Synthesis of Isomeric 3-(2,2-Dichlorovinyl)-2-hydroxymethyl-2-methylcyclopropanecarboxylic Acids and Other Permethrin Metabolites

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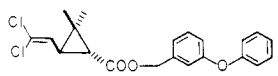
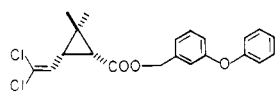
Synthesis procedures are given for the following [1*RS*,*trans*]- and [1*RS*,*cis*]permethrin metabolites and related compounds: four isomeric 3-(2,2-dichlorovinyl)-2-hydroxymethyl-2-methylcyclopropanecarboxylic acids and two isomeric γ-lactones derived from the 2-*cis*-hydroxymethyl acids; 2′-, 3′-, 4′-, 4-, 5-, and 6-hydroxy derivatives of 3-phenoxybenzyl alcohol and of 3-phenoxybenzoic acid; ten permethrin isomers mono- or dihydroxylated at one or both of the acid and alcohol moieties; 12 methyl esters of L-amino acid conjugates of the acid moieties and of 3-phenoxybenzoic acid; the ethereal sulfate of 4′-hydroxy-3-phenoxybenzoic acid. Twenty-nine of the compounds synthesized, or their glucuronide or glucoside conjugates, are found as permethrin metabolites in the mouse liver microsomal monooxygenase system or in mammals or insects.

Permethrin (also known as NRDC 143, FMC 33297, S-3151, and PP 557) is a mixture of two highly insecticidal [1*R*] isomers and equal amounts of two essentially non-insecticidal [1*S*] isomers (Burt et al., 1974; Elliott et al., 1973). This insecticide has improved stability in light and



[1R, *trans*]-permethrin



[1R, *cis*]-permethrin

air as compared with earlier pyrethroids and it is very effective in control of a wide range of pest insects.

The sites of metabolic attack on the permethrin isomers anticipated from previous studies on other pyrethroids (Casida, 1973; Casida et al., 1975/1976; Miyamoto, 1976; Miyamoto et al., 1974; Suzuki et al., 1976) include: ester cleavage, more rapid or complete for the trans than for the cis isomer; hydroxylation of the *gem*-dimethyl group of the acid moiety; hydroxylation of the 4′ position of the alcohol moiety; oxidation of the benzyl alcohol derivatives to benzoic acids; conjugation of the phenolic and carboxylic hydroxyl substituents. The metabolic pathways are now established for *trans*- and *cis*-permethrin administered orally to rats (Elliott et al., 1976; Gaughan et al., 1977a,b) by comparing the excreted metabolites or their deconjugated derivatives with standard compounds from synthesis.

This report gives synthesis procedures for the permethrin metabolites and some of their derivatives. It also considers the initial permethrin metabolites formed in the mouse liver microsomal monooxygenase system.

MATERIALS AND METHODS

Designation of Compounds. The following abbreviations are used in structure designations (Figures 1, 2, and 3; Tables I, II, and III): *t*- and *c*-per for [1*RS*,*trans*]- and [1*RS*,*cis*]permethrin, respectively; 4′-HO-per and 4′-MeO-per for the 4′-hydroxy- and 4′-methoxy derivatives of the permethrin isomers; *t*- and *c*-Cl_2CA for the [1*RS*,*trans*] and [1*RS*,*cis*] isomers, respectively, of 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid; the prefix of *t*-HO or *c*-HO for *t*- and *c*-per and for *t*- and *c*-Cl_2CA to indicate 2-*trans*-hydroxymethyl-2-*cis*-methyl or 2-*cis*-hydroxymethyl-2-*trans*-methyl derivatives, respectively, of these compounds; PBalc and PBacid for 3-phenoxybenzyl alcohol and 3-phenoxybenzoic acid, respectively; the suffixes Me, Et, and AcO to indicate methyl, ethyl, and acetyl esters, respectively; appropriate combinations of these abbreviations to designate other compounds.

Chemicals. Starting materials were obtained or synthesized as follows: 3-bromobenzoic acid and 3,5-dihydroxytoluene from Matheson Coleman & Bell Co. (Los Angeles, Calif.); isovanillin and 5-bromosalicylaldehyde from Aldrich Chemical Co. (Milwaukee, Wis.); PBalc and PBacid (Elliott et al., 1976; Lock and Kempter, 1935; Miyamoto et al., 1974); 1,1-dichloro-4-methyl-1,3-pentadiene (dichlorodiene), *t*-Cl_2CA, *t*-Cl_2CA-Et, *c*-Cl_2CA, and *c*-Cl_2CA-Et (Burt et al., 1974; Farkaš et al., 1959) from FMC Corp. (Middleport, N.Y.) or M. Elliott (Rothamsted Experimental Station, Harpenden, Herts., England); methyl esters of L-amino acids HCl from Sigma Chemical Co. (St. Louis, Mo.). Diazomethane was used for methylation of less than 100-mg quantities of carboxylic acids or phenols.

Thin-Layer Chromatography (TLC). Silica gel chromatoplates (precoated, 20 × 20 cm with fluorescent indicator) were used as follows: 60 F-254 (EM Laboratories, Inc., Elmsford, N.Y.) with 0.25 and 0.50 mm gel thickness for analysis and preparative isolations, respectively; GF (Analtech, Inc., Newark, Del.) with 1.0 and 2.0 mm gel thickness for preparative isolations. Ten TLC solvent systems were used: A = benzene (saturated with formic acid)–ether (10:3); B = benzene–ethyl acetate (6:1); C = benzene–ethyl acetate–methanol (15:5:1); D = carbon tetrachloride–ether (3:1); E = benzene–carbon tetrachloride (1:1); F = chloroform (saturated with formic acid)–ether (10:3); G = ether–hexane (1:1); H = hexane–ethyl acetate (7:3); I = hexane–ethyl acetate (4:1); J = carbon tetrachloride–ether (93:7). When two or more solvent developments were involved, this is designated by, e.g., A × 2 for two developments in the same direction with solvent system A; A × 2-B for two-dimensional development using A × 2 as above in the first direction and then B in the second direction. The resolved compounds detected by their quenching of gel fluorescence under short-wavelength ultraviolet (UV) light were recovered by extraction of the silica gel with ether or acetone (for nonpolar compounds) or methanol–ether mixture (for polar compounds). [^{14}C] labeled and unlabeled chemicals on the

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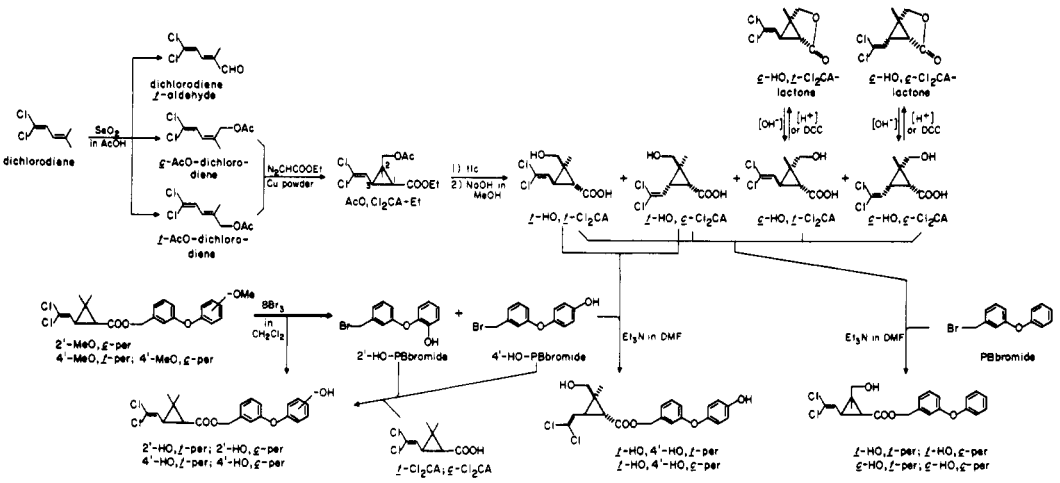

Figure 1. Synthesis of four isomers of 3-(2,2-dichlorovinyl)-2-hydroxymethyl-2-methylcyclopropanecarboxylic acid, the γ-lactones of the 2-*cis*-hydroxymethyl acids, and various mono- and dihydroxy derivatives of permethrin.

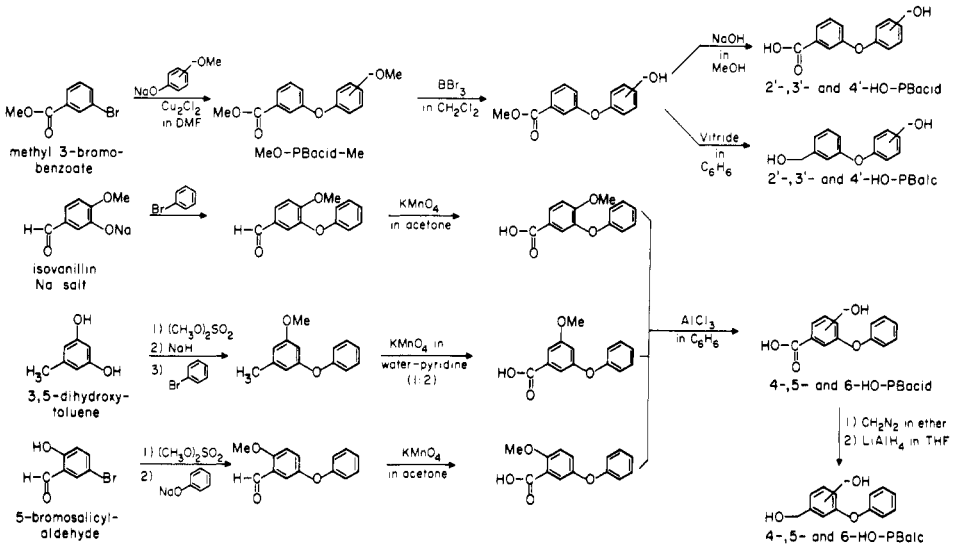

Figure 2. Synthesis of six hydroxy derivatives of 3-phenoxybenzoic acid and six hydroxy derivatives of 3-phenoxybenzyl alcohol.

chromatoplates were detected by autoradiography and chromogenic agents as previously reported (Barton et al., 1952; Gaughan et al., 1977a; Ueda et al., 1975).

Spectroscopy. Nuclear magnetic resonance (NMR) spectra were determined for dilute solutions in chloroform-*d* (for nonpolar compounds) or acetone-d_6 or methanol-d_4 (for polar compounds), using tetramethylsilane (Me_4Si) as the internal standard ($\delta = 0$ ppm), with a Perkin-Elmer Model R12B spectrometer at 60 MHz or R32 spectrometer at 90 MHz. Chemical ionization–mass spectra (CI–MS) were obtained on the Finnigan Corp. Model 1015D mass spectrometer with the System Industries Model 150 control system, using a direct introduction probe and methane, isobutane, or ammonia as the reactant gas at a source pressure of 0.6–1.0 Torr. The quasimolecular ion, $[M + 1]^+$, is reported as m/e (relative intensity) based on the ^{35}chlorine isotope. Infrared (IR) spectra were determined on a Perkin-Elmer Model 457 grating spectrophotometer using Nujol mull, KBr disk, liquid film, or chloroform solution (cm^{-1}).

Microsomal Monooxygenase Reactions. Mouse liver microsomes were prepared and incubated with tetraethyl pyrophosphate (TEPP) to inhibit their esterase activity as described by Soderlund and Casida (1977a). The reaction mixtures in 2.5 mL of 50 mM Tris–HCl buffer, pH 7.5, consisted of the following components added in sequence: TEPP-treated microsomes (1.5–2.5 mg of protein); NADPH (2.2 μmol); [^{14}C-1*R*]-per (13–50 nmol) or [^{14}C-1*RS*]-per (6 nmol) (Gaughan et al., 1977a) introduced in ethanol (5–25 μL). Following 30 min incubation at 37 °C, the reaction mixtures were extracted with *n*-pentane (3 × 8 mL) and then the aqueous phase was adjusted to pH ~2 by addition of HCl, followed by reextraction with ether (3 × 8 mL) after addition of $(NH_4)_2SO_4$ (2 g). [^{14}C] products in the organic extracts were analyzed by TLC, radioautography, and cochromatography. This procedure is essentially the same as that reported by Ueda et al. (1975) in the study of [^{14}C]resmethrin metabolism.

SYNTHESIS

The synthesis routes and chemical structures for permethrin metabolites and important related compounds are shown in Figures 1 and 2. The corresponding analytical data and chromatographic properties are given in Tables I and II.

Four HO-Cl_2CA Isomers and Two Isomeric γ-Lactones. Syntheses from the dichlorodiene are shown in Figure 1.

3-(2,2-Dichlorovinyl)-2-hydroxymethyl-2-methylcyclopropanecarboxylic Acids (*t*-HO,*t*-Cl_2CA; *c*-HO,*t*-Cl_2CA; *t*-HO,*c*-Cl_2CA; and *c*-HO,*c*-Cl_2CA), Their Methyl Esters (*t*-HO,*t*-Cl_2CA-Me; *c*-HO,*t*-Cl_2CA-Me; *t*-HO,*c*-Cl_2CA-Me; and *c*-HO,*c*-Cl_2CA-Me), and γ-Lactones of the 2-*cis*-Hydroxymethyl Acids

Table I. Analytical Data for Permethrin Isomers and Their Mono- and Dihydroxy Derivatives and for Dichlorovinyl Acid Isomers and Their Monohydroxy Derivatives and Lactones

Designations	Mp, °C[a]	NMR chemical shifts, δ ppm, and coupling constants, Hz	TLC R_f values in indicated solvent systems[b] (parentheses designate methylated derivatives)			
		Permethrin Isomers and Their Mono- and Dihydroxy Derivatives				
			A × 2	B	C	D
t-per	43–44	6.8–7.6 (9 H, m), 5.62 (1 H, d, J = 8.5), 5.11 (2 H, s), 2.26 (1 H, dd, J = 8.5 and 5), 1.65 (1 H, d, J = 5), 1.28 (3 H, s), 1.18 (3 H, s)	0.98	0.68	0.94	0.89 E, 0.71
c-per	Liquid	6.8–7.6 (9 H, m), 6.27 (1 H, dd, J = 8.5 and 1.5), 5.09 (2 H, s), 1.75–2.25 (2 H, m), 1.24 (6 H, s)	0.98	0.69	0.94	0.91 E, 0.80
2′-HO,*t*-per	Liquid	6.8–7.5 (8 H, m), 5.64 (1 H, d, J = 8.5), 5.12 (2 H, s), 2.25 (1 H, dd, J = 8.5 and 5), 1.65 (1 H, d, J = 5), 1.26 (3 H, s), 1.18 (3 H, s), 5.60 (OH, s)	0.95	0.55	0.92	0.71
2′HO,*c*-per	Liquid	6.8–7.5 (8 H, m), 6.30 (1 H, d, J = 8.5), 5.10 (2 H, s), 1.70–2.30 (2 H, m), 1.25 (6 H, s), 5.62 (OH, s)	0.95	0.57	0.92	0.75 E (0.42)
4′-HO,*t*-per	Liquid	6.6–7.5 (8 H, m), 5.60 (1 H, d, J = 8.5), 5.10 (2 H, s), 2.25 (1 H, dd, J = 8.5 and 5), 1.65 (1 H, d, J = 5), 1.26 (3 H, s), 1.17 (3 H, s), 5.0 (OH, s)	0.84	0.42	0.84	0.48 E (0.46)
4′-HO,*c*-per	Liquid	6.6–7.5 (8 H, m), 6.26 (1 H, dd, J = 8.5 and 1.5), 5.06 (2 H, s), 1.70–2.30 (2 H, m), 1.23 (6 H, s), 5.15 (OH, s)	0.84	0.43	0.84	0.51 E (0.60)
t-HO,*t*-per	Liquid	6.7–7.6 (9 H, m), 5.75 (1 H, d, J = 8.5), 5.10 (2 H, s), 3.55 (2 H, CH_2OH), 1.6–2.4 (2 H and OH, m), 1.35 (3 H, s)	0.73	0.31	0.80	0.34
c-HO,*t*-per	Liquid	6.7–7.6 (9 H, m), 5.65 (1 H, d, J = 8.5), 5.11 (2 H, s), 3.78 (2 H, CH_2OH), 2.45 (1 H, dd, J = 8.5 and 5), 1.70 (1 H, d, J = 5), 1.24 (3 H, s), 2.30 (OH, s)	0.69	0.27	0.79	0.32
t-HO,*c*-per	Liquid	6.7–7.6 (9 H, m), 6.29 (1 H, dd, J = 8.5 and 1.5), 5.08 (2 H, s), 3.50 (2 H, CH_2OH), 1.80–2.40 (2 H, m), 1.28 (3 H, s), 2.11 (OH, s)	0.74	0.32	0.80	0.35
c-HO,*c*-per	Liquid		0.73	0.30	0.80	0.34
t-HO,4′-HO,*t*-per	Liquid		0.48	0.10	0.56	0.07
t-HO,4′-HO,*c*-per	Liquid	6.6–7.5 (8 H, m), 6.28 (1 H, d, J = 8.5), 5.08 (2 H, s), 3.51 (2 H, CH_2OH), 1.80–2.40 (2 H, m), 1.29 (3 H, s)	0.50	0.11	0.57	0.08
		Dichlorovinyl Acid Isomers and Their Monohydroxy Derivatives and Lactones				
				F		
t-Cl_2CA	91–95 (93–96[c])	5.62 (1 H, d, J = 8.5), 2.28 (1 H, dd, J = 8.5 and 5), 1.61 (1 H, d, J = 5), 1.33 (3 H, s), 1.21 (3 H, s)	0.79	0.66	0.49	
c-Cl_2CA	83–85 (85–88[c])	6.21 (1 H, d, J = 8.5), 1.7–2.3 (2 H, m), 1.27 (6 H, s)	0.86	0.70	0.61	
					G × 2	H
t-HO,*t*-Cl_2CA	119–121	[d]5.78 (1 H, d, J = 8.5), 3.70 (3 H, s), 3.60 (2 H), 2.32 (1 H, dd, J = 8.5 and 5), 1.89 (1 H, d, J = 5), 1.35 (3 H, s), 1.73 (OH, s)	0.39	0.29	(0.45)	(0.28)
c-HO,*t*-Cl_2CA	135–136	[d]5.65 (1 H, d, J = 8.5), 3.80 (2 H, s), 3.71 (3 H, s), 2.45 (1 H, dd, J = 8.5 and 5), 1.66 (1 H, d, J = 5), 1.25 (3 H, s), 2.1 (OH)	0.31	0.24	(0.31)	(0.20)
t-HO,*c*-Cl_2CA	169–170	[d]6.28 (1 H, dd, J = 8.5 and 1.5), 3.68 (3 H, s), 3.52 (2 H, s), 1.9–2.4 (2 H and OH, m), 1.28 (3 H, s),	0.40	0.31	(0.46)	(0.29)
c-HO,*c*-Cl_2CA	107–108	[d]6.20 (1 H, d, J = 8.5), 3.90 (2 H, s), 3.70 (3 H, s), 1.8–2.3 (2 H, m), 1.33 (3 H, s), 1.7 (OH, s)	0.34	0.27	(0.43)	(0.28)
					B	
c-HO,*t*-Cl_2CA-lactone	103	5.55 (1 H, d, J = 8.5), 4.20 (2 H, q), 1.9–2.3 (2 H, m), 1.40 (3 H, s)	0.73	0.63	0.37	0.36
c-HO,*c*-Cl_2CA-lactone	59–62	5.62 (1 H, m), 4.18 (2 H, s), 2.28 (2 H, m), 1.45 (3 H, s)	0.74	0.65	0.37	0.39

[a] Uncorrected. [b] See text for composition of solvent systems. [c] Compounds previously described by Burt et al. (1974). [d] Methyl esters.

Table II. Analytical Data for Hydroxy-3-phenoxybenzyl Alcohols, Hydroxy-3-phenoxybenzoic Acids, and Methyl Esters of L-Amino Acid Conjugates of 3-Phenoxybenzoic Acid and Dichlorovinyl Acid Isomers

Designations	Mp, °C[a]	TLC R_f values in indicated solvent systems[b] (parentheses designate methylated derivatives)		
	Hydroxy-3-phenoxybenzyl Alcohols			
		A × 2	B	C
PBalc	Liquid	0.66	0.27	0.71
2′-HO-PBalc	Liquid	0.43	0.09	0.46
3′-HO-PBalc	Liquid	0.43	0.08	0.43
4′-HO-PBalc	110–111	0.41	0.07	0.40
4-HO-PBalc	131–132	0.47	0.13	0.47
5-HO-PBalc	Liquid	0.40	0.06	0.37
6-HO-PBalc	Liquid	0.51	0.14	0.51
	Hydroxy-3-phenoxybenzoic Acids			
			F	
PBacid	146 (145[c])	0.75	0.65	
2′-HO-PBacid	111 (121–123, 138–140[d])	0.53	0.46	
3′-HO-PBacid	135–136 (130–132[d])	0.51	0.45	
4′-HO-PBacid	177 (169.5–170.5[d])	0.50	0.42	
4-HO-PBacid	192 (187.6–188[d])	0.59	0.53	
5-HO-PBacid	222 (216–217[d])	0.51	0.44	
6-HO-PBacid	133 (132–133[d])	0.75	0.66	
	Methyl Esters of L-Amino Acid Conjugates of 3-Phenoxybenzoic Acid			
PBacid,gly-Me	Liquid[c]	(0.48)	(0.47)	
PBacid,ala-Me	Liquid	(0.60)	(0.59)	
PBacid,ser-Me	Liquid	(0.25)	(0.26)	
PBacid,glut-diMe	Liquid	(0.53)	(0.52)	
	Methyl Esters of L-Amino Acid Conjugates of Dichlorovinyl Acid Isomers			
t-Cl_2CA,gly-Me	121	(0.51)	(0.49)	
c-Cl_2CA,gly-Me	77–79	(0.58)	(0.56)	
t-Cl_2CA,ala-Me	Liquid	(0.62)	(0.60)	
c-Cl_2CA,ala-Me	Liquid	(0.68)	(0.65)	
t-Cl_2CA,ser-Me	107–108	(0.27)	(0.27)	
c-Cl_2CA,ser-Me	87–88	(0.32)	(0.31)	
t-Cl_2CA,glut-diMe	Liquid	(0.52)	(0.53)	
c-Cl_2CA,glut-diMe	Liquid	(0.58)	(0.58)	

[a] Uncorrected. [b] See text for composition of solvent systems. PBalc gives R_f 0.30 in solvent system D. [c] Compounds previously described by Miyamoto et al. (1974). [d] Compounds previously described by Ungnade and Rubin (1951).

(*c*-HO,*t*-Cl_2CA-Lactone and *c*-HO,*c*-Cl_2CA-Lactone). The dichlorodiene [n^{22}_D 1.5218; NMR 6.59 (1 H, d, J = 11.5), 5.95 (1 H, dm, J = 11.5), 1.86 (3 H, s), 1.78 (3 H, s)] (30 g, 0.2 mol) in glacial acetic acid (70 mL) was refluxed with SeO_2 (7.3 g, 0.066 mol) for 1.5 h. The reaction mixture was cooled to room temperature, decanted from the metallic Se, concentrated in vacuo, and poured into saturated NaCl. Distillation of the ether-soluble products after drying (Na_2SO_4) gave the dichlorodiene *trans*-aldehyde and AcO-dichlorodienes [dichlorodiene *trans*-aldehyde; 3.6 g; R_f 0.72 and 0.61 in I and J, respectively; bp 59–70 °C (1 mmHg); mp 60–61 °C; 2,4-dinitrophenylhydrazone mp 236 °C, $\lambda_{max}^{CHCl_3}$ 392 nm (log ϵ = 4.24); IR 1690, 1618; NMR 9.48 (1 H, s), 6.90 (2 H, s), 1.85 (3 H, s); CI–MS (methane) 165 (M + 1, 76%), 129 (M − Cl, 100%): AcO-dichlorodienes (trans/cis = 83/17 mixture; 6.3 g; bp 70–80 °C (1 mmHg)]. Solvent system J was used on a preparative scale (30 mg/0.5 mm plate) to isolate pure *trans*- and *cis*-acetates [*t*-AcO-dichlorodiene, R_f 0.63 and 0.56 in I and J, respectively; liquid; IR 1740; NMR 6.64 (1 H, d, J = 11.5), 6.20 (1 H, dm, J = 11.5), 4.58 (2 H, s), 2.12 (3 H, s), 1.81 (3 H, s); CI–MS (isobutane) 209 (M + 1, 0%), 149 (M − $OCOCH_3$, 100%): *c*-AcO-dichlorodiene, R_f 0.68 and 0.62 in I and J, respectively; liquid; IR 1740; NMR 6.73 (1 H, d, J = 11.5), 6.16 (1 H, dm, J = 11.5), 4.66 (2 H, s), 2.10 (3 H, s), 1.92 (3 H, s); CI–MS (isobutane) 209 (M + 1, 0%), 149 (100%)].

Ethyl diazoacetate prepared from ethyl glycinate HCl (Searle, 1956) (17.3 g, 0.124 mol) was added dropwise over a period of 50 min to the above stirred mixture of dichlorodiene acetates (13 g, 0.062 mol) and Cu powder (0.55 g, washed with dilute HCl) at 120–130 °C. Distillation of the decanted reaction mixture gave the unreacted acetates (~80 °C/1 mmHg, 7.7 g) contaminated with ethyl fumarate and maleate (Burt et al., 1974). On TLC examination (0.25 mm plate, J × 2) of the undistilled fraction (8.5 g) containing the desired diester (AcO,Cl_2CA-Et), four spots (R_f 0.66, 0.56, 0.49, and 0.39) were readily detected with the phosphomolybdic acid reagent but the highest spot was more prominent than the others under UV light. Purification of the diester mixture (620 mg) by preparative TLC (J × 2) (70–80 mg/0.5 mm, 130–160 mg/1 mm, and 200–250 mg/2 mm plates) gave the following product recoveries: R_f 0.66–82 mg, R_f 0.56–114 mg, R_f 0.49–120 mg, and R_f 0.39–126 mg. Dichlorodiene acetates were identified from the R_f 0.66 area by NMR and TLC cochromatography (I and J). *t*-AcO,*c*-Cl_2CA-Et was tentatively identified from the R_f 0.56 area by IR and NMR (stereochemical assignment considered later) [*t*-AcO,*c*-Cl_2CA-Et; IR 1740, 1720; NMR 6.28 (1 H, d, J = 8.5), 4.15 (2 H, q), 3.97 (2 H,

s), 1.9–2.4 (2 H, m), 2.09 (3 H, s), 1.32 (3 H, s), 1.28 (3 H, t)]. The R_f 0.49 area consisted of a mixture of *t*-AcO,*t*-Cl_2CA-Et and *c*-AcO,*c*-Cl_2CA-Et (tentatively identified by NMR) and a contaminant (structure not assigned). Stereochemical assignment of the two isomeric diesters in the mixture was not accomplished due to the very complex NMR signals, inadequate TLC separation, and the presence of an impurity. Therefore, the methyl esters were prepared (diazomethane, 0–5 °C, 2 h) and the structures assigned by NMR comparisons with the methyl esters of the two other isomeric hydroxy acids or by a lactonization reaction (discussed later). *c*-AcO,*t*-Cl_2CA-Et was tentatively identified (NMR, IR) from the R_f 0.39 area [*c*-AcO,*t*-Cl_2CA-Et; IR 1720–1740, broad; NMR 5.64 (1 H, d, J = 8.5), 3.9–4.6 (4 H, m), 2.40 (1 H, dd, J = 8.5 and 5), 2.04 (3 H, s), 1.70 (1 H, d, J = 5), 1.26 (3 H, t), 1.24 (3 H, s)].

Each diester product or isomer mixture from preparative TLC was hydrolyzed (0.5 N NaOH in 7 mL of methanol; 1.5 h reflux) and the desired hydroxy acid or mixture of hydroxy acids recovered by pouring the solution into saturated NaCl (70 mL), acidification (pH ~2) with HCl (6 N) and extraction with ether (3 × 20 mL). A concentrate of the ether extract (dried with Na_2SO_4) held at 5 °C for 0.5 day gave crystalline hydroxy acids from the hydrolysis products of the R_f 0.56 and 0.39 diesters. They were recovered by filtration and washing of the residue with a small amount of cold chloroform (first crop) and further purification of the chloroform wash by preparative TLC (A × 2) (second crop) as follows: *t*-HO,*c*-Cl_2CA-, 48 and 20 mg, respectively; *c*-HO,*t*-Cl_2CA-, 42 and 17 mg, respectively. The hydrolysis products from the R_f 0.49 diester region did not crystallize from ether as above even after 7 days at 5 °C. A minor unidentified impurity was removed and pure crystalline *t*-HO,*t*-Cl_2CA (9 mg) and *c*-HO,*c*-Cl_2CA-lactone (7 mg) were obtained from the mixture by preparative TLC (A × 2). A quantitative yield of *c*-HO,*c*-Cl_2CA was obtained from *c*-HO,*c*-Cl_2CA-lactone by hydrolysis (0.5 N NaOH in methanol) and recovery as above, except the pH was adjusted to 3 and the product was then recovered by immediate ether extraction to minimize the γ-relactonization reaction. *c*-HO,*t*-Cl_2CA-lactone (25 mg) was prepared by heating *c*-HO,*t*-Cl_2CA (30 mg) with 1.2 molar equiv of *N*,*N*′-dicyclohexylcarbodiimide (DCC, 33 mg) in dichloromethane (5 mL) in an ampule (N_2 gas) at 80–90 °C for 2 h. After filtration to remove dicyclohexylurea, the filtrate was purified by preparative TLC (A × 2). This lactone is also formed, but in lower yield, on holding *c*-HO,*t*-Cl_2CA in strong HCl.

Treatment of the four HO-Cl_2CA isomers with diazomethane in ether gave the corresponding methyl esters.

Six Isomeric HO-PBalc Derivatives and the Corresponding HO-PBacid Derivatives. Syntheses from methyl 3-bromobenzoate, isovanillin Na salt, 3,5-dihydroxytoluene, and 5-bromosalicylaldehyde are shown in Figure 2. Different synthesis routes were used in previous preparations of the six isomeric HO-PBacid derivatives, with the exception of 5-HO-PBacid (Ungnade and Rubin, 1951).

3-(Hydroxyphenoxy)benzyl Alcohols (2′-HO-PBalc, 3′-HO-PBalc, and 4′-HO-PBalc), 3-(Hydroxyphenoxy)benzoic Acids (2′-HO-PBacid, 3′-HO-PBacid, and 4′-HO-PBacid), Hydroxy-(3-phenoxy)benzyl Alcohols (4-HO-PBalc, 5-HO-PBalc, and 6-HO-PBalc), and Hydroxy-(3-phenoxy)benzoic Acids (4-HO-PBacid, 5-HO-PBacid, and 6-HO-PBacid). Three isomeric methyl 3-(methoxyphenoxy)benzoates (MeO-PBacid-Me) were synthesized by the general procedure of Miyamoto et al. (1974) using methyl 3-bromobenzoate [mp 31–32 °C, prepared from the acid chloride and methanol (Sudborough, 1895)] and equimolar amounts of the Na salts of 2-, 3-, and 4-methoxyphenols [prepared from the methoxyphenols with 1.2 molar equiv NaH (57% oil dispersion) in dimethylformamide (DMF) solution] and a catalytic amount of Cu_2Cl_2 with N_2 gas at 150–170 °C for 6 h. The methyl 3-(methoxyphenoxy)benzoate in the concentrate of the ether extract from the reaction mixture was purified by distillation or preparative TLC (benzene): 2′-MeO-PBacid-Me, R_f 0.40; liquid; IR 1720, 1580, 1500; NMR 6.8–7.9 (8 H, m), 3.85 (3 H, s), 3.76 (3 H, s); 3′-MeO-PBacid-Me, R_f 0.48; liquid; IR 1720, 1580, 1485; NMR 6.4–7.9 (8 H, m), 3.90 (3 H, s), 3.79 (3 H, s); 4′-MeO-PBacid-Me, R_f 0.51; liquid bp 140–146 °C (0.3 mmHg); IR 1720, 1582, 1500; NMR 6.7–7.8 (8 H, m), 3.83 (3 H, s), 3.75 (3 H, s). These MeO-PBacid-Me derivatives (200 mg, 0.78 mmol) in dichloromethane (2 mL) were treated individually with BBr_3 (200 mg, 0.80 mmol) in dichloromethane (1 mL) at −10 °C, then stirred for an additional 2 min at this temperature. Each mixture was immediately concentrated to dryness in vacuo at room temperature. The clear solution of demethylated product obtained upon slow addition of anhydrous methanol (1 mL) to the reddish residue under ice cooling was further purified by preparative TLC (B) to obtain the desired HO-PBacid-Me derivative (>90% yield): 2′-HO-PBacid-Me, R_f 0.69; mp 108 °C; IR 3340, 1700; NMR 6.7–7.9 (8 H, m), 3.87 (3 H, s); CI–MS (isobutane) 245 (M + 1, 100%); 3′-HO-PBacid-Me, R_f 0.60; liquid; IR 3380, 1720, 1700; NMR 6.3–7.9 (8 H, m), 3.85 (3 H, s); CI–MS (isobutane) 245 (M + 1, 100%); 4′-HO-PBacid-Me, R_f 0.52; mp 94 °C; IR 3360, 1720, 1700; NMR 7.9–6.8 (8 H, m), 3.86 (3 H, s); CI–MS (isobutane) 245 (M + 1, 100%). Each hydroxy carbomethoxy compound (70 mg) in dry benzene (1 mL) was reduced with diluted Vitride [23% solution of Na *bis*(2-methoxyethoxy)aluminum hydride in benzene] (1.5 mL) at room temperature for 1.5 h. The mixture was poured into saturated NaCl solution (80 mL), acidified with HCl (25%, 2 mL), then extracted with ether. A concentrate of the ether extract (dried with Na_2SO_4) gave pure 3-(hydroxyphenoxy)benzyl alcohols. The same hydroxy carbomethoxy compound (75 mg) in 2 N NaOH methanol solution (3 mL) was hydrolyzed at 70 °C for 2 h. Acidification and extraction as above gave pure 3-(hydroxyphenoxy)benzoic acids.

Isovanillin in DMF solution was treated with NaH to obtain the Na salt which was then reacted with bromobenzene in the presence of Cu_2Cl_2 as previously described. 4-Methoxy-3-phenoxybenzaldehyde (0.5 g) [R_f 0.34 (benzene); mp 44–46 °C (49–50 °C, Ungnade and Orwoll, 1943); IR 1690; CI–MS (methane) 229 (M + 1, 100%)] purified by preparative TLC (benzene) was treated with $KMnO_4$ (0.5 g) in 25% aqueous acetone solution (15 mL) at 25 °C for 3 h. After addition of ethanol (1.5 mL), stirring for 40 min to decompose excess $KMnO_4$, and removal of the resulting MnO_2 by filtration, the filtrate was concentrated in vacuo to remove acetone, poured into saturated NaCl (100 mL) and acidified with HCl (25%, 3 mL). A concentrate of the ether extract (dried with Na_2SO_4) gave the pure desired acid (349 mg) [4-MeO-PBacid, R_f 0.60 (F); mp 188.5 °C; IR 1690, 1675; CI–MS (isobutane) 245 (M + 1, 100%)]. The methoxy acid (170 mg) was refluxed with $AlCl_3$ (1.5 g) in thiophene-free benzene (35 mL) for 2 h (Ungnade and Rubin, 1951). The reaction mixture was poured into ice water (100 mL), acidified with HCl (25%, 6 mL), concentrated in vacuo to remove benzene, and the solution was saturated with NaCl

and extracted with 10% methanol–ether mixture. A concentrate of the extract (dried with Na_2SO_4) gave pure 4-HO-PBacid (147 mg). The hydroxy acid (50 mg) was esterified with diazomethane in ether (5 mL) at 0–5 °C for 5 min and the methyl ester was isolated by preparative TLC (B) (38 mg) [4-HO-PBacid-Me; R_f 0.55; mp 122 °C; IR 3330, 1700; CI–MS (isobutane) 245 (M + 1, 39%)]. A minor and less polar material (R_f 0.70, B) from the diazomethane reaction was the dimethylated derivative (4-MeO-PBacid-Me). The hydroxy carbomethoxy compound (35 mg) in tetrahydrofuran (THF) (3 mL) was treated with $LiAlH_4$ (100 mg) at 25 °C for 30 min, then refluxed for 1 h. The reaction mixture was poured into saturated NaCl solution (50 mL), acidified with HCl (25%, 2 mL), and extracted (ether). The concentrate gave pure 4-HO-PBalc (27 mg).

3,5-Dihydroxytoluene was treated with 1.1 molar equiv dimethyl sulfate and 3 molar equiv 10% NaOH aqueous solution to obtain 3-hydroxy-5-methoxytoluene, which was recovered by distillation. Conversion of this phenol to the Na salt (as above) and reaction with bromobenzene in the presence of Cu_2Cl_2 gave 5-methoxy-3-phenoxytoluene (Ungnade and Rubin, 1951). After TLC purification (benzene), 5-methoxy-3-phenoxytoluene (1.7 g) [R_f 0.79; liquid; NMR 6.3–7.5 (8 H, m), 3.75 (3 H, s), 2.30 (3 H, s)] was refluxed with $KMnO_4$ (2.5 g) in water (12 mL) and pyridine (24 mL) for 2 h (Ungnade and Orwoll, 1943). The MnO_2 was filtered off and washed with hot water. The combined filtrate was acidified with HCl (25%, 40 mL), the solution saturated with NaCl, then extracted with ether. The desired acid was recovered in 10% NaOH solution from the ether extract and then liberated by addition of HCl for reextraction into ether. The concentrate of the ether extract gave pure 5-MeO-PBacid after recrystallization from benzene (498 mg) [R_f 0.36 (A); mp 145–146 °C; IR 1690; CI–MS (isobutane) 245 (M + 1, 7%)]. Demethylation of the methoxy acid (150 mg) with $AlCl_3$ in benzene yielded 5-HO-PBacid (130 mg). Methylation of the hydroxy acid (60 mg) [5-HO-PBacid-Me: R_f 0.40 (B); liquid; IR 3370, 1700; NMR 6.6–7.5 (8 H, m), 3.88 (3 H, s)] and reduction of the hydroxy carbomethoxy compound (50 mg) gave 5-HO-PBalc (42 mg).

6-Methoxy-3-phenoxybenzaldehyde was synthesized from 2-methoxy-5-bromobenzaldehyde (mp 117 °C, prepared from 5-bromosalicylaldehyde and dimethyl sulfate as above) and sodium phenolate (prepared with NaH) with Cu_2Cl_2. After TLC purification (benzene), this methoxyphenoxybenzaldehyde (0.4 g) [R_f 0.38; liquid; IR 1685; NMR 10.6 (1 H), 6.8–8.0 (8 H, m), 4.01 (3 H, s); CI–MS (isobutane) 229 (M + 1, 100%)] was treated with $KMnO_4$ (0.4 g) in 25% aqueous acetone solution to yield 6-MeO-PBacid (395 mg) [R_f 0.30 (A); mp 77–92 °C; IR 1700, 1665; NMR 6.9–7.9 (8 H, m), 4.05 (3H); CI–MS (methane) 245 (M + 1, 100%)]. Demethylation of the methoxy acid (210 mg) with $AlCl_3$ in benzene yielded 6-HO-PBacid (175 mg). Methylation of the hydroxy acid (30 mg) and reduction of the hydroxy carbomethoxy compound (31 mg) [6-HO-PBacid-Me: R_f 0.63 (A); liquid; IR 3180, 1680; NMR 10.6 (1 H), 6.8–7.7 (8 H, m), 3.88 (3 H, s)] gave 6-HO-PBalc (25 mg).

Four Isomeric HO-Per Derivatives with Monohydroxylation on the Acid Moiety. Syntheses from the four HO-Cl_2CA isomers and 3-phenoxybenzyl bromide (PBbromide) are shown in Figure 1.

3-Phenoxybenzyl 3-(2,2-Dichlorovinyl)-2-hydroxymethyl-2-methylcyclopropanecarboxylates (*t*-HO,*t*-per; *c*-HO,*t*-per; *t*-HO,*c*-per; and *c*-HO,*c*-per). To prepare PBbromide, PBalc (800 mg, 4 mmol) in dry ether (4 mL) was treated with PBr_3 (360 mg, 1.33 mmol) and dry pyridine (315 mg, 4 mmol) at −25 °C, then stirred for an additional 2 h below 0 °C. The mixture was poured into ice water (70 mL) and extracted with ether (2 × 20 mL). The ether extract was washed with cold water (60 mL) then dried (Na_2SO_4). The resulting PBbromide (752 mg) was used for the esterification without further purification [R_f 0.81 (E); liquid; IR 1585, 1490; NMR 6.7–7.6 (9 H, m), 4.38 (2 H, s)]. The esters were synthesized by heating the four isomeric hydroxy acids (2–40 mg) with 1.5 molar equiv PBbromide and 1.3 molar equiv triethylamine (Et_3N) in DMF solution (0.3–1.5 mL) in an ampule with N_2 gas at 80–90 °C for 2–3 h. Each reaction mixture was directly purified by preparative TLC (C) and the two isomeric esters with monohydroxylation at the *cis*-methyl to the carboxyl were further purified by TLC (B) to remove a small amount of lactone impurity. Isomerization does not occur during reaction or purification but a small portion of the 2-*cis*-hydroxymethyl compounds undergo a lactonization reaction. Under strong acidic conditions, *c*-HO,*t*-per and *c*-HO,*c*-per decompose to the corresponding lactones and PBalc.

Four Isomeric HO-Per Derivatives with Monohydroxylation on the Alcohol Moiety. Syntheses by demethylation of 2′- and 4′-MeO-per with BBr_3 or by reaction of Cl_2CA with 2′- and 4′-HO-PBbromide are shown in Figure 1.

3-(2′- and 4′-Methoxyphenoxy)benzyl and 3-(2′- and 4′-Hydroxyphenoxy)benzyl 3-(2,2-Dichlorovinyl)-2,2-dimethylcyclopropanecarboxylates (2′-MeO,*c*-per; 4′-MeO,*t*-per; 4′-MeO,*c*-per; 2′-HO,*t*-per; 2′-HO,*c*-per; 4′-HO,*t*-per; and 4′-HO,*c*-per) and 3-(2′- and 4′-Hydroxyphenoxy)benzyl Bromides (2′-HO-PBbromide and 4′-HO-PBbromide). To prepare the acid chloride, Cl_2CA (400 mg) and 1.5 molar equiv $SOCl_2$ (340 mg) were refluxed in dry hexane (20 mL) for 3 h. The mixture was concentrated in vacuo at less than 30 °C, then a small amount of dry benzene was added and concentration was continued under the same condition to remove $SOCl_2$. The residue was used for esterification without further purification. The above acid chloride and equimolar 2′- or 4′-methoxy alcohol were esterified with dry pyridine in dry benzene under ice cooling for 10 h. The methoxy esters were purified by preparative TLC (E). Analytical data are as follows: 2′-MeO,*c*-per; liquid; IR 1720; NMR 6.7–7.5 (8 H, m), 6.28 (1 H, d, J = 8.5), 5.07 (2 H, s), 3.82 (3 H, s), 1.7–2.2 (2 H, m), 1.25 (6 H, s): 4′-MeO,*t*-per; liquid; IR 1720; NMR 6.7–7.5 (8 H, m), 5.62 (1 H, d, J = 8.5), 5.08 (2 H, s), 3.76 (2 H, s), 2.26 (1 H, dd, J = 8.5 and 5), 1.66 (1 H, d, J = 5), 1.27 (3 H, s), 1.16 (3 H, s): 4′-MeO,*c*-per; liquid; IR 1720; NMR 6.7–7.5 (8 H, m), 6.28 (1 H, d, J = 8.5), 5.08 (2 H, s), 1.7–2.2 (2 H, m), 1.26 (6 H, s). The methoxy ester (160 mg) in dichloromethane (2 mL) was treated with BBr_3 (160 mg) in dichloromethane (1 mL) at −10 °C, stirred an additional 2 min at −10 °C and the mixture was immediately concentrated to dryness in vacuo at room temperature. Anhydrous methanol (1.5 mL) was slowly added to the reddish residue under ice cooling and the clear solution was immediately subjected to preparative TLC (benzene for 2′ derivative; B for 4′ derivative). Demethylation by BBr_3 in all cases yielded one minor and two major products. The minor product was the desired monohydroxy ester. The major products were the corresponding Cl_2CA and HO-PBbromide derivatives [2′-HO-PBbromide, R_f 0.38 (benzene); liquid; NMR 6.7–7.5 (8 H, m), 5.58 (1 H, broad), 4.46 (2 H, s): 4′-HO-PBbromide, R_f 0.39 (B); mp 86 °C; NMR 6.7–7.5 (8 H, m), 5.75 (1 H, broad), 4.43

(2 H, s); CI–MS (isobutane) 279 (M + 1, 9%), 199 (M – Br, 100%)]. 2′-HO-PBbromide chromatographs above 2′-HO-per with benzene for development whereas 4′-HO-per appears above 4′-HO-PBbromide using solvent system B. Reesterification of the acid and the HO-PBbromide derivatives as above (Et_3N, DMF) also yielded the appropriate ester (TLC, NMR) in each case.

Two Isomeric DiHO-Per Derivatives with Monohydroxylation on Both the Acid and Alcohol Moieties. Syntheses from the two isomeric *t*-HO,Cl_2CA derivatives and 4′-HO-PBbromide are shown in Figure 1.

4′-Hydroxy-3-phenoxybenzyl 3-(2,2-Dichlorovinyl)-2-*trans*-hydroxymethyl-2-*cis*-methylcyclopropanecarboxylates (*t*-HO,4′-HO,*t*-per and *t*-HO,4′-HO,*c*-per). The desired dihydroxy esters were obtained in 25–50% yield by treating *t*-HO,*t*-Cl_2CA (3 mg) and *t*-HO,*c*-Cl_2CA (30 mg) with 4′-HO-PBbromide (from the BBr_3 reaction) by the same esterification method used for monohydroxy esters followed by preparative TLC with benzene–ethyl acetate (3:1).

Methyl Esters of L-Amino Acid Conjugates of Cl_2CA and of PBacid. These compounds were prepared from the acid chlorides and methyl esters of L-amino acids HCl with pyridine in THF–benzene solution. Methyl esters of glycine, alanine, serine, and glutamic acid were used as the amino moieties.

Methyl Esters of 3-(2,2-Dichlorovinyl)-2,2-dimethylcyclopropanecarboxyl- and 3-phenoxybenzoyl-glycine, -alanine, -serine and -glutamic Acid (*t*-Cl_2CA,gly-Me; *c*-Cl_2CA,gly-Me; PBacid,gly-Me; *t*-Cl_2CA,ala-Me; *c*-Cl_2CA,ala-Me; PBacid,ala-Me; *t*-Cl_2CA,ser-Me; *c*-Cl_2CA,ser-Me; PBacid,ser-Me; *t*-Cl_2CA,glut-diMe; *c*-Cl_2CA,glut-diMe; and PBacid,glut-diMe). The acid chlorides of *t*- and *c*-Cl_2CA or of PBacid (200–300 mg) in THF–benzene (1:1) (15–30 mL) were treated with equimolar amounts of the methyl esters of the L-amino acids HCl (glycine, alanine, serine, and glutamic acid) in dry pyridine (2–4 mL) under ice cooling for 5–10 min, then stirred for an additional 2 h at this temperature. 3-Phenoxybenzoyl chloride was obtained by the same method used for the Cl_2CA chloride derivatives with the exception of solvent (benzene). Each reaction mixture was poured into saturated aqueous NaCl, acidified with HCl (pH 1–2), and extracted with ethyl acetate. The concentrate of the ethyl acetate extract (dried with Na_2SO_4) was purified by preparative TLC (A or F) to obtain the 12 amino acid conjugates as their methyl esters in 50–80% yields based on the starting acids.

To prepare the free glycine conjugates of *t*- and *c*-Cl_2CA, the methyl esters (90 mg) were hydrolyzed in 0.5 N NaOH in methanol (2 mL) at 50 °C for 1.5 h. The reaction mixture was acidified and extracted as above to give the pure crystalline compounds (mp 183 °C, 80 mg for trans; mp 153–154 °C, 75 mg for cis).

Ethereal Sulfate of 4′-Hydroxy-3-phenoxybenzoic Acid (4′-HO-PBacid-sulfate). This sulfate was prepared by a procedure based on Feigenbaum and Neuberg (1941). A stirred solution of 4′-HO-PBacid (1 g, 4.3 mmol) in dry pyridine (5 mL) was treated with $ClSO_3H$ (1 g, 8.6 mmol) at 0 °C for 10 min, then stirred for an additional 1.5 h at this temperature. A 50% aqueous NaOH solution (1.5–2 mL) was added to the reaction mixture under ice cooling. The precipitate was collected by filtration, washed with a small amount of cold acetone, and extracted with hot ethanol. The ethanol extract was purified by preparative TLC [1-butanol–acetic acid–water (6:1:1)] to obtain the desired sulfate (52 mg, free acid) from the R_f 0.58 region. This compound on hydrolysis with sulfatase or 3 N HCl (Gaughan et al., 1977a) gave back the starting material, 4′-HO-PBacid (identified by TLC with A × 2 and F).

SPECTRAL IDENTIFICATION AND CHROMATOGRAPHIC PROPERTIES

Spectroscopic Data. Supplementary material (see below) provides appropriate IR, NMR, and CI–MS data supporting the identity of the final products. The $[M + 1]^+$ and appropriate carbonium ion fragments were more prominent with isobutane than with methane. Ammonia was a particularly useful reactant gas with the hydroxy esters since 4′-HO,*t*-per and -*c*-per gave as the base peak the $[M + 18]^+$ ion corresponding to addition of $[NH_4]^+$ to the parent molecule.

Chromatographic Properties. Tables I and II give TLC solvent systems appropriate for separating each of the compounds from closely related materials. Acidic solvent systems (A and F) are required for optimal separation of the carboxylic acids but they also result in some decomposition on holding the developed chromatoplates (as for ^{14}C cochromatography and product recovery) with the esters containing *c*-HO, 2′-HO, and 4′-HO substituents. Solvent systems without acid (e.g., B, C, D, E, G, H, I, and J) provide the best resolution of compounds without carboxylic acid groupings.

All *c*-Cl_2CA and HO-*c*-Cl_2CA derivatives give higher R_f values than the corresponding trans isomers. Derivatives with a hydroxyl group at the *gem*-dimethyl substituent (i.e., HO-Cl_2CA, HO-Cl_2CA-Me, HO-per, and HO,4′-HO-per) show a consistent isomer-dependent order of decreasing R_f values as follows: *t*-HO,*c*-Cl_2CA derivatives; *t*-HO,*t*-Cl_2CA derivatives; *c*-HO,*c*-Cl_2CA derivatives; and *c*-HO,*t*-Cl_2CA derivatives. The HO-PBalc and HO-PBacid isomers also give a uniform pattern of decreasing R_f as follows: $6 > 4 > 2' > 3' > 5 \sim 4'$. The same relationship holds for 2′- and 4′-HO, *t*- and -*c*-per but not for their methoxy derivatives.

Stereochemical Assignments of HO-Cl_2CA Isomers. The stereoselective trans oxidation of *gem*-dimethyl olefins with SeO_2 in ethanol (Bhalerao and Rapoport, 1971) is also apparent from the products recovered on SeO_2 oxidation of the dichlorodiene in glacial acetic acid, i.e., 89% *trans* (dichlorodiene *trans*-aldehyde and *t*-AcO-dichlorodiene) and 11% *cis* (*c*-AcO-dichlorodiene). The exclusive trans configuration of the dichlorodiene aldehyde was established by reduction ($NaBH_4$) and esterification (acetyl chloride) to give a product identical (NMR) with *t*-AcO-dichlorodiene.

On reacting the 83:17 mixture of *t*-AcO- and *c*-AcO-dichlorodienes with ethyl diazoacetate to obtain the desired HO-Cl_2CA derivatives (following appropriate separation and hydrolysis steps), the isomer ratio of *t*-HO,*c*-Cl_2CA:*c*-HO,*t*-Cl_2CA:*t*-HO,*t*-Cl_2CA:*c*-HO,*c*-Cl_2CA ultimately isolated was 47:41:6:6. This ratio is consistent with studies on related compounds (Blatchford and Orchin, 1964; Rilling et al., 1971) which indicate that *t*-AcO-dichlorodiene will give *t*-AcO,*c*-Cl_2CA-Et and *c*-AcO,*t*-Cl_2CA-Et while *c*-AcO-dichlorodiene will give *t*-AcO,*t*-Cl_2CA-Et and *c*-AcO,*c*-Cl_2CA-Et.

NMR spectra (Figure 3) of the HO-Cl_2CA-Me isomers serve to assign the configuration of the 2-hydroxymethyl substituent and the 3-dichlorovinyl side chain relative to the carbomethoxy group on the cyclopropane ring. The cyclopropane ring protons and the olefinic proton of the dimethylvinyl side chain in methyl esters of *trans*- and *cis*-chrysanthemic acids constitute ABX-type systems, which approximate the AMX-case with $J_{AX} \sim 0$ Hz (Bramwell et al., 1969). The same relationships hold for the dichlorovinyl analogues. With HO-Cl_2CA-Me isomers,

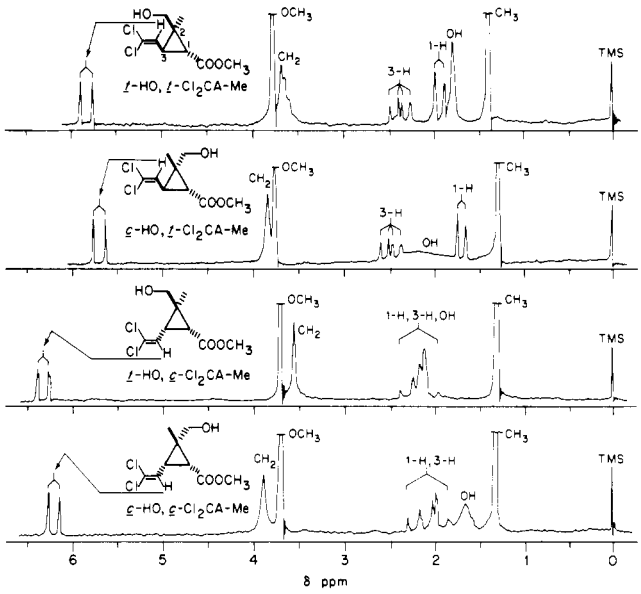

Figure 3. Nuclear magnetic resonance spectra (60 MHz) and assignments for the methyl esters of four isomers of 3-(2,2-dichlorovinyl)-2-hydroxymethyl-2-methylcyclopropanecarboxylic acid.

the cyclopropane ring proton couplings appear at 5.0 Hz (trans) and ~8 Hz (cis) and the olefinic proton is either a doublet at 8.5 Hz (trans) or a doublet-doublet at ~1.5 and 8.5 Hz (cis). The olefinic proton in the cis isomers resonates at lower field (δ = 6.28 and 6.20) than the corresponding proton in the trans isomers (δ = 5.78 and 5.65). The *cis*-methylene protons to the carbomethoxy group on the cyclopropane ring in both isomers resonate at lower field than the corresponding *trans*-methylene protons. The neighboring *cis*-carbomethoxy group clearly deshields the olefinic (Bramwell et al., 1969) and the methylene protons. Additional proof for the stereochemistry of the HO-Cl_2CA isomers comes from the γ-lactonization reaction in the presence of strong acid or DCC to form the γ-lactones from the two isomers with the 2-hydroxymethyl group cis to the carboxy group (Coates and Robinson, 1971).

PERMETHRIN METABOLITES IN MOUSE LIVER MICROSOMAL MONOOXYGENASE SYSTEMS AND IN MAMMALS AND INSECTS

Mouse liver microsomal monooxygenase (TEPP-treated microsomes, NADPH-dependent) reactions with various [^{14}C]-per preparations lead to several HO-per and diHO-per derivatives plus small amounts of an aldehyde ester and of PBalc and the two isomeric γ-lactones from decomposition of *c*-HO,*t*-per and *c*-HO,*c*-per (Table III). Ten hydroxy-ester metabolites, hydroxylated on one or both of the acid and alcohol moieties, are tentatively identified, eight by two-dimensional cochromatography with standards described in this report (Table III). The same metabolites are detected with the [1*R*] and [1*RS*] substrates except as indicated below.

The preferred site of oxidation of [1*R*,*trans*]- and [1*R*,*cis*]-per is the *cis*-methyl group, involving stereoselectivity with the [1*R*,*cis*] isomer and a high degree of stereospecificity with the [1*R*,*trans*] isomer (Table III), confirming the findings of Soderlund and Casida (1977b). The much lower apparent specificity in methyl hydroxylation of [1*RS*,*trans*]-per as compared with [1*R*,*trans*]-per is explained by the stereospecificity for *trans*-methyl hydroxylation with [1*S*,*trans*]-per (Soderlund and Casida, 1977b). A portion of the *c*-HO,*t*-per is further oxidized to the aldehyde (*c*-CHO,*t*-per) and a comparable situation probably exists for *c*-HO,*c*-per (Table III). It appears likely that under certain conditions the aldehyde esters are further oxidized to the corresponding carboxylic acids since large amounts of ester metabolites chromatographing as anticipated for carboxylic acids are detected when very extensive oxidation is involved such as with very low substrate levels. Hydroxylation of the alcohol moiety is much more important with *cis*-per than with *trans*-per and the isomers vary in site specificity, i.e., 6 > 4′ > 2′ for trans and 4′ > 6 > 2′ for cis (Table III). A previous study (Soderlund and Casida, 1977b) using a less sensitive and less specific method of analysis (GLC, trimethylsilyl derivatives) detected the 4′- but not the 2′- and 6-HO-per metabolites. The dihydroxy ester (*t*-HO,4′-HO-per) is detected with [1*R*,*cis*]- but not with [1*R*,*trans*]-per.

Table III. Ester Metabolites of Permethrin Detected in the Mouse Liver Microsomal Monooxygenase System

Derivative	TLC cochrom. system	Relative amount of metabolite from indicated [1*R*] isomer: Trans	Cis
t-HO	A × 2-F; B-C	–[a]	++
c-HO[b]	A × 2-F; B-C	++++	++++
c-CHO		+++[c]	(+)[d]
2′-HO	B-D	–	+
4′-HO	B-D	+	+++
6-HO[e]	B-D	++	++
t-HO,4′-HO	A × 2-F; B-C	–	++

[a] *t*-HO,*t*-Per is detected in amounts equal to *c*-HO,*t*-per with [1*RS*,*trans*]-per as the substrate. [b] Includes PBalc and *c*-HO-Cl_2CA-lactone from lactonization during analysis. [c] R_f 0.62, solvent system B. Not available as an authentic standard. Reduction ($NaBH_4$ in ethanol) of the ^{14}C metabolite yields a product cochromatographing (A × 2-F; B-C) with *c*-HO,*t*-per. [d] Chromatographic position similar to the *c*-CHO metabolite of [1*R*,*trans*]-per (footnote *c*) but reduction with $NaBH_4$ led to product decomposition, suggesting formation of the unstable *c*-HO,*c*-per. [e] Synthesized by a procedure similar to that for the other HO-per derivatives with hydroxylation in the alcohol moiety (Shono et al., 1977). The 6-HO-per isomers chromatograph between the corresponding 2′- and 4′-HO-per isomers in both neutral (B and D) and acidic (A × 2 and F) solvent systems.

Each of the permethrin derivatives synthesized or the corresponding glucuronide or glucoside from conjugation of the hydroxymethyl, phenolic, or carboxylic position is identified as a metabolite of *t*- or *c*-per in rats (Elliott et al., 1976; Gaughan et al., 1977a), in cows (Gaughan et al., 1977b), or in insects (Shono et al., 1977) with the following exceptions: 2′-HO,*t*-per; 2′-HO-PBalc; the 3′-, 4-, and 5-hydroxy derivatives of PBalc and PBacid; the serine conjugate of PBacid; and the alanine conjugates. Thus, 29 of the compounds synthesized in this study are now known to be metabolites of *t*- or *c*-per and in fact these authentic standards were instrumental in establishing the identity of the metabolites.

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Supplementary Material Available: A listing of the available IR, NMR, and CI–MS data on all final products (8 pages). Ordering information is given on any current masthead page.

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1-Phenylcarbamoyl-2-pyrazolines: a New Class of Insecticides. 1. Synthesis and Insecticidal Properties of 3-Phenyl-1-phenylcarbamoyl-2-pyrazolines

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Upon the discovery of the powerful insecticidal properties of 3-(4-chlorophenyl)-1-(4-chlorophenylcarbamoyl)-2-pyrazoline (PH 60-41), a large number of analogues was prepared. The structures of these compounds were confirmed by NMR. Their insecticidal properties were evaluated with larval stages of *Aëdes aegypti* L., *Pieris brassicae* L., and *Leptinotarsa decemlineata* Say. Many of these compounds proved to be excellent insecticides, bringing about the same symptoms as PH 60-41. From the point of view of biological activity and of economic aspects, PH 60-41 appeared to be the most promising compound for several fields of application.

In a recent report from our laboratories (Mulder et al., (1975), the new insecticidal compound 3-(4-chlorophenyl)-1-(4-chlorophenylcarbamoyl)-2-pyrazoline (PH 60-41) was introduced. This compound originated from a research program with derivatives of 3-aryl-2-pyrazolines. On larvae and adults of several insect species this compound proved to be a powerful stomach toxicant with some contact activity. Within 24 h after application, susceptible insects showed convulsions. They lost their grip on the leaves, fell off the plants, and finally died.

In the present paper we report the synthesis of a series of 3-phenyl-1-phenylcarbamoyl-2-pyrazolines and their evaluation as potential insecticides. Parts 2 and 3 of this series of papers (van Hes et al., 1977; Grosscurt et al., 1978) will show that substitution in the 2-pyrazoline nucleus of two phenyl rings at the 3,5 and 3,4 positions, respectively, instead of one at the 3 position gives rise to products with insecticidal properties of the same order or even better.

CHEMICAL METHODS

Microanalyses were carried out in the Analytical Department of the Institute for Organic Chemistry TNO, Utrecht, Netherlands, under the supervision of W. J. Buis. The carbon-13 spectra were measured with a Bruker WH 270 spectrometer, operating at 67.89 MHz. Nuclear magnetic resonance spectra were recorded on a Varian HA 100 spectrometer. Chemical shifts were measured with tetramethylsilane as the internal reference and with deuteriodimethyl sulfoxide as a solvent. The melting points are uncorrected.

One general method was used for the preparation of the various compounds mentioned in Tables I and II. They were prepared according to Scheme I.

4′-Chloro-3-dimethylaminopropiophenone Hydrochloride. A suspension of 30.9 g of 4-chloroacetophenone (0.2 mol), 21.2 g of dimethylamine hydrochloride (0.26 mol), and 7.8 g of paraformaldehyde (0.26 mol) in a mixture of 32 mL of ethanol and 0.4 mL of concentrated hydrochloric acid was refluxed for 2 h. After cooling, 160 mL of acetone was added. The crystals formed were collected, washed with acetone, and dried. Yield 35.8 g (72%), mp 173–175 °C [literature (Nobles, 1958), 176 °C].

3-(4-Chlorophenyl)-2-pyrazoline. A warm solution of 24.8 g of 4′-chloro-3-dimethylaminopropiophenone hydrochloride (0.1 mol) in 70 mL of methanol was added in 10 min to a mixture of 14 mL of hydrazine hydrate, 7.2 mL of 50% sodium hydroxide, and 18 mL of methanol.

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