# On the Metabolism of Hoe 23408 OH in Wheat

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Summer wheat was treated with ^{14}C-labeled Hoe 23408 OH, methyl 2-[4-(2',4'-dichlorophenoxy)-phenoxy-^{14}C]propionate, at a rate of 1 kg/ha. After an 18-day waiting period 3% of the radioactivity present was on the plant surface, 58% could be extracted with organic solvents, 32% could be extracted after alkaline digestion, and 0.6% remained unextracted. The main metabolites were identified as 2-[4-(2',4'-dichlorophenoxy)phenoxy]propionic acid, 2-[4-(2',4'-dichloro-5'-hydroxyphenoxy)phenoxy]propionic acid, and the two possible isomers in respect to the hydroxy substituent, the 3'-hydroxy and 6'-hydroxy derivatives present in the plant as conjugates.

The plant protection chemical designated Hoe 23408 OH is a specific herbicide to control weed grasses (Hoechst AG, 1973; Langelueddeke et al., 1975) and has the following structural formula:
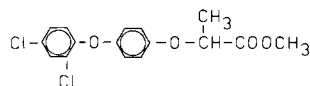
Methyl 2-[4-(2',4'-dichlorophenoxy)phenoxy]propionate

Since previously no information on the metabolism of this compound was available, a postemergent treatment of wheat was carried out to obtain the desired information.

The compounds referred to in the text are identified with Roman numerals as follows:

Hoe 23408 OH

I

II

III
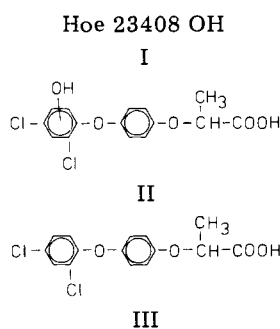
In regard to the position of the hydroxyl group in the dichlorophenyl ring, the isomers of II are designated IIa (-6'-hydroxy-), IIb (-5'-hydroxy-), and IIc (-3'-hydroxy-). Correspondingly, the methyl ether and methyl ester are identified as (II-OCH$_3$) and (III-OCH$_3$), respectively.

EXPERIMENTAL SECTION

^{14}C-Labeled Hoe 23408 OH was composed of methyl 2-[4-(2',4'-dichlorophenoxy)phenoxy-^{14}C]propionate labeled with ^{14}C uniformly in the phenyl ring of the compound adjacent to the oxypropionic acid moiety, with a specific activity of 9.8 μCi/mg. Labeling was carried out by the radiochemical laboratory of Hoechst AG.

Chemical identity with Hoe 23408 OH was confirmed by IR spectroscopic comparison with an analytical grade compound and by the melting point of 39–40 °C. The radiochemical purity of the preparation used was better than 99% (DC radiogram with FP-silica gel-SIF (Riedel de Haen) as the stationary and benzene as the mobile phase).

Preparation of the Methylated Metabolite II (IIb-OCH$_3$), the Methyl 2-[4-(2',4'-dichloro-5'-methoxyphenoxy)phenoxy]propionate. 3-Methoxyphenol is

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chlorinated in the nucleus to 2,4-dichloro-5-methoxy-phenol. This is condensed with 4-chloronitrobenzene to 2,4-dichloro-5-methoxy-4'-nitrodiphenyl ether. The nitro group in the latter compound is reduced and the formed aniline is diazotized and transformed to the corresponding phenol by boiling in aqueous acid solution. The 2,4-dichloro-5-methoxy-4'-hydroxydiphenyl ether is condensed with methyl 2-bromopropionate to the methylated metabolite II.

The purity of the substance was confirmed by gas chromatography and thin-layer chromatography and the identity was confirmed by recording the mass spectrum and determination of C, H, and O.

Preparations Used for Treatment. The preparation used for the tests with plants was a 36% emulsifiable concentrate. The radiolableled compound was mixed with a nonradiolabeled compound in a 1:1 ratio. This mixture (178 mg) was worked up to the above-mentioned formulation. For use the preparation was made up with water to a concentration of 0.18% (with reference to Hoe 23408 OH) in the spray mixture.

Plant Material. Summer wheat (*Triticum aestivum*, Colibri strain) was grown under glass in pots (10 plants/0.5 dm^2 surface). Prior to treatment, the plants were transferred for a number of days to an open greenhouse for purposes of acclimatization. Watering was carried out directly onto the surface of the soil.

Treatments. Sixty pots, each with 10 plants, were treated with a total of 64 mg of ^{14}C-labeled Hoe 23408 OH (314 μCi of ^{14}C $\simeq$ 6.9 × 10^8 dpm) formulated as described above. The suspension was applied to the plants with a Grapho-III sprayer. To avoid contamination with ^{14}C, treatment took place in a box closed on all sides and enveloped in plastic wrap. The treated plants were kept in a screened growing area with the exclusion of rain but subject to the influence of all other climatic conditions. Because of the geometry of the experimental arrangement, the amount of compound per hectare as calculated from the input (64 mg) was not achieved; it amounted to about 1 kg/ha.

Sampling. Eighteen days after treatment all plants were removed (about 600 g) and processed according to the directions below. At different points of time prior to the removal of all the plants, single specimens were analyzed in order to determine the optimal metabolite concentration in the plant material.

Processing of Samples (See Flow Diagram, Figure 1). Immediately after harvesting the plants (600 g) were rinsed with methylene chloride (800 mL) and the drained liquid was concentrated to 100 mL (solution a, residue a). The rinsed plants were extracted, with homogenization, three times with 400 mL of chloroform/methanol (1:1, v/v). The extract was filtered off and concentrated to 250
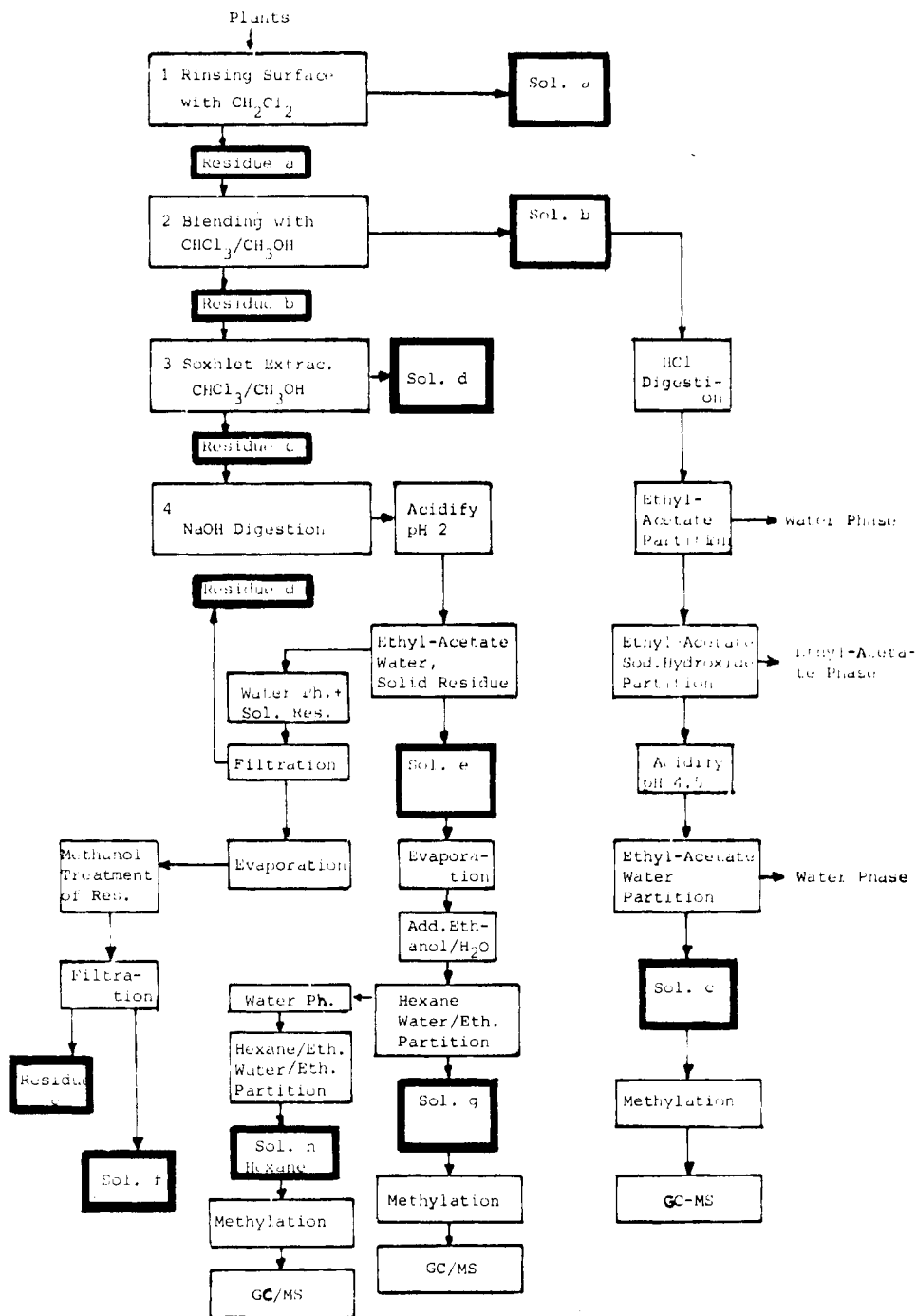
Figure 1. Working-up scheme.

mL (solution b, residue b). Solution b (125 mL) was concentrated to near dryness and refluxed for 1 h with 100 mL of 1 N hydrochloric acid. The hydrolysate was extracted with ethyl acetate (3 × 100 mL) and the ethyl acetate phase reextracted with 2 N sodium hydroxide (3 × 30 mL). The alkaline solution was adjusted to pH 4.5 and extracted with ethyl acetate (3 × 150 mL) and the latter was concentrated to 2 mL (solution c).

The filtercake (residue b) underwent an exhaustive extraction with chloroform/methanol (1:1, v/v) in a Soxhlet extractor. The extract was concentrated to 25 mL (solution d, residue c). A part of the Soxhlet extracted plant material (1/3) was refluxed with 1 N NaOH (500 mL) for 6 h. The products of digestion were acidified with HCl to pH 2 and extracted by shaking with ethyl acetate (3 × 600 mL) and after phase separation the latter was concentrated to 5 mL (solution e). The water phase

containing still some undigested material was filtered off (residue d) and the water phase concentrated to near dryness and taken up with methanol (500 mL) and again filtered off (solution f, residue e). The ethyl acetate solution (solution e) underwent a further cleanup. It was evaporated to dryness, taken up with 50 mL of ethanol/water (1:1, v/v), and extracted with hexane (3 × 75 mL) and the hexane phase concentrated to 1.5 mL (solution g), followed by an extraction with hexane/diethyl ether (3 × 75 mL, 1:1, v/v), the latter being reduced to 1 mL (solution h).

Methylation of Isolated Metabolites with Diazomethane. The following isolates and volumes were used: ethyl acetate phase c, 1.5 mL; hexane phase g, 1.5 mL; hexane–diethyl ether phase h, 0.6 mL. The methylation was carried out according to Deutsche Forschungsgemeinschaft (1969). After methylation the reaction solution

Table I. Radioactivity Recovered by the Consecutively Applied Extraction Steps from Wheat, Sampled 18 Days Posttreatment

Extraction step	Solution designation and solvent (see Fig. 1)	dpm $\times$ 10^6 [a]	% radioact. recovd [a]
1. Rinsing surface with CH_2Cl_2	a, CH_2Cl_2	4.3	3
2. Blending of the surface extracted plants with $CHCl_3/CH_3OH$	b, $CHCl_3/CH_3OH$	77.0	53
3. Soxhlet extraction of blended sample with $CHCl_3/CH_3OH$	d, $CHCl_3/CH_3OH$	9.0	6
4. NaOH digestion of Soxhlet-extracted sample	g, n-hexane	41 ⎫	
	h, n-hexane/ether	5 ⎭	32
	Residue d	0.8	0.6

[a] The total radioactivity in the original sample amounted to 144×10^6 dpm $\simeq 100\%$ (this is the sum of the radioactivity of the surface extract, the $CHCl_3/CH_3OH$ blending extract, and of residue b; see also Figure 1).

Table II. R_f Values of the Radioactive Substances in the Thin-Layer Chromatograms of the Various Isolates from Wheat

Extraction step	Designation of solution in the processing procedure and solvent	R_f values and rel amts of the components in the chromatograms			
1. Rinsing surface with CH_2Cl_2	a, CH_2Cl_2	0.1 6%		0.25 8%	0.34 11%	0.66 75%
2. Blending of surface-extracted plants with $CHCl_3/CH_3OH$	b, $CHCl_3/OH$	0.06 100%				
	c, ethyl acetate HCl, posthydrolysis of b	0.12 14%	0.18 12%	0.30 71%	0.38 3%	
3. Soxhlet extraction of blended sample with $CHCl_3/CH_3OH$	d, CH_3Cl_2/CH_3OH	0.06 100%				
4. NaOH digestion of Soxhlet-extracted sample	g, hexane			0.33 7%	0.43 93%	
	h, hexane/ether			0.33 88%	0.43 12%	
	f, methanol			0.07 100%		
Ref Hoe 23408 OH						0.65 ±0.05
Ref free acid of Hoe 23408 OH (metabolite III)					0.42 ±0.05	

was mixed with 25 mL of ethanol and 25 mL of distilled water, and the solution was shaken three times, each with 30 mL of hexane. The combined hexane phases were concentrated to the commencement of dryness (red light and fan) and the residue was taken up in a small amount of ethyl acetate and then made up to a volume of 1 mL with ethyl acetate in a small graduated measuring cylinder. Aliquots were taken for gas chromatographic and mass spectroscopic examination.

Mass Spectrometric and Gas Chromatographic Analyses of the Isolated Radioactive Substances. A Model 3200 Finnigan mass spectrometer coupled with a Model 9500 Finnigan gas chromatograph was used. Ionized methane was used for chemical ionization of the gas chromatographically separated substances.

Detailed operating conditions were as follows: separation column, 1.2 m, 3% OV-101 on Chromosorb WHP, 80–100 mesh; carrier gas, methane, 15 mL/min; temperatures, sample inlet, 300 °C, column, 150–300 °C, 10 °C/min; transfer, 320 °C; ionization chamber, 150 °C; excitation, 200 eV, 0.8 mA.

Measurement of Radioactivity. Determination of radioactivity of liquid samples followed immediately after mixing an aliquot part of the isolates (0.1 mL as a rule) with 20 mL of dioxane scintillator. Ethyl acetate solutions were always evaporated to the commencement of dryness and taken up in 20 mL of dioxane scintillator.

Solid specimens (e.g. extraction residues) were ignited in a Tricarb Sample Oxidizer, Model 305 (Packard Instruments), and the $^{14}CO_2$ was determined. The gel scraped off from thin-layer plates was suspended in dioxane scintillator for measurement. All measurements were carried out with a Tracerlab scintillation counter,

Model Spectro-coru/matic 100 a. The composition of the dioxane scintillator was as follows: dioxane (extra pure), 89.49%; naphthalene, 10.00%; 2,5-diphenyloxazole, 0.50%; 1,4-bis[2-(5-phenyloxazolyl)benzene, 0.01%.

Thin-Layer Chromatography (TLC). Thin-layer chromatography was carried out on commercially available silica gel plates (type G, 0.3 mm, Merck) and developed with a benzene/methanol/acetic acid solution (85:10:5, v/v). A microsyringe was used to supply a large enough quantity of the extract so that a sufficient amount of radioactivity (at least 1000 cpm) could be detected at the starting spot. For determination of radioactivity the gel was scraped from the developed plates in 5-mm strips.

RESULTS

Eighteen days after treatment of the wheat plants (three-leaf stage) with ^{14}C-labeled Hoe 23408 OH the harvested plants showed a total radioactivity of 144×10^6 dpm (see footnote a, Table I). This corresponds to a total residual quantity of 13.4 mg/600 g of plant material (about 22 ppm, calculated as Hoe 23408 OH). From Table I it can be seen that only 3% of this quantity can be washed from the surface of the plants with methylene chloride.

The greater part of the rinsed off residue consists of unaltered Hoe 23408 OH. This may be concluded from the corresponding peak in the radiogram of the thin-layer chromatogram, Table II. Three other peaks are clearly detectable, but have not been identified so far.

The major part of the radioactivity (53%), which could be extracted by organic solvents, could be dissolved from the substrate at room temperature by simple homogenization, and additional exhaustive extraction with the same solvent in the Soxhlet removed only a further 6%. The
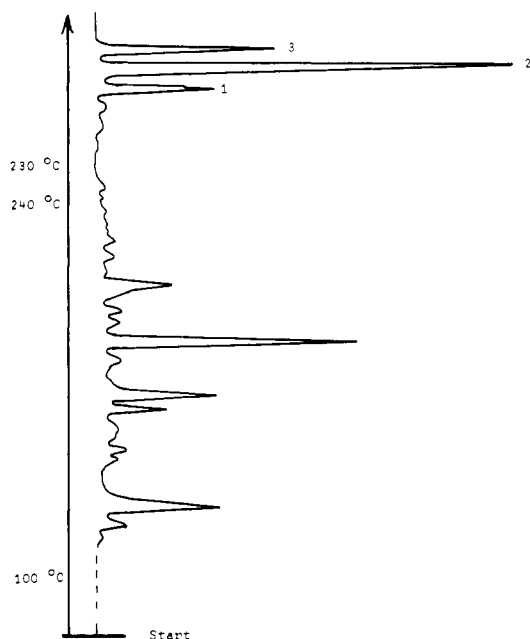
Figure 2. Gas chromatogram of the plant extract, second extraction, ethyl acetate phase c after methylation: experimental conditions, 1.2 m, 3% OV 101 on Chromosorb W HP, 80–100 mesh; detector, mass spectral total ion current.

bulk (32%) of the remaining bound residue could be recovered only by digestion with 1 N sodium hydroxide solution, thus making it accessible for determination (see Table I).

The thin-layer chromatogram of the chloroform/methanol extract (solution b) (Table II) shows a metabolite (or unresolved metabolites) present in a highly polar form (glucoside?). After cleavage of the conjugate with HCl, the bulk of the radioactivity passes through in the process of separation and appears in the ethyl acetate phase, solution c. The thin-layer chromatogram of this solution (Table II) shows that the bulk of the radioactivity (71%) appears in the chromatogram at an R_f value of 0.30. A further, smaller quantity of substance is distributed at the R_f values

of 0.12, 0.18, and 0.38. The R_f value of the principal component has shifted to a higher level as compared with that of the chromatogram of the original solution, indicating the cleavage of the conjugates. After methylation of this principal component the reaction product showed three chlorine containing dominating peaks in the gas chromatogram above 210 °C (Figure 2). There was no indication on other possible metabolites, which could be evaporated without destruction.

The three main components in the gas chromatograms (peaks 1, 2, and 3 in Figure 2) gave mass spectra containing the same parent and fragment ions. This indicated that these three compounds were isomeric.

Figure 3 shows a typical spectrum of the substance which is represented by peak 2 in Figure 2. The spectrum is relatively poor in bands, since the components eluted from the gas chromatograph were chemically ionized and only a few fragments were formed. The bands occurring in Figure 3 were assigned as follows:
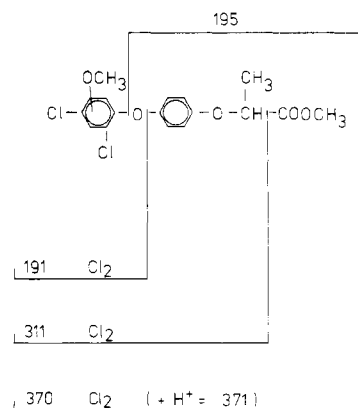
The occurrence of the masses 191/193 at a ratio of about 60:40 identifies this fragment as a carrier of two chlorine atoms. The simultaneous presence of the mass 195 without chlorine content and the absence of masses such as 226:
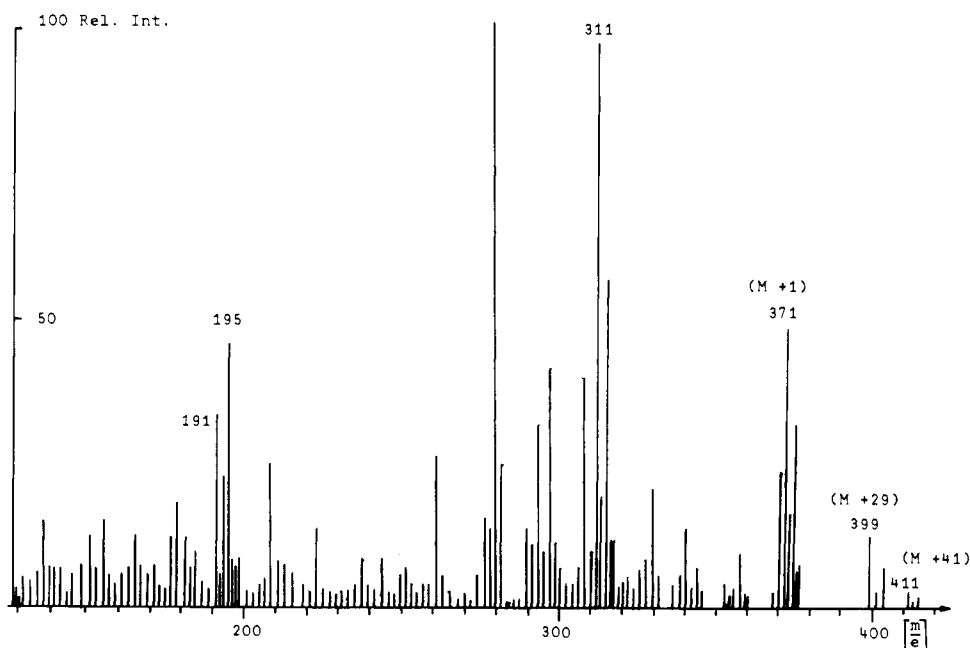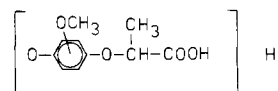

Figure 3. Mass spectrum of the metabolite II (after methylation), record of the substance under peak 2 in the gas chromatogram, Figure 2. For experimental conditions, see text.
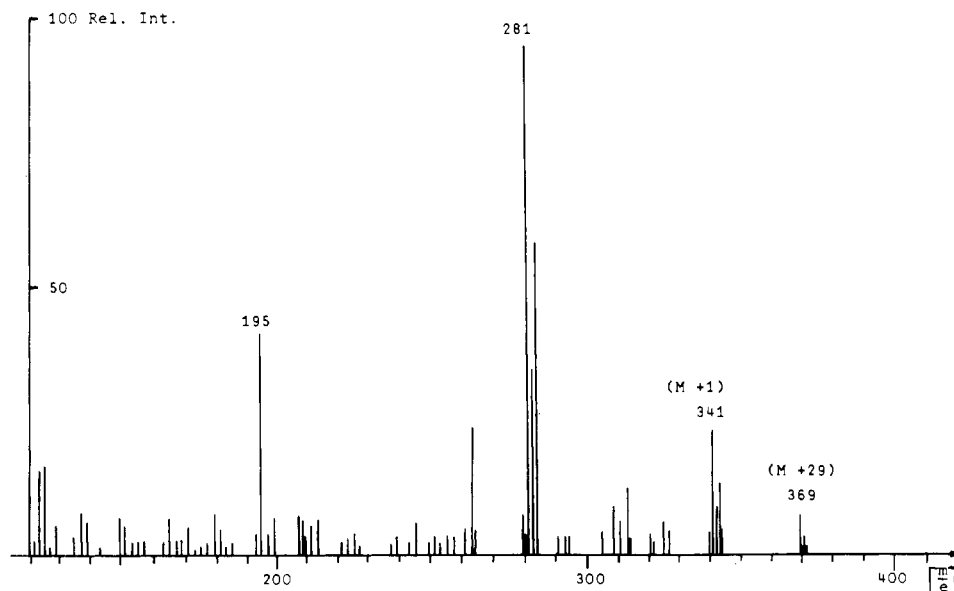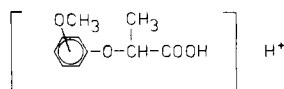
Figure 4. Mass spectrum of Hoe 23408 OH (equivalent to the methylated metabolite III). For experimental conditions, see text.

and 210:

$$\left[\begin{array}{c} \underset{|}{OCH_3} \quad \underset{|}{CH_3} \\ \bigcirc\!\!-O-CH-COOH \end{array} \right] \quad H^+$$

prove the conclusion that the methoxy group is a substituent in the dichlorophenyl ring.

To validate the findings we synthesized methyl 2-[4-(2',4'-dichloro-5'-methoxyphenoxy)phenoxy]propionate (IIb-OCH₃):

$$Cl-\bigcirc\underset{|}{\overset{OCH_3}{\bigcirc}}-O-\bigcirc-O\underset{}{\overset{CH_3}{-CH-COOCH_3}}$$
$$\underset{Cl}{|}$$
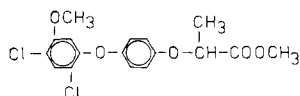
which gave the same mass spectrum as shown in Figure 3. The addition of the compound to the methylated solution c caused peak 2 in the gas chromatogram shown in Figure 2 to increase in height. If the conclusion that the three peaks correspond to the three possible position isomers was correct, then peak 2 would represent the 5'-methoxy compound (IIb-OCH₃). In consequence the 5' substitution, rather than the 3' and 6' substitutions, would be preferred.

The radioactive substances accessible after alkaline digestion (extraction 4 in Table I) were separated into two principal components (hexane phase solution g and hexane/ether phase solution h). The chromatograms of the methylated solution h gave an essentially similar record as shown in Figure 2 and the mass spectra of the equivalent peaks gave the same fragments as shown for the compound

IIb-OCH₃ in Figure 3. The chromatogram of the methylated solution g (hexane) exhibited many peaks and only one dichloro-containing moiety could be detected to a significant amount exhibiting a mass spectrum like that of the unaltered Hoe 23408 OH (Figure 4) in the flank of an interfering peak.

This indicates that prior to methylation the free acid (metabolite III) must have been present in the hexane phase (solution g). This is proved by the R_f value of the reference compound. From the results it may be concluded that the main metabolite in plants is compound II in a conjugated form. Small amounts of compounds III and II are obviously bound to the biomass of the plant and could not be extracted with the organic solvents used; they were only accessible after an alkaline digestion of the already extracted plant homogenate. This should be taken in account in any residue analytical procedure which should be started with an alkaline digestion of the sample.

LITERATURE CITED

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