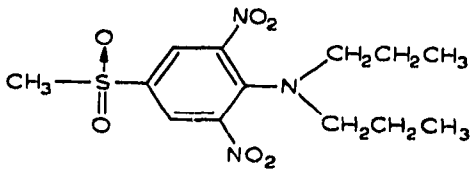
CHROM. 4951

Separation of Planavin® herbicide and some related compounds by two-dimensional thin-layer chromatography

The herbicide, 4-(methylsulfonyl)-2,6-dinitro-N,N-dipropylaniline (I), is marketed under the brandname Planavin® herbicide as a selective pre-emergent herbicide for use on agronomic crops. It is active against a great variety of annual grasses and broadleaf weeds[1].



I

In conjunction with metabolic studies of I in plant and soil systems it was necessary to investigate the chromatographic behavior of this compound, certain related compounds, and possible metabolite or breakdown products. Optimum separation of I and eleven related compounds was obtained using two-dimensional

TABLE I

COLORS AND R_F VALUES OF SELECTED COMPOUNDS IN FOUR SOLVENT SYSTEMS

Solvent systems: (A) hexane–ethyl acetate–tetrahydrofuran (66:30:4); (B) benzene–acetonitrile (3:2); (C) hexane–acetone (3:2); (D) benzene–methanol (9:1).

No.	*Compound*	*Color*	*Solvent system*				
			A (once)	*A (twice)*	*B*	*C*	*D*
1	2-(Methylsulfonyl)-4,6-dinitro-N,N dipropylaniline	Yellow	0.44	0.63	0.69	0.48	0.64
2	4-(Methylsulfonyl)-2,6-dinitro-N,N-dipropylaniline[a]	Bright yellow	0.41	0.60	0.67	0.45	0.62
3	4-(Methylsulfonyl)-2,6-dinitro-N-propylaniline	Bright yellow	0.30	0.48	0.65	0.40	0.59
4	4-(Methylsulfonyl)-2-nitro-N,N-dipropylaniline	Bright yellow	0.23	0.37	0.64	0.40	0.56
5	5-(Methylsulfonyl)-3-nitro-N^2,N^2-dipropyl-*o*-phenylenediamine	Yellow	0.19	0.30	0.62	0.36	0.48
6	4-(Methylsulfonyl)-2,6-dinitroaniline	Pale yellow	0.18	0.30	0.61	0.32	0.52
7	5-(Methylsulfonyl)-N^2-dipropyl-1,2,3-benzenetriamine	Colorless	0.15	0.25	0.56	0.32	0.43
8	5-(Methylsulfonyl)-3-nitro-N^2-propyl-*o*-phenylenediamine	Orange	0.04	0.09	0.56	0.27	0.31
9	4-(Methylsulfonyl)-2-nitroaniline	Yellow	0.04	0.09	0.51	0.21	0.25
10	5-(Methylsulfonyl)-3-nitro-*o*-phenylenediamine	Brown	0.02	0.04	0.40	0.15	0.36
11	4-(Methylsulfonyl)-2-nitrophenol	Yellow	0.02	0.04	0.09	0.05	0.10
12	4-(Methylsulfonyl)-2,6-dinitrophenol	Bright yellow	0.00	0.00	0.09	0.06	0.02

[a] Marketed under brandname Planavin®, herbicide.

thin-layer chromatography on Silica Gel F_{254} precoated plates (Merck) developing twice with a mixture of hexane–ethyl acetate–tetrahydrofuran (66:30:4) in one direction and once with benzene–acetonitrile (3:2) in the perpendicular direction.

The compounds were detected either as colored spots or as blue absorbing areas when the chromatograms were exposed to short-wavelength (254 mμ) UV irradiation.

Experimental

Thin-layer plates (20 × 20 cm) precoated with 0.25 mm silica gel F_{254} (E. Merck AG, Darmstadt, G.F.R., distributed by Brinkman Instruments, Inc., Westbury, N.Y., U.S.A.) were used for the work in this study. The layers were dried but not activated.

Two micrograms of I and each of the eleven compounds (Table I) in methylene chloride solution were applied at a point 2 cm from the bottom of the silica gel layer and developed in one of the following solvent systems: (A) hexane–ethyl acetate–tetrahydrofuran (66:30:4); (B) benzene–acetonitrile (3:2); (C) hexane–acetone (3:2); (D) benzene–methanol (9:1). The chromatograms were dried at room temperature in a forced air hood.

For two-dimensional chromatography, a mixture of 2–5 μg of each of the twelve compounds was spotted 2 cm from the left-hand edge and 2 cm from the bottom of the plate, and developed twice in solvent A, then turned 90° and developed in solvent B. The chromatography tanks were lined with filter paper to insure saturation.

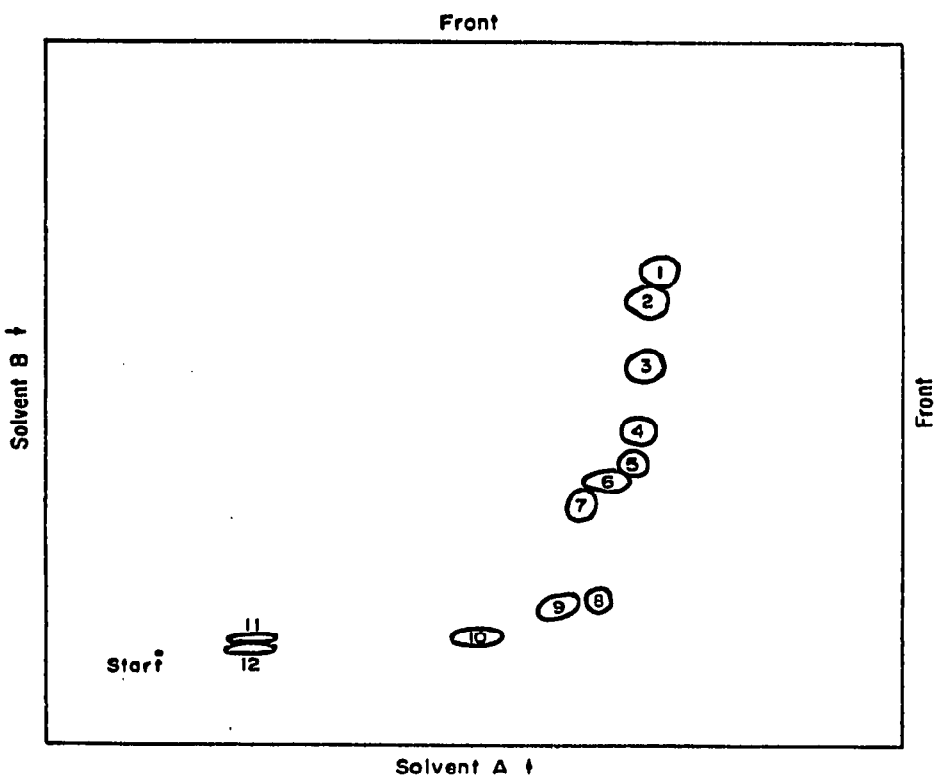


Fig. 1. Two-dimensional thin-layer chromatogram of Planavin® herbicide and eleven related compounds. Solvent systems: (A) hexane–ethyl acetate–tetrahydrofuran (66:30:4) (twice); (B) benzene–acetonitrile (3:2). Compounds: see Table I for identification.

Results and discussion

Table I shows the colors of the twelve compounds, and their approximate R_F values in the four solvent systems used. The detection limit with UV light or the unaided eye was about 0.5 μg or less for compounds 2, 3, 6, 8, 9, 10 and 11, and about 1.0 μg or less for the compounds 1, 4, 5, 7, and 12.

Fig. 1 illustrates the results of the separation obtained using the two-dimensional technique. When radioactive Planavin® herbicide was used, the separated compounds were isolated or eluted and the radioactivity quantified using liquid scintillation counting or scanning methods. In instances where the concentration was below the visible detection limits, the radioactive spots were located by exposing the chromatogram to X-ray film for a specified time, after which the film was developed. The areas were then located and eluted for quantification.

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1 W. J. HUGHES AND R. H. SCHIEFERSTEIN, *Proc. Ann. Mtg. Southern Weed Conf., 19th, 1966*, p. 170.

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The separation of plant glycosides and aglycones using thin-layer chromatography and electrophoresis

Thin-layer chromatographic techniques have been used previously to investigate the phenolic glycosides[1–4] and their hydrolysed aglycones[5] present in plants. In a taxonomic study of the distribution of these compounds in the genus *Coprosma* (Rubiaceae), it became apparent that such chromatography was inadequate. One-dimensional separations failed to separate all the compounds present and two-dimensional separations were difficult to analyse because of the increased spot sizes and concomitant lack of definition (a difficulty also with paper chromatography).

Since a number of methods have been developed to separate organic mixtures by thin-layer electrophoresis[6] it was decided to investigate this technique for these phenolic compounds. Its principal advantage over chromatography is the speed of separation of any ionized compounds formed, thereby decreasing zone distortion and increasing definition.

Materials and methods

Extraction. Leaves from both freshly collected and herbarium specimens of