Severin, Th., Seilmier, W., *Z. Lebensm.-Unters. Forsch.* **137**, 4 (1968).
Shaw, P. E., Tatum, J. H., Berry, R. E., *Carbohydr. Res.* **16**, 207 (1971).
Tatum, J. H., Shaw, P. E., Berry, R. E., *J. Agric. Food Chem.* **15**, 773 (1967).
van den Ouweland, G. A. M., Peer, H. G., *Recl. Trav. Chim. Pays-Bas* **89**, 750 (1970).

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2,4,5-Trichlorophenoxyacetic Acid. Synthesis and Thin-Layer Chromatography Properties of Amino Acid Conjugates and Gas–Liquid Chromatography and Mass Spectra of Methyl Ester Derivatives

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Fourteen amino acid conjugates of 2,4,5-trichlorophenoxyacetic acid were synthesized and characterized by thin-layer chromatography. The methyl esters of the conjugates were prepared and analyzed by gas–liquid chromatography (GLC) and by mass spectrometry. A GLC method was developed employing a 2% OV-1 column and temperature programming conditions that could be used to analyze for 13 of the conjugates. All the methyl ester derivatives of the conjugates exhibited mass spectral fragmentation patterns characteristic of the specific conjugate and most compounds gave molecular ions.

Plants and plant tissue cultures metabolize 2,4-dichlorophenoxyacetic acid (2,4-D) to a number of amino acid conjugates of 2,4-D, primarily the glutamic and aspartic acid conjugates (Andreae and Good, 1957; Klämbt, 1961; Feung et al., 1971, 1972, 1973b, 1975). Similarly, there are reports of amino acid conjugation of indole-3-acetic acid (IAA) by plants (Andreae and Good, 1955; Good, 1956; Good and Andreae, 1956; Row et al., 1961; Tillberg, 1974; Hutzinger and Kosuge, 1968; Feung et al., 1976). No reports of amino acid conjugation of 2,4,5-trichlorophenoxyacetic acid exist. However, in view of the fact that plants form amino acid conjugates of 2,4-D and IAA, it seems likely that amino acid conjugates of 2,4,5-T may exist in plants and are worthy of further examination. To improve the investigator's ability to isolate and identify these potential metabolites and to study their biological properties, this article reports the synthesis and thin-layer chromatography (TLC) of amino acid conjugates of 2,4,5-T and characterization of the methyl esters of these conjugates by gas–liquid chromatography (GLC) and mass spectrometry.

EXPERIMENTAL SECTION

Reagents and Materials. All solvents used were of highest purity. 2,4,5-T was purchased from Nutritional Biochemical Corporation. Amino acids were purchased from J. T. Baker Chemical Co. Diazald was obtained from Aldrich Chemical Co., Inc. 4-Hydroxy-2,5-dichlorophenoxyacetic acid (4OH-2,5-D) was previously synthesized (Hamilton et al., 1971). 5-Hydroxy-2,4-dichlorophenoxyacetic acid (5OH-2,4-D) was obtained from J. Fleeker, Department of Biochemistry, North Dakota State University.

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Preparation of 2,4,5-Trichlorophenoxyacetyl Chloride (2,4,5-T-Cl). 2,4,5-T-Cl was prepared according to Hill et al. (1949) and modifications by Wood and Fontaine (1952). 2,4,5-T (0.1 mol, recrystallized from benzene) was placed in a round-bottom flask equipped with a condenser and thionyl chloride (0.3 mol) was added. The resulting mixture was refluxed for 2 h. At the end of this time the reaction mixture was distilled at atmospheric pressure to remove excess thionyl chloride. The residue was distilled under reduced pressure which yielded 2,4,5-T-Cl as a white, solid product, mp 80–81 °C.

Synthesis of Conjugates. Fourteen 2,4,5-T amino acid conjugates were prepared according to the procedure described by Wood and Fontaine (1952); by the reaction of 2,4,5-T-Cl with the corresponding L-amino acids. Usually, 2,4,5-T-Cl (0.002 mol) was dissolved in 5 mL of benzene, and 0.002 mol of the amino acid was dissolved in 3 mol equiv of 1 N sodium hydroxide and chilled in an ice bath. The benzene solution of acid chloride was added dropwise over a period of 10 min with rapid stirring to the chilled basic amino acid solution. Following the addition of the acid chloride, the ice bath was removed and the stirring was continued for 2 h. The reaction mixture was extracted with ethyl ether, and the aqueous phase was separated and acidified with hydrochloric acid (pH 2). In most cases the crude products precipitated immediately after acidification. With 2,4,5-T-Ser, 2,4,5-T-Pro, 2,4,5-T-Asp, and 2,4,5-T-Glu, overnight refrigeration was necessary for precipitation. 2,4,5-T-Pro was recovered as a white oil which did not crystallize. The L-amino acid conjugates that were synthesized were: 2,4,5-T-Gly, 2,4,5-T-Ala, 2,4,5-T-Ser, 2,4,5-T-Pro, 2,4,5-T-Val, 2,4,5-T-Thr, 2,4,5-T-Leu, 2,4,5-T-Ile, 2,4,5-T-Asp, 2,4,5-T-Met, 2,4,5-T-Glu, 2,4,5-T-Phe, 2,4,5-T-Tyr, and 2,4,5-T-Trp. In the synthesis of the glutamic and aspartic acid conjugates of 2,4,5-T, the acid chloride was dissolved in dioxane rather than benzene. All conjugates were purified by crystallization from 30% methanol by TLC or by preparative

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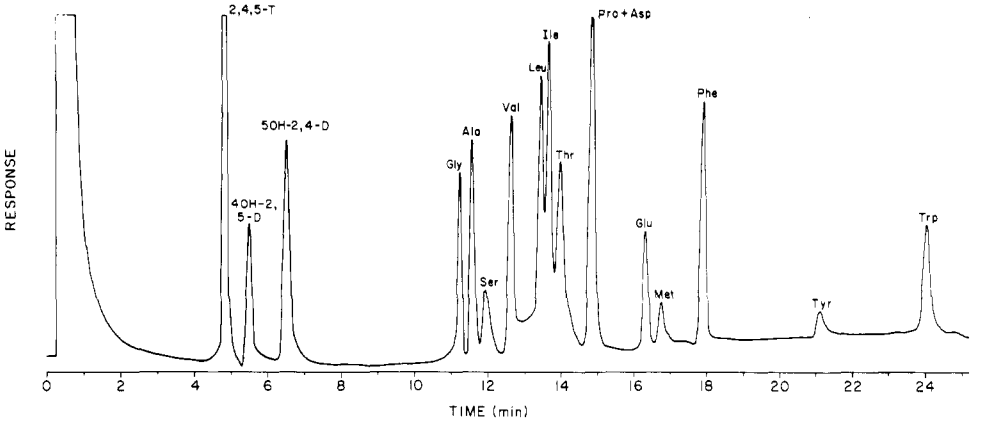

Figure 1. Gas–liquid chromatogram of methyl esters of 2,4,5-T, 4OH-2,5-D, 5OH-2,4-D, and of the amino acid conjugates. Column: 2% OV-1 on 100–120 mesh Supelcoport, 6 ft × 4 mm i.d. glass; temperature programmed at the rate of 5 °C/min up to 280 °C, initial temperature 160 °C; flow rate 60 mL/min; each peak represents ca. 2 μg.

high-pressure liquid chromatography (LC).

The Ala, Gly, Ser, Thr, Asp, and Glu conjugates were partially purified by TLC using solvent system I. The desired band was scratched from the plates and eluted with ethyl acetate. The eluate was then concentrated by means of a rotary evaporator and compounds were recovered as white, solid crystals. 2,4,5-T-Asp and 2,4,5-T-Glu were recovered from the ethyl acetate as oily products which were than crystallized out of 25% methanol. Melting point and GLC analyses were used to measure the purity of the compounds. The main contaminant of the synthetic procedure was unreacted 2,4,5-T which was not detected after purification. The methionine and phenylalanine conjugates required additional purification by LC. This technique offers an alternative method of purification for some of the conjugates. Methanol was used as a solvent for LC and the collected solvent containing the conjugate peak was evaporated, producing pure crystalline conjugate.

Preparation of Methyl Ester Derivatives. Diazomethane was prepared from Diazald (Burke and Gaul, 1968). Stock solutions of each of the compounds were prepared (1 mg/mL) in methanol. One milliliter of each stock solution was added to a 30-mL methylation vial and the methanol was removed under nitrogen at 60 °C. The residue was dissolved in 5 mL of diethyl ether or in diethyl ether–methanol (9:1) and an excess of freshly prepared diazomethane was added. After 1 h the solvent was evaporated under nitrogen and the methylated products were dissolved in 5 mL of dioxane (0.2 μg/μL) of each compound) prior to analysis.

Chromatography. TLC was employed using Supelcosil 12A (Supelco, Inc., Bellefonte, Pa.) as the adsorbent and a zinc phosphor for detection. Six thin-layer solvent systems were used as indicated in Table I.

A Microtek Model 220 gas–liquid chromatograph equipped with a dual-flame ionization detector was used for GLC. The GLC columns consisted of a 6 ft by 4 mm i.d. glass column packed with 2% OV-1 on 100/120 mesh Supelcoport. Nitrogen was used as the carrier gas (50 mL/min) and was predried by passage through a filter dryer. An inlet temperature of 240 °C and a detector temperature of 250 °C was employed. The column was programmed at a rate of 5 °C/min from 160–280 °C.

A Model ALC-GPC 244 high-pressure liquid chromatograph equipped with a 6000A pump, U6K injector, 440 UV detector, and a 660 solvent programmer (Waters Associates, Milford, Mass.) was used. A 61 cm × 17 mm i.d. preparative μBondapak C_{18} column was employed for the purification of selected conjugates (Arjmand et al., 1978).

Table I. R_f Values of 2,4,5-T and Amino Acid Conjugates of 2,4,5-T Obtained by TLC

2,4,5-T or conjugate	Mp, °C	R_f values[a]					
		I	II	III	IV	V	VI
2,4,5-T	152–153	0.70	0.69	0.48	0.19	0.29	0.69
Gly	190–191	0.39	0.68	0.29	0.18	0.23	0.67
Ala	192–195	0.48	0.71	0.33	0.08	0.23	0.71
Ser	138–141	0.19	0.68	0.13	0.21	0.17	0.53
Pro	<90	0.27	0.71	0.29	0.21	0.23	0.59
Val	160–161	0.63	0.75	0.36	0.27	0.26	0.71
Thr	174–175	0.27	0.70	0.17	0.19	0.17	0.53
Leu	165–167	0.60	0.75	0.35	0.21	0.25	0.69
Ile	138–140	0.65	0.77	0.37	0.09	0.27	0.70
Asp	80–81	0.21	0.68	0.19	0.21	0.17	0.53
Met	135–136	0.53	0.74	0.34	0.21	0.24	0.67
Glu	79–80	0.19	0.70	0.17	0.21	0.15	0.51
Phe	186–187	0.55	0.74	0.33	0.12	0.27	0.66
Trp	156–157	0.40	0.72	0.25	0.20	0.23	0.57

[a] I, diethyl ether-petroleum ether (38–46 °C)-formic acid (70:30:3, v/v/v); II, chloroform-methanol-acetic acid (70:20:5, v/v/v); III, benzene-dioxane-formic acid (90:25:2, v/v/v); IV, benzene-triethylamine-methanol-concentrated ammonium hydroxide (85:15:20:2, v/v/v); V, benzene-methanol-cyclohexane-formic acid (80:10:20:2, v/v/v); VI, benzene-dioxane-formic acid (90:75:3, v/v/v).

Mass Spectra. Mass spectra were obtained on a LKB-9000 gas–liquid chromatograph (70 eV) interfaced mass spectrometer using a 6 ft × $^3/_{16}$ in. o.d. column packed with 2% OV-1 on Supelcoport 100/120. Helium was used as carrier gas, and the column temperature was varied between 160–270 °C.

RESULTS AND DISCUSSION

Table I shows the melting points and the TLC properties of 2,4,5-T and 13 amino acid conjugates of 2,4,5-T. Solvent system I gave the best separation, and this solvent system was routinely used for purification of the conjugates. The Glu, Asp, and Ser conjugates could not be resolved on TLC with these solvents.

A gas chromatographic separation on a 2% OV-1 column is presented in Figure 1 of the methyl esters of 14 amino acid conjugates of 2,4,5-T, two hydroxylated derivatives, and 2,4,5-T. Only two conjugates (2,4,5-T-Pro and 2,-4,5-T-Asp) overlapped. All the compounds were eluted within 25 min employing temperature programming conditions (160–280 °C). It was necessary to use a 90% diethyl ether–10% methanol solvent for methylation (CH_2N_2) to obtain complete methylation of the phenolic and carboxyl groups of 4OH-2,5-D, 5OH-2,4-D, and of the tyrosine conjugate. It was difficult to completely methylate

Table II. Mass Spectra of Methyl Esters of 2,4,5-T, 4OH-2,5-D, and of Amino Acid Conjugates of 2,4,5-T

m/e (relative intensity)[a]						
2,4,5-T	264 (40)P	209 (27)	134 (10)	114 (13)	71 (11)	218 (21)
59 (22)	266 (30)	211 (25)	143 (24)	128 (36)	72 (39)	220 (11)
73 (48)	Gly	280 (75)	144 (14)	142 (10)	73 (39)	308 (43)
74 (16)	56 (21)	282 (75)	145 (28)	170 (13)	74 (13)	310 (39)
97 (17)	72 (14)	284 (21)	146 (23)	179 (10)	75 (13)	312 (14)
109 (18)	73 (10)	303 (100)B	147 (16)	181 (11)	77 (10)	332 (43)
143 (14)	74 (13)	304 (20)	148 (11)	209 (15)	83 (84)	334 (29)
144 (11)	88 (43)	305 (68)	167 (12)	211 (13)	84 (38)	355 (5)P
145 (25)	97 (16)	306 (13)	169 (10)	279 (17)	85 (10)	Thr
146 (21)	102 (37)	307 (13)	179 (32)	281 (14)	88 (24)	56 (15)
147 (14)	109 (10)	308 (17)	181 (34)	306 (40)	96 (11)	59 (10)
148 (10)	110 (11)	339 (10)P	183 (14)	308 (38)	97 (47)	62 (10)
167 (20)	130 (48)	Ser	196 (49)	310 (13)	98 (26)	72 (16)
169 (19)	145 (12)	54 (115)	198 (48)	330 (100)B	99 (21)	73 (11)
179 (26)	179 (14)	55 (23)	200 (17)	331 (18)	109 (10)	74 (17)
181 (31)	181 (14)	56 (17)	207 (15)	332 (68)	111 (13)	88 (45)
183 (14)	196 (17)	58 (12)	209 (28)	333 (10)	113 (21)	97 (25)
195 (17)	198 (17)	59 (28)	211 (14)	334 (21)	114 (11)	99 (10)
197 (17)	209 (11)	61 (10)	242 (12)	265 (0.6)P	115 (11)	102 (39)
209 (57)	211 (10)	62 (15)	302 (100)B	Val	130 (23)	109 (12)
211 (52)	290 (100)B	68 (31)	303 (17)	50 (11)	132 (27)	130 (42)
213 (18)	291 (15)	73 (24)	304 (67)	51 (10)	133 (16)	132 (10)
233 (100)B	292 (65)	74 (18)	305 (11)	52 (10)	134 (19)	145 (10)
234 (12)	294 (14)	82 (71)	306 (19)	53 (16)	135 (31)	179 (11)
235 (66)	325 (8)P	83 (10)	337 (32)	54 (14)	139 (21)	181 (14)
237 (13)	Ala	86 (31)	339 (32)	55 (46)	145 (10)	196 (37)
268 (55)P	56 (47)	96 (35)	341 (12)	56 (19)	147 (10)	198 (36)
270 (53)	57 (15)	97 (34)	355 (0)P	57 (11)	162 (10)	200 (12)
272 (21)	59 (14)	99 (14)	Pro	58 (10)	172 (10)	207 (10)
4OH-2,5-D	70 (16)	100 (28)	55 (29)	59 (24)	179 (14)	209 (18)
53 (14)	86 (12)	101 (18)	58 (18)	60 (10)	181 (13)	211 (14)
73 (22)	102 (32)	109 (22)	59 (12)	61 (14)	196 (100)B	267 (10)
97 (13)	115 (14)	110 (20)	68 (18)	62 (20)	197 (24)	290 (100)B
113 (13)	144 (27)	111 (14)	69 (318)	63 (14)	198 (96)	291 (16)
141 (23)	145 (12)	114 (98)	70 (111)	67 (10)	200 (31)	292 (67)
191 (100)B	146 (10)	128 (20)	83 (75)	68 (14)	208 (11)	293 (10)
193 (69)	179 (15)	132 (15)	84 (14)	69 (13)	209 (22)	294 (13)
195 (12)	181 (16)	133 (10)	88 (23)	70 (30)	211 (14)	369 (0)P
Leu	253 (15)	325 (32)	169 (10)	74 (17)	200 (51)	268 (10)
55 (18)	254 (15)	326 (28)	179 (13)	75 (12)	207 (38)	270 (12)
56 (18)	255 (16)	346 (95)	181 (13)	82 (20)	209 (29)	411 (0)P
57 (36)	256 (10)	347 (24)	196 (40)	83 (15)	211 (23)	Phe
58 (11)	322 (55)	348 (63)	197 (10)	88 (15)	213 (10)	63 (12)
59 (26)	324 (53)	349 (13)	198 (37)	96 (47)	256 (12)	65 (16)
68 (10)	325 (12)	350 (14)	200 (13)	97 (59)	281 (16)	73 (19)
69 (100)B	326 (18)	381 (6)P	202 (20)	98 (18)	284 (16)	77 (14)
70 (37)	346 (53)	Asp	209 (27)	99 (28)	292 (12)	88 (16)
73 (12)	347 (10)	59 (23)	211 (23)	100 (14)	294 (11)	89 (16)
84 (28)	348 (34)	60 (10)	302 (10)	109 (19)	316 (22)	90 (16)
86 (41)	381 (3)P	70 (13)	306 (10)	110 (26)	318 (16)	91 (100)B
88 (71)	Ile	72 (13)	338 (20)	111 (12)	319 (21)	92 (14)
96 (14)	55 (10)	74 (17)	340 (20)	115 (19)	320 (11)	97 (16)
97 (27)	56 (15)	75 (10)	362 (100)B	124 (100)B	321 (18)	116 (12)
98 (35)	69 (16)	84 (13)	363 (17)	125 (14)	350 (31)	117 (74)
99 (10)	84 (14)	85 (10)	364 (67)	128 (33)	352 (30)	118 (12)
101 (18)	86 (23)	86 (33)	365 (13)	132 (31)	354 (16)	120 (12)
109 (11)	88 (22)	96 (10)	366 (13)	133 (23)	400 (0)P	131 (44)
112 (10)	97 (14)	97 (23)	397 (3)P	134 (22)	Glu	132 (28)
128 (41)	130 (10)	99 (10)	Met	135 (18)	55 (10)	133 (14)
129 (13)	144 (10)	102 (33)	50 (10)	142 (11)	56 (12)	135 (12)
130 (33)	179 (15)	109 (10)	53 (16)	143 (13)	59 (16)	146 (12)
144 (18)	181 (16)	113 (40)	54 (73)	144 (12)	68 (14)	161 (19)
145 (19)	186 (13)	114 (47)	55 (42)	145 (26)	73 (10)	162 (100)B
146 (17)	196 (21)	115 (13)	56 (26)	146 (21)	84 (55)	163 (19)
147 (12)	198 (20)	128 (30)	57 (12)	147 (18)	97 (12)	179 (14)
179 (20)	209 (24)	132 (10)	58 (18)	156 (52)	128 (10)	181 (14)
181 (23)	211 (23)	133 (10)	59 (24)	162 (20)	179 (10)	187 (28)
183 (10)	218 (12)	135 (13)	60 (10)	167 (13)	181 (12)	196 (42)
186 (15)	254 (11)	142 (10)	61 (18)	169 (14)	184 (100)B	198 (40)
196 (44)	256 (10)	143 (10)	62 (24)	179 (27)	185 (10)	200 (14)
198 (42)	266 (20)	145 (17)	63 (14)	181 (31)	196 (12)	207 (47)
200 (14)	268 (20)	146 (13)	67 (24)	183 (14)	198 (12)	208 (12)
209 (29)	321 (100)B	147 (10)	68 (>100)	196 (>100)	209 (15)	209 (28)
211 (27)	322 (14)	149 (10)	69 (20)	197 (18)	211 (16)	211 (23)
218 (34)	323 (93)	156 (10)	72 (10)	198 (>100)	233 (16)	217 (12)
220 (23)	324 (37)	160 (20)	73 (46)	199 (13)	235 (10)	218 (65)

Table II (*Continued*)

m/e (relative intensity)[a]						
219 (14)	356 (16)	63 (12)	102 (13)	129 (24)	134 (13)	198 (58)
220 (42)	358 (12)	77 (21)	103 (13)	130 (440)	135 (11)	200 (25)
254 (60)	415 (5)P	97 (26)	115 (10)	131 (45)	155 (14)	201 (100)B
256 (56)	Trp	99 (13)	117 (11)	132 (16)	156 (26)	202 (16)
259 (19)	62 (13)	101 (10)	128 (10)	133 (24)	170 (13)	226 (13)
					196 (64)	454 (8)P

[a] Ions greater than *m/e* 50 and 10% relative intensity are presented. The relative intensity of all parent ions are also given. Some of the above compounds possessed very intense ions which were not used as the base ion for calculation of the relative intensities.

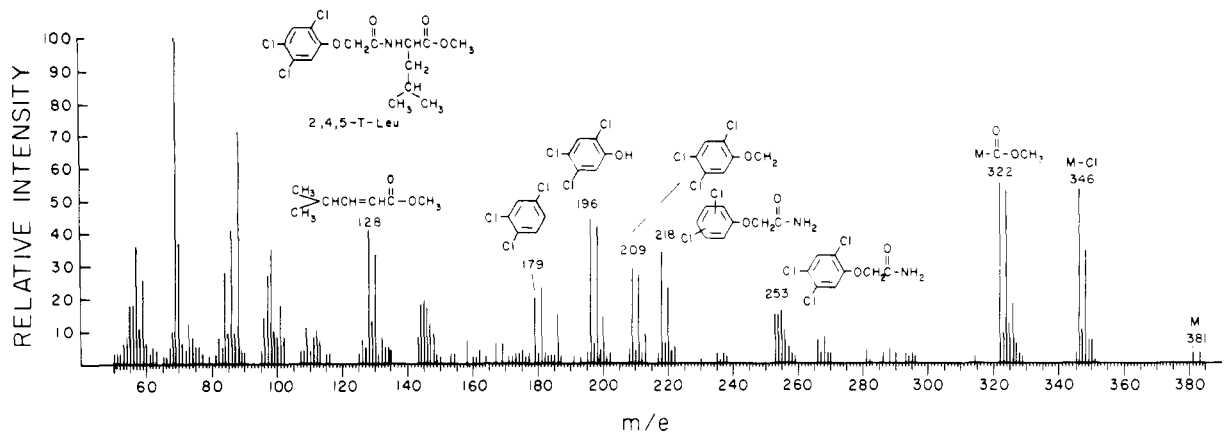

Figure 2. The mass spectra of the methyl ester of 2,4,5-T-Leu.

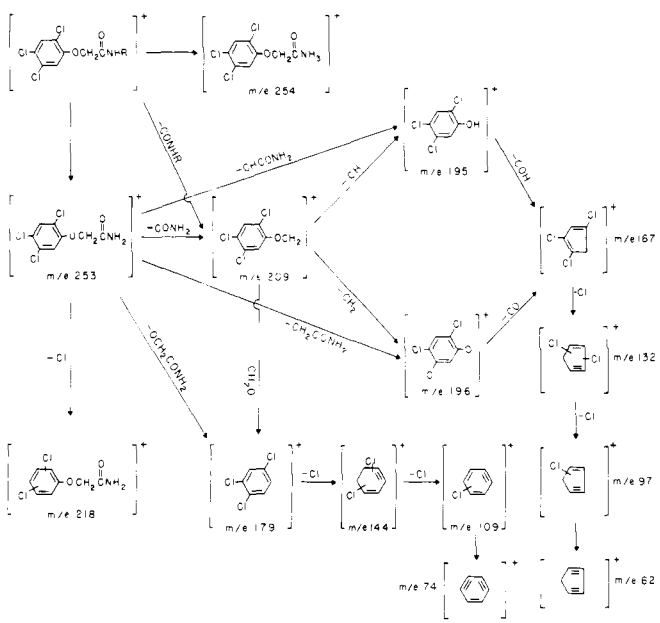

Figure 3. Interpretation of mass spectral fragmentation pattern of the 2,4,5-T portion of the molecule (<260) of the methyl esters of the amino acid conjugates.

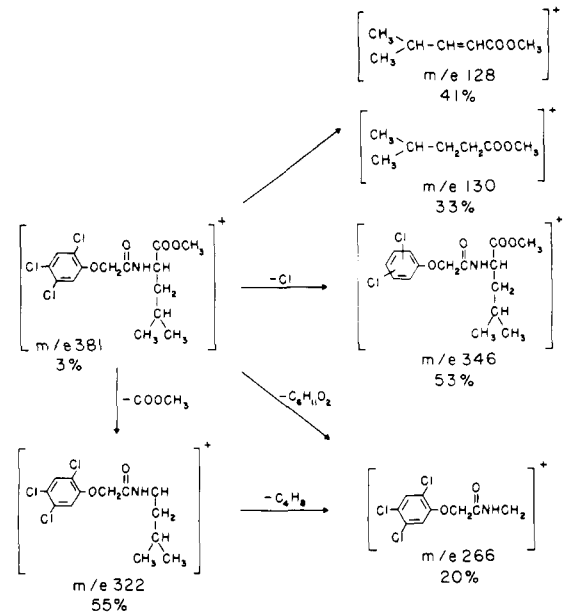

Figure 4. Interpretation of the mass spectral fragmentation pattern of the methyl ester of 2,4,5-T-Leu typical of the amino acid portion of the molecule.

5OH-2,4-D. Hydroxylated 2,4-D derivatives were included because 4OH-2,5-D was identified as a 2,4,5-T metabolite in bean plants (Hamilton et al., 1971).

The mass spectra of the methyl esters of 2,4,5-T, 4OH-2,5-D, and of the amino acid conjugates are presented in Table II. Twelve of the compounds exhibited molecular ions (P). All of the conjugates possess characteristic fragmentation patterns. The mass spectra of 2,4,5-T-Leu is presented in Figure 2 as an example. Prominent ions arising from only the 2,4,5-T portions of the molecule are the following: *m/e* 253, 218, 209, 196, 179, 144, 97, and 74 and an explanation of the origin of these common ions is shown in Figure 3. The upper region of the spectra is characteristic of the specific conjugate and particularly useful for identification. These main fragments can be grouped into three types as follows: (a) parent – Cl (P – 35); (b) P – $COOCH_3$ (P – 59); and (c) P – $RCOOCH_3$. Figure 4 presents an interpretation of the mass spectral fragmentation pattern of the methyl ester of 2,4,5-T-Leu which is characteristic of the amino acid portion of the molecule. A small molecular ion is present (*m/e* 381, P, 3%) which readily loses chlorine (*m/e* 346, 53%), or a $COOCH_3$ group (*m/e* 322, 55%) or $COOCH_3$ plus an amino acid side chain (*m/e* 253, 15%). The amino acid conjugates of 2,4,5-T show similar fragmentation patterns to those exhibited by the amino acid conjugates of 2,4-D (Feung et al., 1973a).

The synthesis and characterization of the amino acid

conjugates of 2,4,5-T and their methyl esters will assist pesticide chemists in their efforts to further elucidate the plant metabolism of 2,4,5-T and permit the evaluation of the biological activity of the amino acid conjugates. In additional work from this laboratory we will report identification of 2,4,5-T amino acid conjugates in plant tissue cultures.

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LITERATURE CITED

Andreae, W. A., Good, N. E., *Plant Physiol.* **30**, 380 (1955).
Andreae, W. A., Good, N. E., *Plant Physiol.* **32**, 556 (1957).
Arjmand, M., Hamilton, R. H., Mumma, R. O., *J. Agric. Food Chem.*, submitted for publication (1978).
Burke, J. A., Gaul, J. A., Ed., "Pesticide Analytical Manual", Vol. 1, U.S. Department of Health, Education, and Welfare, 1968, Sec. 221, 12C.
Feung, C. S., Hamilton, R. H., Witham, F. H., *J. Agric. Food Chem.* **19**, 475 (1971).
Feung, C. S., Hamilton, R. H., Witham, F. H., Mumma, R. O., *Plant Physiol.* **50**, 80 (1972).
Feung, C. S., Hamilton, R. H., Mumma, R. O., *J. Agric. Food Chem.* **21**, 632 (1973a).
Feung, C. S., Hamilton, R. H., Mumma, R. O., *J. Agric. Food Chem.* **21**, 637 (1973b).
Feung, C. S., Mumma, R. O., Hamilton, R. H., *J. Agric. Food Chem.* **23**, 373 (1975).
Feung, C. S., Hamilton, R. H., Mumma, R. O., *Plant Physiol.* **58**, 666 (1976).
Good, N. E., *Can. J. Chem.* **34**, 1356 (1956).
Good, N. E., Andreae, W. A., *Plant Physiol.* **31**, 231 (1956).
Hamilton, R. H., Hurter, J., Hall, J. K., Ercegovich, C. D., *J. Agric. Food Chem.* **19**, 480 (1971).
Hill, C. M., Towns, M. B., Senter, G., *J. Am. Chem. Soc.* **71**, 257 (1949).
Hutzinger, O., Kosuge, T., *Biochemistry* **7**, 601 (1968).
Klämbt, H. D., *Planta* **57**, 339 (1961).
Row, U. U., Sanford, W. W., Hitchcock, A. W., *Contrib. Boyce Thomps. Inst.* **21**, 1 (1961).
Tillberg, E., *Physiol. Plant* **31**, 271 (1974).
Wood, J. W., Fontaine, T. D., *J. Org. Chem.* **17**, 891 (1952).

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Metabolism of Oxamyl and Selected Metabolites in the Rat

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The metabolism of oxamyl and of its principal plant metabolites, methyl *N'*,*N'*-dimethyl-*N*-[1-glucosyl)oxyl]-1-thiooxamimidate (metabolite A) and *N*,*N*-dimethyl-1-cyanoformamide (DMCF), was investigated by incubation with rat liver microsomes and by oral administration to preconditioned rats. Oxamyl was degraded by two major pathways: hydrolysis to the oximino compound (I), or enzymatic conversion via DMCF to *N*,*N*-dimethyloxamic acid (III). Conjugates of I, III, and the monomethyl derivatives II, IV in urine and feces were the major (>70%) elimination products. No oxamyl or other organosoluble metabolites were detected in urine, feces, or tissues. The incorporation of carbon-14 into normal amino acids accounted for most (>50%) of the radioactivity retained in tissues. Metabolite A was somewhat resistant to degradation, 30% of the dose being eliminated unchanged while the remaining ^{14}C was converted to the same conjugates obtained from oxamyl. DMCF was degraded and eliminated mainly as conjugates of III and IV.

This paper is the third in a series to describe the metabolism and biodegradation of oxamyl, which is the active material in Du Pont's Vydate Insecticide/Nematicide. The first paper (Harvey et al., 1978) showed that oxamyl in plants hydrolyzes first to the corresponding oximino compound (I) which in turn is conjugated with glucose to form methyl *N'*,*N'*-dimethyl-*N*-[(1-glucosyl)oxy]-1-thiooxamimidate, designated metabolite A. Additional glucose units may be added to the original glucose of the conjugate, and partial demethylation of the dimethylcarbamoyl group may also occur. In some fruits, metabolism to *N*,*N*-dimethyl-1-cyanoformamide (DMCF) was shown to occur. In soils (Harvey and Han, 1978) hydrolysis to the oximino compound followed by extensive conversion of the carbon-14 to $^{14}CO_2$ was reported. The present paper describes the fate and interrelationships of oxamyl and its principal metabolites in the rat.

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EXPERIMENTAL SECTION

Radiolabeled Materials. *[^{14}C]Oxamyl.* The synthesis of [^{14}C]oxamyl was described earlier (Harvey et al., 1978). *[^{14}C]Oximino compound* (I) is an intermediate in the synthesis of [^{14}C]oxamyl, or may be obtained readily by mild alkaline hydrolysis of [^{14}C]oxamyl.

^{14}C Metabolite A. The thallium salt of I was formed by addition of 68 mg of thallous ethoxide to 2 mL of benzene solution containing 44 mg of [^{14}C]oximino compound (I). After stirring for 30 min, 113 mg of freshly recrystallized tetraacetyl-α-D-bromoglucose (Sigma Chemical) was added, and the mixture was stirred overnight. After filtration, the filtrate was evaporated under a slow stream of nitrogen to give the acetylated glucose derivative as an oil. Hydrolysis of the acetyl groups was accomplished by addition

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