serpentinae radix (Indian drug)
(alkaloid extraction method A, 30 µl)
4 Yohimbe cortex
5 Quebracho cortex
6,7 Catharanthi folium

Reference compound

T1 serpentine
T2 ajmaline
T3 reserpine
T4 rescinnamine
T5 rauwolscine
T6 yohimbine
T7 vincaleucoblastine sulphate (VLB)
T8 vindoline
T9 papaverine (→R_f similar to T8)

Solvent system

Fig. 3,4 A toluene–ethyl acetate–diethylamine (70:20:10)
Fig. 4 B n-butanol–glacial acetic acid–water (40:10:10)

Detection

A UV-365 nm B Dragendorff reagent (DRG No. 13) → vis

Fig. 3

A **Rauvolfiae radix**

The drug extracts 1–3 are generally characterized in UV-365 nm by seven to ten intense blue fluorescent zones from the start till $R_f \sim 0.8$:

$R_f \sim 0.05$ (T1)	Serpentine	[a] Ajmaline shows a prominent quenching in UV-254 nm and only develops a dark blue fluorescence when exposed to UV-365 nm for 40 min.
0.15–0.25	Two to three alkaloids, not identified	
0.30 (T2)	Ajmaline[a]	
0.40 (T5)	Rauwolscine[b]	[b] Rescinnamine and rauwolscine show three to four zones due to artefacts formed in solution and on silica gel.
0.45 (T3, T4)	Reserpine/rescinnamine[b]	
0.6–0.8	Two to three alkaloids, e.g. raubasine	

Rauvolfiae serpentinae radix (1,3) show varying contents of the major alkaloids according to drug origin. The Indian drug mostly has a higher serpentine content than the Siam drug. **Rauvolfiae vomitoriae radix** (2) differs from (1) and (3) by a generally higher content of reserpine, rescinnamine and ajmaline and by the additional compound rauwolscine.

B All Rauwolfia alkaloids give with Dragendorff reagent orange–brown zones (T2/T1). *Note*: Ajmaline immediately turns red when sprayed with concentrated HNO_3.

Fig. 4A **Yohimbe and Quebracho cortex** (4,5)

Both drug extracts are characterized in UV-365 nm by the blue fluorescent zone of yohimbine at $R_f \sim 0.45$ (T6). A variety of additional alkaloids are seen as ten blue zones in the lower R_f range (e.g. quebrachamine, aspidospermine in 5), whereas Yohimbe cortex (4) has two prominent alkaloid zones in the upper R_f range (R_f 0.7–0.75) and one near the solvent front.

B **Catharanthi folium** (6,7)

After treatment with the DRG reagent the extracts reveal five to seven alkaloid zones mainly in the R_f range 0.05–0.75. Two prominent brown zones with vindoline at $R_f \sim 0.7$ (T8) dominate the upper R_f range. Slight differences are noticed in the lower R_f range between the fresh leaf sample (6) and the stored material (7). Vincaleucoblastine (T7) migrates to $R_f \sim 0.2$. It is present at very low concentration in the plant (<0.002%) and therefore not detectable in these drug extracts without prior enrichment.

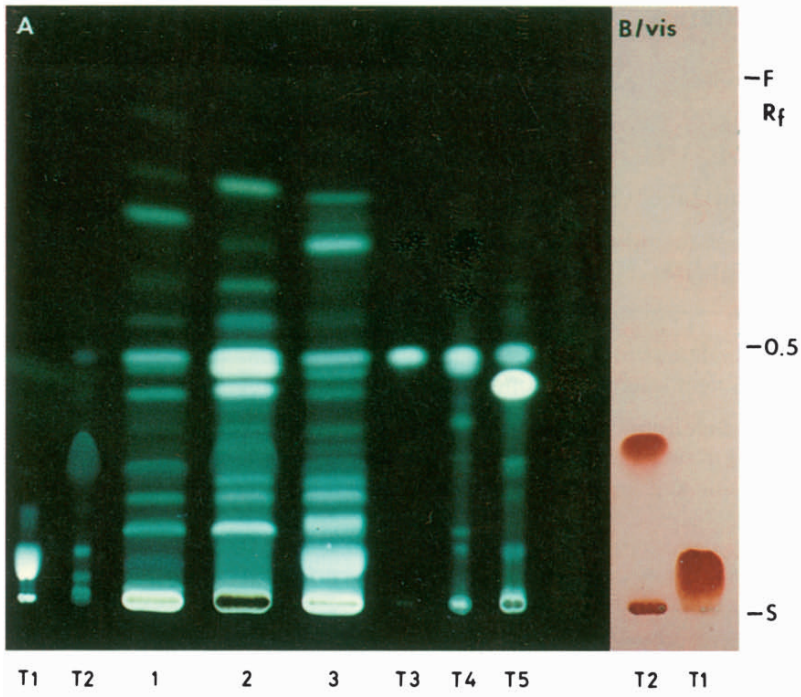


Fig. 3

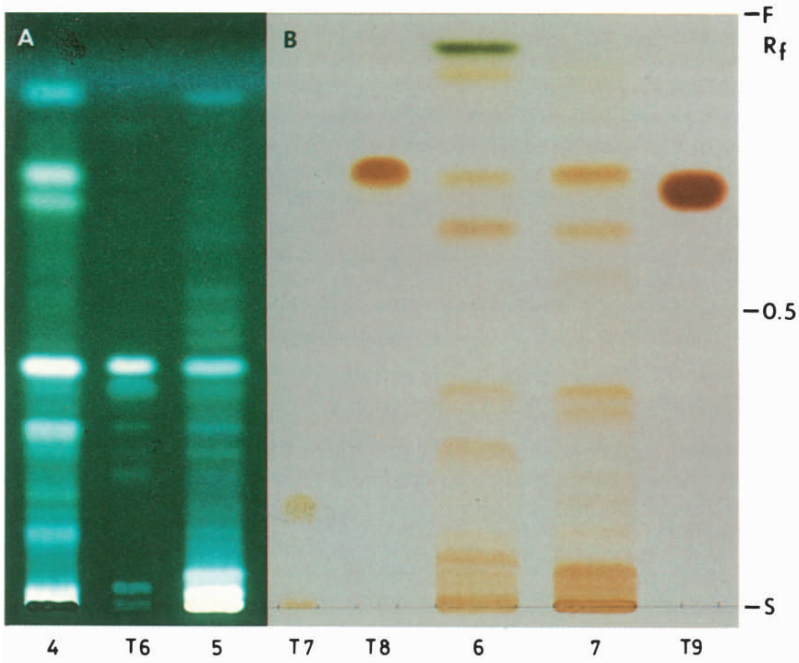


Fig. 4

Vincae minoris folium

Drug sample 1 Vinca minor (fresh leafs), (alkaloid extraction method C, 40 µl)

Reference compound

T1 vincamine	T3 vincine	T5 minovincine
T2 vincaminine	T4 vincamajine	T6 reserpinine

Solvent system Fig. 5 ethyl acetate–methanol (90:10)

Detection A UV-254 nm (without chemical treatment)
B Dragendorff reagent (DRG No. 13B) → vis

Fig. 5A The four principal alkaloids vincamine, vincaminine, vincine and vincamajine (T1-T4) are detected as prominent quenching zones in the R_f range 0.25–0.4.

B The alkaloids of **Vincae folium** (1) show four weak brown zones in the R_f range 0.15–0.45 (T1-T4) and two major zones at $R_f \sim 0.8$–0.85 (T5-T6). The colour obtained with the DRG reagent is unstable and fades easily in vis.

Secale cornutum

Drug sample 1 Secale cornutum (freshly prepared alkaloid fraction)
2 Secale cornutum (stored alkaloid fraction)
(alkaloid extraction method A, 30 µl)

Reference compound

T1 ergocristine	T4 egometrine + artefact▸)
T2 ergotamine	T5 ergotamine + artefact▸)
T3 ergometrine	T6 ergocristine + artefact▸)

Solvent system Fig. 6 toluene–chloroform–ethanol (28.5:57:14.5)

Detection A UV-254 nm (without chemical treatment)
B, C van URK reagent (No. 43) → vis

Fig. 6A The three characteristic **Secale** alkaloids ergometrine at $R_f \sim 0.05$, ergotamine at $R_f \sim 0.25$ and ergocristine at $R_f \sim 0.45$ show prominent quenching in UV-254 nm.

B After treatment with van URK reagent, the Secale extract (1) generates three blue zones of the principal alkaloids (T1-T3) in the R_f range 0.05–0.4.

C Secale alkaloids in solution and exposure to light undergo easy epimerization and also form lumi-compounds. Secale extracts such as sample 2 then show artefacts, such as isolysergic acid derivatives, lumi- and aci-compounds seen as additional, usually weaker zones with *higher* R_f values.
The artefacts (>) are detectable in Secale extract sample 2 as well as in solutions of the reference compounds T4-T6. They also form blue zones with van URK reagent (vis).

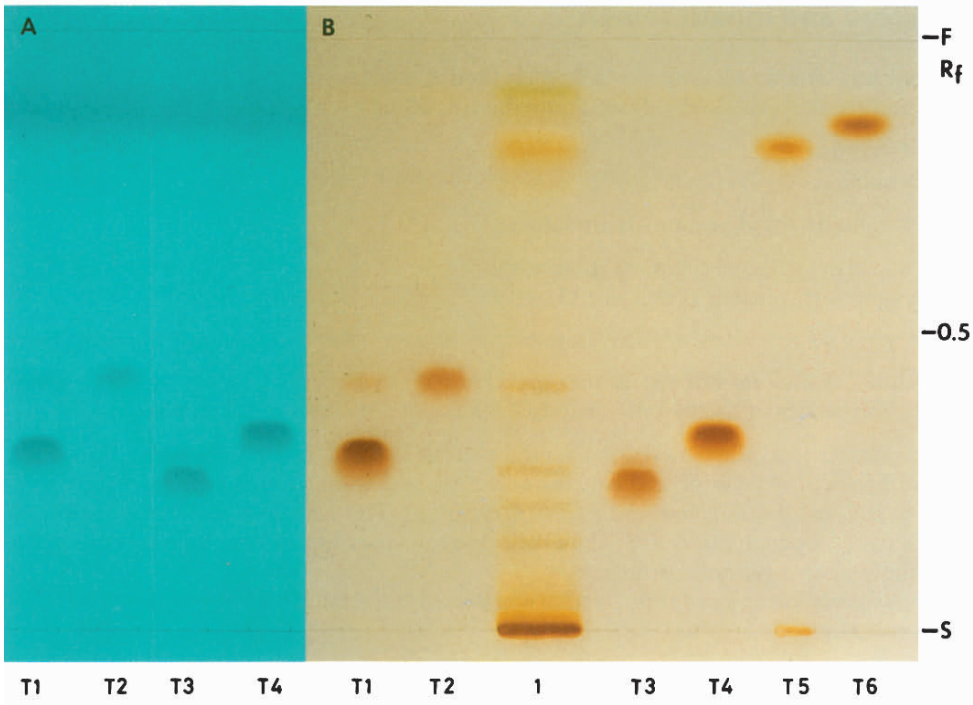

A
B
–F
Rf
–0.5
–S
T1
T2
T3
T4
T1
T2
1
T3
T4
T5
T6

Fig. 5

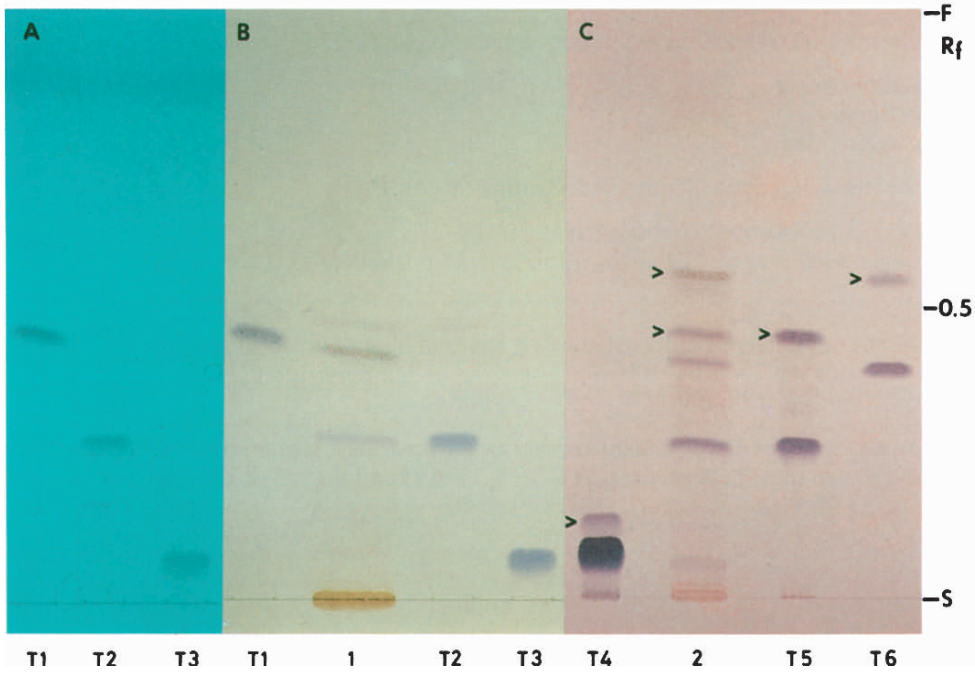

A
B
C
–F
Rf
–0.5
–S
T1
T2
T3
T1
1
T2
T3
T4
2
T5
T6

Fig. 6

Strychni and Ignatii semen

Drug sample 1 Strychni semen (alkaloid extraction method A, 30 µl)
2 Ignatii semen (alkaloid extraction method A, 30 µl)

Reference compound T1 strychnine
T2 brucine

Solvent system Fig. 7 toluene–ethyl acetate–diethylamine (70:20:10)

Detection A UV-254 m (without chemical treatment)
B Dragendorff reagent (DRG No. 13) → vis

Fig. 7A **Strychni** (1) and **Ignatii** (2) **semen** are characterized in UV-254 nm by their strong quenching zones of the two major indole alkaloids strychnine (T1) and brucine (T2).

B Both extracts (1,2) show a similar alkaloid pattern in the R_f range 0.25–0.55 with the two major zones of strychnine and brucine and three additional minor orange-brown zones due to e.g. α-, β-colubrine and pseudostrychnine. The colour of the strychnine zone fades easily when treated with the DRG reagent (vis). Strychnine and brucine occur normally in an equimolar amount.
Note: Brucine forms a red zone (visible when dyed with HNO_3 (25%), whereas strychnine does not react.

Gelsemii radix

Drug sample 1 Gelsemii radix, (alkaloid extraction method B, 40 µl)

Reference compound T1 sempervirine
T2 gelsemine
T3 isogelsemine

Solvent system Fig. 8 aetone–light petroleum–diethylamine (20:70:10)

Detection A UV-365 nm (without chemical treatment)
B Dragendorff reagent (DRG No. 13/followed by 10% $NaNO_2$/13B) → vis

---

Fig. 8A In UV-365 nm **Gelsemii radix** (1) shows a series of blue fluorescent zones in the R_f range 0.05–0.7 with the prominent blue white zone of sempervirine (T1) directly above the start. Gelsemine (T2/→ B: $R_f \sim 0.35$) does not fluoresce.

B Treatment with the DRG reagent reveals as brown zones: sempervirine (directly above the start), two minor alkaloid zones ($R_f \sim 0.15$–0.2) and the major alkaloid gelsemine at $R_f \sim 0.35$ (T2; vis.).

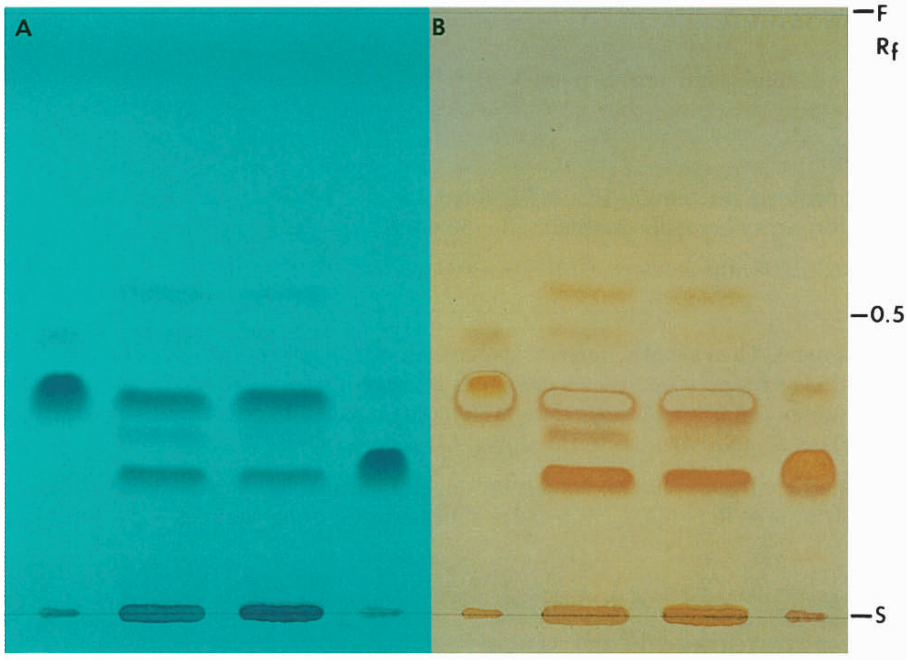


Fig. 7

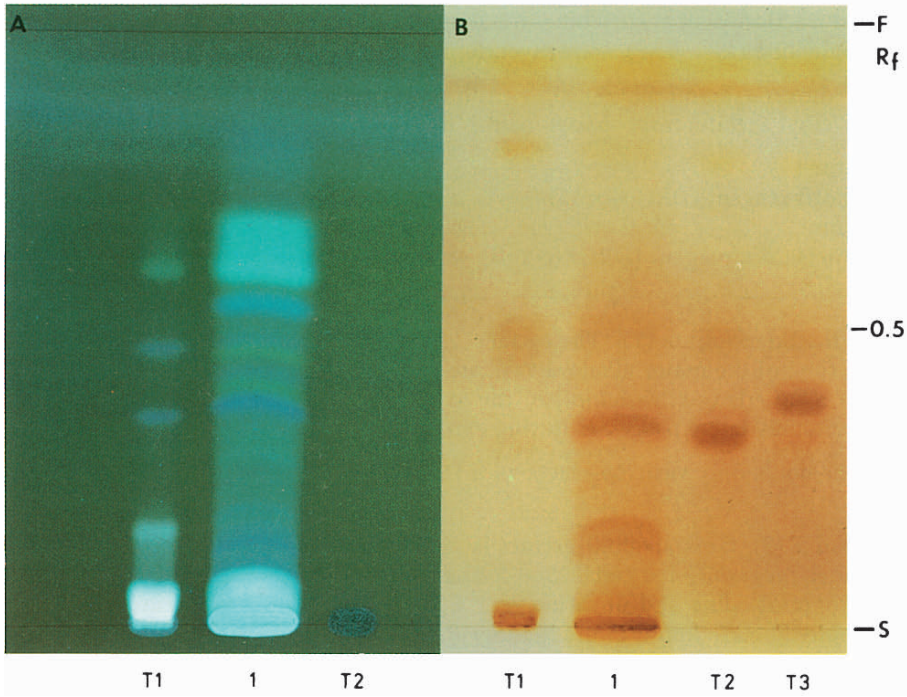


Fig. 8

Harmalae semen

Drug sample 1 Harmalae semen, (methanol extract, 30 µl)

Reference compound
T1 harmalol T3 harmane T5 harmol
T2 harmaline T4 harmine

Solvent system
Fig. 9A chloroform–methanol–10% NH_3 (80:40:1.5)
B chloroform–acetone–diethylamine (50:40:10)

Detection A, B UV-365 nm (without chemical treatment)

Fig. 9A **Harmalae semen.** The carbolin derivatives harmalol (T1), harmaline (T2) and harmine (T4) are found as bright blue fluorescent zones in solvent A in the R_f range 0.1–0.75. The Harmalae semen sample 1 shows as major alkaloids harmalol and harmaline in the low R_f range 0.05–0.25 and harmine in the upper R_f range 0.75.

B Development in solvent system B reveals the zone of harmalol at $R_f \sim 0.05$, harmaline at $R_f \sim 0.4$, harmine at $R_f \sim 0.45$ (T2) besides a low amount of harmane at $R_f \sim 0.55$ (T3).

Justiciae-adhatodae folium, Uncariae radix

Drug sample
1 Adhatodae folium, (alkaloid extraction method B, 30 µl)
2 Uncariae tomentosae cortex, (alkaloid extraction method B, 40 µl)

Reference compound
T1 alkaloid fraction/vasicin enrichment/Adhatodae folium
T2 rychnophylline ($R_f \sim 0.35$) + isorhychnophylline ($R_f \sim 0.75$)

Solvent system
Fig. 10A,B dioxane–ammonia (90:10) → Adhatoda
C,D ethyl acetate–isopropanol–conc.NH_3 (100:2:1) → Uncaria

Detection
A UV-254 nm
B Dragendorff reagent (DRG No. 13) → vis.
C UV-254 nm
D DRG/10% $NaNO_2$ reagent (DRG No 13B) → vis

Fig. 10A **Justiciae-adhatodae-folium** (1). The extract (1) and the alkaloid fraction (T1) show the quenching zone of the major alkaloid vasicine at $R_f \sim 0.55$; vasicinone at $R_f \sim 0.6$ and some other alkaloids (e.g. vasicinol) in the lower R_f range 0.2–0.25. Vasicinone is an artefact due to oxydative processes during extraction.

B From the alkaloids only vasicine reacts with Dragendorff reagent as an orange-brown zone in vis.

C **Uncariae radix** (2). This alkaloid extract is characterized by two pairs of quenching zones in the R_f ranges 0.7–0.8 and 0.25–0.3. The pentacyclic oxindoles, such as isomitraphylline, isopteropodine and uncarine A + F, as well as tetracyclic oxindols such as isorhychnophylline are found in the R_f range 0.7–0.8. The pentacylic mitraphylline and the tetracyclic rhychnophylline give prominent zones in the R_f range 0.25–0.3. The alkaloid distribution is subject to change. The alkaloid pattern of an individual plant changes over the year.

D All alkaloid zones turn orange-brown with Dragendorff/$NaNO_2$ reagent (vis.).

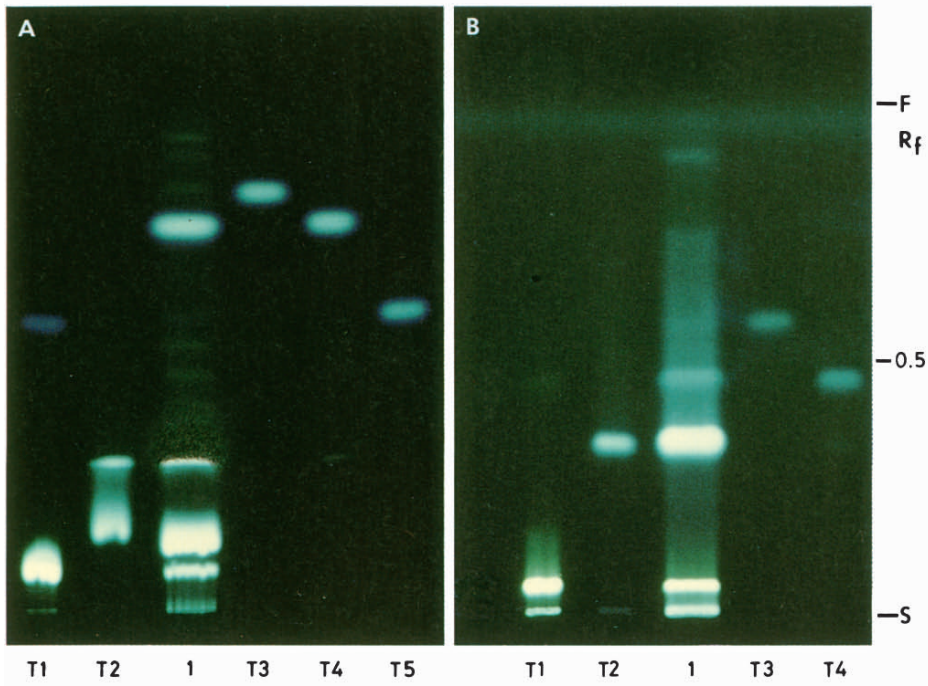

A
B
F
Rf
0.5
S
T1
T2
1
T3
T4
T5
T1
T2
1
T3
T4

Fig. 9

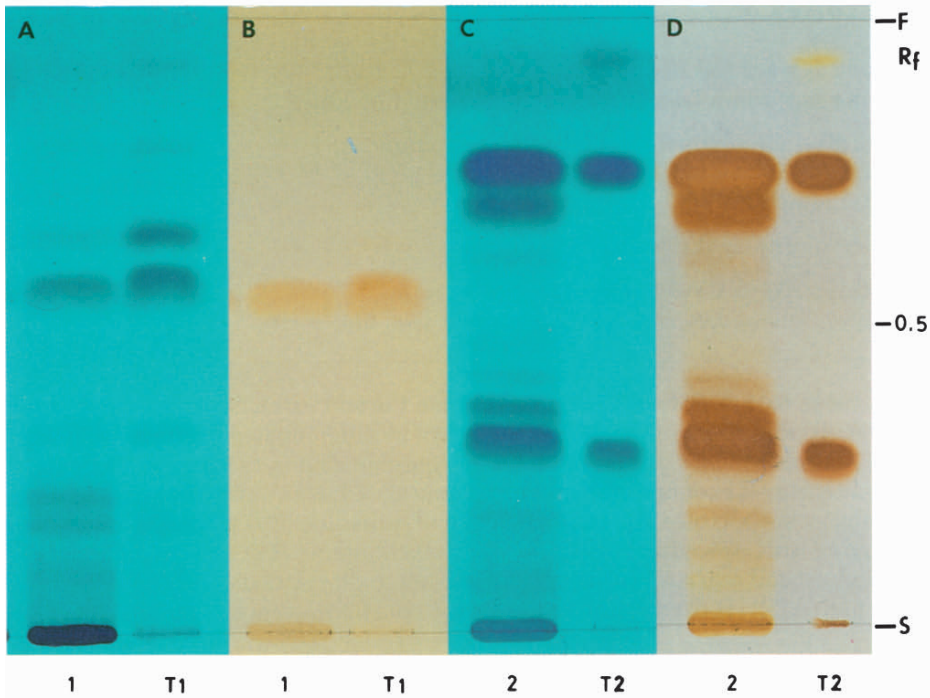

A
B
C
D
F
Rf
0.5
S
1
T1
1
T1
2
T2
2
T2

Fig. 10

Ipecacuanhae radix

Drug sample 1 Cephaelis acuminata "Cartagena/Panama drug"
2 Cephaelis ipecacuanha "Rio/Matto-Grosso drug"
(alkaloid extraction method A, 30 µl)

Reference T1 cephaeline ($R_f \sim 0.2$) ▶ emetine ($R_f \sim 0.4$)

Solvent system Fig. 11 toluene–ethyl acetate–diethylamine (70:20:10)

Detection A, B Iodine/$CHCl_3$ reagent (No. 19) A → UV-365 nm; B → vis
C Dragendorff reagent (DRG No. 13A) → vis

Fig. 11 **Ipecacuanhae radix** (1,2)

A,B Cephaeline ($R_f \sim 0.2$) and emetine ($R_f \sim 0.4$) are the major alkaloids, which fluoresce light blue in UV-365 nm without chemical treatment. With iodine reagent cephaeline fluoresces bright blue and emetine yellow–white in UV-365 nm and they turn red and weak yellow, respectively, in vis. (→ B). Minor alkaloids, e.g. *O*-methylpsychotrine, are found in R_f range of emetine, or psychotrine in the R_f range of cephaeline.
The yellow fluorescence develops after approximately 30 min.

C With DRG reagent the major alkaloids are seen as orange–brown zones (vis).

Chinae cortex

Drug sample 1 Cinchona calisaya (alkaloid extraction method A, 20 µl)
2 Cinchona succirubra (alkaloid extraction method A, 20 µl)

Reference compound

TC China alkaloid mixture	(T1-T4 see section 1.2)
T1 quinine	T3 quinidine
T2 cinchonidine	T4 cinchonine

Solvent system Fig. 12 chloroform–diethylamine (90:10)

Detection A 10% eth. H_2SO_4 → UV-365 nm
B 10% H_2SO_4 followed by iodoplatinate reagent (No. 21) → vis

Fig. 12A In the R_f range 0.05–0.25 both **Cinchona (Chinae Cortex)** extracts show six light blue fluorescent alkaloid zones in UV-365 nm. They can be differentiated on the basis of their quinine (T1) content. In C. calisaya cortex (1) quinine counts as a major alkaloid. C. succirubrae cortex (2) contains the main cinchona alkaloids in approximately the same proportions as test mixture TC. Quinine (T1) and quinidine (T3) fluoresce bright blue after spraying with 10% ethanolic H_2SO_4, while cinchonidine (T2) and cinchonine (T4) turn dark violet and are hardly visible in UV-365 nm. In the extracts (1) and (2) the zone of cinchonidine (T2) is overlapped by the strong blue fluorescence of quinidine (T1).

B Treatment with iodoplatinate reagent results in eight mostly red–violet zones in the R_f range 0.05–0.65 (vis). The violet–brown zone of quinine is followed by the grey–violet zone of cinchonidine, a weak red–violet zone of quinidine and the more prominent brown–red cinchonine (TC). Three additional red–violet zones are found in the R_f range 0.4–0.6.

Remark: The slight variation in R_f values of the cinchona alkaloids (→ A:B) are due to the great sensitivity of the chloroform–diethylamine solvent system to temperature.

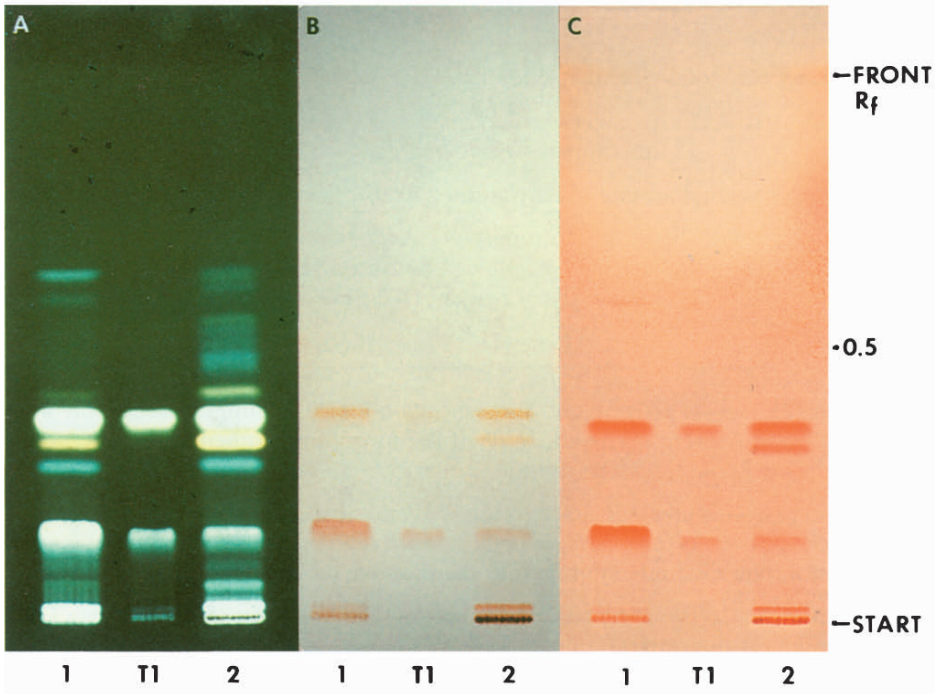

A
B
C
FRONT
Rf
0.5
START
1
T1
2
1
T1
2
1
T1
2

Fig. 11

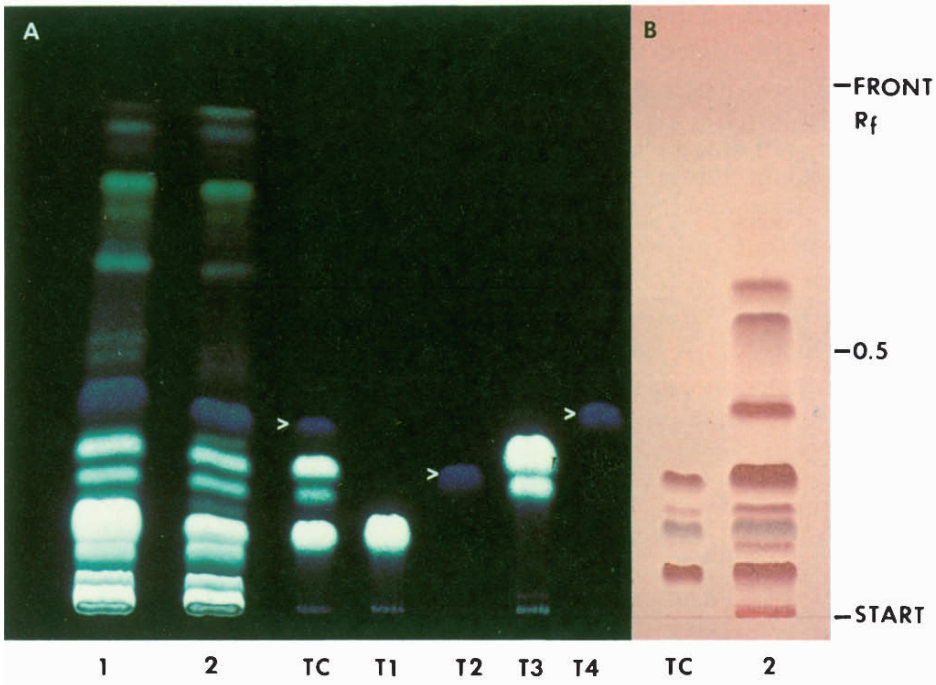

A
B
FRONT
Rf
0.5
START
1
2
TC
T1
T2
T3
T4
TC
2

Fig. 12

Opium

Drug sample 1 Opium extract (5% total alkaloids, 5 µl)

Reference compound
T1 morphine
T2 codeine
T3 papaverine
T4 noscapine

Solvent system Figs. 13, 14 toluene–ethyl acetate–diethylamine (70:20:10)

Detection
A UV-254 nm (without chemical treatment)
B Dragendorff reagent (DRG No. 13A followed by $NaNO_2$; No. 13B) → vis
C Natural products, polyethylene glycol reagent (NP/PEG No. 28) → UV-365 nm
D Marquis reagent (No. 26) → vis

Figs. 13A **Opium extract** (1) shows six to eight fluorescence-quenching zones between the start and $R_f \sim 0.85$ in UV-254 nm. The alkaloids of the morphinane/phenanthrene type are found in the lower R_f range with morphine (T1) at $R_f \sim 0.1$ and codeine (T2) at $R_f \sim 0.2$.

The benzyl isoquinoline alkaloids papaverine (T3) and noscapine (T4) are seen as major quenching zones at $R_f \sim 0.65$ and $R_f \sim 0.85$, respectively.
Thebaine and minor alkaloids migrate into the R_f range 0.3–0.5.

B With Dragendorff–$NaNO_2$ reagent all major opium alkaloids turn orange–brown (vis). Narceine remains at the start.

Fig. 14C Treatment with the NP/PEG reagent reveals a sequence of blue fluorescent zones at the beginning of the R_f range up to $R_f \sim 0.9$ (UV-365 nm).

Except codeine (T2), which does not fluoresce, the main alkaloids morphine (T1), papaverine (T3) and noscapine (T4) give a blue fluorescence in UV-365 nm.

D With Marquis reagent the alkaloids morphine and codeine are immediately stained typically violet.
A nonspecific reaction is given by papaverine, with a weak violet, and by noscapine, with a weak yellow–brown colour.

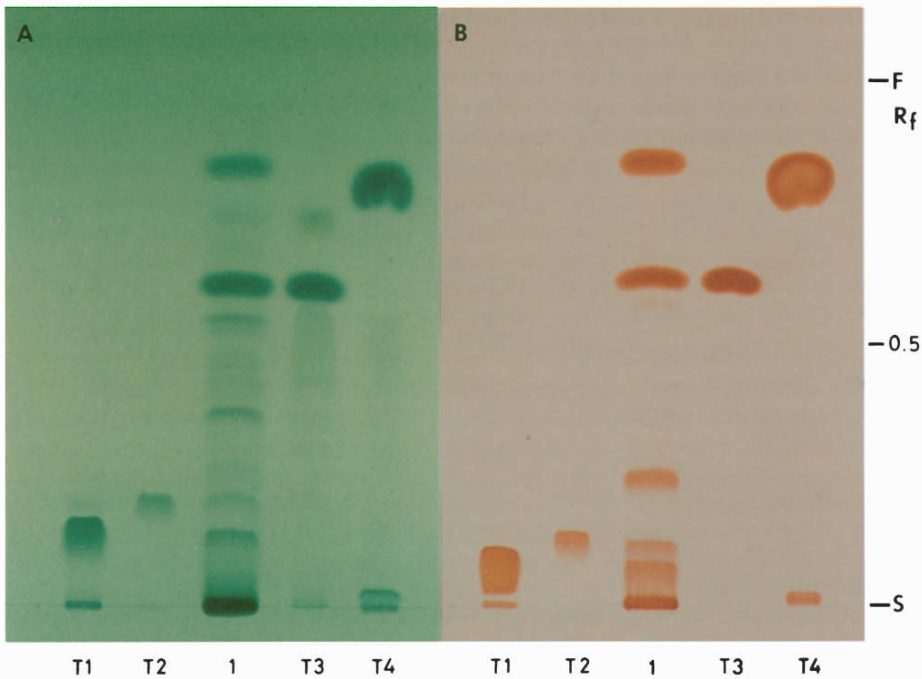

A
B
F
Rf
0.5
S
T1
T2
1
T3
T4
T1
T2
1
T3
T4

Fig. 13

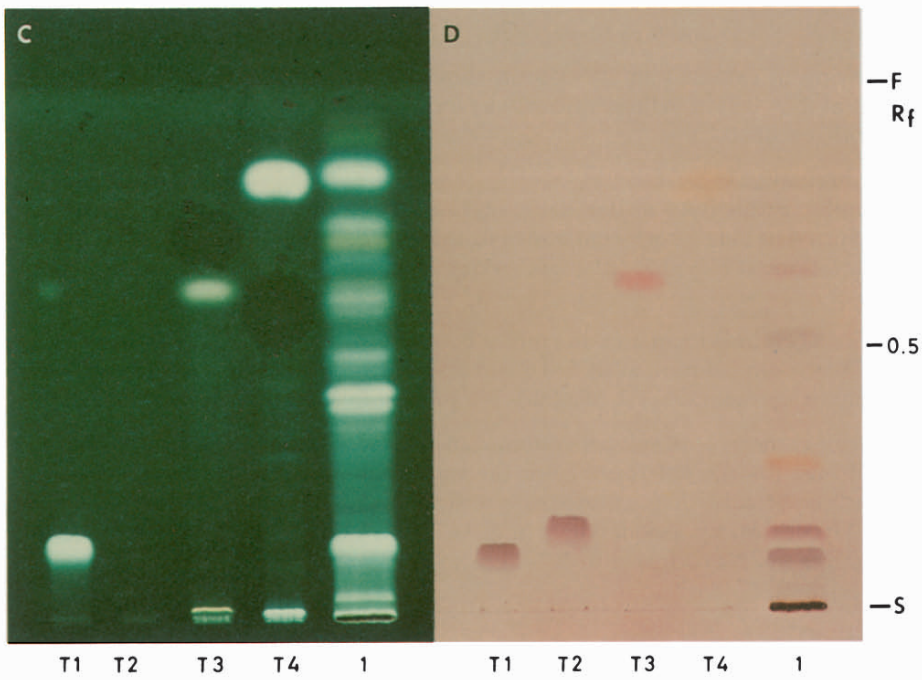

C
D
F
Rf
0.5
S
T1
T2
T3
T4
1
T1
T2
T3
T4
1

Fig. 14

Corydalidis rhizoma, Fumariae herba

Drug sample
1 Corydalidis rhizoma (alkaloid extraction method A, 30 µl)
2 Fumariae herba (methanolic extract 1 g/10 ml, 10 µl)
3 Fumariae herba (alkaloid extraction method C, 30 µl)

Reference Compound
T1 corytuberine T2 corydaline
T3 rutin ($R_f \sim 0.35$) ▶ chlorogenic acid ($R_f \sim 0.4$) ▶ hyperoside ($R_f \sim 0.55$) = Flavonoid test mixture

Solvent system
Fig. 15A–C ethyl acetate–methylethyl ketone–formic acid–water (50:30:10:10) system 1
Fig. 16D,E ethyl acetate–methylethyl ketone–formic acid–water (50:30:10:10) system 1
F ethyl acetate–glacial acetic acid–formic acid–water (100:11:11:26) system 2

Detection
Fig. 15A UV-254 nm
B Dragendorff reagent (No. 13 B) → vis.
C UV-365 nm (without chemical treatment)
Fig. 16D UV-365 nm
E Dragendorff reagent (No. 13 B) → vis.
F Natural products reagent (NP/PEG No. 28) – UV-365 nm

Fig. 15A **Corydalidis rhizoma** (1)
The extract shows seven to eight quenching zones distributed up to R_f 0.75. The prominent zones at $R_f \sim 0.35$ can be identified as corytuberine (T1) and at $R_f \sim 0.7$ as corydaline (T2).

B Most of the major quenching zones react as brown zones with DRG reagent (vis). Corydaline is seen as main zone at $R_f \sim 0.7$, while bulbocapnine and corytuberine (T1) are found at $R_f \sim 0.45$ and 0.35 respectively.

C Direct viewing of extract 1 in UV-365 nm shows a series of predominantly blue (e.g. corydaline at $R_f \sim 0.7$) or yellow–white fluorescent zones (e.g. berberine-type alkaloids) in the R_f range 0.05–0.7.

Fig. 16D **Fumariae herba** (2,3)
A methanolic extraction of the drug (2) and an alkaloid enrichment (3) show in UV-365 nm 4–6 blue fluorescent zones in the R_f range 0.25–0.55 with an additional yellow–white zone at $R_f \sim 0.55$ (phenol carboxylic acids, sanguinarine, protoberberines) in sample 2.

E With DRG reagent two main and one minor brown alkaloid zone (vis) are detectable in sample 3. Protropin is found at $R_f \sim 0.6$ and allocryptopine in the lower $R_f \sim$ range. In the methanolic extract (2) these alkaloids are present in low concentration only.

F Separation of extract (2) in solvent system 2 and spraying with NP/PEG reagent reveals a series of blue fluorescent zones from the start till the solvent front, mostly due to phenol carboxylic acids (e.g. chlorogenic acid at $R_f \sim 0.45$) and a yellow fluorescent flavonoid glycosides, e.g. isoquercitrin at $R_f \sim 0.6$, as well as minor compounds in the lower R_f range (e.g. rutin, quercetin-3,7-diglucosido-3-arabinoside) and the aglycones at the solvent front.

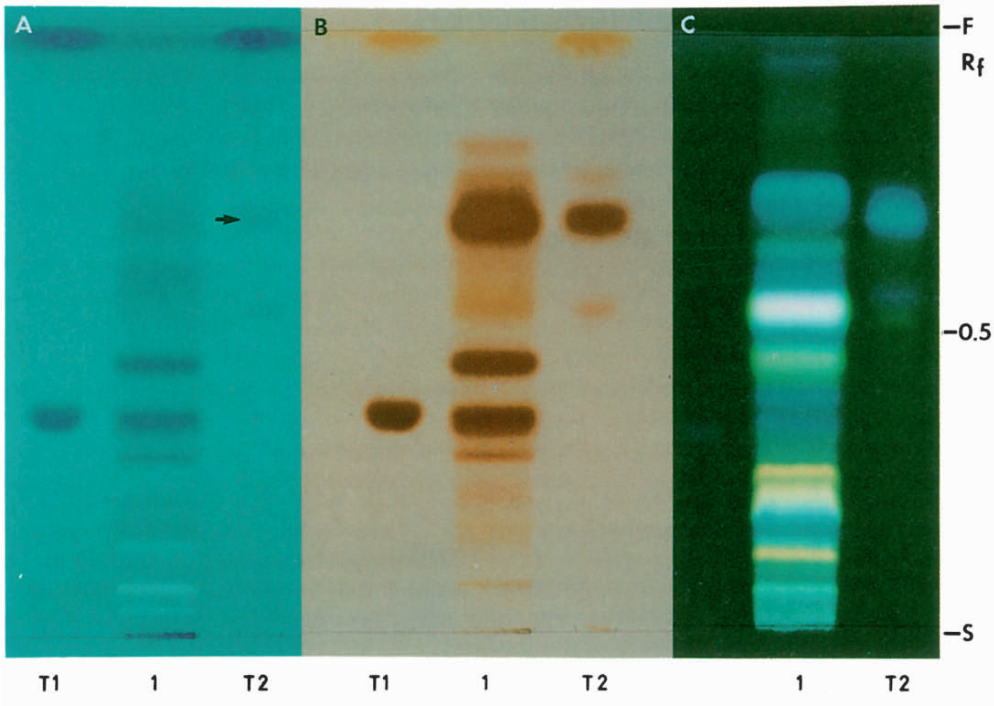


Fig. 15

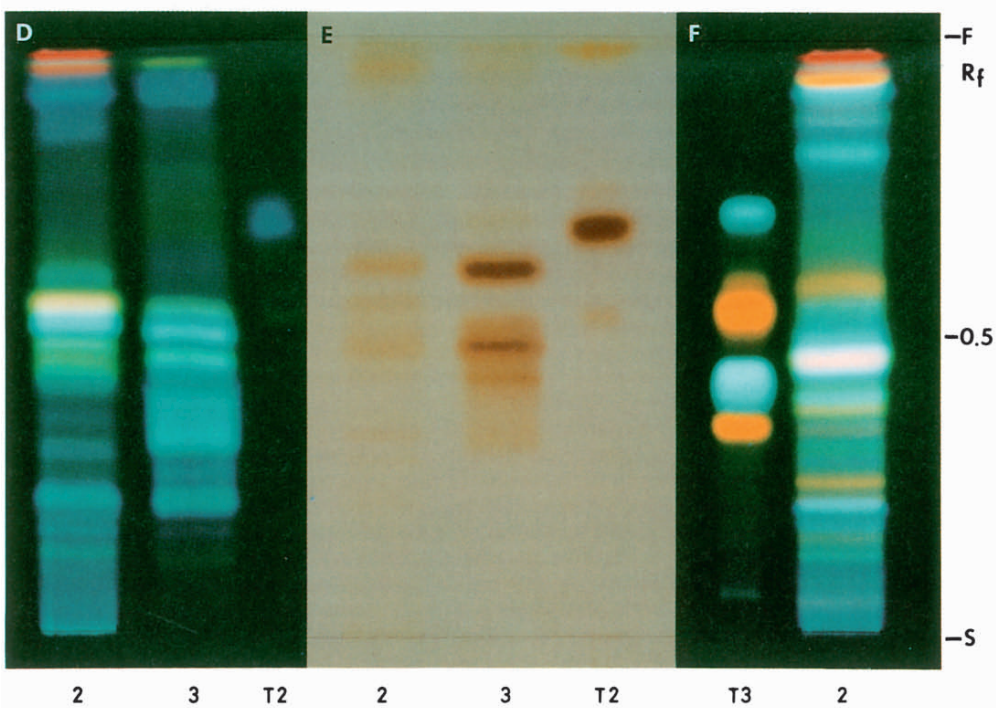


Fig. 16

Spartii flos, Sarothamni (Cytisi) herba

Drug sample
1 Spartii flos (MeOH extract 1 g/10 ml, 10 µl)
1a Spartii flos (alkaloid extraction method A, 50 µl)
2 Sarothamni herba (MeOH extract 1g/10 ml, 10 µl)
2a Sarothamni herba (alkaloid extraction method A, 30 µl)

Reference compound
T1 rutin ($R_f \sim 0.45$) ▶ chlorogenic acid ($R_f \sim 0.5$) ▶ hyperoside ($R_f \sim 0.6$) ▶ isochlorogenic acid = Flavonoid test mixture
T2 sparteine sulphate

Solvent system
Fig. 17A ethyl acetate–glacial acetic acid–formic acid–water (100:11:11:26) → flavonoids
B chloroform–methanol–glacial acetic acid (47.5:47.5:5) → alkaloids

Detection
A Natural products–polyethylene glycol reagent (NP/PG No. 28) → UV-365 nm ▶ flavonoids
B Iodoplatinate reagent (IP No. 21) → vis ▶ alkaloids

Fig. 17A NST/PEG reagent UV-365 nm ▶ Flavonoids
The methanolic extract of **Spartii flos** (1) is characterized by a major orange zone at R_f 0.65 (isoquercitrin, luteolin-4'-O-glucoside), while that of **Sarothamni scopariae herba** (2) shows two yellow-green fluorescent zones of spiraeoside and scoparoside at R_f 0.6–0.7 as well as the aglycone close to the solvent front.

B Iodoplatinate reagent vis. ▶ Alkaloids
Dark blue alkaloid zones are developed with IP reagent. Sparteine (R_f 0.25/T2) is a major alkaloid in Sarothamni scop. herba (2a). Besides sparteine sample 2a shows an additional dark blue zone at R_f 0.15. Cytisine and N-methylcytisine are present in Spartii flos (1a).

Genistae herba

Drug sample
3 Genistae herba (MeOH extract 1 g/10 ml/10 µl)
3a Genistae herba (alkaloid extraction method A, 30 µl)

Reference compound
T1 rutin ▶ chlorogenic acid ▶ hyperoside ▶ isochlorogenic acid
T2 sparteine sulfate

Solvent system
Fig. 18A ethyl acetate–glacial acetic acid–formic acid–water (100:11:11:26) → flavonoids
B chloroform–methanol–glacial acetic acid (47.5:47.5:5) → alkaloids

Detection
A Natural products–polyethylene glycol reagent (NP/PEG No. 28) → UV-365 nm ▶ flavonoids
B Dragendorff reagent (DRG No. 13) followed by $NaNO_2$ (No 13 B) → vis ▶ alkaloids

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Fig. 18A NST/PG reagent, UV-365 nm ▶ Flavonoids
Genistae herba (3) is characterized by a high amount of luteolin glycosides, seen as bright yellow fluorescent zones in the R_f range 0.55–0.8, the aglycone at the front and blue fluorescent isoflavones (e.g. genistin) and phenol carboxylic acids (e.g. chlorogenic acid) at R_f 0.5.

B DRG/$NaNO_2$, vis ▶ Alkaloids
Two brown alkaloid zones in the R_f range 0.1–0.2 of (3a) are due to sparteine type alkaloids such as *N*-methylcytisine, anagyrine and cytisine.

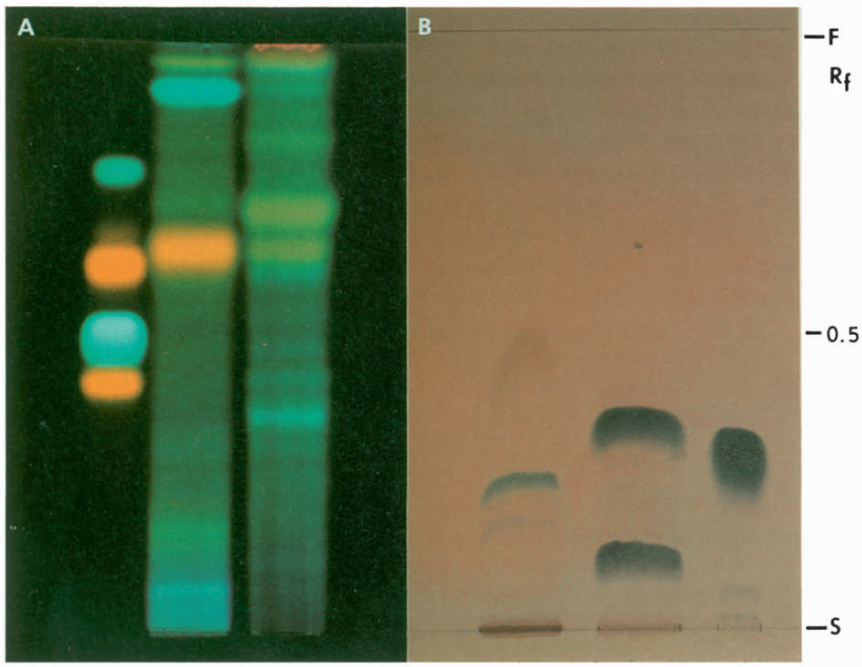

Fig. 17

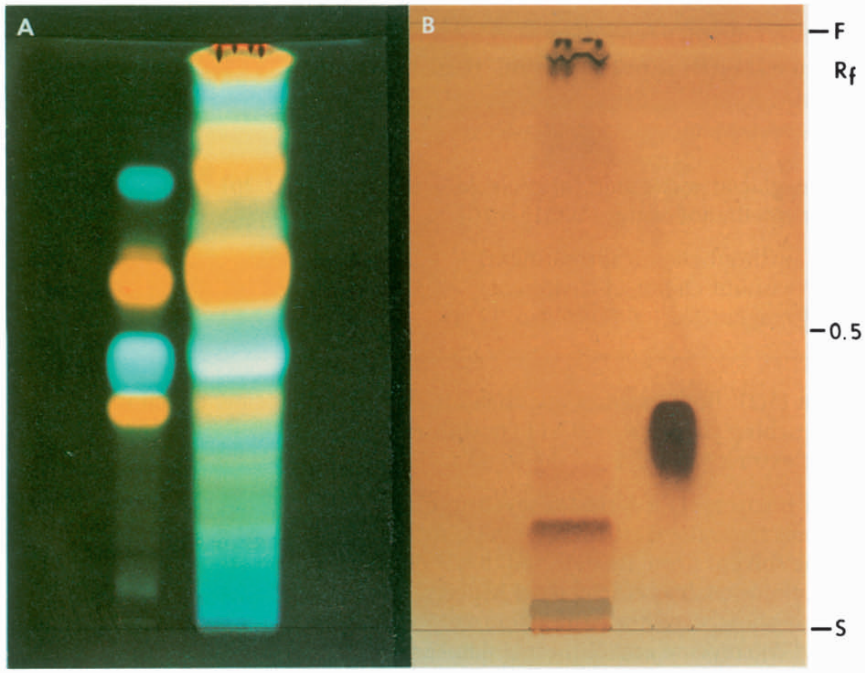

Fig. 18

Chelidonii herba

Drug sample 1–3 Chelidonii herba different trade samples (alkaloid extraction method A, 40 µl)

Reference compound
T1 sanguinarine
T2 papaverine
T3 methyl red

Solvent system Fig. 19 1-propanol–water–formic acid (90:9:1)

Detection
A UV-365 nm (without chemical treatment)
B Dragendorff reagent [DRG reagent No. 13A] → vis

Fig. 19A **Chelidonii herba** (1–3). The extracts of the samples 1–3 are characterized in UV-365 nm by bright yellow fluorescent zones: the major alkaloid coptisin at $R_f \sim 0.15$, followed by minor alkaloids berberine and chelerythrine directly above and sanguinarine (T1) as a broad yellow band in the R_f range 0.3–0.4. In the R_f range 0.75–0.85 weak yellow–green (e.g. chelidonine) and blue–violet zones are found.

B The fluorescent alkaloid zones in the R_f range 0.15–0.85 respond to DRG reagent with brown, rapidly fading colours (vis.). Papaverine (T2) can serve as reference compound for sanguinarine ($R_f \sim 0.4$), and methyl red (T3) for the alkaloidal zones at $R_f \sim 0.8$.

Colchici semen

Drug sample
1 Colchici semen (alkaloid extraction method A, 30 µl)
2 Colchici semen (MeOH extract 3 g/10 ml, 10 µl)

Reference compound
T1 colchicine
T2 colchicoside

Solvent system
A ethyl acetate–glacial acetic acid formic acid–water (100:11:11:26)
B ethyl acetate–methanol–water (100:13.5:10)

Detection
A UV-254 nm (without chemical treatment)
B UV-365 nm (without chemical treatment)
C Dragendorff reagent/$NaNO_2$ (DRG No. 13 B) → vis.

Fig. 20A **Colchici semen** (1,2). Both extracts are characterized by colchicine, which is seen as a prominent quenching zone at $R_f \sim 0.6$ (T1), while colchicoside ($R_f \sim 0.15$/T2) is found in the methanolic extract (2) only.

B In the alkaloid fraction (1) a series of seven to nine prominent blue and yellow–white fluorescent zones from the start till $R_f \sim 0.35$, six weaker blue zones at R_f 0.4–0.85 and two zones at the solvent front are detected in UV-365 nm. Besides colchicine at $R_f \sim 0.25$ (T1) minor alkaloids such as colchiceine, *N*-acetyl demecolcine and 1-ethyl-2-demethyl colchiceine also show a yellow–white fluorescence, while O-benzoyl colchiceine, *N*-formyl-deacetyl colchiceine and *N*-methyl demecolcine fluoresce blue.

C Colchicine and minor alkaloids react as brown zones with DRG reagent (vis). Artefacts of colchicine ($R_f \sim 0.6$) appear as a blue zone at $R_f \sim 0.5$ (vis)

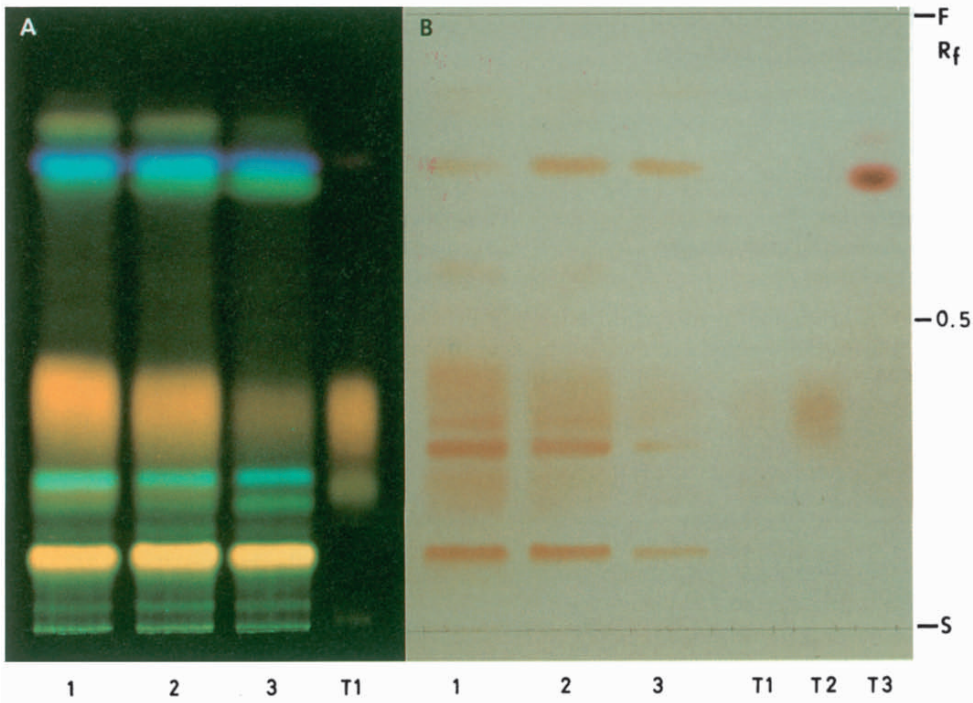

Fig. 19

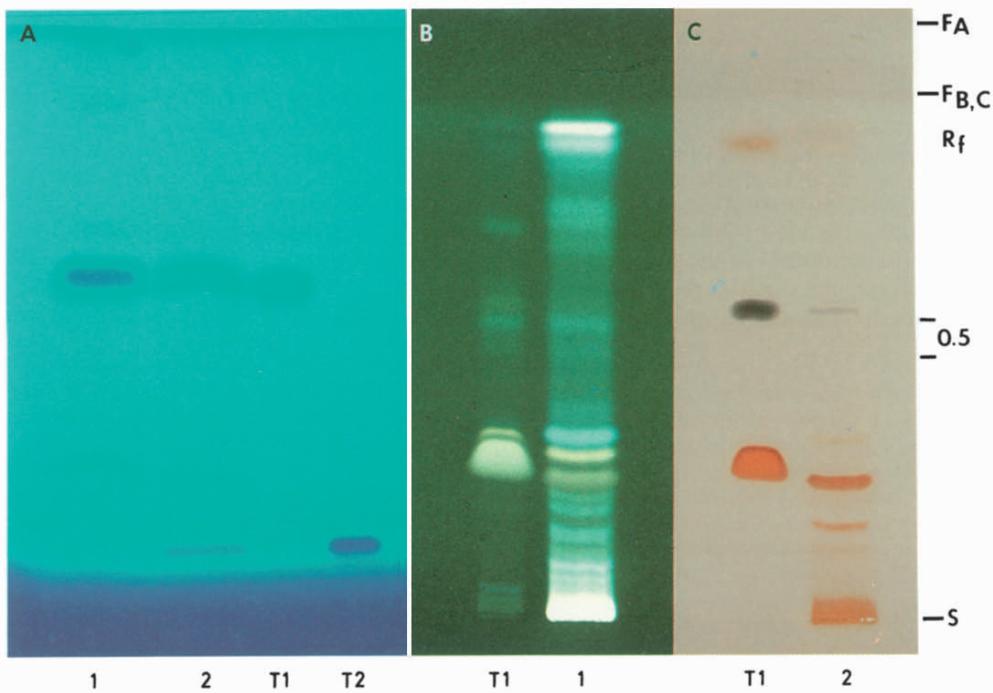

Fig. 20

Berberidis cortex, Colombo radix, Hydrastis rhizoma, Mahoniae radix/cortex

Drug sample
1 Berberidis radix
2 Hydrastis rhizoma
3 Colombo radix
4 Mahoniae radix/cortex
(alkaloid extraction method A, 30 µl)

Reference compound
T1 berberine
T2 palmatine/jatrorrhizine
T3 hydrastine
T4 jatrorrhizine
T5 columbamine
T6 oxyacanthine
T7 berbamine
T8 palmatine

Solvent system
Fig. 21 n-propanol–formic acid–water (90:1:9)
Fig. 22 n-butanol–ethyl acetate–formic acid–water (30:50:10:10)

Detection
A vis (without chemical treatment)
B Dragendorff reagent [DRG No. 13A] → vis
C UV-365 nm (without chemical treatment)
D UV-365 nm (without chemical treatment)

Fig. 21A **Berberidis radixs** (1) shows the characteristic yellow zone of berberine ($R_f \sim 0.2$/T1) on untreated chromatogram (vis.).

B Berberine and the minor alkaloids, such as jatrorrhizine and palmatine, react with a brown–red colour with DRG reagent (vis.).

C Extracts of **Berberidis radix** (1) and **Hydrastis rhizoma** (2) both show the major alkaloid berberine as a prominent lemon-yellow fluorescent zone at $R_f \sim 0.25$.

Hydrastis rhizoma (2) can be differentiated from Berberidis radix (1) by the additional zone of hydrastine, which forms a blue-white fluorescent zone at $R_f \sim 0.03$ and an additional light blue fluorescent zone at $R_f \sim 0.9$ (T3).

Colombo radix (3). The yellow–white alkaloid zone detected in at $R_f \sim 0.15$ represents the unseparated alkaloid mixture of jatrorrhizine, palmatin (T2) and columbamine.

Fig. 22D **Mahoniae radix/cortex** (4) is characterized in the R_f range 0.45–0.5 by the four yellow–green fluorescent protoberberine alkaloids berberine (T1) and jatrorrhizine (T4) as well as columbamine (T5) and palmatine (T8). Magnoflorine is seen as a dark zone at $R_f \sim 0.2$ directly above the blue fluorescent bisbenzylisoquinoline alkaloids oxyacanthine (T6) and berbamine (T7) in the R_f range 0.05–0.1.

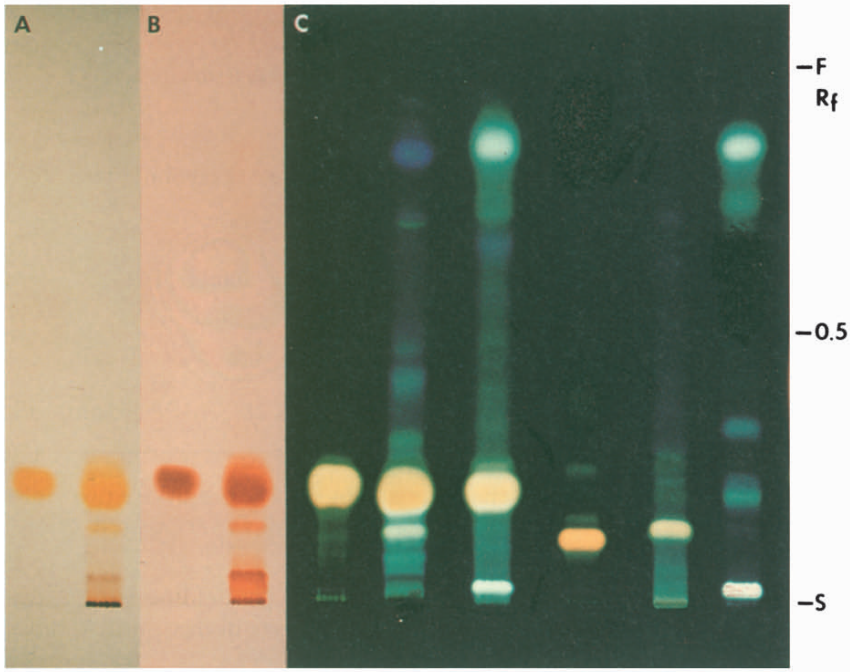

Fig. 21

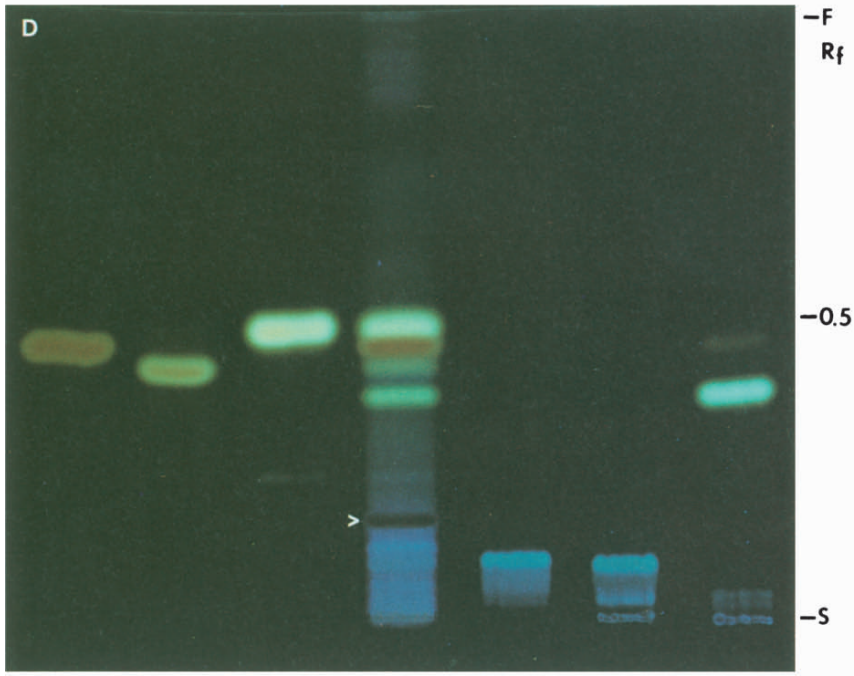

Fig. 22

Boldo folium

Drug samples
1 alkaloid extract (method A, 30 µl)
2 essential oil (TAS method, 100 mg)
3 methanol extract (1 g/10 ml, 10 µl)

Reference compound
T1 boldine
T2 rutin (R_f 0.4) ▶ chlorogenic acid (R_f 0.5) ▶ hyperoside (R_f 0.65) favonoid test

Solvent system
Fig. 23 A,B toluene–ethyl acetate–diethylamine (70:20:10)
C toluene–ethyl acetate (93:7)
D ethyl acetate–formic acid–glacial acetic acid–water (100:11:11:26)

Detection
A UV-365 nm (without chemical treatment)
B Dragendorff reagent (DRG No. 13B) → vis
C Vanillin-H_2SO_4 reagent (VS No. 42) → vis
D Natural products–polyethylene glycol reagent (NP/PEG No. 28) → UV-365 nm

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Fig. 23A **Boldo folium.** The alkaloid extract (1) is characterized in UV-365 nm by the two violet fluorescent zones in the R_f range of the boldine test T1, as well as various red–orange fluorescent chlorophyll zones in the upper R_f range.

B With DRG reagent two dark brown zones in the R_f range of the boldine test T1, two minor alkaloid zones above the start and greenish–brown zones in the upper R_f range due to chlorophyll are detectable.

C The volatile oil compounds (2) yield ten grey or blue zones between the start and R_f 0.85 with 1,4-cineole ($R_f \sim 0.4$) and ascaridole ($R_f \sim 0.8$) as major terpenoides.

D The methanolic extract (3) is characterized by its high amount and variety of flavonol glycosides. Five almost equally concentrated yellow–green fluorescent zones appear in the R_f range 0.4–0.65 (rutin ▶ hyperoside/T2) accompanied by two prominent zones at R_f 0.75–0.8 and three minor zones in the lower R_f range.

Nicotianae folium

Drug samples
1 alkaloid extract (method A, 40 µl)
1a methanol extract (1 g/10 ml, 10 µl)
2 commercial cigarette (method A, 40 µl)
2a methanol extract of (2) (1 g/10 ml, 10 µl)

Reference compound
T1 nicotine
T2 rutin (R_f 0.4) ▶ chlorogenic acid (R_f 0.5) ▶ hyperoside (R_f 0.6) favonoid test

Solvent system
Fig. 24 A,B toluene–ethyl acetate–diethyl amine (70:20:10)
C ethyl acetate–formic acid–glacial acetic acid–water (100:11:11:26)

Detection
A UV-254 nm (without chemical treatment)
B Dragendorff reagent (DRG No. 13B) → vis.
C Natural products–polyethylene glycol reagent (NP/PEG No. 28) → UV-365 nm

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Fig. 24A **Nicotianae folium** (1,2). The major alkaloid nicotine (T1/$R_f \sim 0.75$) shows quenching in UV-254 nm.

B The alkaloid extracts of sample (1) and (2) both contain nicotine and two additional alkaloids at R_f 0.35–0.4 (e.g. nornicotine, anabasine) which turn orange–brown with DRG reagent (vis.).

C The methanolic extracts (1a) and (2a) show, in addition to the alkaloids, the flavonol glycoside rutin and the chlorogenic acid (T2), more highly concentrated in 1a.

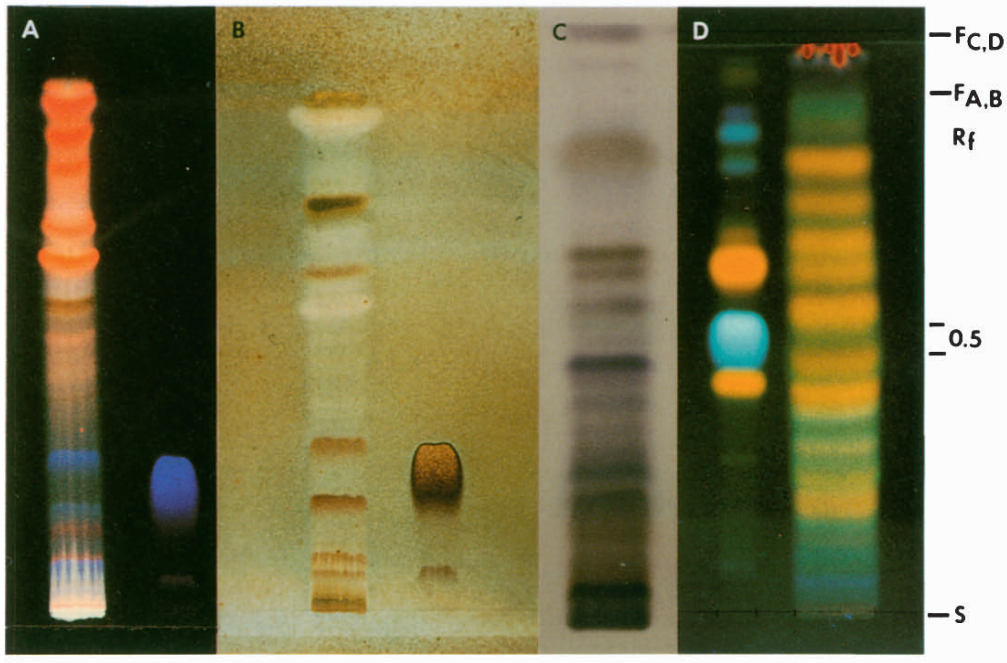

Fig. 23

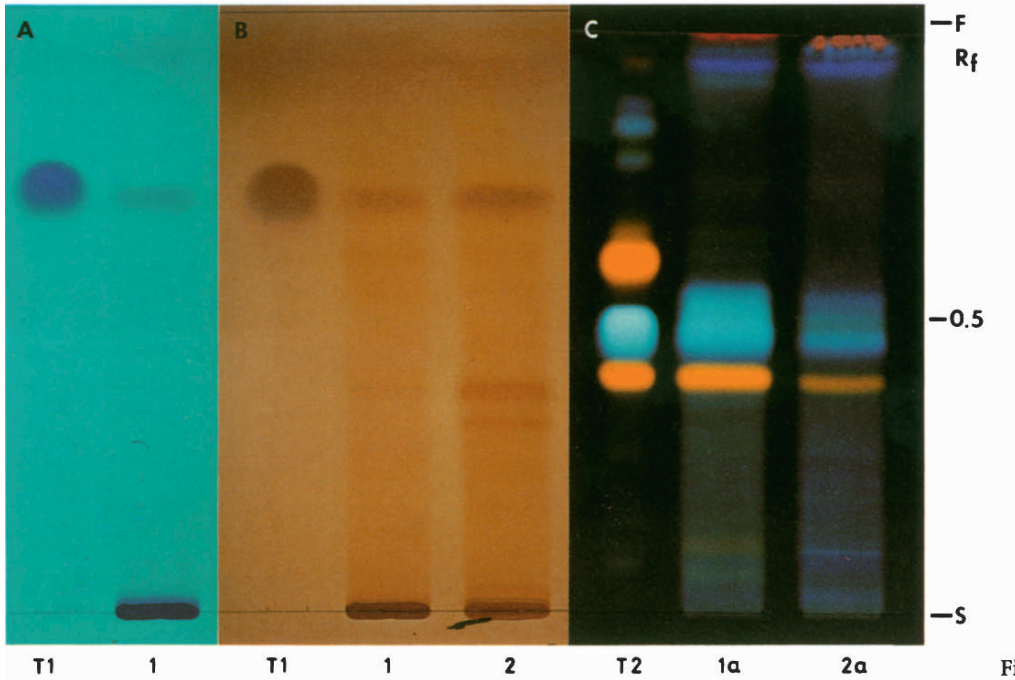

Fig. 24

Aconiti tuber

Drug sample
1 trade sample (1992)
2 A. napellus L. ssp. napellus
3 trade sample (1984)
4 A. paniculatum ssp. paniculatum
(alkaloid extraction method A, 30–40 µl)

Reference compound
T1 aconitine/mesaconitine
T2 aconitine
T3 deoxyaconitine
T4 hypaconitine
T5 benzoylaconine
T6 aconine

Solvent system
Fig. 25 A toluene–ethyl acetate–diethylamine (70:20:10)
B cyclohexane–ethanol–diethyamine (80:10:10)

Detection
Dragendorff reagent (DRG No. 13A) → vis DRG/$NaNO_2$ reagent (No. 13B) → vis

Fig. 25
The European **Aconitum napellus** group comprises three species: A. napellus, A. pentheri and A. angustifolium. The TLC pattern of their alkaloid distribution varies: a dominating aconitine amount, aconitine and mesaconitine as prominent zones or mainly mesaconitine and/or hypaconitine.

A
Extract (1) contains aconitine and mesaconitine (T1) which appear in system A at R_f 0.6–0.75 as brown, fast-fading zones after treatment with DRG reagent (vis).

B
The alkaloids deoxyaconitine (T3) and hypaconitine (T4) and the cleavage products benzoylaconine (T5) and aconine (T6) are separated in system B and show fast-fading zones with DRG–$NaNO_2$ reagent (vis). In samples (1,2) the aconitine/mesaconitine zones at R_f 0.35–0.4 (T1) and in sample (3) various, additional brown zones in the R_f range of benzoylaconine (T5) and aconine (T6) are found. A. paniculatum extract (4) has an obviously different TLC pattern with a main zone in the R_f range of hypaconitine (T4) and at $R_f \sim 0.55$.

Aconiti tuber, Sabadillae semen, Lobeliae herba, Ephedrae herba

Drug sample
1 Aconiti tubera (trade sample)
2 Sabadillae semen
3 Lobeliae herba
4 Ephedrae herba
(alkaloid extraction method A, 30 µl)

Reference compound
T1 aconitine/mesaconitine
T2 veratrine (alkaloid-mixture)
T3 lobeline
T4 ephedrine

Solvent system
Fig. 26 A toluene–ethyl acetate–diethylamine (70:20:10)
B ethyl acetate–cyclohexane–methanol–ammonia (70:15:10:5)
C toluene–chloroform–ethanol (28.5:57:14.5)

Detection
A Iodoplatinate reagent (IP No. 21) → vis
B Ninhydrine reagent (NIH No. 29) → vis
C Dragendorff reagent (DRG No. 13A) → vis

Fig. 26A
Aconiti tuber (1), **Sabadillae semen** (2), **Lobeliae herba** (3). Their major alkaloids are found in the R_f range 0.6–0.65 as white zones against a grey-blue background.
Aconiti tuber (1): aconitine/mesaconitine (T1) and six minor zones (R_f range 0.25–0.7)
Sabadillae semen (2): veratrine (T2) and eight minor zones (R_f 0.5–0.55/0.8).
Lobeliae herba (3): one prominent zone of lobeline (R_f 0.65/ref T3).

B, C
Ephedrae herba (4): ephedrine is detected as a violet-red band (R_f 0.4–0.5) with ninhydrine, or with DRG reagent as a brown zone at $R_f \sim 0.2$ in solvent system C.

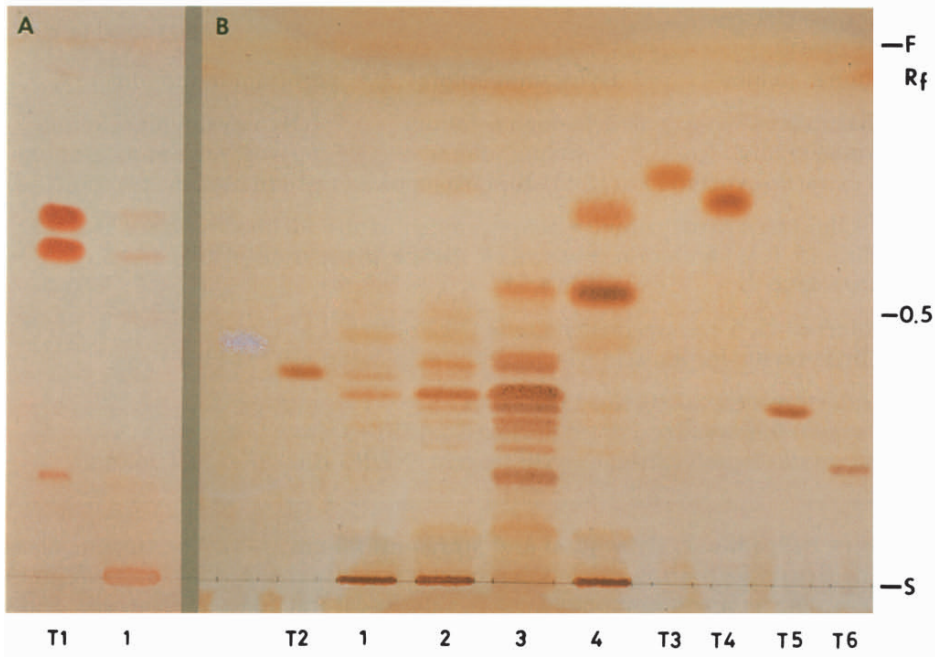

Fig. 25

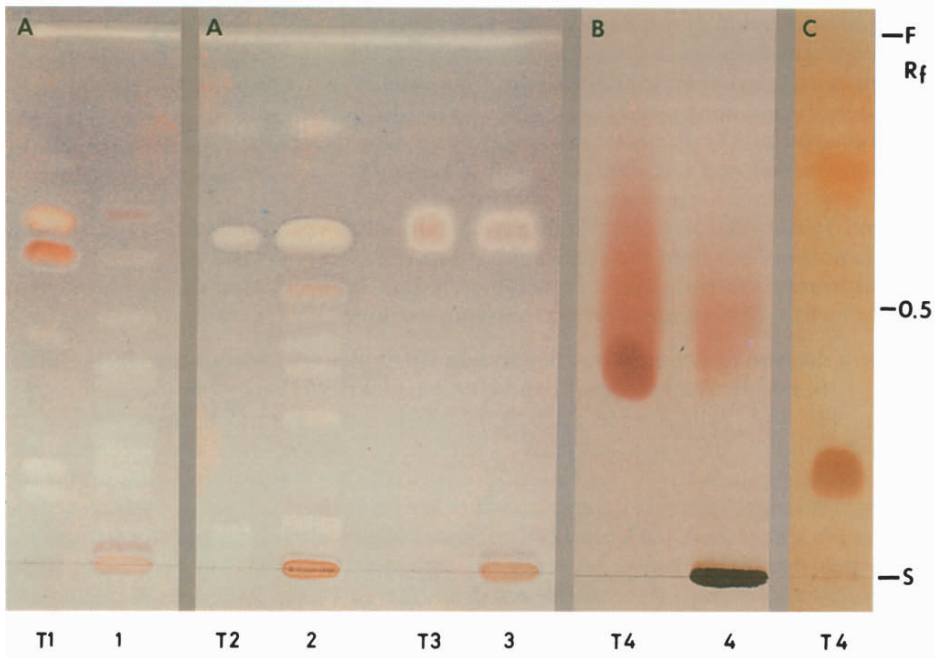

Fig. 26

Solanaceae drugs

Alkaloid extract	1 Belladonnae folium	2 Hyoscyami folium	3 Stramonii folium
Methanol extract	4 Scopoliae radix	6 Belladonnae folium	8 Hyoscyami nigri folium
	5 Belladonnae radix	7 Stramonii folium	9 Hyoscyami mutici folium
	(alkaloid extraction method C: (1)–(3) 30 µl, flavonoids (1g/10ml MeOH): (4)–(9) 20 µl)		
Reference compound	T1–T3 alkaloid test: hyoscyamine ► scopolamine mixture (defined ratio see sect. 1.2)		
	T4 rutin (R_f 0.35) ► chlorogenic acid (R_f 0.45) ► hyperoside (R_f 0.6)		
	T5 scopoletin	T6 caffeic acid	
Solvent system	Fig. 27 toluene–ethyl acetate–diethylamine (70:20:10)		
	Fig. 28 ethyl acetate–formic acid–glacial acetic acid–water (100:11:11:26)		
Detection	A Dragendorff reagent (DRG No. 13A) → vis		
	B DRG reagent followed by sodium nitrite (No. 13B) → vis		
	C Natural products–polyethylene glycol reagent (NP/PG No. 28) → UV 365 nm		

Fig. 27A,B **Alkaloids in Belladonnae, Hyoscyami and Stramonii folium** (1–3). The tropane alkaloids (-)-hyoscyamine (during extraction procedures partly changed into (±) atropine) and scopolamine as major compounds of the alkaloidal fraction of Solanaceae drugs respond to Dragendorff reagent with orange, unstable colour. Treatment with $NaNO_2$ increases the colour stability of the hyoscyamine zones.

A TLC differentiation of the three drugs is based on the hyoscyamine to scopolamine ratio and, to a limited extent, on the contents of the minor alkaloids belladonnine, atropamine and cuskhygrine.

For drug identification and determination of the alkaloid content, DAB 10 describes a TLC comparison with alkaloid mixtures containing defined ratios of atropine-SO_4 to scopolamine-HBr (T1–T3). Identification of the drug is then based on the similarity of colour intensity and zone size between the standard solutions and drug extracts.

Belladonnae folium (1): the ratio of hyoscyamine (R_f 0.25) to scopolamine ($R_f \sim 0.4$) corresponds to that of T1 at about 3:1. Both alkaloids are also present in the roots and seeds.

Hyoscyami folium (2): the ratio of the two main alkaloids is about 1.2:1. The total alkaloid content is less than the standard solution T2.

Stramonii folium (3): a higher scopolamine content than in (1) and (2). The typical hyoscyamine to scopolamine ratio for this drug is about 2:1.

Fig. 28 **Caffeic acid derivatives, coumarins, flavonoids.** The Solanaceae drugs are easily differentiated by their individual flavonoid and coumarin pattern.

Scopoliae- (4) and **Belladonnae radix** (5), which have a similar hyoscyamine to scopolamin content, are characterized by different patterns of blue fluorescent caffeic acid and coumarin derivatives (see Chap. 5 for further information). In **Belladonnae** (6) and **Hyoscyami nigri folium** (8), the main zones are rutin ($R_f \sim 0.4$; orange fluorescence) and chlorogenic acid ($R_f \sim 0.45$; blue fluorescence). In Hyoscyami nigri folium, these are the only two detectable zones, whereas Belladonnae folium shows additional blue, yellow-green and orange fluorescent zones in the R_f range 0.05–0.1 (7-glucosyl-3-rhamnogalactosides of kaempferol and quercetin).

Stramonii folium (7) is characterized by five orange fluorescent quercetin glycosides in the R_f range 0.03–0.25. The absence of rutin and chlorogenic acid clearly distinguishes the drug from Belladonnae and Hyoscyami folium. Hyoscyami mutici folium (9) has only a very low flavonoid content.

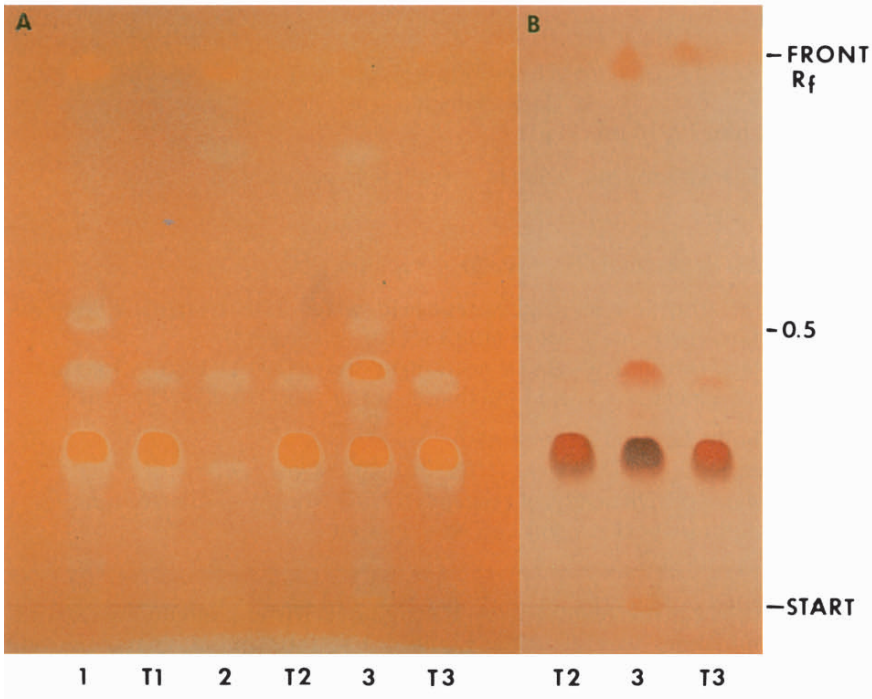
A
B
FRONT
Rf
0.5
START
1
T1
2
T2
3
T3
T2
3
T3

Fig. 27

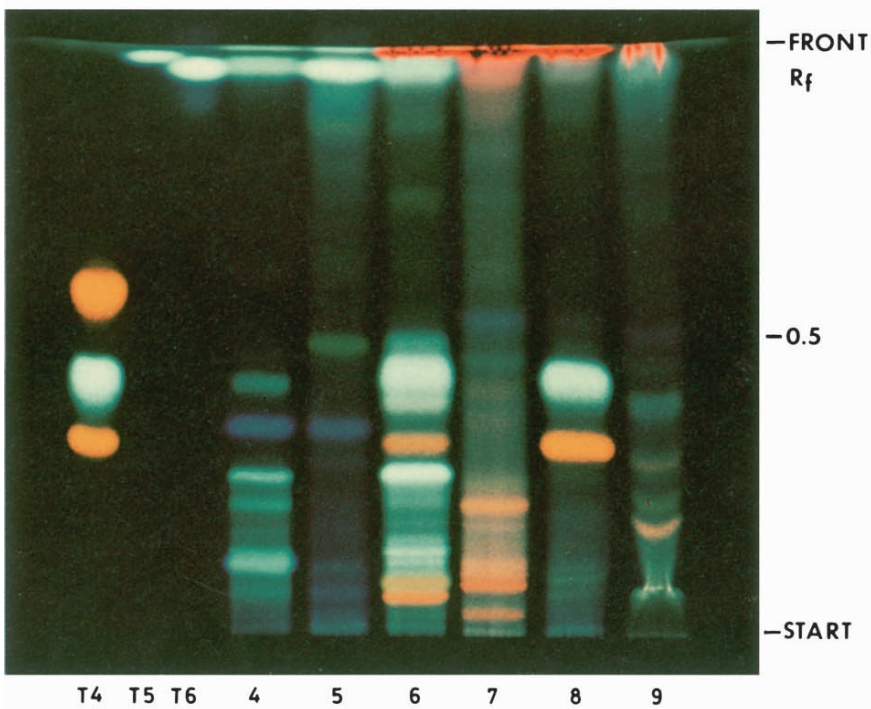
FRONT
Rf
0.5
START
T4
T5
T6
4
5
6
7
8
9

Fig. 28

Purine drugs

Drug sample
1 Coffeae semen
2 Mate folium
3 Theae folium (black tea)
4 Cacao semen
(methanolic extraction, 1 g/10 ml, 30 µl)

Reference compound
T1 rutin ($R_f \sim 0.35$) ▶ chlorogenic acid ($R_f \sim 0.45$) ▶ hyperoside ($R_f \sim 0.6$)
T2 caffeine
T3 theobromine
T4 aescin ($R_f \sim 0.25$) + aescinols ($R_f \sim 0.45$) = saponin test

Solvent system
Fig. 29 A ethyl acetate–formic acid–glacial acetic acid–water (100:11:11:26) → system A
B ethyl acetate–methanol – water (100:13.5:10) → system B
Fig. 30 C ethyl acetate–formic acid–glacial acetic acid–water (100:11:11:26) → system A
D chloroform–glacial acetic acid–methanol–water (60:32:12:8) → system D

Detection
A UV-254 nm (without chemical treatment)
B Iodine–potassium iodide–HCl reagent (I/HCl No. 20) → vis
C Natural products–polyethylene glycol reagent (NP/PG No. 28) → UV-365 nm
D Anisaldehyde–sulphuric acid reagent (AS No. 3) → vis

The Purine drugs 1–4 can be identified by their characteristic contents of caffeine, theobromine, theophylline, various caffeoylquinic acids, flavonoid glycosides and saponines.

Fig. 29A **Puridnerivatives.** (System A). Extracts of Coffeae semen (1), Mate folium (2) and Theae folium (3) show one to four prominent fluorescence-quenching zones in the R_f range 0.4–0.6 with caffeine as the main zone at $R_f \sim 0.60$. Caffeine migrates in this solvent system directly above the hyperoside (T1/$R_f \sim 0.6$). → For detection of caffeoyl quinic acids and flavonoids see reagent C.

B (System B) Treatment with I/HCl reagent generates a dark-brown zone of caffeine at $R_f \sim 0.45$ (T2) in extracts (1) and (3), less concentrated in (2) and (4). Theobromine at $R_f \sim 0.4$ (T3) is detected as a grey, fast-fading zone in Mate folium (2). The concentration of theobromine in Cacao semen (4) is low, the amount of theophylline ($R_f \sim 0.6$) in the extracts 1–4 is not sufficient for detection.

Fig. 30C **Phenol carboxylic acids, flavonoids and saponines.** (System A) Treatment with NP/PEG reagent reveals caffeoyl (CQA) and dicaffeoyl quinic acids as blue and the flavonoid glycosides as orange–yellow or green fluorescent zones in UV-365 nm. Coffeae semen (1) and Mate folium (2): the blue 5-CQA, 3-CQA (R_f 0.45–0.5) and additional dicaffeoyl quinic acids in the upper R_f range are characteristic. One additional orange–yellow zone of rutin at $R_f \sim 0.4$ (T1) is found in Mate folium (2) only.
Theae folium (3): four mainly yellow fluorescent flavonoid glycosides in the R_f range of hyperoside and rutin (T1) and two flavonoid glycoside zones at R_f 0.25–0.3 with yellow and green fluorescence, respectively.

D (System D) **Saponines** (aescin T4) respond as blue–violet zones to AS reagent (vis). In Mate folium (2) the main triterpene saponins are seen as six blue–violet zones in the R_f range 0.4–0.8. In Theae folium (3) broad bands of yellow–brown zones from the start till $R_f \sim 0.4$ ("thea flavines") dominate in the lower R_f range.

Note: Caffeine migrates in solvent system A up to the solvent front.

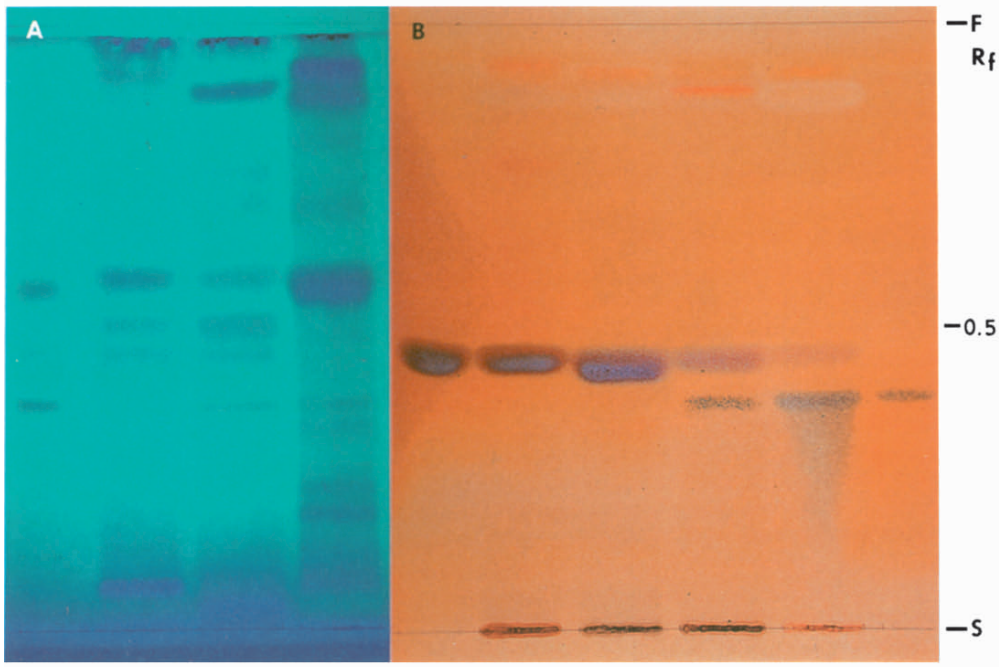

Fig. 29

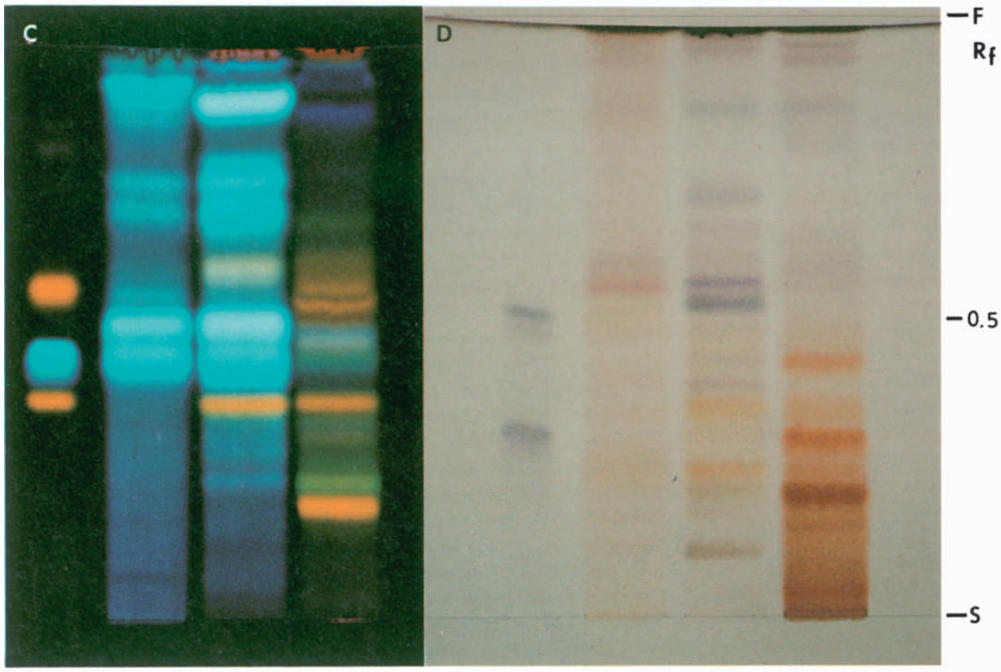

Fig. 30