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Note

Determination of 2,4D in plant tissue. I

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In order to study the penetration of 2,4-dichlorophenoxyacetic acid (2,4D) through leaf surface waxes, it is necessary to use a good method for the quantitative determination of the herbicide. The determination of 2,4D in plant matter can often prove to be a greater problem than its determination in animal tissue because it is difficult to completely remove pigments and other metabolites which interfere with the analysis.

In the past, biological[1], colorimetric[2] and spectroscopic methods[3] have been applied but they are not sufficiently selective to give an accurate estimation of low levels of 2,4D (less than 1 ppm). More recently electron capture detection with gas–liquid chromatography (GLC) has facilitated the work of pesticide residue analysis[4]. Unfortunately, plant matter contains a number of electron capturing metabolites, some of which have been shown to interfere with 2,4D analysis[5], with the result that a rigorous purification of plant extracts is necessary for an accurate estimation.

EXPERIMENTAL

All solvents used were of AnalaR grade; methanol and chloroform were distilled before use. Silica gel (Merck, Darmstadt, G.F.R.) plates (20 × 20 cm) were prepared in the usual manner and activated at 110° for 30 min. The plates were then cooled and washed by allowing chloroform to elute over them for 1–2 h, then reactivated before use.

Gas–liquid chromatography

GLC analyses were performed on a Phillips instrument (Series PV 4000) fitted with a ^{63}Ni electron capture detector, using a 2.74 m × 0.4 mm glass column packed with 3% OV-17 on Gas-Chrom Z (80–100 mesh) and conditioned for 48 h at 320° before use. Dry argon + 10% methane was used as carrier gas.

Conditions for analysis were: column temperature, 200°; injection port temperature, 235°; detector temperature, 250°; pulse interval, 50 μsec; carrier-gas flow-rate, 60 ml/min.

If the detector became contaminated it was heated to 300° for 15 min before proceeding with the analysis.

Extraction of 2,4D from plant tissue

2,4D is normally applied to the plant as a salt or an ester, but hydrolysis is reported to occur rapidly, and it can be present as the free acid. In addition, the her-

bicide can become complexed to plant metabolites, especially proteins[6]. Thus, in order to ensure complete removal of the herbicide from the plant, an exhaustive extraction procedure was used (Fig. 1). The plant matter (20 g) was macerated with warm methanol (100 ml) and conc. HCl (1–2 ml) three times in a Waring blender for 4 min, filtering between each extraction. The solid residue, an off-white powder, was then refluxed with 25% sodium hydroxide (20 ml) for 30 min to hydrolyse any 2,4D protein complexes, filtered and the brownish solution was acidified with hydrochloric acid.

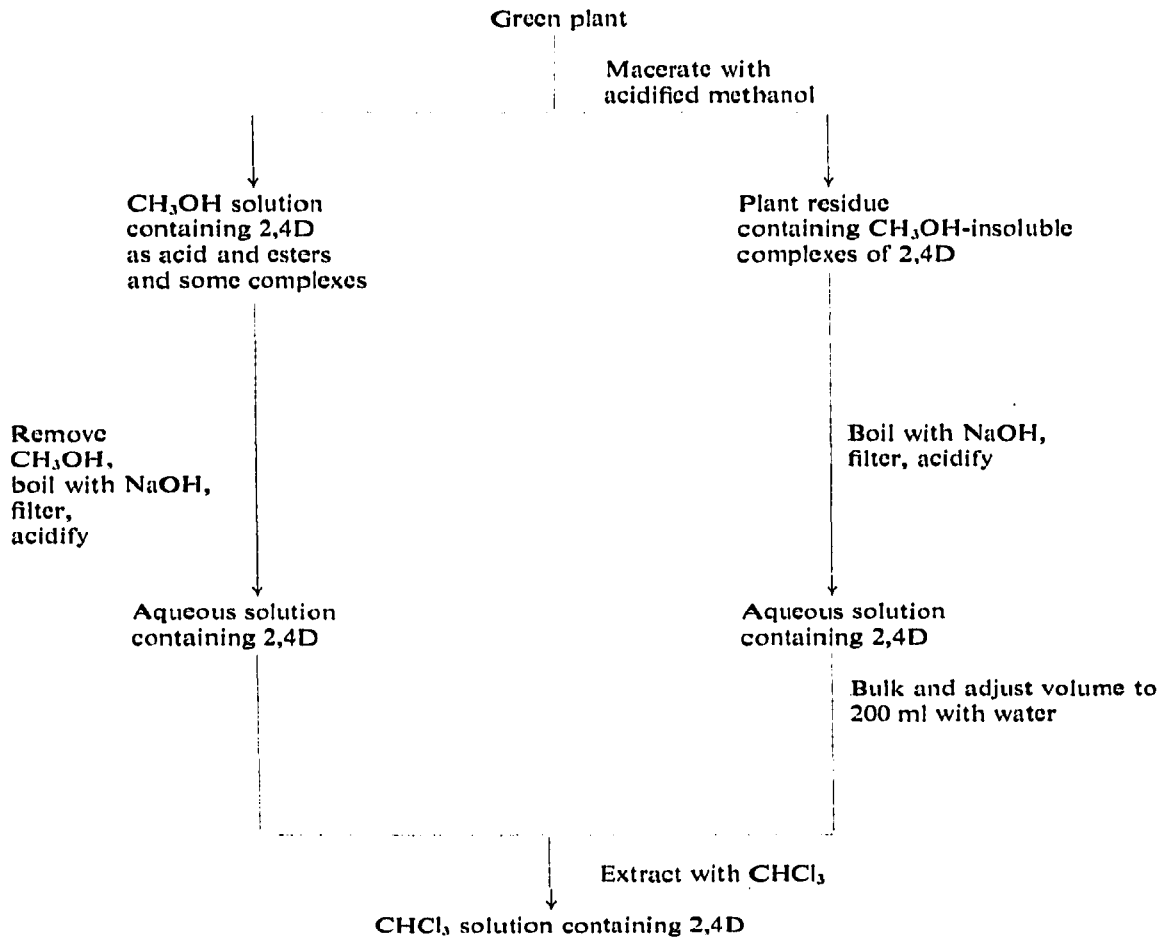

Fig. 1. Extraction of 2,4D.

The original methanol extracts were combined and concentrated to approx. 20 ml under reduced pressure, and then refluxed with 25% sodium hydroxide (20 ml) for 30 min to hydrolyse 2,4D esters and other complexes. The resulting solution was filtered, acidified, and combined with the acidified alkaline extract (above).

The volume of this solution was adjusted to 200 ml with water and extracted with chloroform (4 × 50 ml).

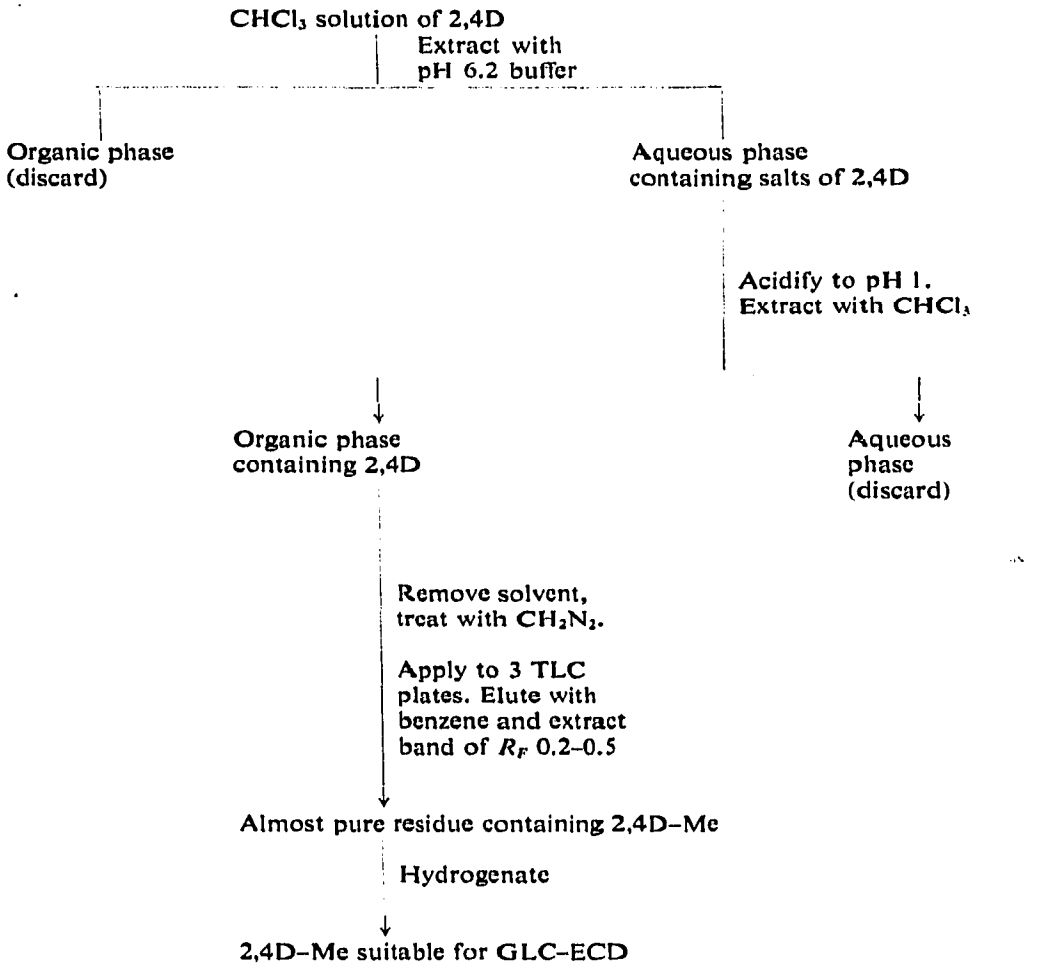

Fig. 2. Purification of 2,4D extracts.

Purification and analysis

It was necessary to purify the dark green chloroform extract before analysis on the electron capture gas–liquid chromatograph because it contained substances which interfered with the analysis. Because of the extreme sensitivity of the electron capture detector, all solvents and reagents were carefully purified and thin-layer chromatographic (TLC) plates were washed with chloroform before use. The purification procedure of the dark green extract is outlined in Fig. 2.

The chloroform solution containing 2,4D was extracted with a phosphate buffer, pH 6.2 (4 × 50 ml), to remove moderately strong acids[7]. The aqueous portion was acidified to pH 1 with conc. HCl and re-extracted with chloroform (4 × 50 ml). The chloroform solution was concentrated to 1–2 ml and excess ethereal diazomethane was added from a small generator until a definite yellow tinge in the solution could be seen. The solution was evaporated to about 0.5 ml in a stream of nitrogen, then ap-

plied to three TLC plates (20 × 20 cm) coated with silica gel (0.25 mm) and eluted with benzene.

The 2,4D methyl ester had an R_F value of 0.35–0.40, but bands with R_F values between 0.2 and 0.5 were scraped from the plates to ensure that the many impurities had not altered the R_F value.

RESULTS AND DISCUSSION

When a solvent partition scheme is introduced into the work-up, there is a marked improvement in the GLC record (Fig. 3). In this experiment with *Rumex*

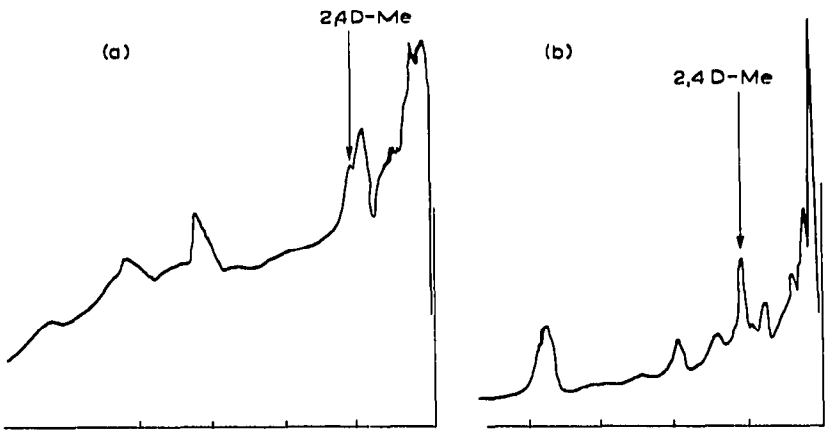

Fig. 3. Electron capture GLC analysis for 2,4D in a total plant extract of *R. obtusifolius* (20 g) fortified with 2,4D (1 mg). The extract had been partially purified by solvent partition using a pH 6.2 buffer with (a) diethyl ether or (b) chloroform.

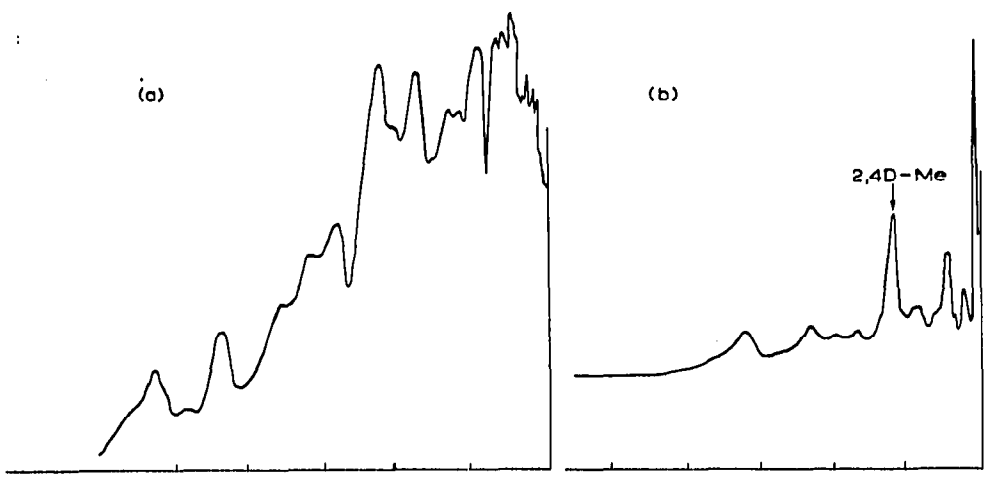

Fig. 4. Electron capture GLC analysis for 2,4D in a total plant extract of *S. media* (20 g) fortified with 2,4D (15 μg). Sample (a) was purified by solvent partition only. Sample (b) was purified by solvent partition followed by TLC.

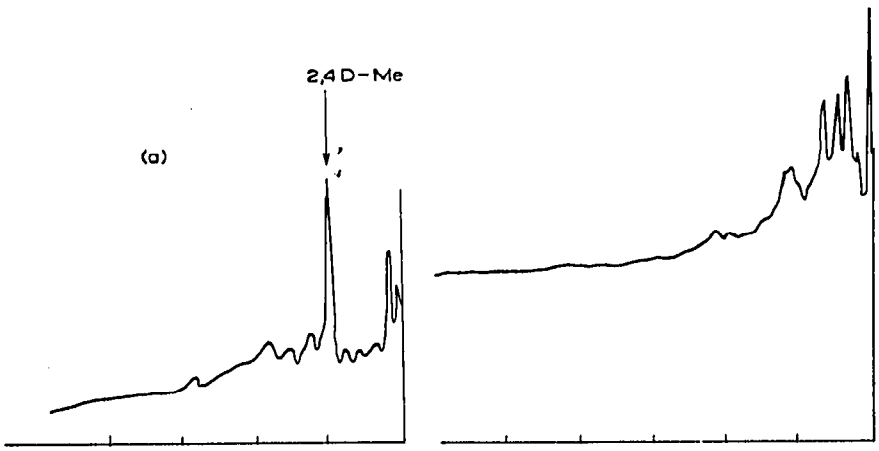

Fig. 5. Electron capture GLC analysis for 2,4D in a total plant extract of *P. major* (20 g) fortified with 2,4D (5 μg). Both samples were purified by solvent partition and TLC, but sample (a) was catalytically hydrogenated before electron capture GLC.

obtusifolius the solvent partition step permits a better peak on the gas–liquid chromatogram to be produced at a level of 1 mg of 2,4D per 20 g of plant material.

When solvent partition is combined with TLC (Fig. 4), a further improvement in the analysis results. The level of detection is 15 μg of 2,4D per 20 g of *Stellaria media*. Only when the sample was catalytically hydrogenated was it possible to reduce the level of detection to 5 μg per 20 g of plant material (Fig. 5). The sample was dissolved in chloroform (3 ml) and the solution placed in a Hirsch tube with Adam's catalyst (Pd/PdO_2, 10 mg). Hydrogen was bubbled into the tube by means of a gas inlet tube so as to agitate the catalyst over a period of 12 h. The side-arm of the Hirsch tube was fitted with a rubber tube which carried the excess hydrogen out of the window. This step, which has not been attempted before, removes the unsaturated compounds, such as plant pigments, which often give rise to interfering peaks on the chromatograms.

By using this improved technique it was possible to show (Table I) that 2,4D could be detected at levels as low as 0.04 ppm with recoveries from 80–96% when it is present with a variety of plant extracts.

TABLE I

RECOVERIES OBTAINED WHEN ANALYSING FOR 2,4D IN FORTIFIED PLANTS

Plant	*Weight (g)*	*Weight of 2,4D added (μg)*	*Level (ppm)*	*2,4D recovered (μg)*	*% recovery*
Rumex obtusifolius	25	1.0	0.04	0.9	90
Rumex obtusifolius	25	200.0	8.00	180.5	90
Stellaria media	22	10.2	0.46	9.8	96
Plantago major	15	1.0	0.07	0.9	90
Lolium perenne	25	48.2	1.92	42.3	88
Chenopodium album	20	1.0	0.05	0.8	80
*Lolium perenne**	25	20.0	1.00	19.2	96

* Plant fortified with the butyl esters of 2,4D.

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