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.

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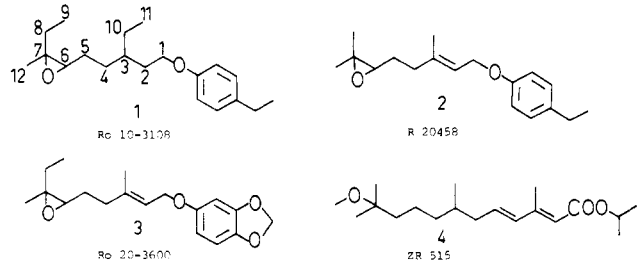
Environmental Degradation of the Insect Growth Regulator 6,7-Epoxy-1-(*p*-ethylphenoxy)-3-ethyl-7-methylnonane (Ro 10-3108) in Polluted Water

Silvia Dorn, Gottfried Oesterhelt, Miloš Suchý,* Karl H. Trautmann, and Hans-Kaspar Wipf

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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was kept constant by adding distilled water as necessary.

For the balance study a sample of labeled [2,3-^{3}H]Ro 10-3108 (1) was metabolized similarly in 1 l. of polluted water and worked up separately. Some of the intermediate metabolites of Ro 10-3108 which were identified in the first test were synthesized and subjected to analogous environmental influences at a later date.

Extraction of Metabolites. After 4 weeks' exposure to the open air the incubated water was lyophilized to a volume of 1 l. and extracted with ether in a Kutscher-Steudel percolator for 72 h. The extract was separated, dried over sodium sulfate, and concentrated in vacuo.

In the degradation experiment with the radioactive sample of Ro 10-3108, 75 g of sodium chloride was added to the incubated water, the solution was extracted with ethyl acetate (1.6 l. in four portions), and the organic phase was dried over sodium sulfate and concentrated in vacuo.

Measurements of Radioactivity. The amounts of tritium in the ethyl acetate extract and in the extracted aqueous phase were determined by standard liquid scintillation counting. The proportion of unaltered Ro 10-3108 (1) in the ethyl acetate extract was determined by thin-layer chromatography. A known amount of the extract was spotted on a silica gel plate (F-254, 20 × 20 cm, E. Merck, Darmstadt) which was developed with benzene–ethyl acetate (1:1). The band corresponding to Ro 10-3108 (1) was scraped off, eluted, and quantified by liquid scintillation counting.

Chromatographic Conditions. The gas chromatographic data were obtained with a Fractovap Model G I gas chromatograph (Carlo Erba), equipped with a flame ionization detector. A 25 m (0.32 mm i.d.) glass capillary column coated with SF-96 silicone oil was used (H. Jaeggi, 9043 Trogen, Switzerland). Operating conditions were: isothermal at 170 °C column temperature; carrier gas, helium at 2 kg/cm^2 (flow rate, 4 ml/min).

For the coupled gas chromatographic–mass spectrometric determinations (GC–MS) a Perkin-Elmer 990 gas chromatograph was interfaced with a Varian MAT Model CH-7 mass spectrometer. A 2 m (2.2 mm i.d.) glass column, packed with SE-30 (5%) on Gas-Chrom Q (80–100 mesh), was used for these experiments. Operating conditions were: column temperature, linear temperature program, 150–280 °C; 6 °C/min; carrier gas, helium (flow rate, 30 ml/min).

Identification by GC–MS was carried out on the trimethylsilyl (Me_3Si) derivatives of the corresponding compounds. Derivatives of the crude extract and (where applicable) of the authentic samples were prepared by standard silylation techniques using bis(trimethylsilyl)-acetamide, trimethylchlorosilane, and pyridine.

It was ascertained that the two metabolites 8 and 9 are not artefacts. They might possibly have originated from thermal decomposition of 5 or rearrangement of 1 (Anderson et al., 1972) in the gas chromatograph, although this process was not observed to occur with the pure products 1 and 5. The degradation experiment with intermediate metabolite 5 showed unambiguously that 8 and 9 are secondary degradation products and can be observed in the absence of 1. 8 and 9 were then separated from the diol 5 and the majority of other components of this crude extract by high-pressure liquid chromatography at room temperature (25 cm glass column, 3 mm i.d., packed with silica gel, particle size ca. 8 μm, at 75 kg/cm^2 with 3% methanol in hexane as eluent) and identified separately.

Synthesis. (a) 6,7-Epoxy-1-(*p*-ethylphenoxy)-3-ethyl-7-methylnonane (Ro 10-3108, 1) was synthesized as described earlier (Hangartner et al., 1976). The tritium-labeled compound ([2,3-^{3}H]1) was obtained by reduction of the 6,7-epoxy-3-ethyl-1-(*p*-ethylphenoxy)-7-methyl-2-nonene with tritium and platinum as catalyst. The raw product was purified by thin-layer chromatography to give the gas chromatographically pure product with a specific radioactivity of 1.66 × 10^4 dpm/ng (5.05 × 10^9 dpm/μmol).

(b) Metabolites with 6,7-Epoxide Function. The metabolites 10, 11, and 14 which contain the 6,7-epoxy group were obtained by treating the corresponding parent unsaturated compounds with equimolar amounts of 3-chloroperbenzoic acid in dichloromethane.

(c) Metabolites with 6,7-Diol Function. The metabolites 5, 6, 7, and 15 which contain the 6,7-diol group were obtained by treating the corresponding parent 6,7-epoxides 1, 10, 11, and 14 with 0.1 N sulfuric acid in 40% aqueous tetrahydrofuran at room temperature for 6 h (Nakanishi et al., 1971).

(d) Metabolites with Allylic 6-Alcohol Function. Treatment of Ro 10-3108 (1) with 0.1 N sulfuric acid in tetrahydrofuran at room temperature for 24 h gives a mixture of the metabolites 5, 8, and 9. Column chromatography of the mixture on silica gel yielded the $\Delta^{7,12}$ isomer 8 and the $\Delta^{7,8}$ isomer 9 gas chromatographically pure.

Only the $\Delta^{7,8}$ isomers of the metabolites 12 and 13 were prepared. They were obtained by treating the corresponding parent 6,7-diols 6 and 7 with neutral aluminum oxide (activity grade I) in ether for 12 h at room temperature (Joshi et al., 1971). Column chromatography of the reaction mixture on silica gel afforded the pure $\Delta^{7,8}$ isomers of 12 and 13.

The spectral data for all metabolites synthesized (5 through 15) will appear in the microfilm edition of this journal (see Supplementary Material Available paragraph at end of paper).

Biological Laboratory Tests. (a) Morphogenetic Effect on Yellow Mealworm (*Tenebrio molitor*). The test compounds in acetone were applied topically (1 μl) to the abdomens of yellow mealworm pupae less than 24 h old. Ten treated pupae were incubated for 10 days in plastic petri dishes at 25 °C and 60% relative humidity. Abnormal developmental effects considered in the assay include: retention of urogomphi, gin traps, pupal cuticle, and formation of adult–larval intermediates. The activity was calculated as percent of normal imagines (Abbott, 1925).

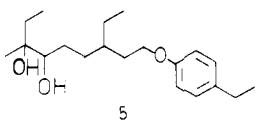
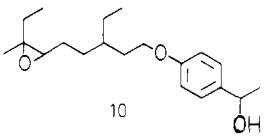
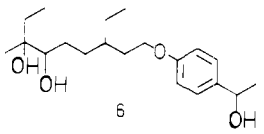
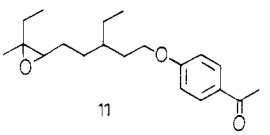
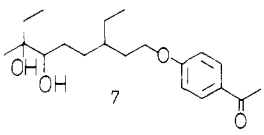
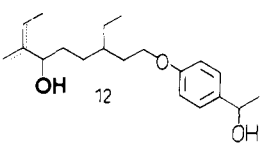
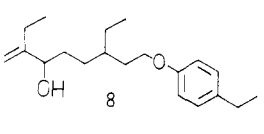
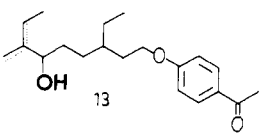
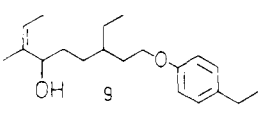
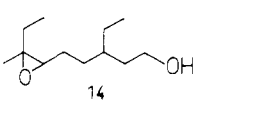
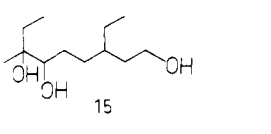
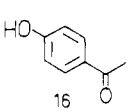
(b) Morphogenetic Effect on Yellow Fever Mosquito (*Aedes aegypti*). Ten fourth instar mosquito larvae were placed in 20 ml of tap water containing 0.2 ml of an acetone solution of the test compound. Small pieces of dog biscuit were offered as food. The treated larvae were maintained at 25 °C and 60% relative humidity until the control mosquitoes had emerged as adults. Percent reduction of normal adults is recorded (Abbott, 1925).

(c) Morphogenetic Effect on Summerfruit Tortrix Moth (*Adoxophyes orana*). The bottom of a petri dish was treated with an acetone solution of the test compound. After 1 h, ten last instar larvae were introduced into the dish, fed with artificial diet, and incubated at 25 °C and 60% relative humidity. After hatching of the adult controls, the activity was calculated as percent reduction of normal imagines (Abbott, 1925).

RESULTS AND DISCUSSION

Balance Study. The total recovery of ^{3}H, 4 weeks after incubating polluted water with ^{3}H-labeled Ro 10-3108 (1), was 82%. The loss of 18% is probably due to evaporation of unaltered compound 1. Of the radioactivity recovered

Table I. Structure Assignments of Environmental Degradation Products of Ro 10-3108 in Polluted Water

Major metabolites	Minor metabolites
 5	 10
 6	 11
 7	 12
 8	 13
 9	 14
	 15
	 16

95% was extractable with ethyl acetate while 5% remained in the aqueous phase. Unaltered Ro 10-3108 accounted for 78% of the radioactivity in the ethyl acetate extract (corresponding to 74% of total radioactivity recovered) as determined by TLC.

Distillation of an aliquot of the extracted water yielded only traces of radioactivity in the distillate, demonstrating that no significant tritium exchange or formation of tritiated water occurred during the experimental period.

Identification of Environmental Degradation Products of Ro 10-3108. The isolation and characterization of individual metabolites of Ro 10-3108 were accomplished by GC–MS analysis of the silylated crude ethyl acetate extract. Positive identification was achieved by synthesis and comparison of retention times (GLC) and corresponding mass spectra (GC–MS). The 12 positively identified degradation products are listed in Table I.

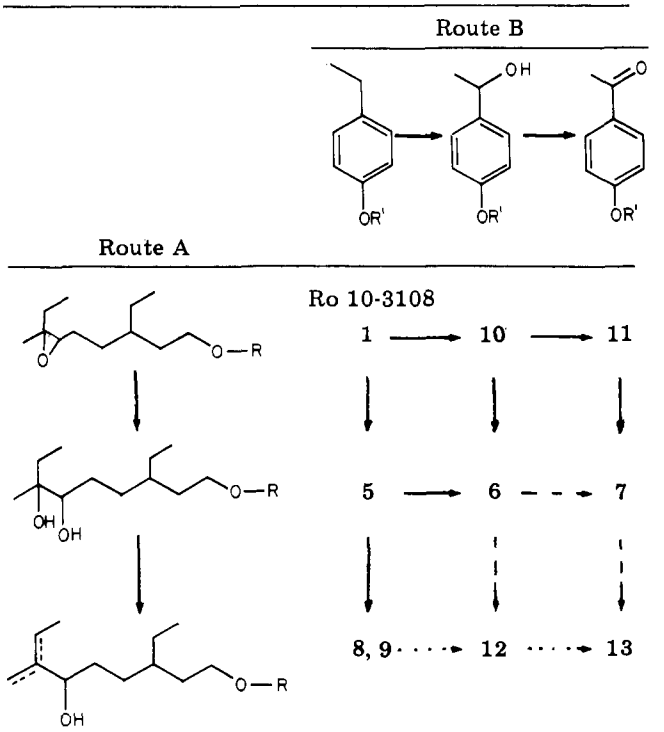
Due to the small amounts of **12** and **13** and to the limited resolution of the packed column used for GC–MS the position of the double bond in these two compounds could not be determined unambiguously, although the mass spectra were identical with those of the corresponding $\Delta^{7,8}$ isomers.

Pathways of Degradation. In order to elucidate the pathways of degradation, several of the intermediate degradation products of Ro 10-3108 which had been synthesized for identification were subjected to the same environmental degradation conditions as the parent compound. The metabolites studied in this manner and their respective degradation products are listed in Table II.

Table II. Environmental Degradation Products from Some of the Intermediate Metabolites of Ro 10-3108 in Polluted Water

Intermediate metabolite	Degradation products observed
5	→ 8, 9, 6, 7, 15
10	→ 6, 12, 7, 13
11	→ 7, 13
14	→ 15

Table III. Major Pathways of Environmental Degradation Operative on Ro 10-3108 and Its Intermediate Metabolites[a]



[a] A solid arrow represents single steps directly observed in the degradation studies with **1**, **5**, **10**, and **11**. A dashed arrow represents single steps indirectly observed in the degradation of **5**, **10**, and **11**. A dotted arrow represents single steps induced by analogy.

Clearly, there are two major routes of environmental degradation operating on Ro 10-3108 and its intermediary metabolites. Route A involves the 6,7-epoxide function and proceeds through the 6,7-diol to an allylic alcohol as indicated in Table III. Although the occurrence of both individual steps has been demonstrated by our experiments, the possibility that in addition to this process a fraction of the epoxide might be converted directly to the allylic alcohol by rearrangement (Anderson et al., 1972) cannot be strictly ruled out.

Route B, the benzylic oxidation of the *p*-ethyl group, proceeds through the intermediary α-hydroxy ethyl moiety to the corresponding acetophenyl derivative as indicated in Table III.

These two predominant modes of environmental degradation seem to be operating independently of each other and can account for 9 of the 12 degradation products identified, among them all five major metabolites. All the metabolites expected according to this scheme have actually been observed (Table III).

Table IV. Morphogenetic Activities of the Metabolites Ro 10-3108 in 3 Standard Tests[a]

Compd	*Tenebrio molitor*,[b] dosage (10^{-x} g/pupa) at x =				*Aedes aegypti*,[b] dosage (10^{-x} g/cm³) at x =				*Adoxophyes orana*,[b] dosage (10^{-x} g/cm²) at x =			
	7	8	9	10	5	6	7	8	6	7	8	9
10-3108 (1)	100	100	60	0	100	100	100	0	100	100	93	11
5	30				90	0			0			
6	0				0				0			
7	0				0				2			
8	60				57				2			
9	0				0				25			
10	100	100	20		100	11			11			
11	100	90	0		100	38			73	0		
12	0				0				0			
13	100	0			90	17			4			
14	0				0				0			
15	0				0				0			
16	0				26	0			0			

[a] For details see Materials and Methods. [b] Test species.

The additional degradation products identified (**14**, **15**, and **16**) are compounds which can be derived from Ro 10-3108 or its major metabolites by ether cleavage. The 12 components identified account for about 80–90% of all the metabolites in the ether extract. Only one other component, which could not be definitely identified, reached a relative abundance of ~5% in the 4-week exposure test. The important degradation mechanisms which operate under the environmental conditions specified have therefore been identified.

Morphogenetic Activities of the Metabolites. All the metabolites identified were tested in three standard tests for their morphogenetic activities (Table IV). None of the major metabolites **5**, **6**, **7**, **8**, and **9** showed any appreciable morphogenetic activity on any of the three test species. Of the minor metabolites only **10** and **11** showed some activity but even these compounds are less active than Ro 10-3108.

Toxicology. Preliminary toxicological data indicate that Ro 10-3108 (1) as well as its major metabolites (**5**, **6**, **7**, **8**, and **9**) are very little toxic to mammals (LD_{50} in mouse >5000 mg/kg per os). Ro 10-3108 shows a negligible acute toxicity to fish. Even after 96 h exposure to a 5000-ppm dispersion of Ro 10-3108 (water solubility 2 ppm) the mortality of all species tested (harlequin fish (*Rusbosa heteromorpha*), Guppy (*Lebestes reticulatus*) and rainbow trout (*Salmo gairdneri*)) was low (LC_{50} >5000 ppm).

CONCLUSION

The pathways for environmental degradation of Ro 10-3108 under natural conditions have been elucidated. The structure assignments of identified degradation products are summarized in Table I. The two major routes of degradation involve modification of the 6,7-epoxy group (route A, Table III) and benzylic oxidation (route B, Table III). These results establish that 6,7-epoxy-3-ethyl-1-(*p*-ethylphenoxy)-7-methylnonane (1) (Ro 10-3108) is degradated in polluted water in analogy to the chemically related compound 6,7-epoxy-1-(*p*-ethylphenoxy)-3,7-dimethyl-*trans*-2-octene (**2**) (R 20458), the metabolism of which has been studied extensively in rats, in algae, and in vitro by enzyme preparations (Hoffmann et al., 1973; Gill et al., 1974). The smaller variety of degradation products of Ro 10-3108 as compared to compound **2** (R 20458) reflects the increased stability due to the absence of the $\Delta^{2,3}$ double bond in the main chain. Preliminary toxicological data show that Ro 10-3108 as well as its major degradation products **5**, **6**, **7**, **8**, and **9** are essentially nontoxic to mammals. The fish toxicity of Ro 10-3108 is extremely low. Even the morphogenetic activity against insects (Table IV) is greatly reduced by the dominant hydrolysis of the 6,7-epoxy moiety. The readily biodegradable new IGR compound Ro 10-3108 promises therefore to show very few side effects on nontarget organisms and should become an ecologically acceptable new tool in insect control.

ACKNOWLEDGMENT

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Supplementary Material Available: Spectral data for all metabolites synthesized (**5**–**15**) (4 pages). Ordering information is given on any current masthead page.

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