10:10:20 gave the best resolution. For recovery of the heat-labile MMS, propanol–30% ammonia systems are more desirable, since these solvents are readily removed under vacuum at room temperatures. The use of TLC for resolution of these compounds offers the advantages of speed and sensitivity over paper chromatography. Development of plates was usually completed within 4 h and as little as 1 μg of MMS or homoserine was detectable.

These solvent systems have recently been utilized in the identification of an MMS salt occurring in milk and for following its conversion to homoserine on heating[5].

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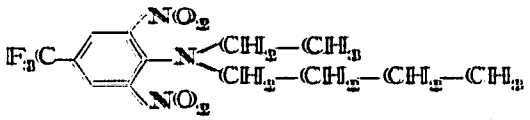
Thin-layer chromatographic separation of benefin and related substances

Benefin (I) (Balan®, N-(*n*-butyl)-N-ethyl-2,6-dinitro-α,α,α-trifluoro-*p*-toluidine) is a selective pre-emergent, soil incorporated herbicide for many agronomic and horticultural crops which controls a wide variety of annual grasses and broadleaf weeds[1]. In its herbicidal activities benefin complements another similar compound, trifluralin (α,α,α-trifluoro-2,6-dinitro-N,N-di-(*n*-propyl)-*p*-toluidine). Chromatographic separation of the latter substance and its related compounds was described previously[2].



(I)

Metabolic studies in plants and soils required knowledge of the chromatographic behavior of benefin and ten related compounds. Two-dimensional thin-layer chromatography on Silica Gel GF permitted useful separations. The best separation was obtained with benzene–methylcellosolve (96:4) and cyclohexane–ethyl acetate (95:5).

The compounds were detected by their natural colors (yellow, orange, brown),

and those not colored were located as blue absorbing spots by scanning the plate with an ultraviolet source.

Experimental

Standard 20 × 20 cm glass plates, coated with a 250 μ layer of Silica Gel GF 254 prepared in the normal manner[3,4], were employed. The plates were activated at 110° for one hour. A standard mixture containing 5 μg each of benefin and ten related compounds (Table I) in benzene was spotted on the plate. The spot was positioned 3 cm from the edge and 3 cm from the bottom. The solvents were poured into the clean, dry chromatographic jars just prior to running the chromatograms. To assure a good separation presaturation of the chromatographic chambers should be avoided.

TABLE I

BENEFIN AND RELATED SUBSTANCES

Compound No.	*Name*	*Color on TLC*
33	Benefin	intense orange yellow
3	2,6-Dinitro-4-trifluoromethylaniline	green yellow
4	α,α,α-Trifluoro-5-nitrotoluene-3,4-diamine	orange yellow
26	α,α,α-Trifluorotoluene-3,4,5-triamine	colorless
34	N-(*n*-Butyl)-2,6-dinitro-α,α,α-trifluoro-*p*-toluidine	intense yellow
35	N-Ethyl-α,α,α-trifluoro-2,6-dinitro-*p*-toluidine	intense yellow
36	N^4-(*n*-Butyl)-N^4-ethyl-α,α,α-trifluoro-5-nitrotoluene-3,4-diamine	pale yellow
37	N^4-(*n*-Butyl)-N^4-ethyl-α,α,α-trifluorotoluene-3,4,5-triamine	colorless
38	N^4-(*n*-Butyl)-α,α,α-trifluoro-5-nitrotoluene-3,4-diamine	orange brown
39	N^4-(*n*-Butyl)-α,α,α-trifluorotoluene-3,4,5-triamine	colorless
40	N^4-Ethyl-α,α,α-trifluoro-5-nitrotoluene-3,4-diamine	brown orange

After development in solvent I, benzene–methylcellosolve (96:4, v/v), the plate was dried at room temperature for 20 min, rotated 90° and developed in solvent II, cyclohexane–ethyl acetate (95:5, v/v).

Results and discussion

The separation of the compounds listed in Table I is shown in Fig. 1. The detection limit of each compound is approximately 0.5 μg. Qualitative identification of benefin and its metabolic products in soil and plant extracts was achieved at concentrations far below this detection limit when ^{14}C-labeled benefin was used. This type of extract was co-chromatographed with a standard mixture of the unlabeled reference substances listed in Table I. The plates were developed in solvents I and II as described. After development the standard compounds were located and marked on the chromatographic plates. Then the plates were covered with X-Ray Medical Film (Eastman Kodak Corporation, Rochester, N.Y.) and stored in darkness. The exposure time, depending on the amount of radioactive material present, ranged from a few days to a few months. Developed X-ray films showed black spots, some of which matched the position of the unlabeled reference substances, suggesting these as probable degradation products. This aided the identification of possible degradation and metabolic products of benefin.

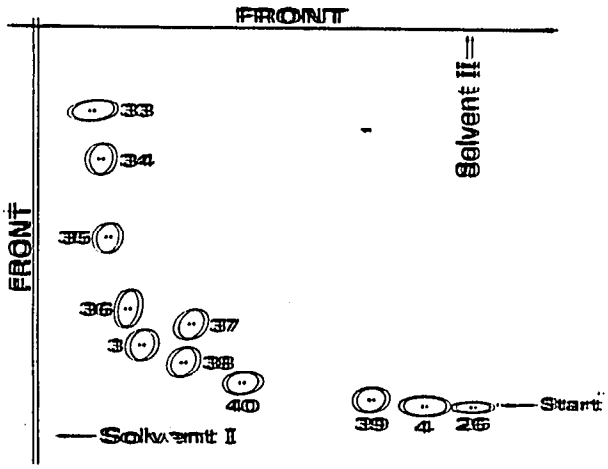


Fig. 1. Thin-layer chromatogram of benefin and ten related compounds. Silica Gel GF. Solvent I: benzene–methylcellosolve (96:4); solvent III: cyclohexane–ethyl acetate (95:5). For compound numbers, see Table II.

Quantitative analysis of these substances, suggested by thin-layer radioautographs, can be obtained with supplementary methods. Some of the probable metabolic substances can be separated with one-dimensional thin-layer chromatography using solvent I or III. Substances recovered from pre-selected zones of the thin-layer plates can be determined by direct radioactive counting, isotope dilution methods or gas–liquid chromatography. Preparative thin-layer chromatography can provide material for infrared spectroscopy, X-ray crystallography or other recognized methods of compound identification.

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