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DETECTION AND THIN-LAYER CHROMATOGRAPHY OF SESQUITERPENE LACTONES FROM GEIGERIA SPECIES

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SUMMARY

Three solvent systems for the thin-layer chromatographic separation of sesquiterpene lactones from *Geigeria aspera* and *Geigeria filifolia* have been investigated. Eighteen different colouring reagents were tested. Reagents containing strong mineral acids were found to be the most suitable for locating the sesquiterpene lactones.

INTRODUCTION

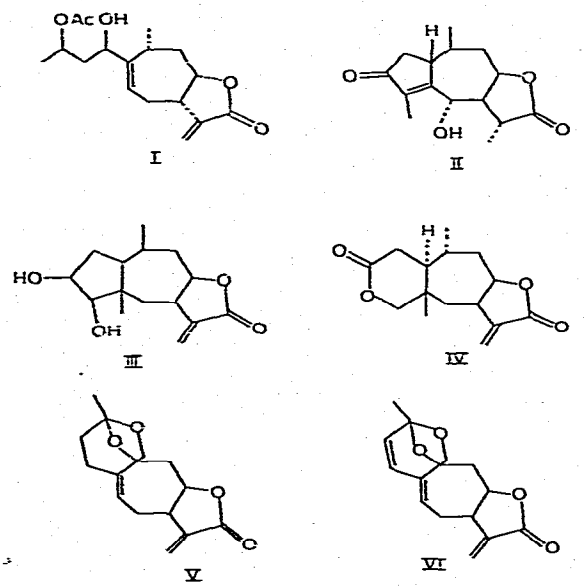
Sesquiterpene lactones are currently being investigated for a variety of reasons. The following properties of these compounds have been described: carcinogenicity[1–3], cytotoxicity[4] and antitumor activity[5–9], allergic contact dermatitis and toxicity to livestock[10–12]. Other properties ascribed to sesquiterpene lactones include skin irritation, sternutative and antibacterial action and vermifugal and insecticidal activity[13]. Its use as fish poison, cordial drug and stomachic and as a remedy for a number of ailments have also been described[13]. Numerous workers have described thin-layer chromatographic (TLC) procedures for the identification of sesquiterpene lactones[14–19]. In the course of a study of sesquiterpene lactones present in *Geigeria aspera* and *Geigeria filifolia*, however, it was necessary to develop a reliable TLC system for the separation and identification of known and unknown sesquiterpene lactones[20].

This paper reports on TLC systems developed for the separation of known sesquiterpene lactones, namely gaffrinin (I), geigerin (II), geigerinin (III), vermeerin (IV), griesenin (V) and dihydrogriesenin (VI)[16,19,21–26], from *Geigeria aspera* and *Geigeria filifolia* and on the location of these lactones with eighteen different reagent sprays.

EXPERIMENTAL

TLC plates

A mixture of silica gel G (according to Stahl, E. Merck, Darmstadt, G.F.R.) and distilled water in mass proportions of 1:2 was vigorously shaken for 60 sec. This mixture was spread on clean 13 × 9 cm glass plates to a thickness of approximately



200 μm by means of an applicator. The plates were allowed to set and were then dried and activated for 60 min in an oven at 110°. After cooling, the plates were stored in a desiccator. Pre-coated plates (Merck $60F_{254}$) were also used.

Solvent systems

Three combinations were examined: (A) chloroform–methanol, 96:4; (B) hexane fraction (68–70°)–isobutanol, 7:3; and (C) 2-butanone–light petroleum (b.p. 60–80°), 1:1.

Chromatographic procedure

Volumes of 2 μl, representing 10 μg of compound, were applied 1 cm from the edge of the plate. The plate was placed in a chromatographic tank lined with filter paper, previously saturated with the solvent system, for at least 10 min and developed over a distance of 10 cm in about 10–15 min (ambient temperature 18–22°).

Detection

The developed plates were first placed in a closed rectangular jar containing a few iodine crystals. Yellow spots developed and these spots were circled with a dotted line. Subsequently a dyeing reagent prepared according to Stahl[27], Merck[28] and Randerath[29] was applied with a glass spraying apparatus. The colours that developed at room temperature and after heating at 110° for 10 min and for longer periods were recorded. In some instances the dyed spots displayed characteristic fluorescence when viewed under UV light.

RESULTS AND DISCUSSION

R_F values are given in Table I. The R_F values for the sesquiterpene lactones separated on silica gel G plates prepared in our laboratory were slightly higher than

TABLE I

R_f VALUES OF SESQUITERPENE LACTONES ON SILICA GEL G OR $60F_{254}$ WITH THREE MOBILE PHASES

Compound	*A*		*B*		*C*	
	G	$60F_{254}$	*G*	$60F_{254}$	*G*	$60F_{254}$
Gafrinin* (I)	0.44	0.38	0.45	0.45	0.37	0.31
Geigerin* (II)	0.36	0.31	0.30	0.32	0.30	0.26
Geigerinin** (III)	0.20	0.19	0.15	0.12	0.11	0.06
Vermeerin* (IV)	0.26	0.25	0.61	0.53	0.35	0.29
Dihydrogriesenin** (V)	0.61	0.49	0.68	0.61	0.51	0.43
Griesenin** (VI)	0.51	0.43	0.66	0.57	0.46	0.41

* A generous gift from Dr. L. A. P. Anderson.
** Isolated in our laboratory, see ref. 20.

those obtained on silica gel $60F_{254}$. With the aid of the three solvent systems and appropriate colouring reagents, each of the six compounds could be identified. All three eluents proved especially useful for the identification of vermeerin, which is relatively difficult to colour.

The results on the detection of some sesquiterpene lactones are given in Table II. A number of detection reagents were found to possess special colouring capacities. Dyeing with iodine vapour gave rise to yellow spots that gradually became dark brown. An obvious advantage of this method is that the plates become colourless after removal of the iodine by exposure to the atmosphere. It was found that isobutanol (b.p. 99°), if not completely evaporated from plates before exposure to iodine vapour, adversely affected the effectiveness of this colouring reagent.

With anisaldehyde–sulphuric acid, griesenin gave a red colour at room temperature, while gafrinin appeared only when the plates were heated slightly with hot air from a hair-drier. Vermeerin appeared only after heating at 110° for 30–60 min.

3,5-Dinitrobenzoic acid, used in the Kedde reaction for digitaloid five-membered ring lactones, gave a purple colour with geigerin only, both at room temperature and at 110°. This reagent was employed to characterise geigerin in complex mixtures.

Antimony trichloride gave only a slightly purple colour at 110° with gafrinin.

In general, the results indicate that the stronger mineral acids possess good colouring properties. This is in accordance with results in the literature, where Lee *et al.*[14] used 50% sulphuric acid to detect helenalin esters and related derivatives. Geissman and co-workers[15,30], on the other hand, employed concentrated sulphuric acid to detect a variety of sesquiterpene lactones. Griffin *et al.*[30] developed a colouring reaction using equal volumes of ethanol and concentrated hydrochloric acid. The colour produced could be measured spectrophotometrically and was used as an aid in structure determinations. The red and blue colours were correlated[15,30] with a number of structural parameters.

In the present study, all of the experiments were carried out with 10-μg amounts, but the detection limits are estimated to be lower for most of the colouring reagents. Inspection under short-wavelength UV light or at 360 nm after spraying with colouring reagents lowered the detection limits in some instances.

It was found that the following detection sequence was the most reliable for

TABLE II

DETECTION CHARACTERISTICS OF SOME SESQUITERPENE LACTONES

Dyeing reagent	*No. of reagent in*		*Temperature of treatment of plate (°C)**	*Wave-length at which viewed (nm)***	*Colour developed by compound on silica gel G****					
	Ref. 28	*Ref. 27*			*Gafrinin (I)*	*Geigerin (II)*	*Geigerinin (III)*	*Vermeerin (IV)*	*Dihydro-griesenin (V)*	*Griesenin (VI)*
Vanillin-ortho-phosphoric acid	—	149	RT	DL	Light purple		—	—	—	—
			110	DL	(Brownish) purple	Light orange	—	—	Brown	Purple
Orthophosphoric acid–water (1:1)	174	—	110	DL	Brown		—	—	Beige	Purple
			110	360	White	Blue-purple	White	—	Yellow	Orange-red
Cinnamaldehyde	214	—	RT	DL	Light blue		—	—	Yellow	Red
			110	DL	Blue		—	—	Brown	Red
Cinnamaldehyde	215	—	RT	DL	—	—	—	—	Yellow	Blue
Sulphuric acid–water (65:35)	—	—	110	DL	Orange	Yellow (brown)	Red (brown)	(Brown)	Yellow	Brown
			110	360	Whitish blue	(Purple)	Light blue	(Light blue)	Dark spot	Yellow
Anisaldehyde, glacial acetic acid, sulphuric acid	11	9	RT	DL	—	—	—	—	—	Red
			RT	360	Light yellow	—	—	—	Light beige	Orange
			110	DL	Blue-purple (brown)	Yellow (orange)	Yellow-green (green)	(Beige)	Yellow brown (brown)	Red
			110	360	—	(Yellow)	—	—	Yellow-green	—
4-Dimethylaminobenz-aldehyde, phosphoric acid	62	47	110	DL	Brown-purple	Light yellow	—	Light yellow	Light brown	Blue
			110	360	Yellow	—	—	—	Light yellow	Yellow
3,5-Dinitrobenzoic acid	72	51	RT	DL	—	Purple	—	—	—	—
			110	DL	—	Purple	—	—	—	—
Trichloroacetic acid	208	143	110	DL	Grey	—	—	—	—	Yellow
			110	UV	Yellow	—	—	—	Light yellow	Yellow

TABLE II *(continued)*

Dyeing reagent	*No. of reagent in*		*Temperature of treatment of plate (°C)**	*Wave-length at which viewed (nm)***	*Colour development by compound on silica gel G****					
	Ref. 28	*Ref. 27*			*Gafrinin (I)*	*Geigerin (II)*	*Geigerinin (III)*	*Vermeerin (IV)*	*Dihydro-griesenin (V)*	*Griesenin (VI)*
Antimony trichloride	–	11	110	DL	Light purple	–	–	–	–	–
			110	360	Yellow	–	–	–	Blue-white	Yellow
Potassium permanganate, copper acetate		See ref. 17	RT	DL	Yellow	Yellow	Yellow	Yellow	Yellow	Yellow
			110	DL	–	–	–	–	–	–
Hydrochloric acid	188	–	110	DL	Light red-purple	–	–	–	–	–
p-Toluenesulphuric acid	207	142	110	DL	Brown-purple	Light yellow	–	–	Yellow	Black-purple
			110	360	Orange-yellow	Blue	Light yellow	–	Green	Yellow-brown
Ceric sulphate, sulphuric acid	33	–	110	DL§	Purple	White	Light yellow	White	Brown	Brown
			110	360	Orange	Blue	Yellow	Light blue	Blue-purple	Yellow
Phosphomolybdic acid	172	–	110	DL§	Blue-purple	Blue	Purple-brown	Blue-green	Blue-purple	Blue-purple
Chloramine, trichloroacetic acid	39	31	110	DL	Light yellow	–	–	–	Light yellow	Light yellow
			110	360	Light yellow	–	–	Light beige	Light yellow	Yellow
Iodine vapour	109	–	RT	DL	Yellow-brown	Yellow-brown	Yellow-brown	Yellow-brown	Yellow-brown	Yellow-brown
			110	DL	–	–	–	–	–	–
Nitroprusside, sodium hydroxide	163		110	DL	–	–	–	Light yellow	–	–

* RT = room temperature.
** DL = daylight.
*** Parentheses indicate colour after prolonged heating at 110°.
§ Colour of background remains after heating and the colours of compounds are contrasted against it.

identification purposes: UV inspection (254 and 360 nm) for absorption or fluorescence, followed by staining with iodine vapour and subsequent removal of iodine by heating. This is followed by spraying with the appropriate dyeing reagent (anisaldehyde–sulphuric acid or 65% sulphuric acid), heating at 110° for 10 min and then UV inspection.

For the detection of known and unknown sesquiterpene lactones, dyeing reagents that contain strong mineral acids are recommended because they are sensitive and give rise to fairly distinctive colours.

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