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### Thin-layer chromatography of *Thalictrum* species alkaloids

Plants belonging to the *Thalictrum* L. species contain numerous basic components representing various types of structure. The identified compounds belong to the following groups: (I) isoquinoline alkaloids, 2 substances; (II) benzylisoquinoline alkaloids, 2 substances; (III) aporphine alkaloids, 8 substances; (IV) bisbenzylisoquinoline alkaloids, 18 substances; (V) aporphinobenzylisoquinoline alkaloids, 4 substances; (VI) hexahydrotriphenilidine alkaloids, 2 substances; (VII) protoberberine alkaloids, 9 substances, and (VIII) glucoalkaloids, at least 46 substances altogether.

The majority of the alkaloids consists of tertiary compounds, the rest is composed of quaternary substances; the separation of both types takes place during extraction or purification of the extracts.

In the course of investigating the compounds contained in the roots of *Thalictrum minus* L. var. *elatum* Koch. a fraction of tertiary products was obtained which showed 11 spots on the chromatograms. The fraction yielded two products identified as aporphinobenzylisoquinoline alkaloids: a new alkaloid thalmineline, the structure of which could be fully elucidated<sup>5</sup>, and another product designated 'compound C'<sup>3</sup>.

The eleven-spot fraction of quaternary bases yielded berberine, magnoflorine, jatrorrhizine, and thaliphenidine<sup>3</sup> as pure substances.

The initial tests on the constituents of the above-mentioned plant were carried out by thin-layer chromatography (TLC), the details of the procedure being worked out with standard substances, including 16 tertiary bases and 6 quaternary ones, obtained from various research centres.

#### Experimental

The plates covered with Silica Gel G or Aluminium Oxide according to Stahl were activated at 120° for 2 h.

The following systems were found in the course of numerous experiments as the most convenient ones for the separation of tertiary alkaloids.

For plates covered with Silica Gel G:

System A, benzene-chloroform-isopropanol-ammonia (90:90:16:20).

System B, benzene-ethyl acetate-methanol (75:75:100); a modification of PFEIFER's<sup>4</sup> system.

System C, benzene-chloroform-methanol-ethyl acetate (2:7:3:1); a system developed by PFEIFER<sup>4</sup>.

For plates covered with Aluminium Oxide G:

System D, benzene-chloroform (1:9) (ref. 1).

System E, ether.

The quaternary alkaloids were chromatographed on Silica Gel G according to Stahl in the solvent chloroform-methanol-ammonia (75:30:5) (system F). Berberine, palmatine, and columbamine which cannot be separated with system F were chromatographed on the same sorbent in system G which consisted of chloroform-methanol-pyridine-monoethylamine (50% aqueous solution) (100:40:10:5) (ref. 2).

The following detection reagents were employed: (a) Dragendorff's reagent;

TABLE I

*R<sub>F</sub>* VALUES OF SELECTED TERTIARY ALKALOIDS FOUND IN THE THALICTRUM SPECIES

Designation	System				
	A	B	C	D	E
Thaliphendlerine (II)	0.43	0.69	0.77	0.10	0.37
Laudanosine <sup>a</sup> (II)	0.97	0.49	0.72	0.78	0.71
Isocorydine (III)	0.95	0.72	0.85	0.89	0.89
Glaucine (III)	0.98	0.69	0.89	0.87	0.85
Obamegine (IV)	0.35	0.23	0.48	0.10	0.08
Berberamine (IV)	0.48	0.29	0.56	0.10	0.22
Hernandesine (IV)	0.79	0.45	0.73	0.65	0.00
Thalidasine (IV)	0.80	0.78	0.89	0.55	0.80
Thalidesine (IV)	0.50	0.00	0.63	0.00	0.00
Thalmetine (IV)	0.60	0.79	0.85	0.00	0.00
O-Methylthalmetine (IV)	0.70	0.83	0.68	0.69	0.15
Thalmelatine (V)	0.55	0.30	0.59	0.09	0.09
Thalicarpine (V)	0.70	0.30	0.67	0.70	0.70
Thalmineline (V)	0.45	0.71	0.81	0.21	0.10
Tetrahydrothalifendine (VII)	0.50	0.43	0.24	0.00	0.00
Veronamine (VIII)	0.07	0.54	0.51	0.00	0.00

<sup>a</sup> Has not been discovered in *Thalictrum* sp. up to the present.

(b) diazotized *p*-nitraniline (aromatic hydroxy groups); (c) Labat reagent (dioxy-methylene groups).

The alkaloids were spotted on the plates in 20  $\mu$ g amounts as chloroform or methanolic solutions.

The chromatograms were developed for 12 cm. After drying, they were inspected in UV light and sprayed with the Dragendorff reagent, Labat reagent, and with diazotized *p*-nitraniline. The *R<sub>F</sub>* values are reported in Tables I and II.

The results of two-dimensional chromatography are shown in Fig. 1.

### Discussion

Tables I and II report all the *R<sub>F</sub>* values found for the alkaloids studied. Each of the systems recommended for the tertiary alkaloids may be successfully used for the separation of a mixture consisting of several compounds, *e.g.* as a fraction isolated from plant material.

TABLE II

*R<sub>F</sub>* VALUES OF SOME QUATERNARY ALKALOIDS FOUND IN THE THALICTRUM SPECIES

Designation	<i>R<sub>F</sub></i> value in system F	-OH	-CH <sub>2</sub> -O-CH <sub>2</sub> -
Berberine	0.75		+
Palmatine	0.75		
Columbamine	0.75	+	
Magnoflorine	0.43	+	
Jatrorrhizine	0.65	+	
Thaliphendine	0.88	+	+

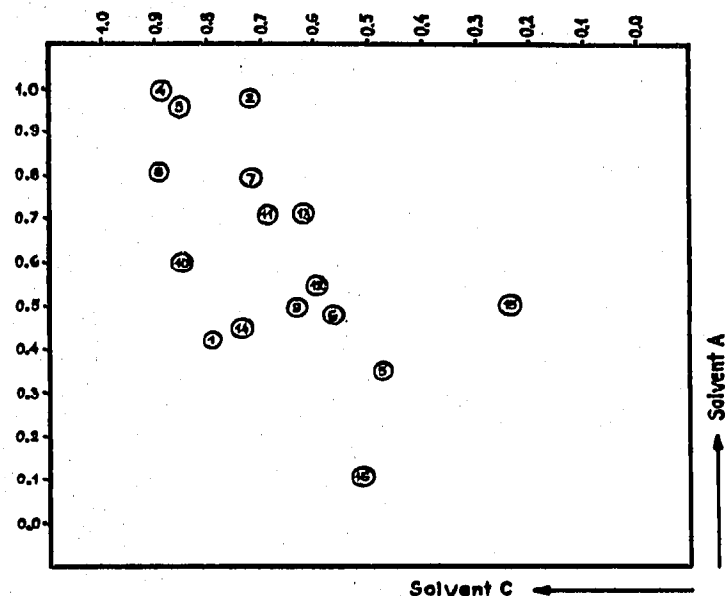


Fig. 1. Two-dimensional chromatogram of a mixture of tertiary alkaloids. 1 = Thaliphendlerine; 2 = laudanosine; 3 = isocozydine; 4 = glaucine; 5 = obamegine; 6 = barbamine; 7 = hernandesine; 8 = thalidasine; 9 = thalidesine; 10 = thalmetine; 11 = O-methylthalmetine; 12 = thalmelatine; 13 = thalicarpine; 14 = thalmineline; 15 = tetrahydrothalifendine; 16 = veronamine. Solvent systems: (A) benzene-chloroform-isopropanol-ammonia (90:90:16:20); (C) benzene-chloroform-methanol-ethyl acetate (2:7:3:1).

In practice, phenolic bases and non-phenolic ones were chromatographed separately.

Application of two-dimensional chromatography with systems A and C, or A and B, permits the separation of 16 alkaloids, as shown in Fig. 1.

In our experiments on the isolation of the alkaloids from a fraction obtained from *Th. minus* var. *elatum* Koch, some bases were found which had identical  $R_F$  values to standard substances, sometimes even in two solvent systems. Further tests, however, did not confirm their identity. For full chromatographic identification it is thus necessary to test all five of the recommended systems.

System F, developed for the quaternary alkaloids, is unsuitable for berberine, palmatine, and columbamine which, in this system, have identical  $R_F$  values.

System F may, however, be used successfully for the separation of the majority of the alkaloids present in various genera of the *Thalictrum* species. It was tested for *Th. elegans* Wall. (5 spots); *Th. fendleri* Engelm. (11 spots); *Th. minus* var. *elatum* Koch. (11 spots); *Th. minus* var. *nanus* (10 spots); *Th. pedunculatum* Edgew. (11 spots); *Th. rhinocarpum* Dill. et Rich. (9 spots); and *Th. squarosum* (9 spots). The largest spots were always those of magnoflorine and berberine.

Berberine, palmatine, and columbamine were identified in system G, the alkaloids showing  $R_F$  values of 0.48, 0.40, and 0.15, respectively.

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