Table I. DBCP Residues in Peaches Harvested from Orchards Treated with DBCP

location	DBCP treatment, av no. of L/treated ha	no. sites	mean DBCP concn, ppb (3 replicates/site)
Coastal area	control	3	n.d.[a]
	50.5	3	n.d.
	101.0	3	0.27
Sandridge area	control	2[b]	n.d.
	51.4	2	0.13
	102.9	2	0.24
Piedmont area	control	3	n.d.
	69.2	3	0.41
	137.5	3	0.72
overall	control	8	n.d.
	58.0	8	0.19
	115.1	8	0.43

[a] n.d. = none detected. [b] No residue data to report for one site because entire peach crop was destroyed by freeze.

residues of 0.19 ppb of DBCP while an average application rate of 115.1 L/treated ha resulted in residues of 0.43 ppb of DBCP (Table I).

DBCP residues were higher in samples collected from the Piedmont area of South Carolina (Table I) than from samples collected from either the Sandridge or Coastal area. This may have resulted from higher application rates in this area. Additionally, the presence of clay in these soils may have affected adsorption and subsequent uptake and/or volatilization of DBCP. However, this is speculative since factors other than application rates that might affect fruit residue were not examined.

Previous suppositions that DBCP volatilizes and becomes adsorbed onto the developing peach or that contaminated dust settles on the fruit (K. Maddy, personal communication) seem less credible by the appearance of DBCP residues in fruit following a postharvest, fall fumigation treatment. Contamination by uptake, translocation, and accumulation of DBCP in the developing peach appears to ensue. Earlier pilot studies indicated that DBCP residues can be found in the peach pit and conductive tissues of the tree (G. Carter, unpublished experiments).

In individual plots where application rates were 46.8 L/treated ha or less residues of DBCP were not found in the fruit, an observation not reflected by the reporting of average values. This is a key point since 46.8 L/treated ha was the recommended application rate prior to cancellation. Potential for residues in fruit, therefore, would probably not limit the use of an effective concentration of DBCP in peach orchards if other problems, such as the tendency of DBCP to contaminate shallow wells, could be resolved.

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We thank R. W. Miller, Department of Plant Pathology and Physiology, Clemson University, and cooperating peach growers for providing field plots used in this study and Jane E. Lawrence for valuable technical assistance.

Registry No. 1,2-Dibromo-3-chloropropane, 96-12-8.

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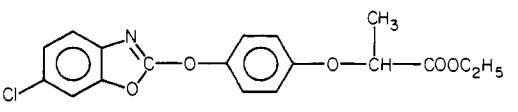
Metabolism of the Herbicide Hoe 33171 in Soybeans

Ottmar Wink, Erich Dorn, and Klaus Beyermann*

Soybean plants were treated with formulated ^{14}C-labeled Hoe 33171, ethyl 2-[4-[(6-chloro-2-benzoxazolyl)oxy]phenoxy]propanoate. At the end of a 15-day degradation period 75% of the radioactivity in/on the leaves was characterized as water-soluble metabolites apart from 25% unchanged parent compound present mainly on the leaf surface. The free carboxylic acid of Hoe 33171 (5%) did not accumulate because of rapid conversion to bound residues (30%) and more than 10 polar conjugates, some of which contained the structural elements of hydroxylated (4-hydroxy and 5-hydroxy isomer; 12.8%) and nonhydroxylated 6-chloro-2,3-dihydrobenzoxazol-2-one. The latter is the common structure of 43% of the total residue and is suitable to be quantified in routine residue analysis. For the identification of the hydroxylated metabolites 4-hydroxy-6-chloro-2,3-dihydrobenzoxazol-2-one was synthesized.

The plant protection compound designated Hoe 33171 is a selective herbicide to control a broad spectrum of grass weeds in dicotyledonous crops (Hoechst AG, 1976; Bieringer et al., 1982) and has the structural formula



ethyl 2-[4-[(6-chloro-2-benzoxazolyl)oxy]phenoxy]-propanoate

Previously reported results on the mode of action by Köcher et al. (1982) indicate rapid destruction of plant

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Table I. Distribution of Radioactivity throughout the Different Parts of Soybean Plants, 15 Days Posttreatment, and Radioactivity Balance of the Extracted, Directly Hit Leaves

part of the harvested plants	fresh weight, g	distribution of radioactivity, %
leaves, directly hit during spraying	220.7	87
stems, directly hit during spraying	208.5	7
newly grown stems and beans	63.4	1
newly grown leaves	152.0	5

Fractionation of Directly Hit Leaves

extraction step	fraction (see Figure 2)	distribution of radioactivity, %
total		100
(1) rinsing surface with diethyl ether	S	23.6
(2) hexane-soluble products	A	5.1
(3) water-soluble products	B	41.3
(4) nonextractable, bound residue	R	30.0

meristems in spite of very low translocation rates of the applied radioactivity to these meristems. In this respect it was important to know which metabolites are formed from Hoe 33171.

The compounds referred to in the text are identified with Roman numerals as explained by structural formulas and chemical names in Figure 10. The two methyl derivatives of III are designated as III-N-CH_3 and III-O-CH_3. Correspondingly, the methyl ethers of IV and V are identified as IV-methyl-N-CH_3 and IV-methyl-O-CH_3 and V-methyl-N-CH_3 and V-methyl-O-CH_3.

EXPERIMENTAL SECTION

Hoe 33171-^{14}C was synthesized by D. Gantz, Hoechst AG, with >98% radiochemical purity and 975 MBq/g (≅26.35 mCi/g) specific radioactivity. The position of carbon-14 labeling was chlorophenyl uniformly.

Preparation Used for Plant Treatment. The formulation emulsifiable concentrate used for the treatment of plants was prepared by mixing the following components: 26.0 mg of Hoe 33171-^{14}C plus 16.7 mg of Hoe 33171 (nonlabeled) plus 220.0 mg of formulation ingredients plus 56 mL of water. Main formulation ingredients were Xylol (27.5%), DMF (25.0%), Genapol (16.0%), and some additional emulsifying agents. The concentration of Hoe 33171 in the formulated preparation was 15.8% (w/w) of 100% pure active ingredient.

Plant Material. Soybeans (*Gylcine max*), variety Evans, were grown in pots of 13-cm diameter (density of 10 beans per pot). After the emergence the plant density was reduced to four plants per pot. Growth conditions in the greenhouse were 24 °C during the daytime and 16 °C during the night. Artificial sunlight (mercury lamp, Philips FPL deluxe; 7000 lx at the plant surface) was provided during days with a cloudy sky. The plants were irrigated by watering the soil from below.

Treatments. The plants were treated with a total of 43 mg of formulated ^{14}C-labeled Hoe 33171 (594 MBq/g ≅ 16 mCi/g). The suspension was applied to the plants by means of a Grapho-Sprayer. The spraying device

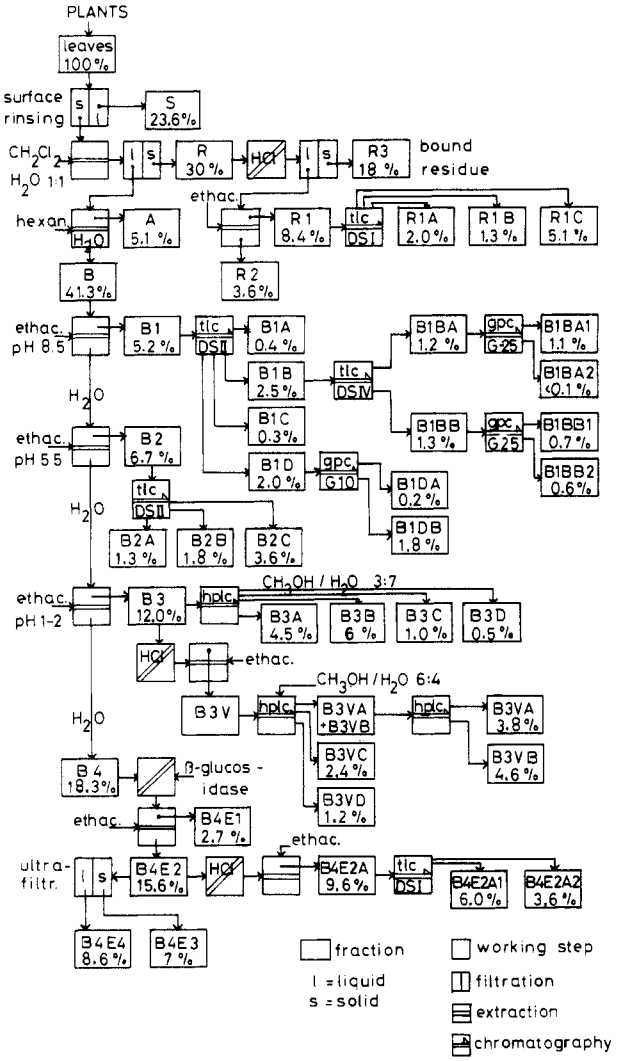

Figure 1. Flow scheme of the processing of the leaves.

generates droplet sizes similar to those of field sprayers. The spraying mixture, under atmospheric pressure, is added to a nitrogen stream (2 bar). The rate was 0.3 kg of a.i./ha and 400 L of water/ha. The total experimental area equaled 1.4 m^2.

Sampling. The plants were harvested 15 days after treatment. The leaves and stems, which had been hit during treatment, were separated from newly grown leaves and stems. Samples from each part were homogenized for the measurement of the distribution of the radioactivity (Table I). All the metabolite identification reported below were carried out with the directly sprayed leaves.

Processing of Samples (See Flow Scheme, Figure 1). Immediately after the plants were harvested, the leaves (220.7 g) were rinsed with diethyl ether (1.0 L), the latter of which was concentrated to 50 mL (fraction S). The rinsed leaves were extracted with homogenization for 3 times with 500 mL of CH_2Cl_2–H_2O (1:1, v/v). The extraction mixture filtered and the two liquid phases were separated. The water phase was extracted again with *n*-hexane, the organic phases were combined and evaporated (fraction A). The filter cake (fraction R) was refluxed with HCl (c = 3 mol/L) for 6 h. The solid material was filtered off (fraction R3). The acidic solution was extracted by shaking with ethyl acetate. The ethyl acetate phase (Fraction R1) was subjected to preparative TLC. The extracted water phase (fraction B, pH 6), containing the polar metabolites, was first extracted at pH 8.5 with ethyl acetate. After separation of the organic layer (fraction B1)

the pH value was adjusted to pH 5.5, followed by the extraction with ethyl acetate (fraction B2). Subsequently the pH value was adjusted to pH 1–2 and the extraction with ethyl acetate led to the fractions B3 (organic) and B4 (aqueous). The organic phases were investigated by TLC, GPC, and HPLC to separate and isolate the different metabolites for GC–MS analysis before and after hydrolysis with HCl (c = 1 mol/L). The water phase (fraction B4), containing the highly polar transformation products, was incubated with β-glucosidase, and after extraction with ethyl acetate (fraction B4E1) the liberated exocons were identified.

Methylation with Diazomethane. The methylation was carried out according to the method of Deutsche Forschungsgemeinschaft (1969). Sample preparation involved evaporation to dryness and redissolving in methanol and ethyl acetate, respectively. Diazomethane was passed through the solution with a stream of nitrogen.

β-Glucosidase Treatment. An aliquot fraction was evaporated to dryness at <40 °C and incubated with 50 mg of β-glucosidase (Fluka AG, Buchs Switzerland, 1000 units/mg) in 2 mL of 0.1 mol/L sodium acetate, pH 5.0 (4 h, 25 °C). After addition of 20 μL of 10% hydrochloric acid, radioactive cleavage products could be extracted with several portions of ethyl acetate for further examination.

Gas Chromatographic Analyses with a Radioactivity Detector (GC-14-C). GC-14-C analyses were carried out by use of a gas chromatograph, Model 427, from Packard Instruments by means of a separation column, 0.8 m × 2 mm i.d., 10% OV-101 on Chromosorb WAW DMCS, 80–100 mesh. Two detectors were used: detector 1, a heat conductivity detector; detector 2, mounted serially subsequent to a combustion furnace (filled with copper oxide, 800 °C), for the detection of radioactivity (model: gas proportional counter 894, from Packard Instruments). All separations were carried out by means of a temperature program: from 100 to 300 °C, 10 °C/min.

Mass Spectrometric and Gas Chromatographic Analyses (GC–MS). A mass spectrometer, Model MAT 44 (Varian), coupled with a gas chromatograph, Model 1400 (Varian), was used. An FUSI-OV-1 column and electron impact (70 eV) ionization mode were used in all experiments. The detailed conditions during the measurements are given under Results, where the measurements are reported.

Measurement of Radioactivity. Aliquot portions of the solid samples were combusted in a Tricarb sample oxidizer (Model 305, Packard Instruments); the $^{14}CO_2$ was absorbed in 8 mL of Carbo-Sorb (Packard) and mixed with 13 mL of scintillation cocktail XAA 8 (Riedel de Haen AG). Aliquot portions of liquid fractions (about 0.1 mL) were added to 10 mL of scintillation cocktail XAA 8. The gel scraped off from thin-layer plates was suspended in the same scintillation cocktail for measurement. All measurements were carried out with two liquid scintillation counters (Model Betaszint BF 5000, Fa. Berthold, and Model Spectro-coru/matic 100a from Tracerlab). Quenching effects were determined by use of the method of external standard in the case of the BF 5000 and internal standard in the case of Spectro-coru/matic 100a.

Thin-Layer Chromatography (TLC). Commerically available silica plates (0.25 mm, Merck 60, GF-254, Fa. Merck) were used. The solutions were applied to the plates by using microsyringes. For development the following solvent mixtures were applied: DS I, toluene–ethyl acetate–water–acetic acid (50:50:0.5:1 v/v/v/v); DS II, isopropyl alcohol–cyclohexane–water–acetic acid (70:30:1:1 v/v/v/v); DS III, chloroform–ethyl acetate–acetic acid (70:30:3 v/v/v); DS IV, ethyl acetate–isopropyl alcohol–water (65:23:12 v/v/v).

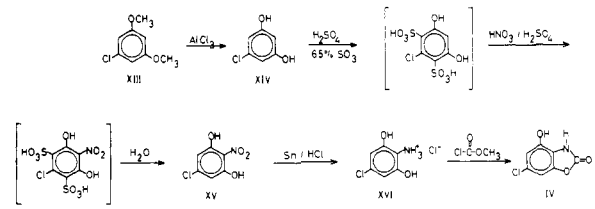

Figure 2. Scheme of synthesis of 4-hydroxy-6-chloro-2,3-dihydrobenzoxazol-2-one (IV).

Gel Permeation Chromatography (GPC). GPC separations were carried out by means of a separation column with adapter (Pharmacia), bed height 30 cm, 2.5-cm i.d., filled with (a) Sephadex G-10 or (b) Sephadex G-25 fine (Pharmacia). The elution mixture contained isopropyl alcohol/water in the ratio 1:1. The flow rate was 0.1 mL min^{-1} cm^{-2}. The effluent was passed through a flow cell for recording the UV absorbance and subsequently 2-mL fractions were collected for measuring the radioactivity distribution.

High-Performance Liquid Chromatography (HPLC). The instrument was a Waters HPLC pump unit, Model M-45, and the following conditions were used: column, LiChrosorb from Merck, static phase RP-18, 7 μm, length 24 cm, i.d. 0.4 cm; mobile phase, different ratios of methanol and water; detector, UV detector, wavelength 254 nm, from Waters Associates, Model 440. The effluent was collected in 0.4-mL fractions for measurement of the radioactivity.

Synthesis of the Reference Compound for Metabolite IV (4-Hydroxy-6-chloro-2,3-dihydrobenzoxazol-2-one) (Figure 2). 1-Chloro-3,5-dimethoxybenzene (0.12 mol) (EGA-Chemie) was treated with 0.38 mol of $AlCl_3$ in toluene (Russel and Gulledge, 1942), to cleave the two ether bonds.

Nitration of 1-chloro-3,5-dihydroxybenzene was carried out according to Kauffmann and de Pay (1904). At first the substance was sulfonated with concentrated sulfuric acid (65% SO_3) at 50 °C. Then a mixture, containing nitric acid (96%) and sulfuric acid (65% SO_3) in the ratio 1:1 (w/w), was added under ice cooling. The mixture was stirred for 3 h at 0 °C. After hydrolysis of the sulfonated product, the formed 1-chloro-4-nitro-3,5-dihydroxybenzene was separated by water–steam distillation. The yellow crude product was chromatographed on thin-layer plates of silica gel GF 254 (Merck, Darmstadt) by using the solvent mixture DS I. The desired product showed a R_f value of 0.65. The dinitro product had a R_f value of 0.19. In the preparative scale of pure mononitro product, the crude mixture was chromatographed on a 22 × 200 mm column of 0.05–0.2-mm mesh silica gel (Merck) with solvent DS I.

The reduction of the red mononitro product was carried out in hydrochloric acid (c = 8 mol/L) with tin powder according to Kauffmann and de Pay (1906).

To remove excess tin, hydrogen sulfide was bubbled through the solution. The white amine hydrochloride (highly susceptible to air oxidation) was converted with chloromethyl formate in toluene in the presence of triethylamine under stirring at room temperature.

After 1 day of incubation the reaction mixture was refluxed with hydrochloric acid (c = 1 mol/L) for 2 h. The product was extracted with ethyl acetate. After evaporation the residue was chromatographed on thin-layer plates of silica gel by using developing solvent DS I. That synthetic product, which showed the same R_f value as the

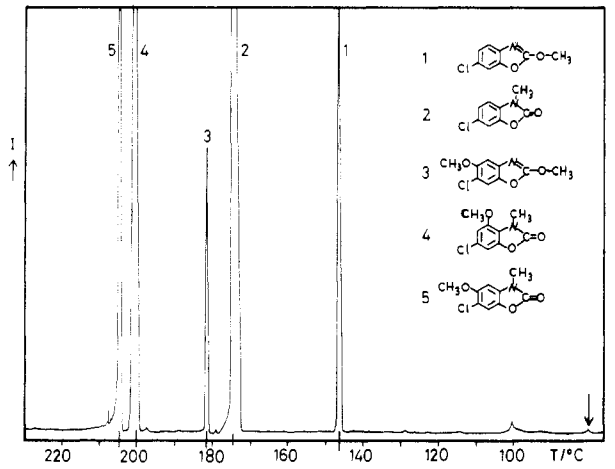

Figure 3. Total ion monitoring chromatogramm of the methyl derivatives of the synthetic reference compounds (III–V). Experimental conditions: FUSI-OV-1 column, 25 m, i.d. 0.22 mm; split 10 mL/min; temperature program 100–300 °C, 10 °C/min. Retention temperatures of the compounds: 1 = 149 °C; 2 = 177 °C; 3 = 182 °C; 4 = 200 °C; 5 = 205 °C.

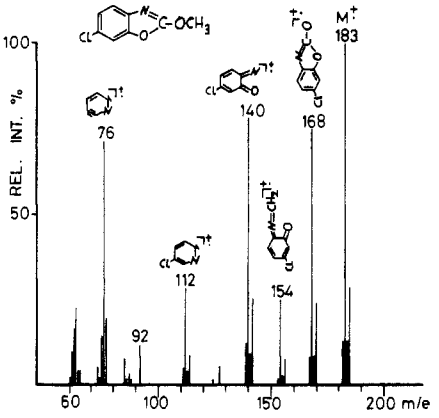

Figure 4. Mass spectrum of the *O*-methyl isomer of synthetic compound III (III-O-CH_3).

compound, isolated from plants, was eluted from the thin-layer plates to yield 4-hydroxy-6-chloro-2,3-dihydrobenzoxazol-2-one.

The mass spectral data of the synthetic compound and its methyl derivatives, as well as its TLC and HPLC properties, were identical with those of the isolated metabolite, confirming the identity of isolated and authentic synthetic compound.

RESULTS

Preliminary investigations on the authentic reference compounds (III–V) were necessary for the interpretation of the GC–MS data obtained from several isolated metabolites. Methylation of the reference substance III resulted in the formation of *N*- and *O*-methyl derivative (III-N-CH_3 and III-O-CH_3) in the ratio 2.5:1, which can be separated by use of GC (Figure 3). NMR analyses of the two isomers separated by use of HPLC allowed to identify the early eluted GC peak as the *O*-methyl isomer and the subsequently eluted one as the *N*-methyl isomer. In the HPLC analysis with a methanol–water mixture (8:2) on the RP-18 column the *N*-methyl isomer was eluted prior to the *O*-methyl product. The two isomers showed a very different mass spectrometric fragmentation pattern (Figures 4 and 5). The accurate masses of the purposed fragments were determined with high-resolution mass spectrometric analysis.

For the identification of the hydroxylated metabolites, the 4-hydroxy-6-chloro-2,3-dihydrobenzoxazol-2-one (IV),

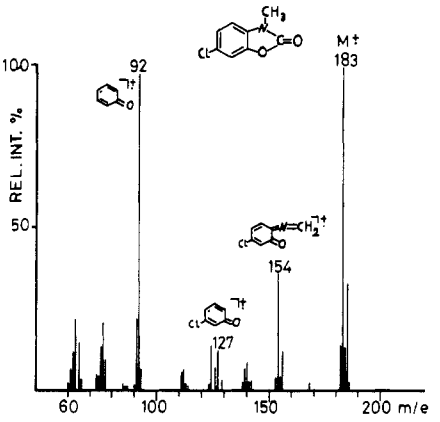

Figure 5. Mass spectrum of the *N*-methyl isomer of synthetic compound III (III-N-CH_3).

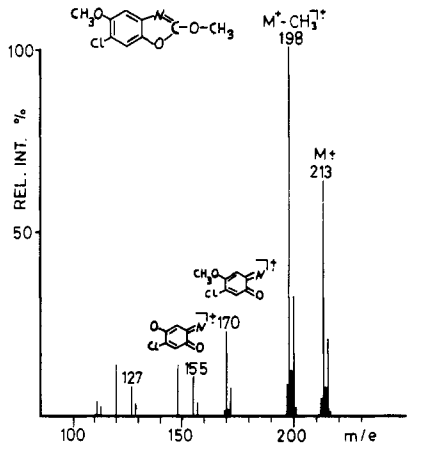

Figure 6. Mass spectrum of the *O*-methyl isomer of methylated synthetic reference compound V (V-methyl-O-CH_3).

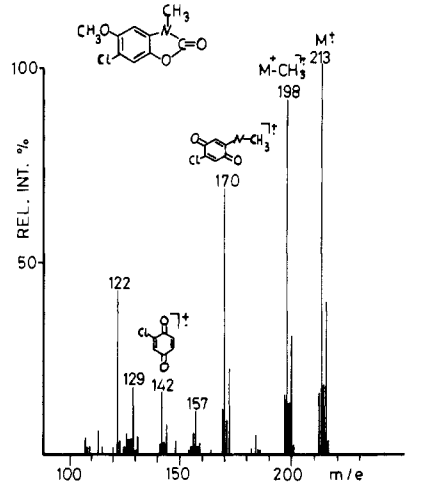

Figure 7. Mass spectrum of the *N*-methyl isomer of methylated synthetic reference compound V (V-methyl-N-CH_3).

not described in the literature before, was synthesized (see Figure 2). Together with the 5-hydroxy-6-chloro-2,3-dihydrobenzoxazol-2-one (V), synthesized by R. Handte (Hoechst AG), the HPLC, TLC, and derivatization behavior and mass spectrometric fragmentation were studied. The two isomers could not be separated by use of TLC (DS I; R_f value of both compounds are 0.24), but a clear HPLC separation is possible on the RP-18 phase with the elution solvent methanol–water (6:4) and a flow rate of 0.4 mL/min. Methylation of compound V resulted in the formation of *N*-methyl and 2-*O*-methyl derivaties (V-methyl-N-CH_3 and V-methyl-O-CH_3) separable by means of GC

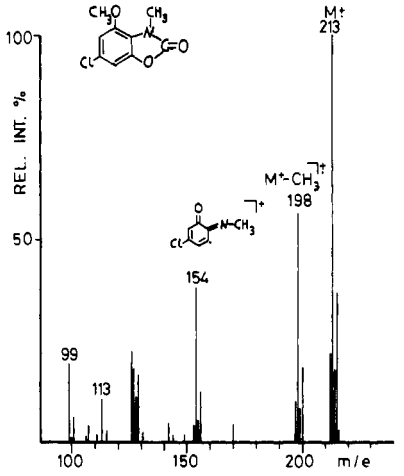

Figure 8. Mass spectrum of the methylated synthetic reference compound IV (IV-methyl-N-CH_3).

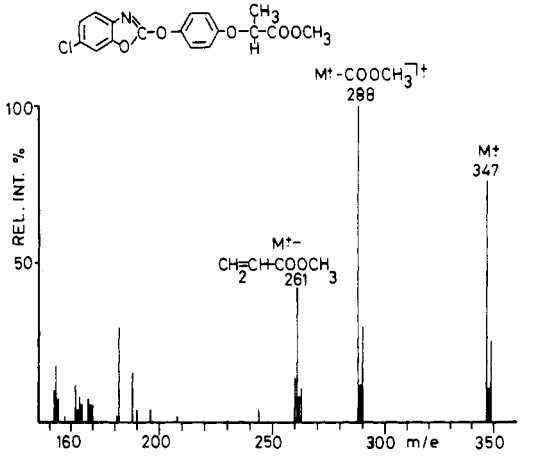

Figure 9. Mass spectrum of the methylated free carboxylic acid (II) of Hoe 33171.

(Figure 3), showing different fragmentation patterns in the mass spectrum (Figures 6 and 7).

After the treatment of compound IV with diazomethane in methanol only one methylation product occurs in GC–MS analysis (see Figures 3 and 8). Because of the analytical data, this is assumed to be the *N*-methyl isomer (IV-methyl-N-CH_3). Comparison of the mass spectra in Figures 6–8 showed significant differences in the fragmentation pattern (qualitative and quantitative).

For the metabolism study the soybean plants were harvested 15 days after treatment with ^{14}C-labeled Hoe 33171 and separated in four parts (see Table I). The distribution of the radioactivity in the different plant parts showed that only 6% of the applied radioactivity was translocated into newly grown parts.

Only the directly sprayed leaves were investigated for metabolites (Table I). The identified transformation products in the different fractions are given in the flow scheme of the workup procedure (see Figure 1).

The analytical data, which led to the identification of the radioactive metabolites in each fraction, are given below. The values in parentheses show which percentage of the total radioactivity was contained in the respective fraction.

Fraction S (23.6% surface rinse) was identified to be the parent compound Hoe 33171 by use of TLC and concomitant chromatography with the authentic parent compound using three developing solvents DS I (R_f 0.66), DS II (R_f 0.69), and DS III (R_f 0.69).

Fraction A (5.1%) was identified to be the parent compound by use of TLC and GC-14-C analysis and comparison with the authentic material.

Fraction B1BA1 (1.1%) and fraction B2C (3.6%) were identified to be compound II (free carboxylic acid) based on the following properties: the bulk is extracted at pH 5.5 (fraction B2C), the smaller portion at pH 8.5 (fraction B1BA1); R_f values, DS I 0.17 and DS II 0.46. MS direct inlet data at 280 °C ion source, M^+ ion at *m/e* 333/335 (62/20%), *m/e* 288/290 (40/13%) loss of CO_2 and H, *m/e* 261/263 (100/32%) loss of CH_2=CH–COOH. After methylation and subsequent GC–MS analysis, the mass spectrum is equivalent to that of the methylated reference substance II (see Figure 9). Because of the lability of compound II under acidic conditions with formation of compound III as an artifact, only slightly acidic media were applied during isolation.

Fraction B1DB (1.8%). After TLC with DS I (R_f 0.24) and DS II (R_f 0.70) and GPC separation, the GC–MS data of the methylated metabolite were found to be identical with those of 4-hydroxy-6-chloro-2,3-dihydrobenzoxazol-2-one (compound IV) (see Figure 8).

Fraction B3VB (4.6%) was identified to be the 5-hydroxy-6-chloro-2,3-dihydrobenzoxazol-2-one (V), which is a structural element of a conjugate. Properties were as follows: The latter one was extractable at pH values below 2 only. After cleavage of the conjugate by use of acid treatment (c_{HCl} = 1 mol/L, 4 h, refluxing), extraction from the reaction mixture, chromatographic separation by use of HPLC (RP-18), and elution prior to the 4-hydroxy isomer (IV) and the nonhydroxylated one (III), the two methylated derivatives were separated by means of GC and showed different mass spectrometric fragmentation. Their GC and MS properties were demonstrated to be identical with those of the authentic reference compound (V).

Fraction B3VC (2.4%) was identified to be the 4-hydroxy isomer (IV) as a structural element of a polar conjugate, extracted at pH 1–2. Properties were as follows: After cleavage of the conjugate by use of acid treatment (c_{HCl} = 1 mol/L, 4 h, refluxing), extraction from the reaction mixture, chromatographic separation by use of HPLC (RP-18), and elution subsequent to the 5-hydroxy isomer (V) and prior to the nonhydroxylated compound (III), there was only one dominating radioactive peak on GC analysis after methylation, and the fragmentation pattern is the same as that one of the synthetic product (IV) (Figure 8).

Fraction B3VD (1.2%) was identified to be 6-chloro-2,3-dihydrobenzoxazol-2-one (III) as a structural element of a conjugate. Properties were as follows: GC–MS analyses of the methylated derivatives revealed identity with the authentic reference compound. The compound was released form its conjugate by acid hydrolysis.

Fraction B4E1 (2.7%) was identified to be the 5-hydroxy isomer (V). This compound was conjugated as a glucoside, which was cleaved by means of β-glucosidase. GC–MS data of the methylated authentic reference compound (V) were identical with those of the isolated metabolite (Figures 6 and 7).

Fraction B4E2A (9.6%). One portion (3.6%) of the radioactivity in this fraction could be attributed to compound III after GC–MS analysis of the methylated derivative and comparison with the data of the authentic compound III. The structural element was liberated from its conjugate on acid treatment.

Fraction B4E3 (7%). The finding that the radioactive compounds could not pass a filter with an exclusion pore size of molar mass >1000 daltons (ultrafiltration) allows us to conclude that these compounds are cellular components synthesized from completely degraded Hoe 33171

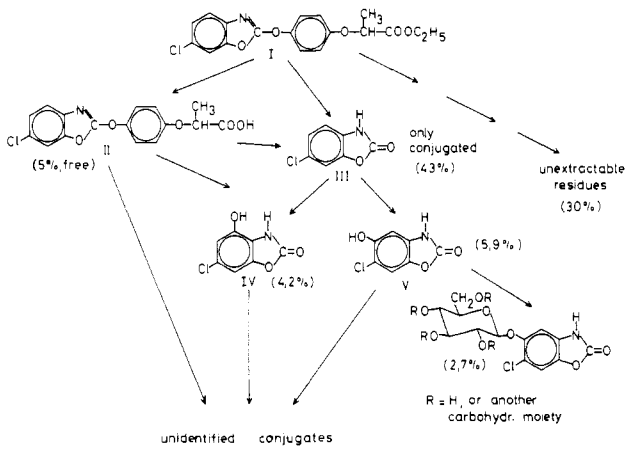

Figure 10. Proposed degradation scheme of Hoe 33171 in soybean with structural formula and chemical names of the compounds: I = Hoe 33171; II = 2-[4-[(6-chloro-2-benzoxazolyl)-oxy]phenoxy]propionic acid; III = 6-chloro-2,3-dihydrobenzoxazol-2-one; IV = 4-hydroxy-6-chloro-2,3-dihydrobenzoxazol-2-one; V = 5-hydroxy-6-chloro-2,3-dihydrobenzoxazol-2-one.

or degradation products bound to polymer cell constituents.

Fraction R1B (1.3%) was identified to be the 5-hydroxy isomer (V) by use of GC–MS analysis of the methylated derivatives (Figures 6 and 7). This structural element was bound in the solid residues even after exhaustive extraction and was released from the solid residues after acid treatment (c_{HCl} = 3 mol/L, 6 h, refluxing).

Fraction R1C (5.1%) was identified to be nonhydroxylated product III as revealed by analysis of the methylated derivatives and comparison with the reference compound. This metabolite was also released from the solid residues after acid cleavage.

CONCLUSION

This report provides information on the quality of the residues in soybean plants that originated from Hoe 33171 application (see degradation scheme, Figure 10). The very low residue level in the beans at the normal day of harvest does not enable identification; thus, we characterized the metabolites at that growth stage (day 15) when the ratio of metabolite amount to plant material was the most favorable from an analytical point of view.

In a parallel experiment, Dorn et al. (1983), soybean plants were cultivated post day 15 until maturity. In the fully developed beans no radioactive residues were detected above the limit of quantitation (LOQ = 0.005 μg/g, calculated as μg of active ingredient equiv/g).

Registry No. I, 66441-23-4; II, 73519-55-8; III, 19932-84-4; IV, 88412-28-6; V, 88412-29-7; 1-chloro-3,5-dimethoxybenzene, 7051-16-3; 1-chloro-3,5-dihydroxybenzene, 52780-23-1; 1-chloro-4-nitro-3,5-dihydroxybenzene, 88412-30-0.

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A Simple Single-Step Derivatization Method for the Gas Chromatographic Analysis of the Herbicide Glyphosate and Its Metabolite

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A single-step derivatization method for the herbicide glyphosate [*N*-(phosphonomethyl)glycine] and its major metabolite (aminomethyl)phosphonic acid is reported that allows for their analysis at the μg/mL level by gas chromatography with flame photometric detection. Derivatization is achieved with the reagent *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide, which introduces the dimethyl-*tert*-butylsilyl group at active hydrogens.

Glyphosate [*N*-(phosphonomethyl)glycine] (GLYPH) is a nonselective postemergence herbicide with a growing list of international uses. When used in weed control, it does not injure crops planted immediately after treatment (Sprankle et al., 1975). (Aminomethyl)phosphonic acid (AMPA) has been shown to be the major metabolite in plants, water, and soil (Sprankle et al., 1978).

Various approaches to the analysis of GLYPH and AMPA have been taken. These include gas chromatography (GC) after chemical derivatization ("Pesticide Analytical Manual", 1980; Guinivan et al., 1982), high-performance liquid chromatography (HPLC) utilizing postcolumn fluorogenic labeling (Moye and St. John, 1980; Moye et al., 1983), and thin-layer chromatography (TLC; Sprankle et al., 1978; Young et al., 1977). While the postcolumn fluorogenic labeling procedure has performed well in our hands, some laboratories do not have the instrumentation required and would prefer a GC procedure. However, both published GC procedures require double derivatizations, and one ("Pesticide Analytical Manual", 1980) requires the preparation and use of diazomethane, a highly toxic and explosive reagent.

This report describes the preparation and analysis of derivatives of GLYPH and AMPA using a single reaction, which is rapid, clean, and accomplished with a relatively

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