periment in Germany, whereas extractable hydrophilic metabolites constituted the main part of the radioactivity in the haulm of plants from England. The deeper soil layers, especially in the experiment in England, contained mostly compounds which were not extractable by organic solvents and probably were hydrophilic products, as considered previously.

The residues in the weeds (0.14 ppm) collected in Germany consisted of 20% of aldrin, 2.3% of metabolite X, 38.2% of dieldrin, 32.8% of hydrophilic metabolites, and 6.7% of unextractable residues. Generally, the proportion of hydrophilic metabolites and the proportion of unextractable compounds in soils increased with increasing depth of the samples. In the case of soil samples from England, the sum of hydrophilic metabolites and unextractable residue increased with increasing depth.

These two experiments show that the conversion of aldrin and its epoxide, dieldrin, to hydrophilic products and unextractable residues was slightly higher in the experiment in England than that carried out in Germany, with the exception of tuber and peel. The differences are caused by several factors which were not investigated separately in these experiments.

CONCLUSIONS

It is shown that under outdoor conditions aldrin is transformed mainly to dieldrin but also in varying amounts to acidic metabolites, the main product being dihydrochlordene dicarboxylic acid.

In our Institute, studies are in progress to investigate whether hydrophilic metabolites occur, besides dieldrin, in other edible crops. It is possible that they exist in many edible crops containing dieldrin resulting from aldrin application. It seems to be useful to develop analytical methods to detect these residues in market samples.

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Metabolism of 1-(4′-Ethylphenoxy)-6,7-epoxy-3,7-dimethyl-2-octene (R 20458) in the Rat

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Oral administration of *trans*-1-(4′-ethylphenoxy-^{14}C)-6,7-epoxy-3,7-dimethyl-2-octene (R 20458), an insect juvenile hormone analog, to rats resulted in the excretion of numerous urinary and fecal metabolites. Approximately 100% of the administered dose was recovered in equal amounts in urine and feces. No significant radiocarbon was detected in tissues or expired air. Metabolites were identified by chromatographic and spectrometric analyses. The chemical nature of the metabolites indicates that R 20458 is metabolized *via* the following biotransformations: α and ω oxidation of the 4′-ethyl moiety; hydration of the trans olefin; hydration of the 6,7-epoxy group; and ether cleavage. The results of this study indicate that R 20458 is a highly biodegradable compound which is unlikely to leave persistent or toxic residues in animals or the environment.

The insect juvenile hormone analog *trans*-1-(4′-ethylphenoxy)-6,7-epoxy-3,7-dimethyl-2-octene (R 20458 of Stauffer Chemical Company) has shown promise as a selective insect control agent (Pallos and Menn, 1972; Pallos *et al.*, 1971). This terpene derivative represents a novel class of insect growth regulators.

The metabolic fate of terpenes has not been extensively studied. Gill *et al.* (1972) showed that R 20458 administered intraperitoneally (ip) to rats gave rise to numerous relatively polar products in urine and feces. One metabolite detected in feces cochromatographed with 1-(4′-ethyl-

Stauffer Chemical Company, Agricultural Research Center, Mountain View, California 94040.

phenoxy) - 6,7 - dihydroxy-3,7-dimethyl-2-octene (diol). Slade and Zibitt (1972) reported the metabolism in mice of methyl - *trans,trans,cis* - 10,11 - epoxy - 7 - ethyl - 3,11 - dimethyltrideca-2,6-dienoate (the juvenile hormone of the silk moth, *Hyalophora cecropia*) labeled with ^{14}C in the 2 position. Although the urinary metabolites were only partially characterized, it appeared that the epoxide group was hydrated and the ester group was hydrolyzed, leading to the dihydroxy acid as a key biotransformation product in the metabolism of this sesquiterpene derivative. These biotransformation steps also occur in several diverse insect species (Slade and Zibitt, 1972). Geraniol is known to be metabolized in *Pseudomonas citronellois*, a soil inhabiting bacteria, to form acetate, acetyl-CoA, and dimethylacrylyl-CoA (Seubert and Fass, 1964).

This paper describes the metabolism of R 20458 in the rat and the chemical identity of urinary and fecal metabolites. The compound labeled with ^{14}C in the phenyl moiety was used to trace the intact molecule, biotransformations involving the uncleaved geranylphenyl ether, and the metabolites arising from the cleaved ethylphenyl moiety.

MATERIALS AND METHODS

Chemicals. The chemicals and their sources are listed in Table I. R 20458-phenyl-^{14}C (17.0 mCi/mmol), synthesized by Kalbfeld *et al.* (1973), had a radiochemical purity greater than 98%, as determined in the benzene–ether (20:1) thin-layer chromatography (tlc) solvent system. (Reference to tlc solvent systems is made by code number, as defined in Table II.) Metabolite reference standards had greater than 95% chemical purity.

Balance Study. Four male Simonsen albino rats (Simonsen Laboratories, Gilroy, Calif.) weighing 270–296 g, were dosed orally with the radiochemical (0.3 mCi/mmol) at an average dose of 46 mg/kg. This dose yielded no evidence of toxic effects; the acute oral LD_{50} of R 20458 to male rats is >4640 mg/kg (Pallos and Menn, 1972). Rats remained active and healthy throughout the 4-day period. Food and water consumption and excretion were observed to be normal. Treated rats were fitted with polyethylene tail cups and placed in metabolism cages (Ford *et al.*, 1966), enabling the separate collection of urine, feces, and expired CO_2. Ninety-six hours after a single oral dose, the animals were sacrificed and the following tissues were collected, weighed, and frozen: brain, fat (surrounding the *vas deferens*), gonads, heart, hide, kidney, leg muscle, liver, lung, spleen, and remaining carcass. Gross pathology at sacrifice indicated no abnormalities.

Feces were homogenized in 50% aqueous methanol and centrifuged. Aliquots of the fecal precipitate and the tissues were separately combusted using a Model 305 Sample Oxidizer (Packard Instrument Co., Downers Grove, Ill.). The oxidized fecal precipitate, tissues, and duplicate aliquots of the fecal supernatant, urine, and expired air trapping solution were suspended in Instagel scintillation fluid (Packard Instrument Co.), and radioassayed by liquid scintillation counting (lsc) using a Model 3375 Tri-Carb (Packard Instrument Co.) with external standardization.

Metabolite Isolation. To facilitate the identification of metabolites, 12 male rats (220 g average weight) were dosed ip with a corn oil solution of R 20458-phenyl-^{14}C (3.2 μCi/mmol) at 1800 mg/kg, and the rats were held in metabolism cages as described by Ford *et al.* (1966). Urine and feces were collected separately for 72 hr after treatment.

Urinary Metabolites. Pooled urine was lyophilized to dryness; the residue was extracted with methanol and the extract containing the radiocarbon was applied to 1.0 mm silica gel GF chromatoplates (Analtech Inc., Newark, Del.). These plates were developed in tlc solvent system 2, and the radioactive gel regions were detected by autoradiography. The initial chromatographic separation made possible the division of R 20458-phenyl-^{14}C urinary metabolites into three groups based on polarity. Gel regions containing metabolites were scraped free from tlc plates and eluted from the gel with acetone or methanol. Purification for spectrometric analyses was accomplished by repeated tlc in appropriate solvent systems (Table II, Figure 1). Radiochemical and chemical purities for the metabolites were determined by autoradiography and tlc–fluorescence quenching, respectively. Quantitation of metabolite groups and isolated metabolites was accomplished by lsc.

Table I. Names, Sources, and Corresponding Metabolite Codes of Compounds Used

Compound	Metabolite code	Source
trans-1-(4′-Ethylphenoxy)-6,7-epoxy-3,7-dimethyl-2-octene	F-2	Stauffer Chemical Co., Richmond, Calif.
cis-1-(4′-Ethylphenoxy)-6,7-epoxy-3,7-dimethyl-2-octene		Stauffer Chemical Co., Richmond, Calif.
1-(4′-Ethylphenoxy)-3,7-dimethyl-2,6-octadiene	F-1a	Stauffer Chemical Co., Richmond, Calif.
1-(4′-Ethylphenoxy)-6,7-dihydroxy-3,7-dimethyl-2-octene	F-5b	Stauffer Chemical Co., Richmond, Calif.
1-(4′-Acetylphenoxy)-6,7-epoxy-3,7-dimethyl-2-octene		Stauffer Chemical Co., Richmond, Calif.
1-(4′-Acetylphenoxy)-6,7-dihydroxy-3,7-dimethyl-2-octene	F-5c	Stauffer Chemical Co., Mt. View, Calif.
4-Hydroxybenzoic acid	U-3c	J. T. Baker Chemical Co., Phillipsburg, N. J.
4-Hydroxyphenyl acetic acid	U-3b	J. T. Baker Chemical Co.
4-Ethylphenol		J. T. Baker Chemical Co.
1,4-Dihydroquinone		J. T. Baker Chemical Co.
4-Acetylphenol	U-1a	Aldrich Chemical Co., Milwaukee, Wis.
4-Hydroxyhippuric acid		Aldrich Chemical Co.
4-Hydroxymandelic acid		Aldrich Chemical Co.
1-(4′-Hydroxyphenyl)-ethanol		K & K Laboratory, Inc., Hollywood, Calif.

Table II. Tlc Solvent Systems Used for Separation of R 20458 Metabolites

Designation	Solvent components, v/v
1	Benzene–ether, 20:1
2	Ethyl acetate–methanol, 15:1
3	Benzene–ether, 1:1
4	Benzene–ether, 1:4
5	Benzene–ether–acetic acid, 300:100:1
6	Ethyl acetate–*n*-propyl alcohol, 5:1
7	Pentanone–methanol, 2:1
8	Benzene–ether, 2:3
9	Chloroform–hexane, 2:3
10	Chloroform–ethyl acetate, 1:1
11	Chloroform–acetic acid, 6:1
12	Ethyl acetate–*n*-propyl alcohol–1% ammonium hydroxide, 10:5:1
13	Chloroform–dichloromethane–ethyl acetate–*n*-propyl alcohol, 10:10:1:1

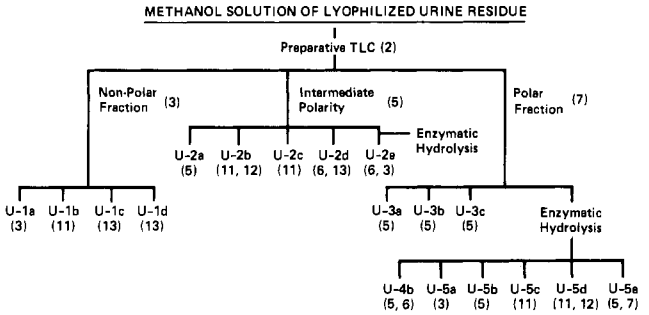


Figure 1. Flow diagram for isolation and purification of R 20458-phenyl-^{14}C urinary metabolites with tlc solvent system code for each metabolite indicated in parentheses.

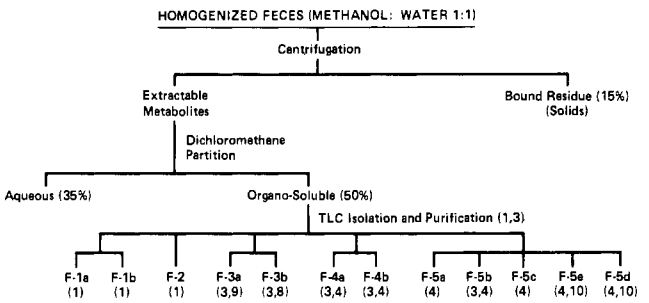


Figure 2. Flow diagram for isolation and purification of R 20458-phenyl-^{14}C fecal metabolites with tlc solvent system code for each metabolite indicated in parentheses.

Aliquots of certain isolated metabolites and, in some cases, mixtures of metabolites were analyzed for the presence of conjugated products by enzymatic hydrolyses, as follows: A, incubated with 50 mg of β-glucuronidase (pH 4.5) or with 50 μl of β-glucuronidase-aryl sulfatase (pH 5.5) with shaking for 24 hr at 37°; B, incubated with buffer only (pH 4.5) with shaking for 24 hr at 37°.

The hydrolysates were exhaustively extracted with ethyl ether, and the ether extracts and aqueous residues were quantified by lsc and compared chromatographically. The resulting deconjugated metabolites were preparatively chromatographed to obtain the desired chemical purity, as described above.

Fecal Metabolites. Feces were homogenized and centrifuged, as described in the Balance Study section and Figure 2, to facilitate separation of the R 20458-phenyl-^{14}C metabolites from the solids. The fecal supernatant was partitioned with dichloromethane, resulting in aqueous and organosoluble metabolite fractions which were subsequently quantified for ^{14}C content by lsc. In this study efforts were directed toward elucidation of the organosoluble metabolites.

Aliquots of the organosoluble ^{14}C-labeled metabolites were chromatographed in tlc solvent systems 1, 3, and 8, and the percent distribution of the metabolites was determined by lsc of radioactive gel regions. Preliminary separation of metabolites into groups was accomplished by preparative chromatography in tlc solvent systems 1 and 3 (Figure 2). Gel regions containing metabolite groups were scraped free from tlc plates and eluted with acetone. Individual metabolites were separated by repeated tlc. The isolated fecal metabolites were then purified for spectrometric analyses in a manner similar to that used with the urinary metabolites (Table II, Figure 2).

Spectrometric Analysis. Whenever a sufficient amount of a purified R 20458-phenyl-^{14}C metabolite was available, one or more of the following analyses was performed as an aid in structure identification.

Infrared spectra (ir) were obtained using a Perkin-Elmer Model 457 spectrophotometer. All metabolites and

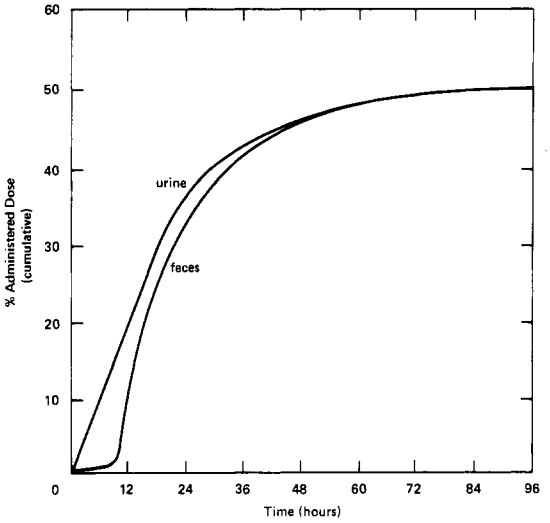


Figure 3. Rate of radiocarbon excretion in the urine and feces from rats receiving a single oral dose of R 20458.

reference standards, unless otherwise indicated, were analyzed as a thin film on a potassium bromide crystal using fast scan and normal slit opening.

Mass spectrometric analyses (ms) were accomplished on a Varian MAT CH-5 mass spectrometer at an electron energy of 70 eV. Where noted, spectra of certain metabolites and reference standards were alternately obtained by gas chromatography-mass spectrometry (gc-ms) on a Finnigan Model 1015 gas chromatograph-mass spectrometer (Sunnyvale, Calif.) at 70 eV, which was interfaced with a Varian 1700 gas chromatograph by a Gohlke separator. When necessary, metabolites and authentic reference standards were derivatized using TRI SIL-Z reagent (Pierce Chemical Co., Rockford, Ill.) prior to gc-ms.

Nuclear magnetic resonance spectra (nmr) were recorded on a Varian HA-60-IL or XL-100. Samples were prepared by dissolving the metabolite or reference standard in the appropriate deuterated solvent.

RESULTS

Balance Study. The average total recovery of administered radiocarbon, 96 hr after treatment, was 101.8% (±2.2), of which 51.5% (±4.1) was recovered in the urine and 50.4% (±4.9) was recovered in the feces (Figure 3). No significant radiocarbon (<0.1% of the oral dose) was detected in the expired air and tissues.

Isolation and Identification of Urinary Metabolites. Essentially all of the radioactive metabolites were soluble in methanol after lyophilization of the pooled urine. The isolation of individual metabolites from the methanol solution was accomplished according to the flow diagram presented in Figure 1, which also lists the tlc solvent systems used for these separations. Table III lists each metabolite, its structure when known, and the relative percent of urinary radiocarbon as determined by lsc.

Metabolites U-1a and 4-acetylphenol have similar ir and gc-ms features [mass spectra *m/e* (parent) 136, 121, 93, and 43]. On enzymatic hydrolysis, both U-2e and U-5a yield the same ether-extractable product, which is identified as 4-acetylphenol by cochromatography in tlc solvent system 3 and gc-ms. Metabolite U-2e deconjugates on incubation with β-glucuronidase-aryl sulfatase, but not with β-glucuronidase alone, which indicates that this metabolite is a sulfate conjugate (Table IV). Metabolite U-5a yields 4-acetylphenol on incubation with either enzyme preparation, indicating glucuronide conjugation (Table IV). These metabolites comprise 30.5% of the urinary radiocarbon (Table III).

Metabolite U-1b has been identified as 1-(4′-hydroxyphenoxy)-6,7-dihydroxy-3,7-dimethyl-2-octene. No reference standard was available; however, the nmr and ms are consistent with this assignment (Figure 4a). Metabolite U-5c yielded an ether-soluble deconjugated product after incubation with either enzyme preparation that cochromatographs in tlc solvent systems 5 and 11 with metabolite U-1b. Furthermore, its nmr and ms are similar to those of metabolite U-1b. These two metabolites comprise 5.4% of the urinary radiocarbon.

Metabolite U-1c is not completely characterized; however, nmr indicates hydroxylation of the octene moiety at positions 2, 6, and 7 (determined by exchange with D_2O), and oxidation of the 4′-ethyl moiety to the 4′-acetyl (singlet at 2.55 ppm).

Metabolite U-1d gives ms fragments corresponding to both the phenyl and the geranyl moieties (m/e 121 and 158, respectively), indicating that the phenyl-ether linkage is still intact. Spectral analyses also indicate the oxidation of the 4′-ethyl to the 4′-acetyl moiety (ir absorption at μ 6.1 and m/e 121), and hydroxylation of the octene moiety in positions 3, 6, and 7 (nmr,ms).

Metabolite U-2b has been identified as 1-(4′-carboxyphenoxy)-2,3,6,7-tetrahydroxy-3,7-dimethyloctane based on nmr. Metabolite U-5d yielded an ether-soluble product after incubation with β-glucuronidase and β-glucuronidase–aryl sulfatase, indicating the U-5d is excreted as a glucuronide (Table IV). This aglycone is identical to metabolite U-2b (cochromatography in tlc solvent systems 11 and 12; similar nmr). Both metabolites account for 7.5% of the urinary radiocarbon.

Metabolite U-2d has been assigned the structure 1-(4′-acetylphenoxy) - 2,3,6,7 - tetrahydroxy-3,7-dimethyloctane based on ir and nmr; the ms features are also consistent with this structure (Figure 4b).

Metabolite U-3b has been identified as 4-hydroxyphenyl acetic acid. It is excreted as the unconjugated acid. The structure of this metabolite was established by cochromatography with authentic 4-hydroxyphenyl acetic acid in tlc solvent systems 5 and 6 and gc–ms of the trimethylsilyl (TMS) derivatives [m/e (parent) 296 and m/e 281, 252, 225, 179, 164, and 74].

Metabolite U-3c has been identified as 4-hydroxybenzoic acid based on comparison with an authentic reference standard (cochromatography in the tlc solvent systems 5 and 6; gc–ms of the TMS derivatives: m/e (parent) 282, 223, 193, 126, and 73). Metabolite U-4b was deconjugated after incubation with β-glucuronidase–aryl sulfatase, but not with β-glucuronidase alone; whereas metabolite U-5b yielded a deconjugated product after incubation with either enzyme preparation, indicating that U-4b was a sulfate conjugate and U-5b was a glucuronide conjugate of metabolite U-3c (Table IV). The aggregate of these three products represents 20.5% of the urinary radiocarbon.

Isolation and Identification of Fecal Metabolites. The distribution of radiocarbon in the aqueous, organosoluble, and nonextractable fractions is 35, 50, and 15%, respectively. The separation and subsequent isolation of individual fecal metabolites are described in Figure 2. The structure and percent distribution of organosoluble fecal metabolites is presented in Table V.

Metabolite F-1a, the least polar fecal metabolite, has been identified as 1-(4′-ethylphenoxy)-3,7-dimethyl-2,6-octadiene by comparison with a reference standard

Table III. Structure Assignment and % Distribution of R 20458–Phenyl-^{14}C Rat Urinary Metabolites

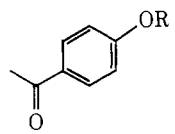
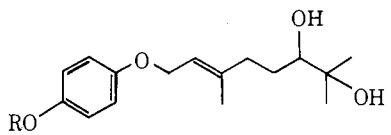
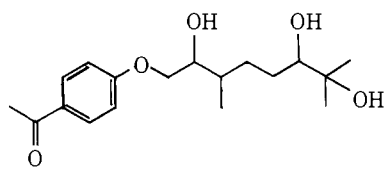
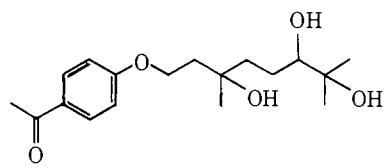
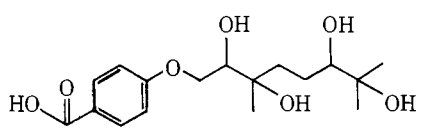
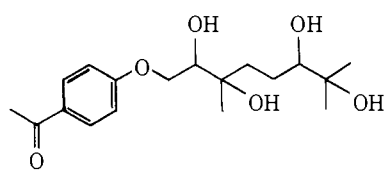
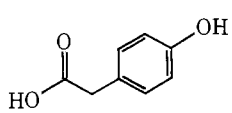
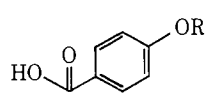
Metabolite no.	Structure identification	% urinary radiocarbon
U-1a, U-2e, U-5a	 R = H, 0.6%; R = SO_3H, 20.6%; R = glucuronide, 9.3%	30.5
U-1b, U-5c	 R = H, 1.3%; R = glucuronide, 4.1%	5.4
U-1c		1.1
U-1d		4.1
U-2a Unknown		0.7
U-2b, U-5d	 4.2%, and glucuronide, 3.3%	7.5
U-2c Unknown		3.9
U-2d		12.5
U-3a Unknown		3.4
U-3b		3.3
U-3c, U-4b, U-5b	 R = H, 5.7%; R = SO_3H, 6.3%; R = glucuronide, 8.5%	20.5
U-5e Unknown		7.1
Total		100.0

Table IV. Enzyme Cleavage of R 20458–Phenyl-^{14}C Urinary Metabolites

Incubation conditions	Metabolite designation and % hydrolysis					
	U-2e	U-4b	U-5a	U-5b	U-5c	U-5d
Buffer only (control)	5	5	7	5	4	7
β-Glucuronidase–aryl sulfatase	87	87	84	86	95	90
β-Glucuronidase	6	9	89	91	88	85

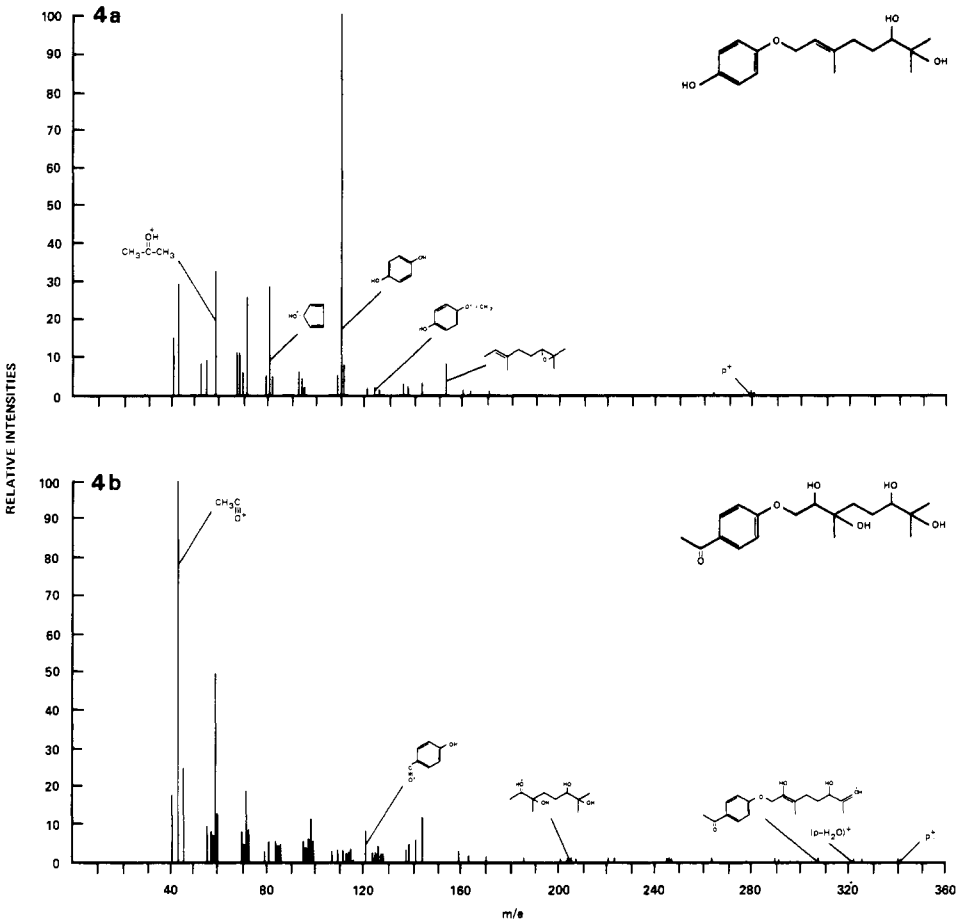


Figure 4. Mass spectra of R 20458–phenyl-^{14}C urinary metabolites U-1b (4a) and U-2d (4b).

[cochromatography in tlc system 1, and similar ms: *m/e* (parent) 258, 136, 122, 107, and 93].

Metabolite F-1b has been identified as 1-[4′-(1-hydroxyethyl)phenoxy]-3,7-dimethyl-2,6-octadiene because prominent ms fragments appear at *m/e* (parent) 274, and *m/e* 138, 123, and 95; the *m/e* fragment 123 is indicative of the methylphenyl carbinol moiety (Figure 5a).

Product F-2, the most abundant radiolabeled organosoluble fecal product (Table V), is unmetabolized R 20458 [cochromatography in tlc system 1; ir, nmr, and ms: *m/e* (parent) 274, 153, 122, 93, and 71].

Metabolite F-3a was assigned the structure of 1-(4′-hydroxyphenoxy)-3,7-dimethyl-2,6-octadiene. Although no standard was available for this metabolite, the nmr and ms are consistent with this assignment (Figure 5b).

Metabolite F-3b has a ms (Figure 5c) which suggests several possible structures (Table V). The fragment at *m/e* 135 indicates that the phenyl–ether linkage remains intact, *m/e* 122 indicates that no modification has occurred involving the 4′-ethylphenoxy moiety, and *m/e* 155 indicates hydroxylation of the geranyl moiety and the presence of the 6,7-epoxide.

Metabolite F-4a has been assigned the structure 1-(4′-acetylphenoxy)-4-hydroxy-3,7-dimethyl-2,6-octadiene, based on ir, nmr, and ms (Figure 5d).

Metabolite F-4b could be one of two possible structures differing only in position of the hydroxyl; *i.e.*, a 5-hydroxy-2,6-octadiene or a 3-hydroxymethyl-2,6-octadiene derivative (Table V). These assignments are based on nmr and ms (Figure 5e).

Metabolite F-5a was assigned the structure 1-(4′-ethylphenoxy)-6,7-epoxy-2,3-dihydroxy-3,7-dimethyloctane based on the ms, which is clearly consistent with the proposed assignment (Figure 5f).

Metabolite F-5b has been identified as 1-(4′-ethylphenoxy)-6,7-dihydroxy-3,7-dimethyl-2-octene (Table V) by comparison with a reference standard [cochromatography in tlc solvent systems 3 and 4; similar nmr and ms: *m/e* (parent) 292, and *m/e* 171, 122, 107, and 93].

Metabolite F-5c differs from F-5b only in an α oxidation to the 4′-acetylphenoxy moiety. This metabolite was identified as 1-(4′-acetylphenoxy)-6,7-dihydroxy-3,7-dimethyl-2-octene by comparison with authentic reference standard [cochromatography in tlc solvent system 3; nmr and ms: *m/e* (parent) 306, 171, 153, 121, and 71].

Metabolites F-5d and F-5e were not obtained in sufficient purity to determine whether or not the phenyl–ether linkage remains intact; however, their ir spectra show strong absorption at μ 6.1, indicating that in each case the 4′-ethyl moiety may have been oxidized to the 4′-acetyl moiety.

DISCUSSION

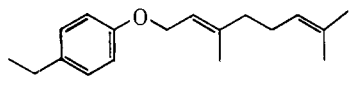
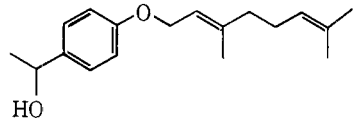
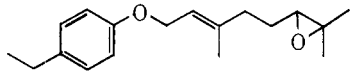
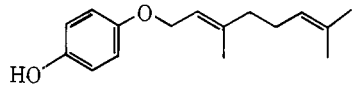
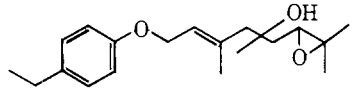
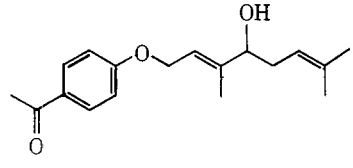
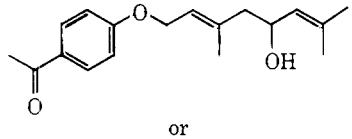
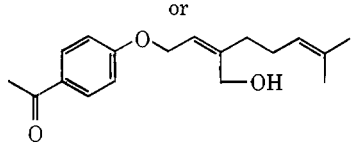
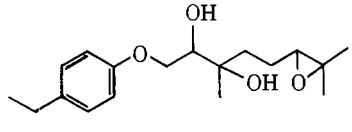
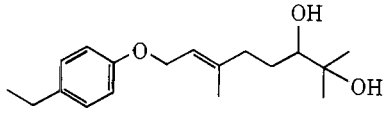
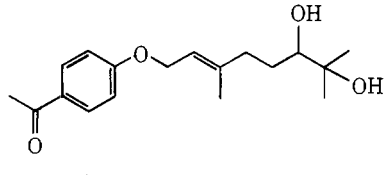
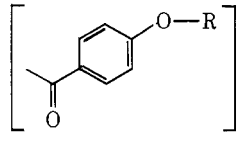
This study clearly establishes that R 20458 is extensively metabolized and readily excreted in rats, and that the compound *per se* and its phenyl-labeled metabolites do not persist in tissues and organs following a single oral dosing. The identified urinary metabolites account for 85% of the urinary radiocarbon.

The large number and variety of R 20458 urinary metabolites indicate the great lability of the terpene and alkylbenzene moieties to metabolic attack. The ease of metabolism results from metabolic attack at several sites on the molecule; these include the epoxide, the olefin, the ether linkage, and the ethyl side chain of the phenyl moiety.

An examination of the biotransformation steps indicates that the 4′-ethyl moiety is transformed primarily *via* α ox-

idation (with metabolites U-1a, U-1c, U-1d, U-2d, U-2e, and U-5a accounting for 48.2% of the urinary radiocarbon) and secondarily *via* ω oxidation which accounts for 31.3% of the urinary radiocarbon (metabolites U-2b, U-3b, U-3c, U-4b, U-5b, and U-5d).

Table V. Structure Assignment and % Distribution of R 20458-Phenyl-^{14}C Organosoluble Fecal Metabolites

Metabolite no.	Structure identification	% organo-soluble fecal radiocarbon
F-1a		1.2
F-1b		1.2
F-2		28.4
F-3a		4.3
F-3b		3.4
F-4a		5.0
F-4b	 or 	6.9
F-5a		3.0
F-5b		7.7
F-5c		13.3
F-5d, F-5e		25.8
Total		100.0

It is known that ethyl benzene is metabolized in rabbits first to methylphenyl carbinol *via* α oxidation and then to mandelic and benzoic acids *via* ω oxidation (Smith *et al.*, 1954a). However, methylphenyl carbinol appears as a urinary metabolite of methylphenyl ketone in rabbits (Smith *et al.*, 1954b). In our studies we have not isolated metabolites containing the carbinol moiety. Conceivably, carbinol metabolites, if formed *in vivo* by the rat, may have been rapidly oxidized to the corresponding ketone derivatives. The latter explanation is in accord with the findings of Eto *et al.* (1971), who showed that with tri-*p*-ethylphenyl phosphate the *p*-ethyl group is hydroxylated in living rats and mice in the α position and subsequently transformed to the acetyl group by the action of a soluble dehydrogenase. Although the distribution of radiocarbon in the urinary phenolic metabolites does not indicate the predominance of any one biotransformation step, El Masry and coworkers (1956) found that in rabbits 60–70% of administered ethylbenzene undergoes α and ω oxidations to yield methylphenyl carbinyl glucosiduronic and hippuric acids, respectively.

One of the more unusual biotransformations is associated with formation of the 4′-hydroxy metabolites F-3a and U-1b, which also appears as a conjugate U-5c. A chemical analogy for this biotransformation can be derived from the Baeyer–Villiger oxidation reaction involving alkyl aryl ketones containing hydroxy or alkoxy groups in the ortho or para positions, which are converted to polyhydric phenols by hydrogen peroxide in alkaline solution (Hassall, 1957). More specifically, *p*-hydroxyacetophenone is converted to 1,4-dihydroxybenzene by gently boiling the ketone for a few minutes with a slight excess of ammonia and hydrogen peroxide (Dakin, 1909). Thus, it appears possible that metabolites containing the acetophenone moiety might undergo further *in vivo* oxidative cleavage, giving rise to the 4′-hydroxyphenyl metabolites.

A yet unexplained biotransformation involves the fecal diene metabolites (F-1a and F-1b). It is not clear by which mechanism the 6,7-epoxide moiety is converted to the olefin. Possibly these minor products represent concentrated impurities which occur as trace contaminants in the original R 20458-phenyl-^{14}C preparation.

Ether cleavage is an important biotransformation step in the metabolism of R 20458. Approximately 54% of the identified urinary metabolites are accounted for as para-substituted phenolic products. Aromatic ethers are cleaved *in vivo* into phenols and alcohols; their rate of cleavage depends on the nature of the para substituent (Williams, 1959). It appears that the α oxidation of the ethyl moiety increases the rate of ether cleavage. Examination of the identified products reveals that all the chemical modifications involving the 4′-ethyl moiety in the cleaved metabolites, with the exception of U-3b, are also present in the uncleaved metabolites. This leads us to conclude that biotransformations of the 4′-ethyl moiety occurred primarily prior to splitting of the ether linkage.

The geranyl portion of the R 20458 molecule contains two labile sites for metabolic attack, the trans olefin and the epoxide ring, which is rapidly hydrated to the corresponding diol. Cleavage of epoxides by hydration occurs with triethyleneglycol-bis-2,3-epoxypropyl ether (Epodyl) after intravenous administration to rats (James and Solheim, 1971). Similarly, the 10,11-epoxide of the *H. cecropia* juvenile hormone is hydrated after oral administration to mice (Slade and Zibitt, 1972).

Only metabolite U-1b and its conjugate U-5c retain the olefinic configuration. All other geranyl-containing metabolites occur with the hydrated double bond. Leibman and Ortiz (1970) have demonstrated the formation of labile

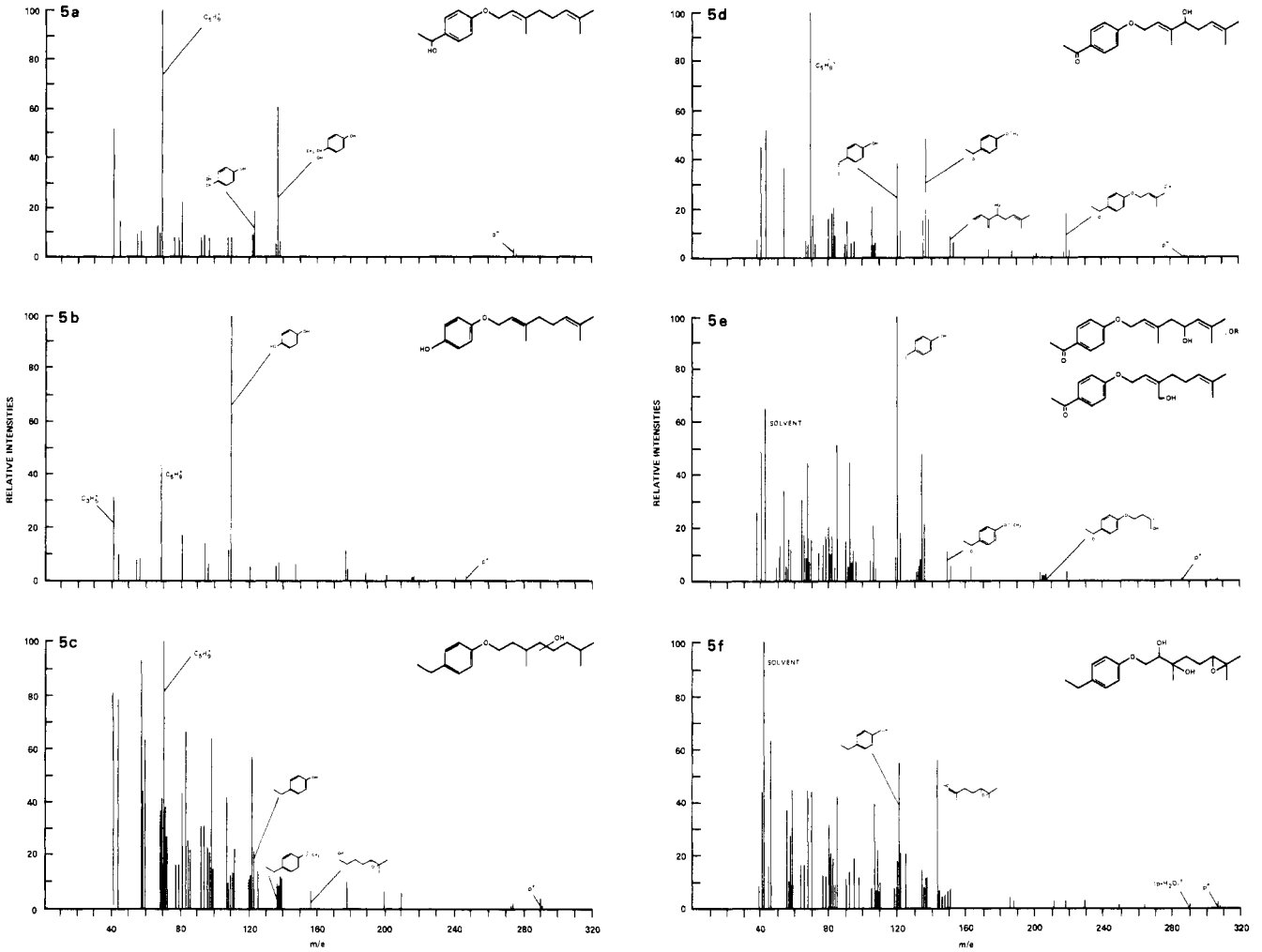


Figure 5. Mass spectra of R 20458-phenyl-^{14}C fecal metabolites F-1b (5a), F-3a (5b), F-3b (5c), F-4a (5d), F-4b (5e), and F-5a (5f).

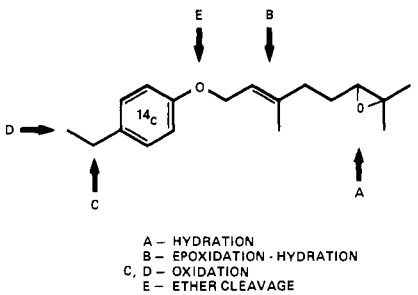


Figure 6. Biotransformation steps of 1-(4′-ethylphenoxy)-6,7-epoxy-3,7-dimethyl-2-octene in the rat.

epoxide intermediates in microsomal oxidation of certain olefins to glycols. Indeed, no epoxide derived from the trans olefin has been isolated in the present study. Although in this study the fate of the cleaved geraniol portion of R 20458 was not determined, it has been shown that geraniol undergoes double ω oxidation and asymmetric reduction of the 2,3 double bond after oral administration to dogs and rabbits (Kühn *et al.*, 1936).

Only the organosoluble radiolabeled fecal metabolites have been characterized and identified in this study. These represent 49.6% of the total fecal radiocarbon. Fecal excretion most likely arose *via* biliary transfer rather than passing directly through the gut, as the ^{14}C dose was administered ip. Relatively few polar compounds are excreted *via* the bile (Stowe and Plaa, 1968); thus, it is not surprising to find unchanged R 20458 as a major fecal metabolite.

The organosoluble fecal metabolites are formed by similar types of biotransformations shown to occur with the urinary metabolites; *i.e.*, α oxidation of the 4′-ethyl moiety, hydration of the trans olefin, and hydration of the epoxy group.

Figure 6 summarizes the types of biotransformations occurring with R 20458 in the living rat. No attempt is made to present a metabolic pathway leading to the various metabolites because of the great variety of compounds and multiple sites of metabolic attack. In conclusion, the biodegradability of R 20458 further suggests that this compound will not concentrate in food webs and that it will not remain unmetabolized in mammalian species.

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Carbon-14 Balance and Residues of Dichlorvos and Its Metabolites in Pigs Dosed with Dichlorvos-^{14}C

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One dose of dichlorvos-^{14}C (2,2-dichlorovinyl-*1*-^{14}C dimethyl phosphate) formulated as slow-release polyvinylchloride (PVC) pellets was fed as a top dressing to each of nine young male Yorkshire pigs. After treatment, three of the pigs were killed at each of the following intervals: 2 days, 7 days, and 14 days. In the 14-day trial, 61.8% of the ^{14}C administered was found in the pellets recovered from the feces, 5.6% was found in the remainder of the feces, 3.6% was found in the urine, 14.1% was recovered from the expired air, and 9.6% remained in the carcass. The ^{14}C content of the tissues from the pigs in all the treatments ranged from 2 to 33 ppm equivalents of dichlorvos, but no dichlorvos, demethyl dichlorvos, dichloroacetaldehyde, and dichloroacetic acid were found in the tissues of the pigs. It is concluded that the ^{14}C present in the tissues is the result of incorporation of C-1 and C-2 fragments from the vinyl moiety of dichlorvos into normal tissue constituents.

In vivo and *in vitro* studies have shown that the insecticidal and anthelmintic compound dichlorvos (2,2-dichlorovinyl dimethyl phosphate) is degraded enzymatically by animal tissue, and in whole animals is degraded to dichloroacetaldehyde (DCA), dichloroethanol (DCE), dichloroacetic acid (DCAA), demethyl dichlorvos (DMD), dimethyl hydrogen phosphate, and methyl dihydrogen phosphate (Bull and Ridgway, 1969; Casida *et al.*, 1962; Hodgson and Casida, 1962). Rats dosed with dichlorvos labeled with ^{14}C in the 1 position of the vinyl group excreted part of the ^{14}C as carbon dioxide in the expired air.

Because no information was available on the fate of the dichlorovinyl part of the molecule after dechlorination other than the evolution of carbon dioxide, a study was initiated to determine the degradative fate of dichlorvos in young pigs, one of the animals in which dichlorvos is effective as an anthelmintic.

Dichlorvos labeled with ^{14}C on the 1 position of the vinyl group was prepared as described by Burton (1971) and formulated as a slow-release formulation in polyvinylchloride (PVC) pellets. The pellets were fed to pigs and the residues of the ^{14}C, dichlorvos, and known dichlorvos metabolites were measured.

MATERIALS AND METHODS

Preparation of PVC Pellets. The PVC pellets were prepared by extruding a mixture of PVC resin (63.75 wt %), plasticizer and stabilizer (13.25 wt %), and dichlorvos (23 wt %) into a strand 0.06-in. in diameter and by cutting off ⅛-in. long segments. The conditions employed have been given by Menn *et al.* (1965) and Folckemer *et al.* (1967). Analysis of the pellets showed that they contained ^{14}C equivalent to 21.3 wt % of dichlorvos. Tlc analysis of the pellet extracts gave only one radioactive spot, which had an R_f value corresponding to that of a dichlorvos standard.

Feeding Experiment. Nine Yorkshire cross male pigs were employed. Three days prior to the start of the test the pigs were placed in individual closed metabolism cages that allowed the separate collection of the urine and feces. The carbon dioxide in the expired air was collected by countercurrent extraction with 8% sodium hydroxide. After a 48-hr acclimatization period, the pigs were fasted for 24 hr and then given a standard pig mash containing 19.85% dehydrated alfalfa meal, 2.48% meat and bone, 2.48% fish meal, 74.4% ground milo and wheat, 0.50% salt, and 0.25% Microfac supplement. (The Microfac supplement was supplied by Dawes Laboratories, Chicago, Ill.) A single dose of PVC pellets containing 21.3% dichlorvos-^{14}C was given to each pig as a top dressing at a rate of approximately 40 mg of dichlorvos per kilogram, the therapeutic dose of dichlorvos. The specific activity of the dichlorvos was 205 dpm per microgram. After the pigs consumed the initial portion of feed and top dressing, they were given water and feed *ad libitum* for the duration of the experiment. Three of the pigs were killed 2 days after treatment, three were killed 7 days after treatment, and three were killed 14 days after treatment.

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