Gas Chromatographic Determination of 2,4-Dichlorophenoxyacetic Acid (2,4-D) by Mass Fragmentography with a Deuterated Internal Standard

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A method for the quantitative determination of 2,4-dichlorophenoxyacetic acid (2,4-D) in urine by means of mass fragmentography is described. The deuterated methyl ester of the compound is used as the internal standard. Its molecular ion at m/e 237 and that of the methyl ester of 2,4-D (m/e 234), formed by diazomethane treatment of the extract, are selected for mass fragmentographic registration. The detector has a linear response in the 10–300 ppb and 0.3–10 ppm ranges after corrections for overlapping of the internal standard signal by the 2,4-D H_3-methyl ester signal. More reproducible results are obtained with the deuterated derivative of the compound being measured as the internal standard than with a conventional internal standard.

Among chlorophenoxy acid herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) is probably the most widely used and best known representative. A great number of papers, dealing with analysis, residues, and metabolism, have been published, an excellent review of which has appeared recently (Cochrane and Purkayastha, 1973). Due to the presence of chlorine atoms, gas chromatography with electron capture detection was preferred in most of the analytical procedures. The technique of mass fragmentography, introduced by Sweeley et al. (1966), not only matches its sensitivity, but exceeds by far its selectivity. The best internal standard for any gas chromatographic analysis is one that is as similar as possible to the compound being measured.

Therefore, incorporation of stable isotopes (^{15}N, D) in the molecule of interest has found wide acceptance in mass fragmentography, as the GC–MS combination is able to distinguish between chemically identical compounds on a purely physical basis (Samuelson et al., 1970; Gafney et al., 1971; Bertillson et al., 1972; Sjoquist and Änggard, 1972; Rosenfeld et al., 1974).

In the present work we report a procedure for the quantitative determination of 2,4-D by means of mass fragmentography, which has the same sensitivity as the conventional electron capture detection techniques, but exceeds them all in selectivity, thus enabling a reduced number of cleanup steps, which in turn offers higher extraction efficiencies and thus extrasensitivity.

EXPERIMENTAL SECTION

Apparatus. A Varian Model 1400 gas chromatograph was fitted with a glass column of 1.5 m × 0.25 in. o.d., 2 mm i.d., packed with 2.5% DC-11 on Varaport AW-DMCS 100–120 mesh. The flow rate of the carrier gas (helium) was 25–30 ml/min. Temperatures were: injection port, 200 °C; column, 175 °C; transfer line, 190 °C. The gas chromatograph was connected by a Gohlke jet separator to a Finnigan Model 3000 Quadrupole mass spectrometer. The temperature of the ion source was kept at 130 °C, the pressure at 5×10^{-5} Torr. The ionizing energy was 70 eV. Beam current, ion energy, extractor voltage, and resolution were adjusted to yield maximal sensitivity. The electron multiplier voltage was kept at 3.3 kV. All data from the GC–MS combination were stored in a Finnigan Model 6000 Interactive Data System, provided with a mass fragmentography optional program.

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Reagents. Reagents were all analytical reagent grade; solvents were freshly distilled before use. Diazomethane was prepared from *N,N'*-dimethyl-*N,N'*-dinitrosoterephthalamide in alkaline medium and stored as an ethereal solution at −18 °C, although this is no longer considered safe.

All manipulations with diazomethane as well as the preparation were carried out in a fume cupboard. The use of ground glass stoppers to seal containers should be avoided as this could cause an explosion.

The D_3-methyl ester of 2,4-D was used as the internal standard. One hundred milligrams of 2,4-D acid (Polyscience Corp.) was dissolved in 4 ml of CD_3OD (isotopic purity 99%, Merck) plus 0.15 ml of CH_3COCl. The reaction was allowed to proceed for 18 h. After evaporation to dryness, 1 ml of water was added plus NaOH, 0.45 N to slightly alkaline. Next, the solution was extracted three times with 2 ml of ethyl acetate. After drying over anhydrous sodium sulfate, the solvent was evaporated, yielding 96 mg of a thick oil, which crystallized into white crystals (mp 39 °C) after cooling down.

Upon mass spectrometric examination no peaks at m/e 234, 235, and 236, indicating impurities of the parent compound (molecular ion at m/e 237), could be detected, so that it was assumed that the reaction product had at least the same isotopic purity (99%) as the starting reagent CD_3OD.

Procedure. In a conical glass centrifuge tube 1 ml of urine was acidified with 10% H_3PO_4 and extracted with 2 and 1 ml of diethyl ether, respectively. To the combined organic layers ethereal diazomethane solution was added until a yellow color persisted in the reaction tube. After 5 min reaction time with occasional shaking up, the solution was immersed for a while in a warm water bath at 40 °C in order to remove excess diazomethane, and subsequently passed onto a small glass column (70 mm × 6 mm i.d.), plugged with glass wool and filled with 150 mg of Al_2O_3 (activity grade, II–III) and 150 mg of anhydrous Na_2SO_4 (upper layer).

The column was rinsed with two 1-ml portions of diethyl ether. Next the internal standard solution (100 ppb or 5 ppm) in isooctane (100 μl) was added and the mixture concentrated to about 100 μl in a nitrogen stream.

The isooctane served as a keeper as volatilization of the methyl esters occurs after complete evaporation of the solvent. A 1-μl aliquot was injected into the gas chromatograph. The mass spectrometer was focused alternately on the molecular ion at m/e 234 of the compound being measured and on the molecular ion of the deuterated methyl ester at m/e 237. The intensity of these ions was

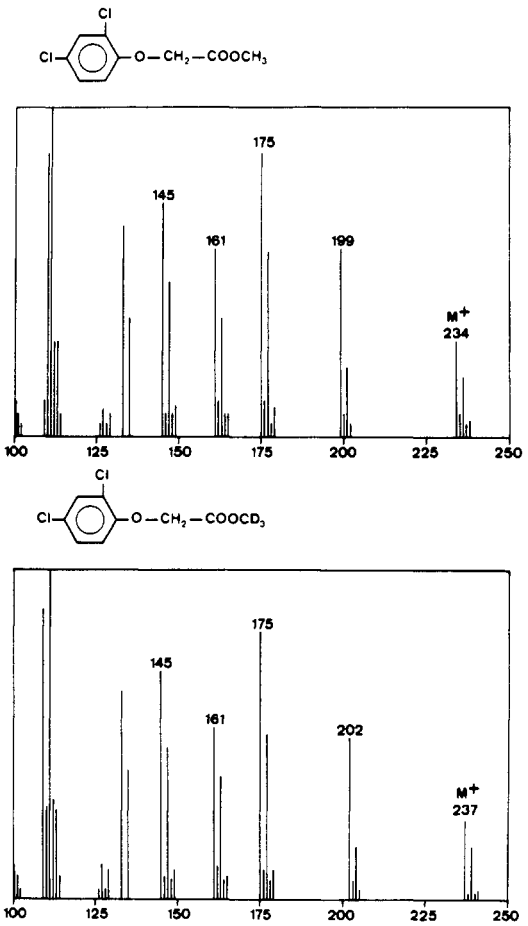

Figure 1. Mass spectra (70 eV) of 2,4-D H_3-methyl ester and 2,4-D D_3-methyl ester.

simultaneously displayed on an oscilloscope and could be reconstructed afterwards by the data system.

Standard mixtures in isooctane, each containing 1 ng of 2,4-D D_3-methyl ester and 0.1, 0.5, 1, 3, and 5 ng, respectively, of 2,4-D H_3-methyl ester per microliter were injected in duplicate into the gas chromatograph. Those mixtures correspond with 10, 50, 100, 300, and 500 ppb of 2,4-D H_3-methyl ester in urine.

The ratios of the 2,4-D H_3-methyl ester peak area to the corrected internal standard peak area were plotted vs. the corresponding urine concentrations between 10 and 500 ppb.

A similar procedure was employed for the calibration curve in the 0.3–10 ppm range with 5 ppm of 2,4-D H_3-methyl ester as the internal standard.

Blank urine samples, spiked with known amounts of 2,4-D acid, were analyzed by the procedure described. The results were used to determine the extraction efficiency.

RESULTS AND DISCUSSION

The mass spectra of 2,4-D H_3-methyl ester and 2,4-D D_3-methyl ester are shown in Figure 1. The molecular ions of both compounds, although they are not the base peaks, were selected for mass fragmentographic registration, because of their elevated specificity. The higher the selected m/e values, the less it is probable that a substance which elutes at the same time as the compound being measured has those same ions in its fragmentation pattern.

As 2,4-D H_3-methyl ester and the deuterated internal standard are chemically identical, they eluted at the same retention time, so that peak heights could be used instead of areas for quantitative determinations. There were two main reasons for not doing so: the data system has a

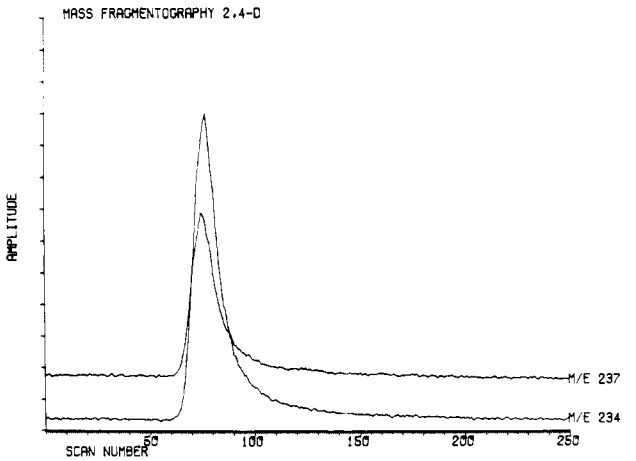

Figure 2. Reconstructed mass chromatogram of a urine extract, each peak corresponding to ~50 ng of 2,4-D H_3-methyl ester (m/e 234) and ~50 ng of 2,4-D D_3-methyl ester (internal standard).

built-in peak area integrator with automatic subtraction of the counts under the baseline and secondly there is an overlapping of the internal standard by the compound being measured. As can be seen from Figure 1, the molecular ion of 2,4-D H_3-methyl ester at m/e 234 has a P + 3 peak at m/e 237, due to ^{37}Cl and ^{13}C isotopes. It means that the amount of internal standard which was estimated by the intensity of its m/e 237 fragment ion was erroneously increased by the presence of 2,4-D H_3-methyl ester in the final extract and that abnormally lowered peak area ratios were calculated. To eliminate this error, a certain area had to be subtracted from the internal standard area depending on the relative intensity of the 237 peak to the 234 peak in the mass spectrum of 2,4-D H_3-methyl ester. The normal value of 7% could be increased due to the intentionally lowered resolution of the mass spectrometer when used in the mass fragmentography mode. Its actual value was established on injection of a 2,4-D H_3-methyl ester solution and measurement of the 237/234 ratio. Under the conditions of our experiments it was found to amount to 9%, so that an area corresponding to 9% of the area of the peak of the unknown had to be subtracted from the area of the internal standard peak. This 9% value was constant if the operating parameters of the mass spectrometer, mainly resolution and ion energy, remain unchanged. On the other hand, the absolute amount of area in digital counts to be subtracted from the internal standard peak area depended on the peak area of the 2,4-D H_3-methyl ester.

As the internal standard is of very high purity no peak at m/e 234 indicating the completely nondeuterated 2,4-D methyl ester could be detected, so that it had no influence at all on the 2,4-D H_3-methyl ester peak. After correction of the areas, calibration curves were straight lines in the 10–300 ppb range with 100 ppb 2,4-D D_3-methyl ester as the internal standard and in the 0.3–10 ppm range with 5 ppm 2,4-D D_3-methyl ester as the internal standard. The average extraction efficiency in the 10–500 ppb range was found to be 92% and it was 95% in the 0.5–50 ppm range.

Figure 2 shows a computer reconstructed mass chromatogram of a sample extract, each peak corresponding to ~50 ng of compound. The precision of the technique was distinctly enhanced when using a deuterated internal standard. We carried out an analysis of a urine sample fortified with 10 ppm of 2,4-D acid, once with 2,4-D D_3-methyl ester as the internal standard and once with 2-(2-methyl-4-chlorophenoxy)propionic acid methyl ester as the internal standard. The final extracts were both

injected several times into the gas chromatograph.

The relative standard deviations (number of injections = 9) were 2 and 16.7%, respectively. In our opinion this was due to unavoidable fluctuations of the emission current of the ion source and therefore of the detector sensitivity during the analysis. It means that the emission current had a different value when the compound was being measured and the internal standard eluted, so that the relative sensitivity toward both substances had been altered. Therefore, it is advisable to adjust manually the emission current after the first peak has eluted, when using a structural homologue as an internal standard. With a deuterated homologue both co-elute and the influence of the relative sensitivity is eliminated.

CONCLUSION

The proposed method appears to be useful for the determination of 2,4-D in other kinds of samples as well. Due to the high sensitivity and specificity of both gas chromatography and mass spectrometry, minute amounts will be detected in samples where no suitable cleanup will be available.

LITERATURE CITED

Bertillson, L., Atkinson, A. J., Jr., Althaus, J. R., Härfast, A., Lindgren, J.-E., Holmstedt, B., *Anal. Chem.* **44**, 1434 (1972).
Cochrane, W. P., Purkayastha, R., *Toxicol. Environ. Chem. Rev.* **1**, 137 (1973).
Gafney, T. E., Hammar, C.-G., Holmstedt, B., McMahon, R. E., *Anal. Chem.* **43**, 307 (1971).
Rosenfeld, J. J., Bowins, B., Roberts, J., Perkins, J., Macpherson, A. S., *Anal. Chem.* **46**, 2232 (1974).
Samuelson, B., Hamberg, M., Sweeley, C. C., *Anal. Biochem.* **38**, 301 (1970).
Sjöquist, B., Änggard, E., *Anal. Chem.* **44**, 2297 (1972).
Sweeley, C. C., Elliott, W. H., Fries, I., Ryhage, R., *Anal. Chem.* **38**, 1549 (1966).

Received for review July 22, 1975. Accepted November 14, 1975.

Environmental Degradation of the Insect Growth Regulator 6,7-Epoxy-1-(*p*-ethylphenoxy)-3-ethyl-7-methylnonane (Ro 10-3108) in Polluted Water

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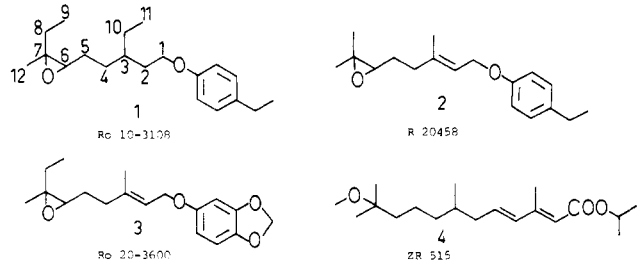
The insect growth regulator (IGR) Ro 10-3108 was subjected to degradation in polluted water under natural environmental conditions. Analysis of the degradation products by gas–liquid chromatography (GLC), high-pressure liquid chromatography (HLC), and combined gas chromatography–mass spectrometry (GC–MS) led to the identification of 12 metabolites which were synthesized and confirmed by GC–MS and cochromatography. The major routes of degradation involve hydration of the 6,7-epoxy group, α oxidation of the *p*-ethyl moiety, and ether cleavage. None of the metabolites shows morphogenetic activity comparable to that of Ro 10-3108 in the *Tenebrio molitor*, *Aedes aegypti*, and *Adoxophyes orana* bioassays.

In a recent report from our laboratory (Hangartner et al., 1976) it was demonstrated that the insect growth regulator 6,7-epoxy-1-(*p*-ethylphenoxy)-3-ethyl-7-methylnonane (1) (Ro 10-3108) is an efficient agent for practical plant protection. Ro 10-3108 gave good control of natural populations of summerfruit tortrix moth, aphids, mealy bugs, and San José scale in the field. Ro 10-3108 is clearly superior to the previously described IGR candidates **2** (Pallos et al., 1971), **3** (Bowers, 1971), and **4** (Henrick et al., 1973) with respect to stability and persistency in the natural environment, while it is fortunately still not very persistent by more conventional standards (e.g. DDT). This advantage, along with the low mammalian toxicity of Ro 10-3108 and its metabolites, make the compound a promising and ecologically acceptable new tool in insect control. In the present paper we report on the degradation of Ro 10-3108 in polluted water under natural environmental conditions. It is shown that the environmental degradation of Ro 10-3108 proceeds in close analogy to that of the related compound 6,7-epoxy-1-(*p*-ethylphenoxy)-3,7-dimethyl-*trans*-2-octene (**2**) (R 20458, Stauffer Chemical Co.), the metabolism of which is known in great detail (Hoffmann et al., 1973; Gill et al., 1974; Hammock et al., 1974).



MATERIALS AND METHODS

Environmental Degradation Conditions. The polluted water used for the environmental degradation studies was taken from the Glatt river at Dübendorf, Switzerland. It had a pH of 7.9 and contained the following impurities (in milligrams per liter): nitrogen (NO_3^-), 1–2; nitrogen (NH_4^+), 0.2–0.8; phosphorus, 0.1–0.3; organic carbon, 6–8; oxygen, 10–12; and calcium carbonate, 200. Four 5-l. dishes were filled with a total of 16 l. of this water, incubated with Ro 10-3108 (1% in acetone) at the 10-ppm level, covered with a nylon mull shelter, and exposed to the open air for a period of 4 weeks in June 1974. The volume of the water

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