2 Drugs Containing Anthracene Derivatives

The characteristic constituents of this drug group are anthraquinones, oxanthrones, anthranols and anthrones with laxative properties. The anthraquinones possess phenolic groups on C-1 and C-8 and keto groups on C-9 and C-10. In the anthrones and anthranols, only C-9 carries an oxygen function. In addition, a methyl, oxymethyl or carboxyl group may be present on C-3, and a hydroxy or methoxy group on C-6. Most compounds in this group are present in the plant as O-glycosides. The glycoside linkage is usually at C-1, C-8 or C-6-OH. C-Glycosides occur as anthrones only, with the C–C bond always at C-10. In the O- and C-glycosides, the only sugars found so far are glucose, rhamnose and apiose.

2.1 Preparation of Extracts

General method, methanolic extract

Powdered drug (0.5 g) is extracted for 5 min on a water bath with 5 ml methanol. The filtrate is used for TLC: 5 µl (Aloe) and 20 µl (Rheum, Frangula, Cascara).

Senna

Sennae folium or fructus are extracted with 50% methanol; 20 µl is used for TLC.

Hydrolysis of anthraquinone glycosides

Powdered drug (0.5 g) and 25 ml 7.5% hydrochloric acid are heated under reflux for 15 min. After cooling, the mixture is extracted by shaking with 20 ml ether. The ether phase is concentrated to about 1 ml, and 10 µl is used for TLC (e.g. Rhei radix).

2.2 Thin-Layer Chromatography

Reference solutions

Aloin, frangulin A/B, glucofrangulin A/B, rhein, aloe-emodin and rhaponticoside (stilbene glucoside) are applied as 0.1% methanolic solutions.
Sennosides A and B are prepared as a 0.1% solution in methanol-water (1:1).
A total of 10 µl of each reference solution is used for TLC.

Adsorbent

Chromatography is performed on silica gel $60F_{254}$ precoated plates (Merck, Germany).

Chromatography solvents

- Ethyl acetate-methanol-water (100:13.5:10)
  With the exception of Senna preparations, the solvent system is suitable for the chromatography of all anthracene drug extracts.
- n-propanol-ethyl acetate-water-glacial acetic acid (40:40:29:1) ▶ Senna
- light petroleum-ethyl acetate-formic acid (75:25:1) ▶ anthraquinone aglycones

- toluene-ethyl formiate-formic acid (50:40:10) or (50:20:10) ► for the non-laxative dehydrodianthrones of Hyperici herba

2.3 Detection

- UV 254 nm All anthracene derivatives quench fluorescence
- UV 365 nm All anthracene derivatives give yellow or red-brown fluorescence

- Spray reagents (See Appendix A)

  - Potassium hydroxide (KOH No. 35; → Bornträger reaction)
    After spraying with 5% or 10% ethanolic KOH, anthraquinones appear red in the visible and show red fluorescence in UV-365 nm.
    Anthrones and anthranols: yellow (vis.), bright yellow fluorescence (UV-365 nm).
    Dianthrones do not react.

  - Natural products-polyethylene glycol reagent (NP/PEG No.28)
    Anthrones and anthranols: intense yellow fluorescence (UV-365 nm).

  - Sennoside detection
    The TLC plate is sprayed with concentrated HNO_3 and then heated for 10 min at 120°C. It is then sprayed with 5% ethanolic KOH. After further heating, sennosides appear as brown-red zones in UV-365 nm and brown zones in the visible.
    Sennosides can also be detected with a 1% solution of sodium metaperiodate in 10% ethanolic KOH. After spraying and heating (approximately 5 min), yellow-brown zones are obtained in UV-365 nm.

  - Rhaponticoside detection
    Phosphomolybdate-H_2SO_4 reagent (PMA-H_2SO_4 No.36)
    Rhaponticoside gives dark blue zones in the visible

  - Hypericin detection
    A 10% solution of pyridine in acetone intensifies the red fluorescence of hypericin in UV-365 nm.

  - "Isobarbaloin" test for the differentiation of Aloe capensis and Aloe barbadensis.
    One drop of saturated $CuSO_4$ solution, 1 g NaCl and 10 ml 90% ethanol are added to 20 ml of an aqueous solution of Aloe barbadensis (Curacao aloe, 1:200). A wine-red colour is produced, which is stable for at least 12 h. Solutions of Aloe capensis fade rapidly to yellow.

2.4 Circular TLC in Addition to the Ascending TLC

This method is generally useful for the separation of drug extracts containing a high proportion of ballast substances, e.g. mucilages from Sennae folium.

Application

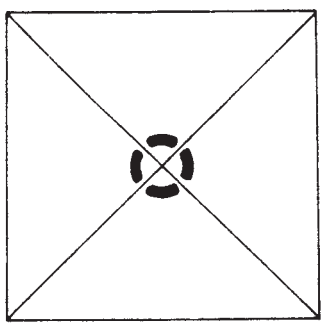
Two diagonal pencil lines are drawn from the corners of the TLC plate. The centre point of the plate is marked and a circle is drawn around it with a diameter of approximately 2 cm. The circle is thus divided into four segments by the diagonals. The perimeter of each segment serves for the application of drug extracts or reference solutions (see figure below).

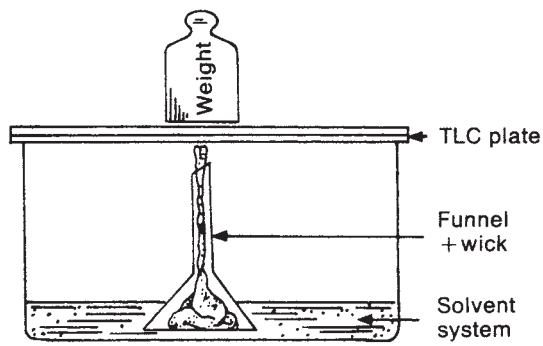
Procedure

100 ml of solvent are placed in a round, straight-sided chamber (glass trough, 10 cm high, 20 cm in diameter). A glass funnel is loosely packed with cotton, which extends as a wick through the tube of the funnel. The funnel is placed in the solvent system, so that the solvent soaks into the cotton. With the loaded side facing downwards, the TLC plate is placed over the top of the trough, so that the cotton makes contact exactly at the marked centre.

The solvent migrates circularly from the point of application. The zones of the separating substances form single arcs, which increase in length from the starting point to the periphery of the spreading solvent.

The same adsorbent (silica gel 60 F_{254} precoated plates, 20 × 20 cm; Merck, Darmstadt), the solvent systems and detection methods can be used as described for ascending TLC. Good separations are obtained by solvent migrations of 6 cm only.





Glass trough

2.5 Drug List

	Drug/plant source Family/pharmacopoeia	Main constituents Hydroxyanthracenes
Fig. 1,2	**Aloes** Various types such as: Cape and Curacao aloes Socotrine aloes DAB 10, Helv VII, USP XXII, MD Uganda, Kenya aloe, Indian aloe Asphodelaceae (Liliaceae)	Dried juice of aloe leaves. Aloin A, B (10-C-β-D glucopyranoside of aloe emodin-anthrone), α- and β-stereoisomers Aloinoside A and B (stereoisomers of aloin-11-α-L-rhamnoside), aloe-emodin (aglyone) Aloeresins (non-laxative compounds): aloesin A (chromone-C-glucoside), aloesin B (p-coumaric acid ester of aloeresin A), aloesin C (glucoside of aloesin B)
Fig. 1,2	**Aloe capensis** Cape aloes Aloe ferox MILLER and hybrids DAB 10, BHP 90, ÖAB 90, USP XXII, Helv VII, Jap XI	Not less than 18% hydroxyanthracenes calculated as aloin (e.g. DAB 10) Aloin A/B, aloeresins A/B (type I) Aloin A/B, aloinosides A/B, aloesin A/B (type II), 5-hydroxyaloin A/B, aloe-emodin (<1%)
Fig. 1,2	**Aloe barbadensis** Curacao aloes, Aloe vera Aloe barbadensis MILL. DAB 10, BHP 90, Helv VII, ÖAB 90, USP XXII, MD	Not less than 28% hydroxyanthracenes calculated as aloin (DAB 10) Aloin A/B, 7-hydroxyaloin A/B (3%) 8-Methyl-7-hydroxyaloin A/B, aloesin B/D
Fig. 2	**Aloe perryi** Socotrine aloes Aloe perryi BAK. MC	Up to 14% hydroxyanthracene derivatives calculated as aloin Aloin A/B, aloinosides A/B, aloeresins A/B
Fig. 4	**Rhamni purshiani cortex** **Cascarae sagradae cortex** Cascara sagrada bark Sacred bark, chitten bark Rhamnus purshianus D.C. Rhamnaceae DAB 10, PhEur II, ÖAB 90, Helv VII, MD USP XXII (extract)	Not less than 8% hydroxyanthracenes with at least 60% cascarosides calc. as cascaroside A (DAB 10) Cascarosides A and B (diastereoisomers of aloin-8-O-β-D-glucoside); cascarosides C and D (diastereoisomers of deoxyaloin-8-O-β-D-glucoside); Aloin, deoxyaloin (10%–20%), small amounts of emodine; frangula-emodin-O-glycosides (10%–20%)
Fig. 3	**Frangulae cortex** Rhamni frangulae cortex Alder buckthorn bark Rhamnus frangula L. Rhamnaceae DAB 10, PhEur II, Helv VII, MD	Not less than 6% anthraquinone glycosides Glucofrangulin A and B (emodin-6-O-α-L-rhamnosyl-8-O-β-D-glucoside and -6-O-α-L-apiosyl-8-O-β-D-glucoside). Frangulin A and B (emodin-6-O-α-L-rhamnoside and emodin-6-O-α-L-apioside). Emodin-8-β-O-glucoside, -diglucoside Physcion, chrysophanol glycosides

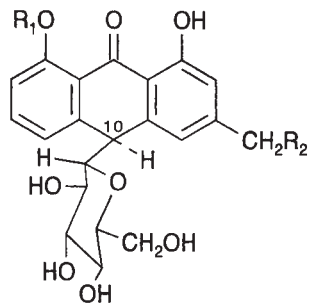
Drug/plant source Family/pharmacopoeia	Main constituents Hydroxyanthracenes	
Frangulae fructus Alder buckthorn fruits Rhamnus frangula L. Rhamnaceae ÖAB	Low concentrations of anthraquinone aglycones and traces of anthraquinone glycosides.	Fig. 3,4
Oreoherzogiae cortex Rhamni fallaci cortex Rhamnus alpinus L. ssp. fallax (BOISS.) PETITMAIRE Rhamnaceae	1%–3% Hydroxyanthracene derivatives Emodin-glucoside, physcion-rutinoside Flavonoids: e.g. xanthorhamnin ▶ adulterant of Frangulae cortex	Fig. 3
Rhamni cathartici fructus Buckthorn berries Rhamnus catharticus L. MD	Low contents of anthraquinones in fruit flesh, 0.7%–1.4% hydroxyanthracenes in semen: frangulaemodin, -emodinanthrons Flavonol glycosides >1%: xanthorhamnines = triglycosides of rhamnocitrin (7-methyl-kaempferol and 7-methyl-quercetin) Catharticin (rhamnocitrin-3-O-β-rhamnoside)	Fig. 3,4
Rhei radix Rhubarb rhizome Rheum officinale BAILLON Rheum palmatum L. and hybrids Polygonaceae DAB 10, ÖAB. MD, Japan, China	1%–6% Hydroxyanthracenes (not less than 2.5%): 60%–80% of mono- and diglucosides of physcion, chrysophanol and rhein (e.g. physcion-8-O-gentiobioside); rhein, physcion, chrysophanol, emodin, aloe-emodin; bianthronglycosides: rheidin A–C, sennidin C,D, galloyl-β-D-glucose	Fig. 5,6
Rhei rhapontici radix Garden rhubarb Rheum rhaponticum L. Polygonaceae	0.3%–0.5% anthraquinone aglycones and glucosides, 7%–10% stilbene derivatives: rhaponticoside 5%, desoxyrhaponticoside, Adulterant of Rhei radix	
Sennae folium Senna leaves Cassia senna L. (Alexandrian senna) Cassia angustifolia VAHL (Tinnevelly senna) Caesalpiniaceae DAB 10, ÖAB 90, Helv VII, Jap XI, MD	2%–3.5% dianthrone glycosides (not less than 2.5%). calc. as sennoside B for Alexandrian and Tinevelly senna (e.g. DAB 10). As principal active compounds: sennoside A and B as 8,8′-diglucosides of sennidin A/B (= stereoisomeric 10-10′-dimers of rhein anthrone) Sennoside A (dextrorotary), sennoside A_1 (optical isomer), sennoside B (optically inactive mesoform) low amounts of Sennoside C and D (=heterodianthrons), rhein, emodin and their mono- and diglycosides	Fig. 7,8

Drug/plant source Family/pharmacopoeia	Main constituents Hydroxyanthracenes
Sennae fructus Senna pods Cassia senna L. (Alexandrian senna) Cassia angustifolia VAHL (Tinevelly senna) Caesalpiniaceae DAB 10, PhEur I, ÖAB, Helv VII, MD, USP XXII	2.2%–3.4% dianthrone glycosides Alexandrian senna pods > 3.4% (DAB 10) Tinnevelly senna pods > 2.2% (DAB 10) Sennoside A,B besides C,D; rhein, mono- and diglycosides of emodin and rhein Naphthalenes: 6-hydroxy musizin glucoside (C. senna); tinevellin-glucoside (C. angustifolia)
Hyperici herba St. John's wort Hypericum perforatum L. Hypericaceae (Glusiaceae) DAC 86, Helv VII, MD	0.05–0.6% dehydrodianthrones Hypericin, pseudohypericin, protohypericin Flavonol glycosides: rutin, hyperoside, quercitrin, isoquercitrin; quercetin; biapigenin Chlorogenic acid. Hyperforin (fresh plant)

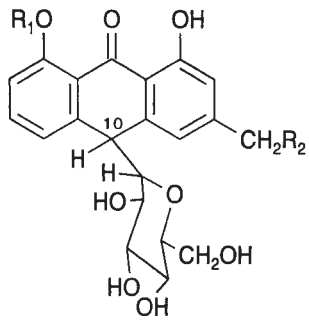
Fig. 7,8

Fig. 9,10

2.6 Formulae

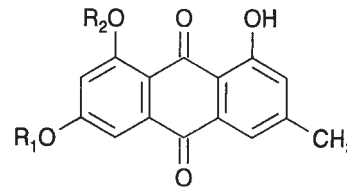


	R_1	R_2
Aloin A	H	OH
(−)-11-Desoxyaloin	H	H
Aloinoside A	H	O-α-L-rhamnose
Cascaroside A	β-D-glucose	OH
Cascaroside C	β-D-glucose	H

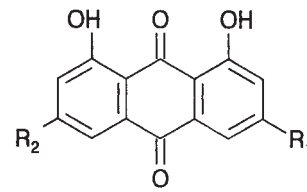


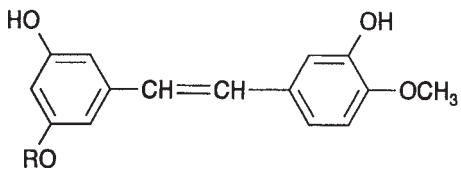
	R_1	R_2
Aloin B	H	OH
(−)-11-Desoxyaloin	H	H
Aloinoside B	H	O-α-L-rhamnose
Cascaroside B	β-D-glucose	OH
Cascaroside D	β-D-glucose	H

	R_1	R_2
Glucofrangulin A	α-L-rhamnose	β-D-glucose
Glucofrangulin B	β-D-apiose	β-D-glucose
Frangulin A	α-L-rhamnose	H
Frangulin B	β-D-apiose	H
Frangula emodin	H	H

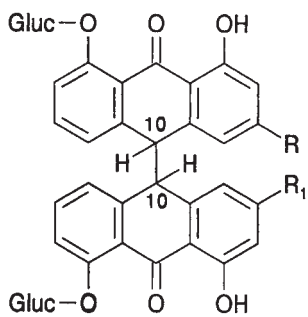


	R_1	R_2
Rheum emodin	CH_3	OH
Aloe emodin	CH_2OH	H
Rhein	COOH	H
Chrysophanol	CH_3	H
Physcion	CH_3	OCH_3



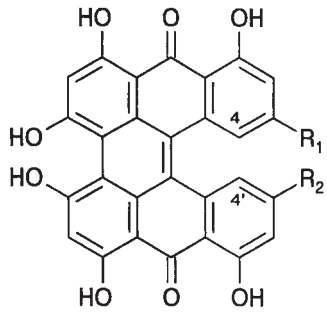


Rhaponticoside R = β-D-glucose
Rhapontigenin R = H

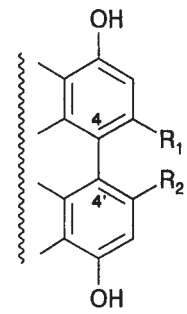


Sennoside A: R, R_1 = COOH (+)-form
Sennoside B: R, R_1 = COOH mesoform

Sennoside C: R = COOH R_1 = CH_2OH (+) form
Sennoside D: R = COOH R_1 = CH_2OH mesoform



Protohypericin
$R_1 = R_2 = CH_3$
Protopseudohypericin
$R_1 = CH_3 \quad R_2 = CH_2OH$



4-4′: Hypericin
$R_1 = R_2 = CH_3$
4-4′: Pseudohypericin
$R_1 = CH_3 \quad R_2 = CH_2OH$

2.7 Chromatograms

Aloes

Drug sample	1 Aloe capensis (type I) 2 Aloe capensis (type II) 3 Aloe barbadensis (Curacao aloe) (methanolic extracts, 5 µl)	4 Aloe perryi (Socotrine aloe) 5 Aloe of Kenian origin 6 Aloe of Ugandan origin
Reference Compound	T1 aloin T2 7-hydroxyaloin	T3 aloin ($R_f \sim 0.45$) ▶ aloe emodin ($R_f \sim 0.95$)
Solvent system	ethyl acetate-methanol-water (100:13.5:10)	
Detection	Fig. 1 Without chemical treatment → A UV-365 nm, B UV-254 nm Fig. 2 10% ethanolic KOH reagent (No. 35) → C UV-365 nm, D vis	

Aloe species are characterized by aloin A/B, aloe-emodin and the non-laxative aloeresins (aloesin A–C). In addition some aloes contain aloinosides and substituted aloins (5- or 7-hydroxyaloin A/B).

Fig. 1 **Aloe capensis** (1,2)

A Cape Aloe (1) is characterized by the yellow fluorescent zone of aloin ($R_f \sim 0.5$/T2) and aloe-emodin (solvent front). The zones of aloeresins such as aloesin A and B ($R_f \sim 0.55$ and $R_f \sim 0.25$, respectively) fluoresce light blue.
Trade samples of Cape aloe (2) can show besides the yellow fluorescent aloin and aloe-emodin, additional yellow zones of the aloinosides A/B (R_f 0.25–0.3) and additional glycosides (e.g. $R_f \sim 0.75$). The blue fluorescent zones are less prominent than in sample 1 (e.g. aloe resins).

B All major compounds, such as aloins or aloinosides and specifically the aloesins show quenching in UV-254 nm.
Note: 7-hydroxyaloin (T2) a characteristic compound in Curacao aloes (3) is absent in Cape aloes (1,2).

Fig. 2 **TLC synopsis of aloes** (1–6)

C Treatment with KOH reagent intensifies the yellow fluorescence of aloin and aloinosides as well as the blue fluorescence of the aloe resins. Aloe-emodin shows a typical red Bornträger reaction in UV-365 nm.

Aloe resins	Aloin	Aloinosides	Aloesins	Remarks
1 Cape aloe	+	++	+	Cape and Curacao aloes are differentiated
2 Cape aloe	+	−−	++	by the "isobarbaloin-test" of KLUNGE
3 Curacao aloes	++	−−	++	(see section 2.3) which gives yellow or
4 Socotrine aloes	+	+	++	wine red colour, respectively
5 Kenya aloes	++	+	(+)	Socotrine and Curacao aloes show a dark
6 Uganda aloes	+	(+)	(+)	zone directly below aloin, e.g. 7-hydroxyaloin in 3

D All Aloe (1–4) samples show aloin as prominent yellow zones (vis.). The samples 2 and 4 contain, in addition, aloinosides (yellow/R_f 0.25–0.3), and a dark violet-red zone (vis.) characterizes Curacao (3) and Scocotrine aloe (4). This zone directly below aloin can be identified in (3) as 7-hydroxyaloin.

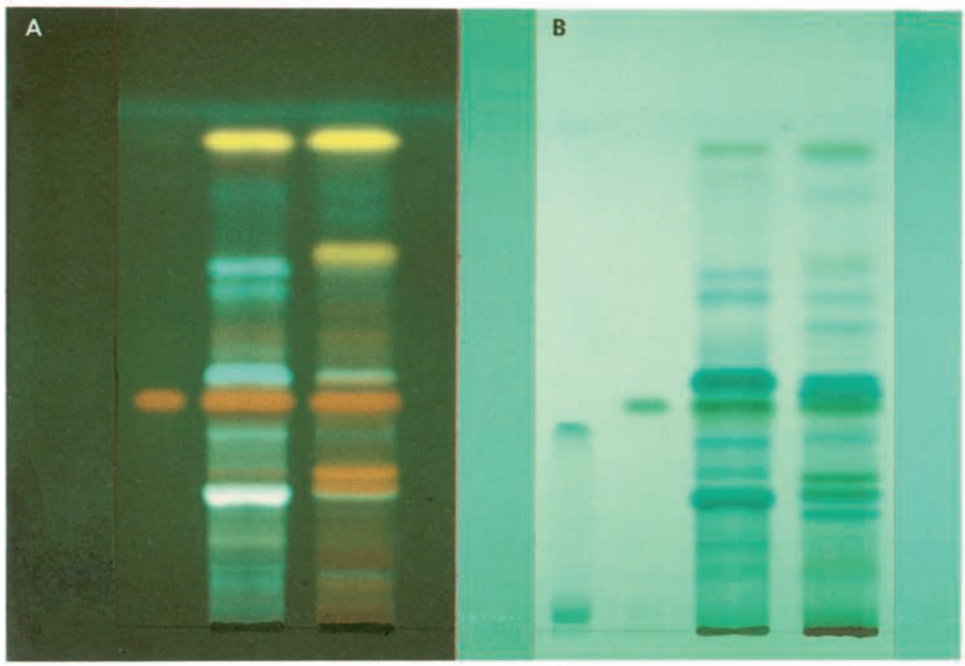


Fig. 1

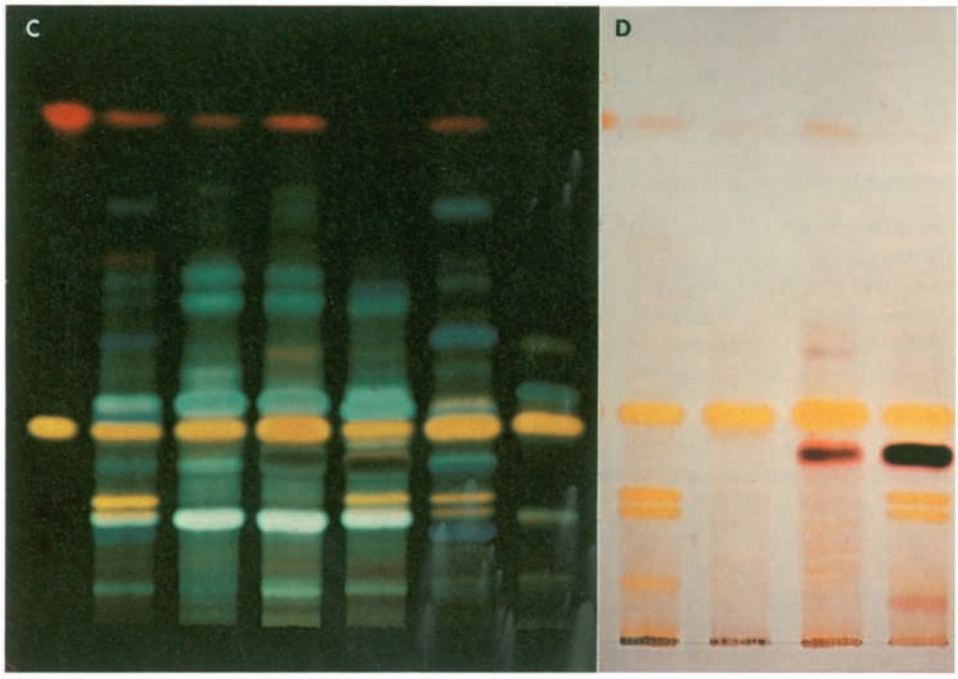


Fig. 2

Rhamnus species

Drug sample

1 Frangulae cortex (Rhamnus frangula)
2 Oreoherzogiae cortex (Rhamnus alpinus ssp. fallax)
3 Frangulae fructus (Rhamnus frangula)
4 Rhamni carthartici fructus (Rhamnus cartharticus)
5–7 Cascarae cortex (Rhamnus purshianus-trade samples)
(methanolic extracts, 20 µl)

Reference compound

T1 glucofrangulin A (R_f 0.25) ▶ aloin (R_f 0.45) ▶ frangulin A (R_f 0.75) ▶ emodin (front)
T2 aloin

Solvent system

ethyl acetate-methanol-water (100:13.5:10)

Detection

Fig. 3 KOH reagent (No. 35) A → vis; B, C → UV-365 nm
Fig. 4 Natural products-polyethylene glycol reag. (NP/PEG No. 28) D, E → UV-365 nm

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Fig. 3 **Anthraquinones**

A **Frangulae cortex** (1) is characterized by two pairs of red-brown anthraquinone glycosides (vis.): glucofrangulin A (R_f 0.2), B (R_f 0.3) and frangulin A (R_f 0.75), B (R_f 0.8). Aglycones such as emodin, physcion and chrysophanol move with the solvent front.
Oreoherzogiae cortex (2) counts as an adulterant of Frangulae cortex: glucofrangulin A/B present in considerably lower concentration, only traces of frangulin A/B, additional anthraquinone glycosides such as physcion-rutinoside ($R_f \sim 0.3$) and emodin-glucoside ($R_f \sim 0.5$) dominate. A yellow zone at $R_f \sim 0.2$ in both samples (1,2) is due to flavonol glycosides see Fig. 4 D.

B All anthraquinones of Frangulae and Oreoherzogiae cortex (1,2) show a bright orange-red fluorescence in UV-365 nm.

C **Frangulae fructus** (3) shows only traces of frangula-emodin at the solvent front.
Rhamni carthartici fructus (4). Four to five orange-red zones are detectable in the R_f range of glucofrangulin ($R_f \sim 0.25$), frangulin ($R_f \sim 0.8$) and above.

Fig 4 **Flavonoids and cascarosides**

D **Frangulae cortex** (1): one green fluorescent flavonoid glycoside ($R_f \sim 0.2$) and the zones of frangulin A/B with brown fluorescence.
Frangulae fructus (3): two yellow orange fluorescent flavonol glycosides (R_f 0.15/0.45).
Rhamni cathartici fructus (4): a band of prominent orange-yellow fluorescent xanthorhamnins (triglycosides, see 2.5 Drug List) between the start and $R_f \sim 0.25$, and between $R_f \sim 0.75$ up to the solvent front. Xanthorhamnin ($R_f \sim 0.2$) is found in (3) and (4).

E **Cascarae cortex** (5–7) samples are characterized by anthrone glycosides: two pairs of yellow fluorescent cascarosides A/B (R_f 0.05–0.15) and cascarosides C/D (R_f 0.2–0.25). The cascarosides A/B dominate. The amount of yellow fluorescent aloin (T2), deoxyaloin (R_f 0.65) and the red-brown fluorescent aglycones emodin, aloe-emodin, chrysophanol (solvent front) varies. Four blue fluorescent naphthalide derivatives are detectable in the R_f range 0.3–0.45.

Note: Cascarosides A–C also fluoresce bright yellow when treated with the KOH reagent.

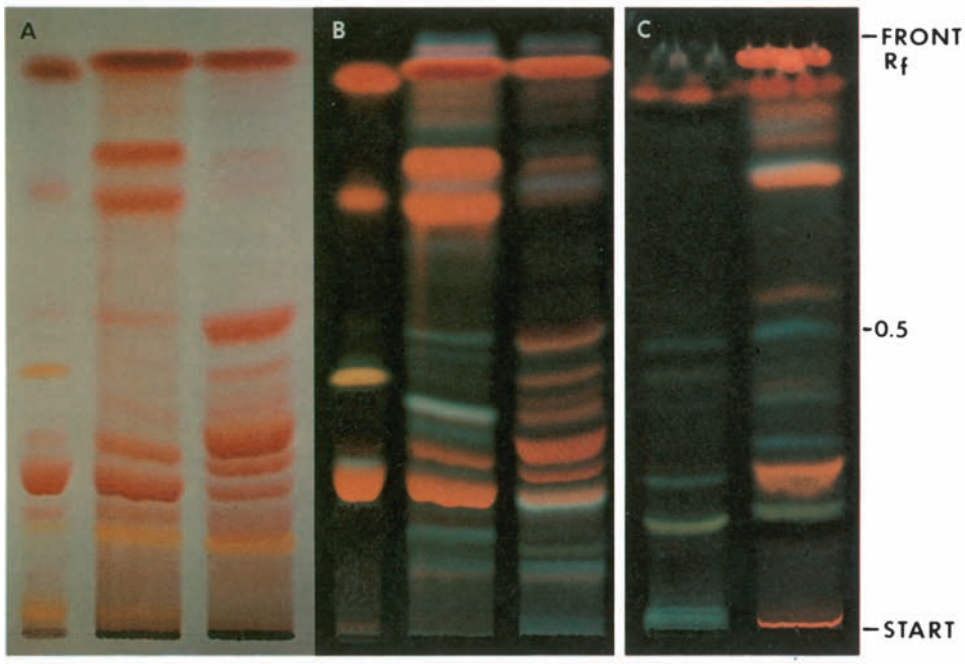
A
B
C
FRONT
Rf
0.5
START
T1
1
2
T1
1
2
3
4

Fig. 3

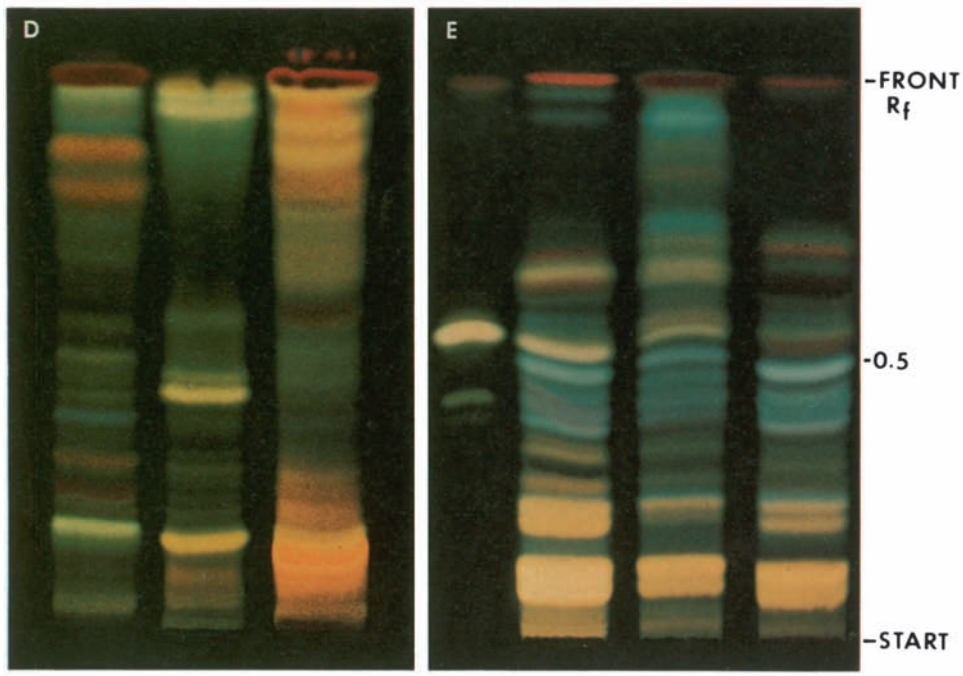
D
E
FRONT
Rf
0.5
START
1
3
4
T2
5
6
7

Fig. 4

Rhei radix

Drug sample
1 Rhei palmati radix (methanolic extract, 20 µl)
2 Rhei rhapontici radix (methanolic extract, 20 µl)
3 Rhei palmati radix (hydrolysate, 10 µl)
4 Rhei rhapontici radix (hydrolysate, 10 µl)

Reference compound
T1 rhein
T2 rhaponticoside
T3 emodin ($R_f \sim 0.4$)

Solvent system
Fig. 5 ethyl acetate-methanol-water (100:13.5:10) → glycosides
Fig. 6 light petroleum-ethyl acetate-formic acid (75:25:1) → aglycones

Detection
A Without chemical treatment → UV-365 nm
B Phosphomolybdic acid/H_2SO_4 reagent (PMS No. 34) → vis
C Without chemical treatment → UV-254 nm
D Without chemical treatment → UV 365 nm

Fig. 5 **Glycosides**

A **Rhei radix** (1) is characterized in UV-365 nm by the prominent yellow fluorescent authraquinone aglycone zone (emodin, aloe-emodin, physcion, chrysophanol) at the solvent front. Their 8-O-monoglucosides migrate as a brown-red band to R_f 0.45–0.55. The corresponding diglycosides are present as minor compounds in the R_f range 0.1–0.3. The polar aglycone rhein (T1) at $R_f \sim 0.4$ is overlapped by blue fluorescent zones.
Rhei rhapontici radix (2) contains anthraquinone aglycones and monoglucosides in low concentration only. In addition the prominent violet-blue fluorescent stilbene derivatives rhaponticoside/deoxyrhaponticoside (R_f 0.45–0.55/T2) are present. They overlap the antraquinone monoglucoside zone.

B Treatment with the PMA reagent produces light yellow zones of anthraquinones (1) and a characteristic dark blue band of rhaponticoside/deoxyrhaponticoside (T2) and rhapontigenin (solvent front) in sample 2.

Fig. 6 **Aglycones**

C,D The aglycone mixtures (3,4) obtained by HCl hydrolysis of Rheum extracts (1,2) are separated in the lipophilic solvent system and evaluated in UV-254 nm and UV-365 nm. All aglycones show fluorescence quenching in UV-254 nm and uniformly yellow or orange-brown fluorescence in UV-365 nm.
Rhei palmati radix (3). Aloe-emodin and rhein (R_f 0.15–0.25/T1), emodin ($R_f \sim 0.3$/T3), chrysophanol and physcion (R_f 0.6–0.7) are characteristic aglycones.
Rhei rhapontici radix (4). The hydrolysate shows a qualitatively similar, but quantitatively different aglycone pattern with traces of rhein (T1) only. In addition blue fluorescent stilbene aglycones are found at R_f 0.05–0.1.

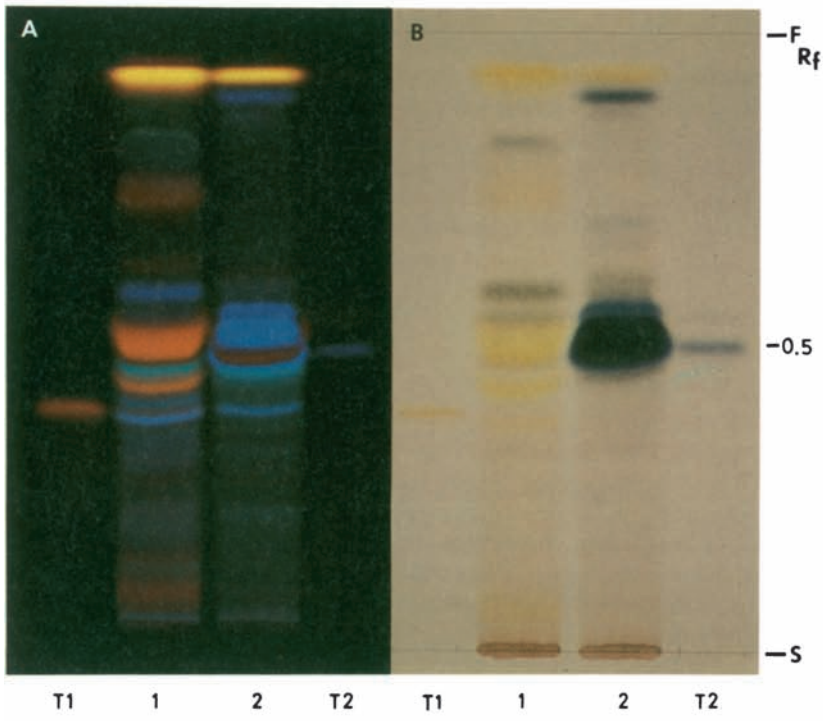

Fig. 5

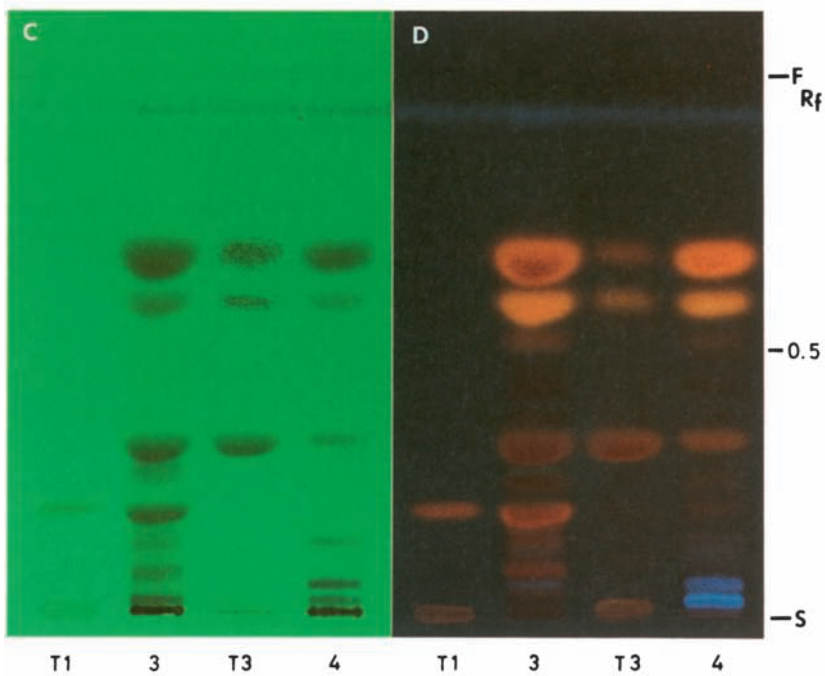

Fig. 6

Sennae folium, fructus

Drug sample
1 Sennae fructus (methanolic extract, 20 µl)
2 Sennae folium (methanolic extract, 20 µl)

Reference compound
T1 sennoside A*)
T2 sennoside B*)

Solvent system
n-propanol-ethyl acetate-water-glacial acetic acid (40:40:29:1)

Detection
Fig. 7 HNO_3-potassium hydroxide reagent (HNO_3/KOH No.30) → vis
Fig. 8 A HNO_3-potassium hydroxide reagent (HNO_3/KOH No.30) → UV-365 nm
B Sodium metaperiodate reagent (see 2.3 Detection) → UV-365 nm

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Fig. 7 **Sennae fructus** (1) and **folium** (2)
Treatment of the TLC plate with concentrated HNO_3, heating for approximately 30 min at 150°C and spraying with KOH reagent produces six to eight brownish and yellow zones (vis) in the R_f range 0.1 up to the solvent front.
The dark-brown zones are due to the sennosides B,A (R_f 0.25 and R_f 0.4) and the sennosides D,C (R_f 0.5 and R_f 0.7). The yellow zones indicate anthraquinone aglycones (e.g. rhein/R_f ~ 0.8; emodine/solvent front) and their glucosides (R_f ~ 0.3/R_f ~ 0.6).

Fig. 8A Evaluation under UV-365 nm light is more sensitive. The main brown zones (vis.) of Sennae extracts (1,2) now appear light brown to orange-brown. The minor compounds of the R_f range 0.5–0.9 are also more easily detectable.
The two dianthron glycosides, sennoside A (R_f 0.4/T1) and sennoside B (R_f 0.25/T2) are the major compounds in Sennae fructus (1) and S. folium (2).
In Sennae folium extract (2) a R_f value depression of sennoside A and specifically of sennoside B occur, caused by the mucilages also extracted from the plant material with 50% methanol. To avoid this effect the circular TLC method can be used (see Fig. 9).
Sennoside D (R_f ~ 0.55) is more highly concentrated in Sennae folium extracts (2) than in Sennae fructus extracts (1). Sennoside C can be localized at R_f ~ 0.7. Rhein is detectable as a yellow zone at R_f ~ 0.8 and its 8-O-glucoside is found between sennoside D and C.

B Direct treatment of the TLC plate with the sodium metaperiodate reagent and heating for 5 min under observation at 100°C reveals green-yellow or dark brownish zones when evaluated under UV-365 nm. It is a fast detection method, but less sensitive compared with the HNO_3/KOH reagent.

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*) The commercial reference compound "sennoside A" contains small amounts of sennoside C and D. The reference compound "sennoside B" shows, in addition, sennoside A as minor component.

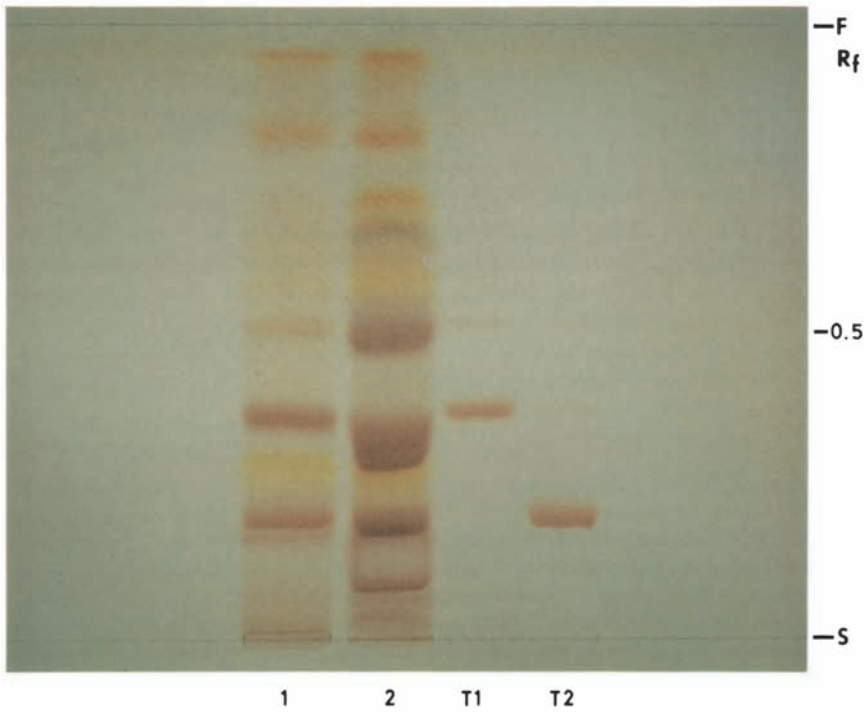

Fig. 7

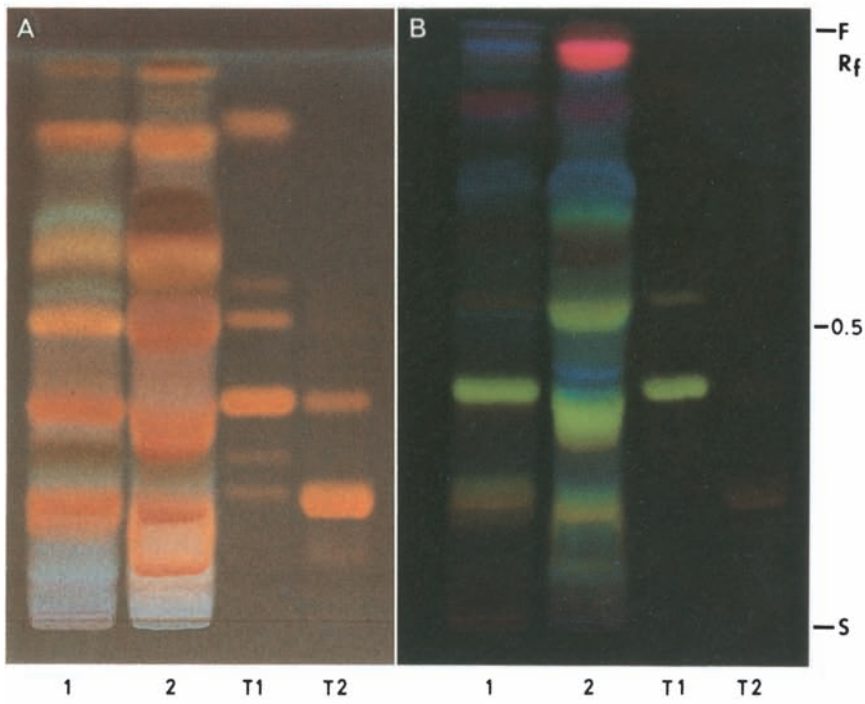

Fig. 8

Circular TLC (CTLC) in comparison to ascending TLC of Senna extracts

Drug sample, segment
Sennae folium (upper segment) *A* sennoside A *D* sennoside D
Sennae fructus (lower segment) *B* sennoside B *Al* aloin *Rh* rhein

Solvent system n-propanol-ethyl acetate-water-glacial acetic acid (40:40:29:1)

Detection Fig. 9 CTLC Sodium metaperiodate reagent (see 2.3 Detection) → vis
Asc. TLC HNO_3-potassium hydroxide reagent (HNO_3/KOH No. 30) → vis

Description The CTLC in general is a convenient method to achieve good separations over the short distance of 5–6 cm. Extracts and reference compounds are applied in the inner circle (start) in an overlapping mode, to make sure that compounds are clearly identified by references. Ballast substances of the extracts such as mucilagines are diluted in the circular separation lines. The disturbance and R_f value depression of sennoside A,B are reduced (preparation see 2.4 Circular TLC).

Fig. 9 The sennosides are detected as bright yellow-brown bands with sodium metaperiodate (CTLC) and as darker brown zones with the HNO_3-KOH reagent (asc. TLC).
The CTLC of Sennae folium und Sennae fructus shows as two prominent circles sennoside A and B (→ test *A/B*) in the inner parts of both segments. The bands of sennoside D (→*D*) and C are found slightly below the aloin test (→ test *Al*). Rhein (test *Rh*) is clearly seen in Sennae fructus extracts. The influence of mucilagines on the R_f value of sennoside B results in a dwelling circle (CTLC) and causes an R_f value depression in the picture of the ascending TLC (compare with Figs. 7,8).

Hyperici herba

Drug sample
1 Hyperici herba (Hypericum perforatum) (methanolic extracts, 25 µl)
2 Hyperici herba (commercial trade sample)

Reference compound
T1 hypericin
T2 rutin (R_f 0.35) ▶ chlorogenic acid (R_f 0.4) ▶ hyperoside (R_f 0.5) ▶ isochlorogenic acid

Solvent system Fig. 10 A,B ethyl acetate-formic acid-glacial acetic acid-water (100:11:11:26)
C toluene-ethyl formate-formic acid (50:40:10)

Detection
A,B Natural products-polyethylene glycol reagent (NP/PEG No. 28);
A UV-365 nm, B vis.
C 10% pyridine in ethanol → vis

Fig. 10A **Hyperici herba** (1,2) is characterized in UV-365 nm after treatment with NP/PEG reagent by the prominent red-violet fluorescent zones of the non-laxative dehydrodianthrons, the hypericins (R_f 0.75–0.8), five bright yellow fluorescent flavonolglycosides (R_f 0.35–0.7) and blue fluorescent phenol carboxylic acids such as chlorogenic acid (R_f ~ 0.4/T2). The flavonolglycosides are identified as rutin (R_f ~ 0.35/T2), hyperoside (R_f ~ 0.5/T2), isoquercitrin (R_f ~ 0.6) and quercitrin (R_f ~ 0.7). The aglycones, e.g. quercetin, migrate with the red fluorescent chlorophylls to the solvent front.

B Hypericins are seen as green-brown and the flavonolglycosides as orange-yellow zones (vis).

C Variation of the solvent system and the detection with pyridine reagent reveals a broad band of red zones in the R_f range 0.5–0.6 (T1). Red zones at R_f 0.9–0.95 show chlorophyll compounds.

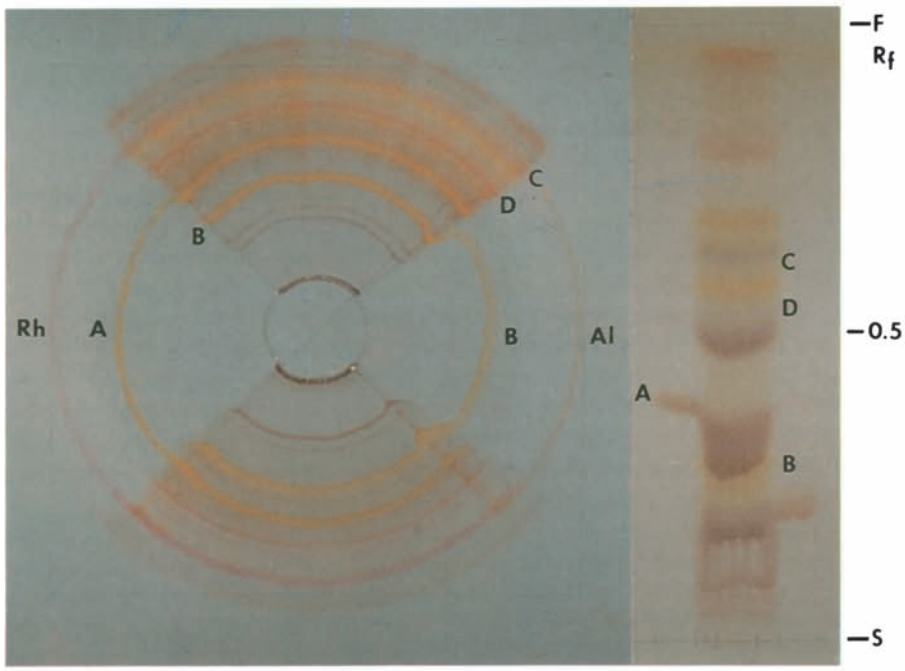

Fig. 9

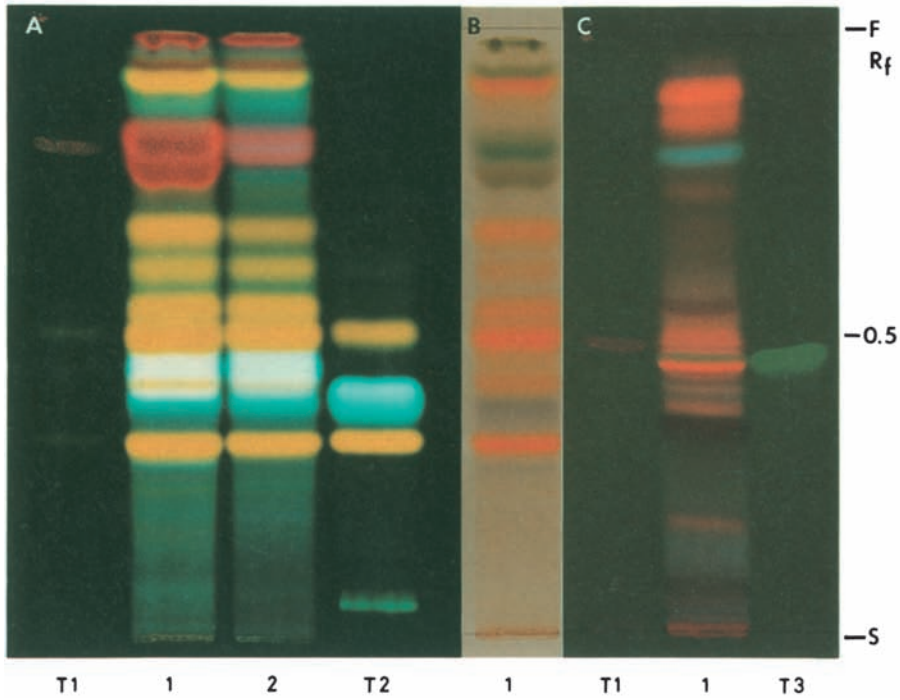

Fig. 10

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