

CHROM. 12,437

VISUALIZATION REAGENTS FOR SESQUITERPENE LACTONES AND POLYACETYLENES ON THIN-LAYER CHROMATOGRAMS

ANNA K. PICMAN*

Department of Botany, University of British Columbia, Vancouver, B.C. V6T 1W5 (Canada)

R. L. RANIERI

W. R. Grace & Co., Washington Research Center, 7379 Route 32, Columbia, Md. 21044 (U.S.A.)

G. H. N. TOWERS*

Department of Botany, University of British Columbia, Vancouver, B.C. V6T 1W5 (Canada)

and

JØRGEN LAM

Department of Organic Chemistry, University of Aarhus, 8000 Aarhus C. (Denmark)

(Received October 12th, 1979)

SUMMARY

Vanillin and *p*-dimethylaminobenzaldehyde were found to be the most useful of several benzaldehyde and benzoic acid derivatives for the detection of sesquiterpene lactones and polyacetylenic compounds on thin-layer chromatography (TLC). Colors produced with these two reagents and with 70 sesquiterpene lactones and 25 polyacetylenes were of all shades of the spectrum and were specific for individual compounds. Therefore these reagents can be used as an aid in the identification of these compounds on TLC. These spray reagents are the most sensitive of all reagents which have so far been used for the detection of sesquiterpene lactones or polyacetylenes on TLC.

INTRODUCTION

The *Asteraceae* (*Compositae*) family of plants characteristically contain two biologically active types of secondary metabolites, namely sesquiterpene lactones and polyacetylenes¹. In spite of an increasing interest in the cytotoxic, antibiotic, phototoxic and antineoplastic activities of these compounds^{2,3} satisfactory reagents for their visualization on thin-layer chromatograms have not been reported. The techniques generally used for the detection of sesquiterpene lactones on thin-layer (TLC) or paper chromatography (PC) are UV light^{4,5}, exposure to iodine vapours^{6,7}, spraying with a solution of KMnO_4 ⁷ or with concentrated H_2SO_4 ⁸, followed by heating⁹. These methods are generally unspecific giving the same color reaction with almost all unsaturated compounds. Acid catalyzed color reactions of certain ses-

* To whom correspondence should be addressed.

quiterpene lactones have been described^{8,10}, but the technique is restricted to those lactones which form color complexes in acidic solution. Polyacetylenic compounds are often identified by their highly characteristic UV spectra¹¹ but, with the exception of thiophenes¹², no simple reliable method for the visualization of these compounds by TLC or PC has been described.

We report here the use of several acidic reagents containing either vanillin or related benzaldehyde or benzoic acid derivatives which are highly sensitive for these compounds and which give characteristic colors with individual sesquiterpene lactones or polyacetylenes after TLC visualization on silica gel.

Spray reagents containing vanillin in mineral acids are commonly used in the detection of steroids¹³⁻¹⁵, higher alcohols, phenols, essential oils¹⁵, catechols, ketones¹⁶ and cannabinoids (Duquenois reagent¹⁷). An acidic solution of *p*-dimethylaminobenzaldehyde is a well-known spray reagent for azulenes, indole derivatives, urea and ureides, and nitro compounds¹⁶. Other structurally similar benzaldehydes have also been reported for use as spray reagents although mostly for steroid and alkaloid detection¹⁶.

EXPERIMENTAL

Chemicals

Sesquiterpene lactones, melcanthin-B, confertiflorin, cinerenin, melampodin-A and -E, enhydrin and melampodinin were kindly provided by Dr. N. H. Fisher (Louisiana State University, Baton Rouge, La., U.S.A.), helenalin, cumarin, eupatoriopicrin and santamarine by Dr. A. Romo de Vivar (Universidad Nacional Autonoma de Mexico, Mexico), ambrosin, damsine, tenulin, isotenulin, tetraeurin-A, -B, -D and -E, conchosin-B, and hymenin by Dr. E. Rodriguez (University of California, Irvine, Calif., U.S.A.), glaucolide-A, -B, -D, -E, -F and -G and marginatin by Drs. T. J. Mabry and M. Betkouski (University of Texas, Austin, Texas, U.S.A.), quadron by W. R. Grace & Co. (Columbia, Md., U.S.A.) and frullania lactone by Dr. G. Ourisson (University of Strasbourg, France). Alantolactone and isoalantolactone (as a mixture "Helenin") were purchased from Sigma (St. Louis, Mo., U.S.A.) and santonin from Fluka, (Buchs, Switzerland). All other sesquiterpene lactones were provided by the late Professor T. A. Geissman (University of California, Los Angeles, Calif., U.S.A.) or were previously isolated in our laboratory (University of British Columbia) from various plant sources. Methyl 2-thienyl ketone was purchased from Eastman-Kodak (Rochester, N.Y., U.S.A.) and thiophene from ICN Pharmaceuticals (Cleveland, Ohio, U.S.A.). All sesquiterpene lactones and polyacetylenes were used without further purification. Other chemicals which were used as standards or in spray solutions were obtained from common commercial sources and were used without further purification. Polyacetylenes were isolated from various plant materials by one of us (I.L.).

Spray reagents

0.5 g of vanillin, *p*-dimethylaminobenzaldehyde, *p*-hydroxybenzaldehyde, salicylaldehyde, *m*-anisaldehyde, cinnamaldehyde, *p*-hydroxybenzoic acid or vanillic acid, was dissolved in a solution consisting of 9 ml of ethanol (95%), 0.5 ml of concentrated sulphuric acid and three drops of acetic acid. This reagent should be freshly prepared before use.

TLC plates

Silica gel plates, without gypsum and with fluorescent indicator (Polygram; Brinkmann, Westbury, N.Y., U.S.A.), were spotted with 5–10 μg of each compound. Sesquiterpene lactones were dissolved in chloroform or acetone, polyacetylenes in ethanol, other compounds (Table IV) in various solvents. The plates were developed in a standard chamber (without chamber saturation) with chloroform–acetone (6:1). After chromatography the plates were air dried, sprayed with the reagent solution and directly placed on a hot plate (TekPro HeatStir 36, Scientific Products) and slowly heated (temperature 70°). When vanillin was used as a spray reagent the background become yellowish and with continued heating slightly violet. Colors formed by reaction with the compounds tested were recorded at 10 min after this treatment (Table IV) and also 24 h later (Table I). Some polyacetylenes changed colors after 48 h (Table II) where there was no change of color after 24 h. The colors formed are unstable and change on standing. Some preservation was achieved by wrapping plates in plastic film (Saran Wrap) and storing plates in dark. The colors were recorded using the Methuen Handbook of Colour¹⁸.

Reagent sensitivity test

Each of five sesquiterpene lactones (Table III), in acetone solution, was applied in quantities ranging from 0.05 to 20.0 μg on TLC plates and developed as described above. Developed plates were visualized under UV light or exposed to iodine vapours or sprayed with a solution of either KMnO_4 , vanillin or *p*-dimethylaminobenzaldehyde reagent followed by heating. The minimum quantities of sesquiterpene lactones forming visible spots on the chromatograms were recorded. In the same way the sensitivities of vanillin and *p*-dimethylaminobenzaldehyde were tested with two natural polyacetylenes, phenylheptatriyne (20 in Table II) and the thiophene derivative, α -terthienyl (25 in Table II). The sensitivity of isatin was tested on the latter compound. The minimum quantities of both polyacetylenes (in ethanol) still showing distinct characteristic curves of their UV spectra were recorded (Unicam Sp. 800).

Test of various reagents for their reactions with sesquiterpene lactones

5–10 μg of each of twelve selected sesquiterpene lactones (tamaulipin-A, alantolactone, isoalantolactone, pulchellin-C, parthenin, coronopilin, helenalin, desacetoxymatricarin, coumambriin-B acetate, tenulin, isotenulin and xanthinin) in chloroform or acetone solution were spotted on thirteen silica gel plates. Each plate was directly (without developing) sprayed with one of the spray reagents listed above or 5% aqueous solution of KMnO_4 , concentrated H_2SO_4 , or ethanol–hydrochloric acid (1:1) followed by heating to obtain the most intensive colors or was exposed to iodine vapours. Colors were recorded during first 10 min and after 24 h.

RESULTS

Of the reagents tested for TLC visualization of sesquiterpene lactones in acidic solutions only benzaldehydes and vanillic acid produced distinct colors with individually tested compounds. In contrast to this other reagents (iodine vapours, KMnO_4 solution, concentrated H_2SO_4 and ethanol with hydrochloric acid) produced

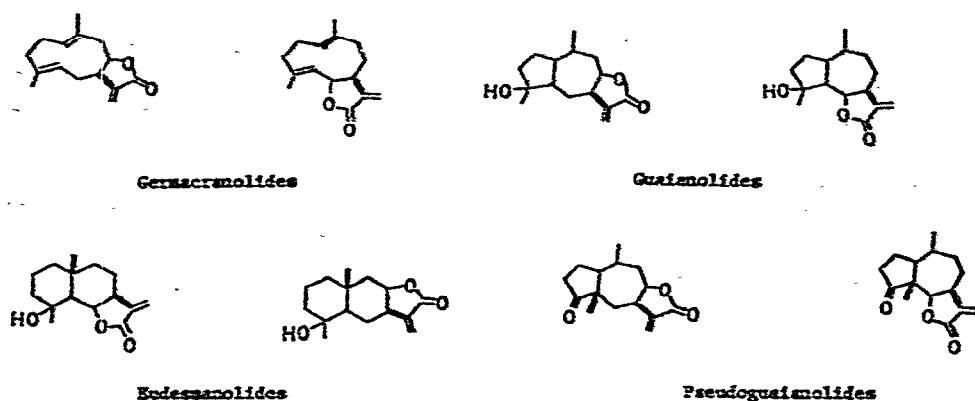


Fig. 1. Basic skeletal classes of sesquiterpene lactones.

only greyish to brownish colors with sesquiterpene lactones. The ethanol-HCl spray gave distinct colors with some sesquiterpene lactones, but, however, did not give any color with others. Sesquiterpene lactones representing all the basic skeletal classes (Fig. 1) and a few modified sesquiterpene lactones (altogether 70; Table I)

TABLE I

COLOR REACTIONS OF SESQUITERPENE LACTONES WITH VANILLIN OR *p*-DIMETHYLAMINO-BENZALDEHYDE SPRAY REAGENT

Arrows indicate changes during heating of the plate. First color recorded after 10 min; second after 24 h.

Sesquiterpene lactone	Reagent		R_F (chloroform-acetone, 6:1)	Structure (ref.)
	Vanillin	<i>p</i> -Dimethylamino-benzaldehyde		
<i>Germacranolides</i>				
Parthenolide	Dull blue	Greyish ruby	0.83	19
	Dull violet	Light brown		
Pyrethrosin	Dull green	Brownish red	0.80	19
	Light brown	Orange		
Mikanolide, dihydro-	Bluish grey	Brownish orange	0.52	19
	Grey	Brownish orange		
Chamissonin, diaceto-	Blue	Brownish grey	0.83	19
	Greyish yellow	Brownish grey		
Tamaulipin-A	Greyish violet	Dark brown	0.52	19
	Greenish grey	Dark brown		
Tamaulipin-B	Grey	Brown	0.57	19
	Grey	Light brown		
Eupatoriopicrin	Dull green	Dark brown	0.15	19
	Greyish brown	Dark brown		
Melampodin-A	Dull blue	Greyish brown	0.43	20
	Bluish grey	Olive brown		
Melampodin-B	Greyish green	Brownish orange	0.60	21
	Dull blue	Brownish orange		

TABLE I (continued)

Enhydrin	Violet	Light brown	0.77	20
	Pastel violet	Greyish green		
Cimerenin	Grey	Brownish orange	0.19	21
	Grey	Brownish orange		
Melampodimin	Greyish blue	Greyish brown	0.50	20
	Grey	Greyish brown		
Melcanthin-B	Dull blue	Greyish brown	0.07	22
	Reddish grey	Greyish brown		
Glaucolide-A	Olive grey	Purple→light brown	0.75	23
	Greyish violet	Brownish grey		
Glaucolide-B	Olive grey	Light brown	0.68	23
	Greyish violet	Brownish grey		
Marginatin	Dark ruby	Olive brown	0.86	24
	Violet-brown	Olive brown		
Glaucolide-D	Dark violet	Light brown	0.70	25
	Dark violet	Light brown		
Glaucolide-E	Dark violet	Light brown	0.80	25
	Dark violet	Light brown		
Glaucolide-F	Olive grey	Purple→light brown	0.74	28
	Greyish violet	Brownish grey		
Glaucolide-G	Dark ruby	Olive brown	0.87	28
	Violet-brown	Olive brown		
<i>Guaianolides</i>				
Coumambrin-A	Dark blue	Reddish brown	0.59	19
	Dull green	Light brown		
Coumambrin-B	Greyish turquoise	Light brown	0.42	19
	Dull green	Light brown		
Coumambrin-B, dihydro-	Dark blue	Reddish brown	0.32	29
	Dull green	Light brown		
Coumambrin-B, tetrahydro-	Dark blue	Brownish red	0.14	29
	Dark purple	Reddish brown		
Coumambrin-B, acetate	Greyish turquoise	Reddish brown	0.61	29
	Dull green	Light brown		
Coumambrin-B, formyl	Greyish turquoise	Light brown	0.55	29
	Dull green	Light brown		
Matricarin	Purplish red	Greyish yellow	0.84	19
	Greyish red	Greyish yellow		
Matricarin, desacetoxy-	Greyish ruby	Yellow	0.83	19
	Purplish pink	Yellow		
Grossheimin	Greyish yellow	Greyish yellow	0.43	19
	Greyish yellow	Greyish yellow		
<i>Eudesmanolides</i>				
Alantolactone	Dull blue	Violet-brown	0.91	19
	Grey	Greyish magenta		
Alantolactone, tetrahydro-	Dull blue	Violet-brown	0.91	19
	Blue	Greyish magenta		
Alantolactone, iso-	Dull blue	Violet-brown	0.91	19
	Bluish grey	Greyish magenta		
Ivalin	Purple	Dark brown	0.51	19
	Greenish grey	Purplish grey		
Ivasperin	Violet-blue	Light brown	0.15	19
	Greenish grey	Light brown		

(Continued on p. 192)

TABLE I (continued)

Pinnatifidin	Bluish red→red Yellowish orange	Vivid yellow Vivid yellow	0.65	19
Pulchellin-C	Brownish red Greyish ruby	Greyish orange Greyish orange	0.14	19
α-Santonin	Greyish brown→ dull green	Brownish orange	0.81	19
Ludovicin-A	Greyish orange Bluish grey	Brownish orange Dark brown	0.58	19
Ludovicin-B	Brownish grey Bluish grey	Brownish grey Dark brown	0.41	19
Ludovicin-C	Brownish grey Dark blue	Brownish grey Greyish ruby	0.89	19
Reynosin	Brownish grey Dull blue	Greyish ruby Light brown	0.58, 0.49*	19
Frullania lactone	Violet grey Dark green	Light brown Greyish magenta	0.91	19
Santamarine	Olive Dark blue	Purplish grey Light brown	0.63	19
	Dark violet	Light brown		
<i>Pseudoguianolides</i>				
Ambrosin	Yellow→orange-red	Light orange	0.67	19
Damsin	Greenish yellow Vivid yellow	Light orange Golden yellow	0.73	19
Coronopilin	Yellowish white Blue	Golden yellow Yellow→orange-red	0.46	19
Parthenin	Greyish magenta Bluish green	Red Golden yellow	0.44	19
Hymenin	Greyish yellow Bluish green	Golden yellow Golden yellow	0.44	19
Confertiflorin, desacetyl-	Greyish yellow Greyish yellow	Golden yellow Greyish yellow	0.25	19
Tetraneurin-A	Greyish green Greyish green	Brownish yellow Brownish yellow	0.42	19
Tetraneurin-B	Blue Bluish violet	Brownish red Brownish red	0.44	19
Tetraneurin-D	Dull green Olive brown	Brownish orange Light brown	0.09	19
Tetraneurin-E	Dull green Olive brown	Greyish orange Greyish orange	0.09	19
Conchosin-A	Lilac Greyish blue	Pink→pastel blue Pastel blue	0.05	19
Conchosin-B	Yellowish green Light yellow	Golden Golden	0.42	19
Tenulin	Orange-red Brownish orange	Reddish orange Reddish orange	0.44	19
Tenulin, iso-	Greyish yellow	Yellow→greyish orange	0.71	19
Gaillardilin	Greyish yellow Brownish grey→ dull green	Greyish orange Greyish yellow	0.59	19
Helenaflin	Dull green Yellow→reddish orange	Greyish yellow Greyish yellow	0.42	19

TABLE I (continued)

Flexuosin-B	Greyish yellow Violet-red→ greyish orange Pale yellow	Greyish yellow Yellow→reddish golden Reddish golden	0.53	19
Spathulin	Black Brown	Golden brown Golden brown	0.07	19
Baldwinin	Olive yellow Greyish yellow	Yellow Yellow	0.63	19
Cumanin	Brownish yellow→ greyish brown Greyish brown	Brownish yellow→ greyish brown Light brown	0.21	19
Ivalin, pseudo-	Vivid violet→ dark blue Olive	Reddish brown Reddish brown	0.43, 0.35*	26
<i>Other sesquiterpene lactones</i>				
Xanthinin	Brownish red Ruby	Vivid yellow Orange yellow	0.82	19
Psilostachyin	Dull green Blue	Grey Grey	0.59	19
Psilostachyin-C	Dull green Greenish grey	Blue→grey Grey	0.50	19
Vernolepin	Grey Greyish brown	Dark yellow Dark yellow	0.28	19
Vernomenin	Light brown Grey	Dark yellow Dark yellow	0.29	19
Quadrone	Deep yellow→ light yellow Light yellow	Vivid yellow Yellow	0.84	27

* Two spots of identical color and intensity.

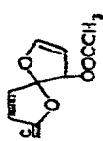
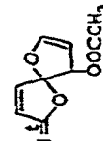

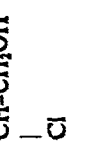
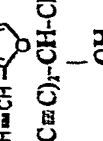

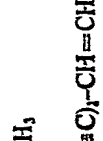
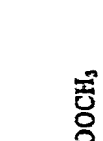


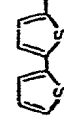

and 25 polyacetylenes (Table II) were tested with the vanillin and *p*-dimethylaminobenzaldehyde reagents. Vanillin was chosen because it produces a great range of colors and *p*-dimethylaminobenzaldehyde because it gives a different spectrum of colors when compared with vanillin. Vanillin was tested with a few representatives of other classes of compounds (Table IV). Monoterpenes, steroids and carotenoids gave very bright colors, fatty acids gave greyish colors and some aromatic acids did not give any color. Flavonoids generally produced yellow colors.

The smallest quantities of sesquiterpene lactones detectable on TLC by various reagents are shown in Table III. The most sensitive were vanillin and *p*-dimethylaminobenzaldehyde which can be used to detect 0.05 μg of certain sesquiterpene lactones. Dimethylaminobenzaldehyde, in some cases, was even more sensitive than vanillin. The smallest quantity of a sesquiterpene lactone which can be detected depends on the brightness and intensity of the colors developed on TLC. Thus tenulin (I) which produces bright orange-red colors with vanillin and *p*-dimethylaminobenzaldehyde is detectable on TLC at 0.05 μg while helenalin (II) which gives a weaker reddish orange color with vanillin and a greyish yellow color with *p*-dimethylaminobenzaldehyde is only detectable at 0.5 and 0.1 μg . Parthenin (V) which forms a bluish green color with vanillin can be detected at 0.5 μg but the golden yellow color with *p*-dimethylaminobenzaldehyde is visible at a concentration ten times lower. α -Terthienyl (25 in Table II) which gives a very bright color with

TABLE II

COLOR REACTIONS OF POLYACETYLENIC COMPOUNDS WITH VANILIN OR *p*-DIMETHYLAMINO-BENZALDEHYDE SPRAY REAGENTSArrows indicate changes during heating of the plate, t = *trans*; c = *cis*.

Compound	Reagent		R_f (chloroform- acetone, 6:1)
	Vanillin	<i>p</i> -Dimethylamino- benzaldehyde	
1 $\text{CH}_2\text{-(C}\equiv\text{C)}_2\text{-(CH}\equiv\text{CH)}_2\text{-CH-CH}_2\text{-CH}_2\text{-OH}$ 	Dull blue	Olive yellow	0.16
2 $\text{CH}_2\text{-(C}\equiv\text{C)}_2\text{-CH=CH}$ 	Greyish brown	Brownish orange	0.71
3 $\text{CH}\equiv\text{C-C}\equiv\text{C-CH=C}$ 	Deep orange	Reddish orange	0.87
4 $\text{CH}_2\text{-CH}_2\text{-CH}_2\text{-C}\equiv\text{C-CH=O}$ 	Bluish violet Blue*	Greyish magenta Violet*	0.83
5 $\text{C}_6\text{H}_5\text{-(C}\equiv\text{C)}_2\text{-CH=CH-CHO}$	Dark yellow	Olive yellow	0.91
6 $\text{C}_6\text{H}_5\text{-(C}\equiv\text{C)}_2\text{-CH=CH-CH}_2\text{OH}$	Brownish beige	Greyish yellow	0.63
7 $\text{CH}_2\text{-CH=CH-(C}\equiv\text{C)}_2\text{-CH=CH-CH}_2\text{CH}_2\text{-OH}$ 	Brownish beige	Olive yellow	0.17
8 $\text{CH}_2\text{-(CH=CH)}_2\text{-C}\equiv\text{C-CH=CH-OOCCH}_3$	Olive brown	Olive brown	0.92
9 $\text{CH}_2\text{-CH=CH-(C}\equiv\text{C)}_2\text{-(CH=CH)}_2\text{-CH=CH}_2$	Grey	Yellowish brown	0.93
10 $\text{CH}_2\text{-CH=CH-(C}\equiv\text{C)}_2\text{-CH}_2\text{-CH=CH-(CH}_2\text{)}_2\text{-CH=CH}_2$	Reddish grey	Light brown	0.93
11 $\text{CH}_2\text{-CH=CH-(C}\equiv\text{C)}_2\text{-(CH=CH)}_2\text{-OOCCH}_3$	Greyish brown	Yellowish brown	0.91
12 $\text{CH}_2\text{-CH=CH-(C}\equiv\text{C)}_2\text{-CH=CH-CH-CH=CH}_2$ 	Dull green	Yellowish brown	0.36
13 $\text{CH}_2\text{-(C}\equiv\text{C)}_2\text{-(CH=CH)}_2\text{-CH-CH}_2\text{-CH}_2\text{-OOCCH}_3$ 	Olive brown and deep magenta	Olive and greyish ruby	0.89, 0.73**

14	$\text{CH}_3-(\text{C}\equiv\text{C})_2-\text{CH}-\text{CH}(\text{OOCCH}_3)$ 	Olive brown	Olive brown	0.90
15	$\text{CH}_3-(\text{C}\equiv\text{C})_2-\text{CH}-\text{CH}(\text{OOCCH}_3)$ 	Deep violet Violet*	Greyish violet	0.79
16	$\text{CH}_3-\text{CH}=\text{CH}-(\text{C}\equiv\text{C})_2-\text{CH}=\text{CH}-\text{CH}-\text{CH}_2\text{OH}$ 	Greyish brown	Olive Greyish brown*	0.20
17	$\text{CH}_3-\text{CH}=\text{CH}-(\text{C}\equiv\text{C})_2-\text{CH}=\text{CH}-\text{CH}-\text{CH}_2\text{OH}$ 	Olive	Greyish brown	0.79
18	$\text{CH}_3-(\text{C}\equiv\text{C})_2-\text{CH}(\text{OH})-\text{CH}(\text{OH})-\text{CH}(\text{OH})-\text{CH}(\text{OH})-\text{CH}_3$ 	Greenish grey	Greenish grey	0.92
19	$\text{CH}_2=\text{CH}-\text{CH}-(\text{C}\equiv\text{C})_2-\text{CH}-\text{CH}=\text{CH}-\text{C}_6\text{H}_{11}$ 	Greyish brown	Dark brown Olive brown*	0.58
20	$\text{C}_6\text{H}_5-(\text{C}\equiv\text{C})_2-\text{CH}(\text{OH})-\text{CH}_3$ 	Dark blue Blue*	Greyish yellow	0.95
21	$\text{CH}_3-(\text{CH}_2)_2-(\text{C}\equiv\text{C})_2-\text{CH}=\text{CH}-\text{COOCH}_3$ 	Dark blue	Grey	0.98
22		Dark blue	Dull blue	0.95
23		Dark blue	Orange-red	0.88
24		Olive → dull blue	Vivid red	0.90
25		Greenish blue	Greenish blue	0.90
		Bluish green	Vivid blue	0.90

* Change of color after 48 h.
** Two spots of identical intensity

TABLE III

MINIMUM QUANTITIES (μg) OF SELECTED SESQUITERPENE LACTONES AND POLY-ACETYLENES DETECTABLE WITH VARIOUS REAGENTS

NT = Not tested.

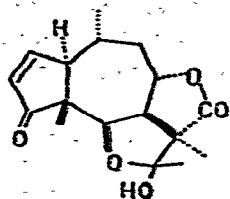
Compound	UV light	Iodine vapours	KMnO_4 (5% water)	Vanillin	<i>p</i> -Dimethyl-amino-benzaldehyde	Isatin (0.4% conc. H_2SO_4)	UV spectrophotometry
Parthenin	1.0	1.0	0.5	0.5	0.05	NT	NT
Coronopilin	5.0	1.0	0.5	0.05	0.05	NT	NT
Teuulin	1.0	1.0	0.5	0.05	0.05	NT	NT
Helenaflin	1.0	1.0	0.5	0.5	0.1	NT	NT
Alantolactone	5.0	1.0	0.5	0.1	0.1	NT	NT
α -Terthienyl	NT	NT	NT	0.05	0.05	0.05	2.5 mg/ml
Phenylheptatriene (PHT)	NT	NT	NT	0.5	0.5	NT	0.5 mg/ml

TABLE IV

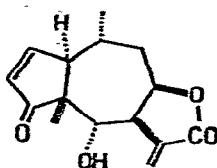
COLOR REACTIONS OF SOME NATURALLY OCCURRING COMPOUNDS WITH VANILLIN SPRAY REAGENT

Arrows indicate changes during heating of the plate; — = no color reaction.

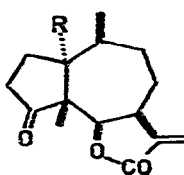
Compound	Color	R_f (chloroform-acetone, 6:1)
<i>Monoterpenes</i>		
d-Limonene	Dark blue	0.94
Citronellol	Dark blue	0.76
Citronellal	Dark blue	0.86
Menthol acetate	Dark blue	0.90
<i>Steroids</i>		
Cholesterol	Purple	0.76
Ergosterol	Purple	0.76
<i>Carotenoids</i>		
β -Carotene	Dark blue	0.93
<i>Fatty acids</i>		
Stearic	Grey	0.79
Oleic	Greyish blue	0.79
<i>Flavonoids</i>		
Quercetin	Yellow \rightarrow orange	0.18
Kaempferol	Yellow \rightarrow orange	0.37
Myricetin-3-O-Arab	Golden yellow	0.00
Kaempferol-3-O-Glu	Golden yellow	0.00
Quercetin-3-O-Glu	Golden yellow	0.00
<i>Aromatic acids</i>		
Anisic	—	
<i>p</i> -Hydroxybenzoic	—	
Vanillic	—	
Caffeic	—	
Ferulic	Pastel violet	0.49
<i>p</i> -Coumaric	Pastel blue	0.47
<i>p</i> -Aminobenzoic	Vivid yellow	0.42
<i>Miscellaneous compounds</i>		
Catechol	Bluish red	0.46
Resorcinol	Vivid red	0.29
Hydroquinone	Deep magenta	0.29
Coumarin	—	
Hydroxycoumarin	—	



I, tenuifin

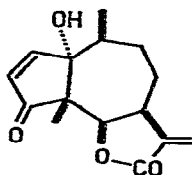


II, helenalin



III, R=H, damsine

IV, R=OH, coronopilin



V, parthenin

VI, hymenin, β -C-1-OH

vanillin, *p*-dimethylaminobenzaldehyde and isatin has a lower limit of detection of 0.05 μg . Phenylheptatriyne (20 in Table II) (forming the least intensive color of all polyacetylenes we tested) can be detected on TLC plates with vanillin or *p*-dimethylaminobenzaldehyde reagent only at the level of 0.5 μg which, however, is still 1000 times lower than the minimum amount needed for its detection by UV spectrophotometry.

DISCUSSION

Some of the reagents which we have tested, particularly acidic solutions of vanillin and *p*-dimethylaminobenzaldehyde are most suitable for the identification of sesquiterpene lactones and of polyacetylenic compounds. These reagents produce a variety of colors with structurally very different compounds. The differences in colors produced by various sesquiterpene lactones and other compounds allows for the ready detection of impurities in crystalline preparations. The TLC method can be made highly specific by: (1) the use of several reagents that form different colors or shades with the same compound, (2) the use of standard compounds and (3) evaluating R_F values.

The wide range of colors produced with all types of sesquiterpene lactones and polyacetylenes makes it difficult to assign the formation of a particular color to a chemical structure. This is supported by the fact that the structurally similar sesquiterpene lactones, such as damsine (III), coronopilin (IV) and parthenin (V), produce quite different colors with the vanillin reagent (Table I) while diastereoisomers produce identical colors as is the case with parthenin and hymenin (V and VI).

Hydroxybenzoic acid did not produce distinct colors with sesquiterpene lactones but *p*-hydroxybenzaldehyde formed a large variety of colors. Both vanillic acid and vanillin, on the other hand, gave a good range of colors. Although the most

distinctive and variable colors were produced with *p*-hydroxybenzaldehyde and vanillin, certain sesquiterpene lactones might be more conveniently detected with a different spray reagent (e.g., cinnamaldehyde forms a very bright stable color with helenalin).

To conclude, we recommend this TLC technique as a simple, fast, sensitive and specific method that can be used in a preliminary search for sesquiterpene lactones and polyacetylenes in crude (plant) extracts of the *Asteraceae* (*Compositae*) and also in the identification of individual compounds. Research in progress supports the practical application of this method (Picman and Towers, in preparation).

ACKNOWLEDGEMENTS

We would like to thank all those who kindly provided samples of sesquiterpene lactones and the National Science and Engineering Research Council of Canada for financial support.

REFERENCES

- 1 V. H. Heywood, J. B. Harborne and B. L. Turner, *The Biology and Chemistry of the Compositae*, Academic Press, New York, 1977.
- 2 E. Rodriguez, G. H. N. Towers and J. C. Mitchell, *Phytochemistry*, 15 (1976) 1573.
- 3 G. H. N. Towers and C. -K. Wat, *Rev. Latinoamer. Quim.*, 9 (1978) 162.
- 4 G. W. Ivie, A. W. Donald, W. Herz, R. Kannan, J. O. Norman and J. A. Weech, *J. Agr. Food Chem.*, 23 (1975) 841.
- 5 G. W. Ivie, D. A. Witzel and D. D. Rushing, *J. Agr. Food Chem.*, 23 (1975) 845.
- 6 T. J. Mabry, in J. B. Harborne (Editor), *Phytochemical Phylogeny*, Academic Press, New York, 1970, p. 286.
- 7 E. Rodriguez, *Phytochem. Bull.*, 8 (1975) 7.
- 8 T. A. Geissman and T. S. Griffin, *Phytochemistry*, 10 (1971) 2475.
- 9 I. H. Hall, K. -H. Lee, E. C. Mar and C. O. Starnes, *J. Med. Chem.*, 20 (1977) 333.
- 10 T. S. Griffin, T. A. Geissman and T. E. Winters, *Phytochemistry*, 10 (1971) 2487.
- 11 F. Bohlmann, T. Burckhardt and C. Zdero, *Naturally occurring Acetylenes*, Academic Press, London, 1973.
- 12 R. F. Curtis and G. T. Phillips, *J. Chromatogr.*, 9 (1962) 366.
- 13 J. S. Matthews, *Biochim. Biophys. Acta*, 69 (1963) 163.
- 14 K. L. Bajaj and K. L. Ahuja, *J. Chromatogr.*, 172 (1979) 417.
- 15 E. Stahl, *Thin-Layer Chromatography*, Springer, New York, 2nd ed., 1969.
- 16 E. Merck, *Dyeing Reagents for Thin Layer and Paper Chromatography*, E. Merck, Darmstadt.
- 17 S. N. Tewari and J. D. Sharma, *Pharmazie*, 34 (1979) 196.
- 18 A. Kornerup and J. H. Wauscher, *Methuen Handbook of Colour*, Methuen & Co., London, 2nd ed., 1967.
- 19 H. Yoshioka, T. J. Mabry and B. N. Timmermann, *Sesquiterpene Lactones: Chemistry, NMR and Plant Distribution*, University of Tokyo Press, Tokyo, 1973.
- 20 N. H. Fisher and R. A. Wiley, *Rev. Latinoamer. Quim.*, 7 (1976) 87.
- 21 D. L. Perry and N. H. Fisher, *J. Org. Chem.*, 40 (1975) 3480.
- 22 N. H. Fisher, F. C. Seaman and R. A. Wiley, *J. Org. Chem.*, 43 (1978) 4984.
- 23 W. G. Padolina, H. Yoshioka, N. Nakatani and T. J. Mabry, *Tetrahedron*, 30 (1974) 1161.
- 24 W. G. Padolina, N. Nakatani, H. Yoshioka, T. J. Mabry and S. A. Monti, *Phytochemistry*, 13 (1974) 2225.
- 25 M. Betkouski and T. J. Mabry, *Rev. Latinoamer. Quim.*, 6 (1975) 191.
- 26 T. K. Devon and A. I. Scott, *Handbook of Naturally Occurring Compounds, Vol. II, Terpenes*, Academic Press, New York, 1972.
- 27 R. L. Ranieri and G. J. Calton, *Tetrahedron Lett.*, (1978) 499.
- 28 M. Betkouski, personal communication.
- 29 T. A. Geissman, personal communication.