Nathan, D. G., Davidson, J. D., Waggoner, J. G., Berlin, N. I., *J. Lab. Clin. Med.* **52**, 915 (1958).
Pfeifer, W. K., unpublished reports of the U.S. Bureau of Sport Fisheries and Wildlife, Division of Wildlife Services, Bismarck, N.D., 1968–1972.
Starr, R. I., Cunningham, D. J., *J. Agric. Food Chem.* **22**, 409 (1974).
Starr, R. I., Cunningham, D. J., *J. Agric. Food Chem.* **23**, 279 (1975a).
Starr, R. I., Cunningham, D. J., *Arch. Environ. Contam. Toxicol.* **3**, 72 (1975b).
Stickley, A. R., Mitchell, R. T., Heath, R. G., Ingram, C. R., Bradley, E. L., *J. Wildl. Manage.* **36**, 1313 (1972).

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Metabolism of 2,4-Dichlorophenoxyacetic Acid. 8. Gas–Liquid Chromatography of Trimethylsilyl Derivatives of Amino Acid Conjugates

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A gas–liquid chromatographic technique was developed for the analysis of 19 metabolites of 2,4-D or potential metabolites, with particular emphasis on the amino acid conjugates of 2,4-D. This technique involves trimethylsilyl derivatization of the metabolites by hexamethyldisilazane, separation by GLC with OV-1 and OV-17 stationary phases, and quantification with a flame ionization detector. The structures of the Me_3Si derivatives were confirmed by mass spectrometry. Preliminary studies on the practical application of this technique for the analysis of plant extracts were conducted.

2,4-Dichlorophenoxyacetic acid (2,4-D) is one of the most widely used herbicides in agricultural production. Methods currently being used for the analysis of 2,4-D residues are on the basis of the conversion of the extracted materials to methyl or butyl esters, separation on gas–liquid chromatography, and detection by electron capture or microcoulometric detectors (Pesticide Analytical Manual, 1969; Schultz, 1973; Yip, 1962, 1971; Yip and Ney, 1966). Residue analysts usually only consider the quantification of free 2,4-D and possibly some hydroxylated metabolites; however, 2,4-D is metabolized by plants to a number of metabolites including the biologically active amino acid conjugates (Feung et al., 1971, 1972, 1973a,b, 1974, 1975). Information on the amount and identification of these metabolites in the natural ecosystems is necessary from both toxicological and ecological standpoints. The overall objective of this study was to develop an analytical method which utilizes GLC for the analysis of 2,4-D metabolites, with special emphasis on the biologically active amino acid conjugates of 2,4-D. Such a technique would provide a means to quantify the metabolites of 2,4-D as well as aid in the identification of structurally unknown metabolites.

EXPERIMENTAL PROCEDURE

Reagents and Materials. All solvents used were of highest purity. 2,4-D was purchased from Aldrich Chemical Co., Inc. *n*-Octacosane and all silylation reagents were purchased from Supelco, Inc.: bis(trimethylsilyl)-trifluoroacetamide (BSTFA), Sylon HTP (a 3:1:9 mixture of hexamethyldisilasane–trimethylchlorosilane–pyridine), Sylon-BTZ (a 3:2:3 mixture of *N,O*-bis(trimethylsilyl)-acetamide–trimethylchlorosilane–trimethylsilylimidazole), hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS).

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4-Hydroxy-2-chlorophenoxyacetic acid (4-OH-2-Cl) was obtained from Dr. J. Fleeker, North Dakota State University, while 4-hydroxy-2,3-dichlorophenoxyacetic acid (4-OH-2,3-D) and 4-hydroxy-2,5-dichlorophenoxyacetic acid (4-OH-2,5-D) were previously synthesized (Hamilton et al., 1971). All amino acid conjugates of 2,4-D were supplied by Dr. C. S. Feung, Pesticide Research Laboratory, The Pennsylvania State University. The amino acid conjugates of 2,4-D were: 2,4-dichlorophenoxyacetylalanine (2,4-D-Ala); 2,4-dichlorophenoxyacetylarginine (2,4-D-Arg), 2,4-dichlorophenoxyacetylaspartic acid (2,4-D-Asp), 2,4-dichlorophenoxyacetylcysteine (2,4-D-Cys), 2,4-dichlorophenoxyacetylglutamic acid (2,4-D-Glu), 2,4-dichlorophenoxyacetylglycine (2,4-D-Gly), 2,4-dichlorophenoxyacetylhistidine (2,4-D-His), 2,4-dichlorophenoxyacetylhydroxyproline (2,4-D-Hyp), 2,4-dichlorophenoxyacetylisoleucine (2,4-D-Ile), 2,4-dichlorophenoxyacetylleucine (2,4-D-Leu), 2,4-dichlorophenoxyacetyllysine (2,4-D-Lys), 2,4-dichlorophenoxyacetylmethionine (2,4-D-Met), 2,4-dichlorophenoxyacetylproline (2,4-D-Pro), 2,4-dichlorophenoxyacetylphenylalanine (2,4-D-Phe) 2,4-dichlorophenoxyacetylserine (2,4-D-Ser), 2,4-dichlorophenoxyacetylthreonine (2,4-D-Thr), 2,4-dichlorophenoxyacetyltryptophan (2,4-D-Trp), 2,4-dichlorophenoxyacetyltyrosine (2,4-D-Tyr), and 2,4-dichlorophenoxyacetylvaline (2,4-D-Val).

Instruments. A MicroTek 220 gas chromatograph equipped with an Infotronics Model II digital integrator was used for this investigation. Silylanized 6 ft × 4 mm i.d. glass columns were employed with various column packings; 1, 2, and 3% OV-1 and 10% OV-7 on 100/120 mesh Supelcoport, and 1% OV-17 on 80/100 mesh Supelcoport. Dual flame ionization detectors were used. The instrument conditions were as follows: column temperature varied from 150 to 280 °C; detector, 280 °C; inlet, 245 °C; and flow of 50–60 ml of N_2/min.

Mass spectra were obtained on a LKB-9000 gas–liquid chromatograph interfaced mass spectrometer using a 6 ft

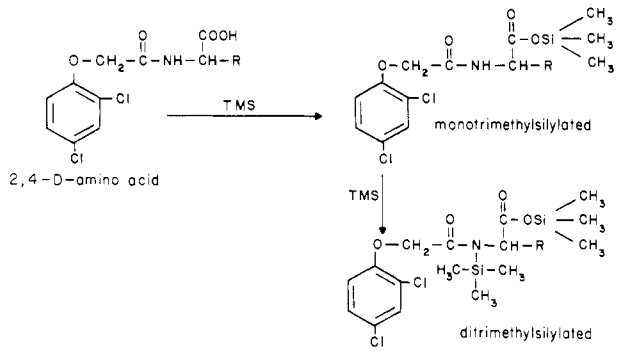

Figure 1. Trimethylsilylation of amino acid conjugates of 2,4-D.

× $^{3}/_{16}$ in. o.d. glass column packed with 2% OV-1 on Supelcoport 100/120, a He flow rate of 30 ml/min, and a variable column oven temperature.

Preparation of Trimethylsilyl Derivatives. Aliquots containing 1 mg of each compound in ethanol were added to a reaction tube (10-ml glass screw cap vial). The ethanol was evaporated by placing the tube in a sand bath at 70 °C and passing a regulated stream of filtered N_2 into the tube. Methylene chloride (2 ml) was then added and evaporated to dryness to ensure the complete azeotropic removal of water. *n*-Octacosane (1 mg) in acetonitrile was placed into the reaction vial and sufficient additional acetonitrile was added to the vial such that there was 0.5 ml of acetonitrile for each milligram of total compound. Then, 0.5 ml of HMDS or TMCS or BSTFA or Sylon-BTZ was added for each milligram of total compound. The vial was closed securely with a Teflon-lined screw cap and placed in an oven for various times and temperatures as indicated in the Results section. When compounds were silylated with Sylon-HTP, no acetonitrile was used as solvent. In all cases for individual compounds a final concentration of 0.5 mg/ml was achieved prior to GLC analysis.

To determine the optimum conditions for silylation, the time and temperature of derivatization were varied for each of the metabolites. Reaction times of 15 min to 4 h and temperatures of 60, 100, and 150 °C were investigated. The stability of the trimethylsilyl derivatives was also determined. Samples of each compound were prepared together with internal standard and were analyzed in duplicate by gas chromatography 0 min, 6 h, 1 day, 4 days, and 8 days after derivatization. The derivatives were quantified relative to an internal standard (Gehrke et al., 1968).

Response. Six milliliters of stock solution containing 1 mg/ml of either 2,4-D, its hydroxylated derivatives, or its amino acid conjugates was placed in a silylation reaction tube and derivatized according to the procedure mentioned previously. One milligram of internal standard was also separately subjected to derivatization conditions. To each of ten small vials was added 0.1 ml of derivatized internal standard and from 0.1 to 1 ml of the derivatized compound, increasing in increments of 0.1 ml, so that in the tenth vial the relative concentration of the compound to internal standard was 10/1. Relative calibration curves in the range of 1 to 10 μg were prepared by injection of 2 μl from the first vial, 3 μl from the second, . . . and 11 μl from the tenth vial. Each injection was repeated two times and the average relative response was calculated.

Practical Application. In order to evaluate the practical application of this method, 100 g of soybean callus tissue was fortified with 3 mg of each compound (2,4-D, 4-OH-2-Cl-P, and 11 amino acid conjugates of 2,4-D: Ala, Gly, Val, Leu, Ile, Ser, Asp, Met, Glu, Phe, and Tyr) and the tissue was extracted according to the procedure used

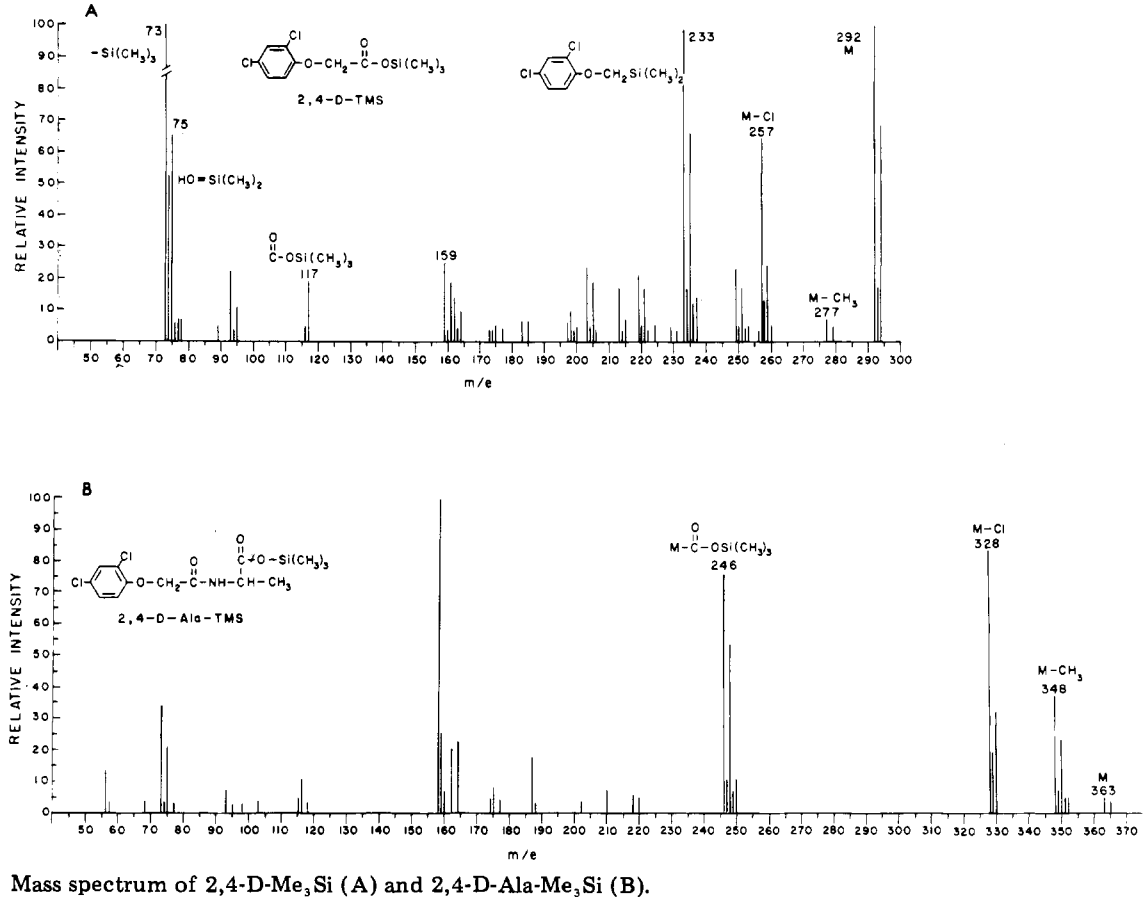

Figure 2. Mass spectrum of 2,4-D-Me_3Si (A) and 2,4-D-Ala-Me_3Si (B).

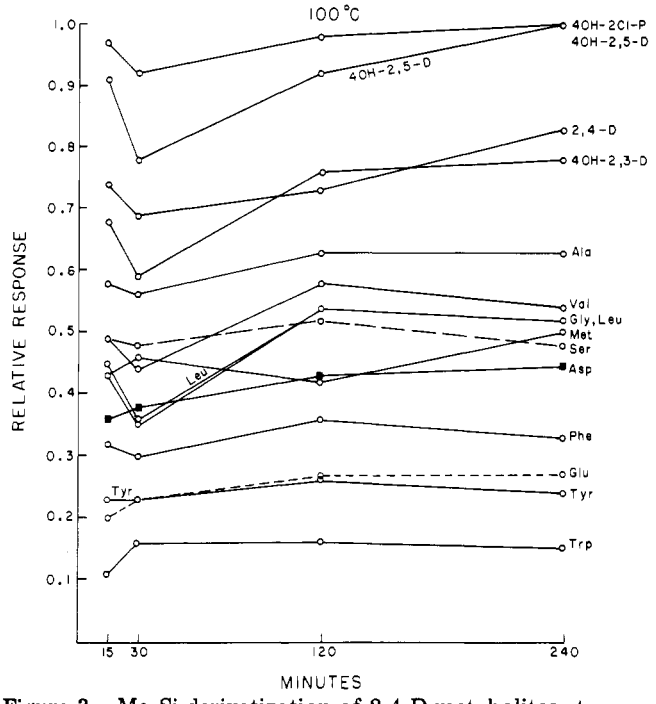

Figure 3. Me_3Si derivatization of 2,4-D-metabolites at 100 °C.

by Feung et al. (1971). The ether fraction was evaporated to dryness and the residue was redissolved in 95% ethanol, transferred to a small vial (20 ml), and evaporated to dryness under nitrogen at 70 °C. The internal standard (0.7 mg) dissolved in acetonitrile was then added to the sample. The sample was derivatized and was brought to a final volume of 2.8 ml. From this solution, 9 μl was injected for quantitative determination. A calibration mixture containing 0.5 mg of each compound plus 0.5 mg of internal standard was also prepared and derivatized.

RESULTS AND DISCUSSION

Preparation of Me_3Si Derivatives. When the amino acid conjugates of 2,4-D were derivatized with the strong silylating agent BSTFA, typically used with amino acids (Gehrke and Leimer, 1971), two distinct gas chromatographic peaks resulted. The presence of the second GLC peak is due to incomplete silylation of the molecule at a second site as proposed in Figure 1. The bis(trimethylsilyl) derivative is more volatile than the mono(trimethylsilylated) product, and elutes earlier on GLC. These results were confirmed by mass spectrometry.

Various parameters of derivatization of the amino acid conjugates of 2,4-D with BSTFA were investigated to determine if reaction conditions could be found that would form only the mono(trimethylsilylated) or bis(trimethylsilylated) product. Neither the mildest conditions (5 min at 0 °C) nor the most extreme reaction conditions (6 h at 150 °C) were adequate to form only one product. For example, the disilylated product predominated when derivatization was performed at 150 °C while the monosilylated product predominated at 0 °C. Multiple products were also formed under all conditions investigated when Sylon-BTZ and Sylon-HTP were used for derivatization. In addition, Sylon-BTZ gave rise to extra impurity peaks on GLC which interfered with the analyses. Since the most active silylation reagents gave incomplete derivatization, milder silylating agents such as hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS) were examined. Derivatization with HMDS or TMCS formed only one product, the monosilylated derivative; thus, derivatization with HMDS was used exclusively for subsequent experiments.

Confirmation of the Structure of the Me_3Si Derivatives by Mass Spectrometry. The mass spectra of trimethylsilylated 2,4-D and 2,4-D-Ala are presented in Figure 2 and are typical of the mass spectra of all silylated derivatives. The mass spectrum of 2,4-D-Me_3Si showed an intense molecular ion at m/e 292 (M) and characteristic fragments at m/e 277 (M – CH_3), 257 (M – Cl), 233 (M – CH_3 – CO_2), and other ions typical of trimethylsilylated products 117, 93, 75, and 73. The presence of the two chlorine atoms in 2,4-D-Me_3Si aids in the interpretation of the spectrum since the fragments possessing chlorine atoms give characteristic isotope patterns. The mass spectra of 2,4-D-Ala-Me_3Si also contain fragments typical of the amino acid conjugate (Figure 2B). The molecular ion of low intensity is observed at m/e 363 (M) and other prominent characteristic ions at m/e 348 (M – CH_3, M –

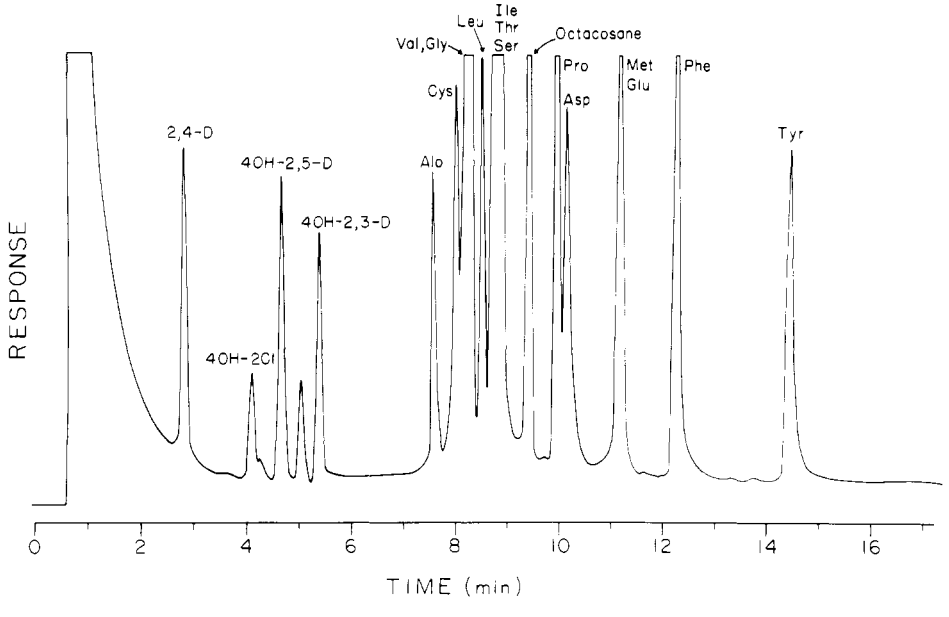

Figure 4. GLC separation of Me_3Si derivatives of 2,4-D-metabolites. Column: 1% OV-17 on 80/100 mesh Supelcoport, 6 ft × 4 mm i.d. glass. Temperature programmed at a rate of 5 °C/min up to 280 °C, initial temperature 180 °C. Each peak represents ca. 2 μg; flow rate, 60 ml/min.

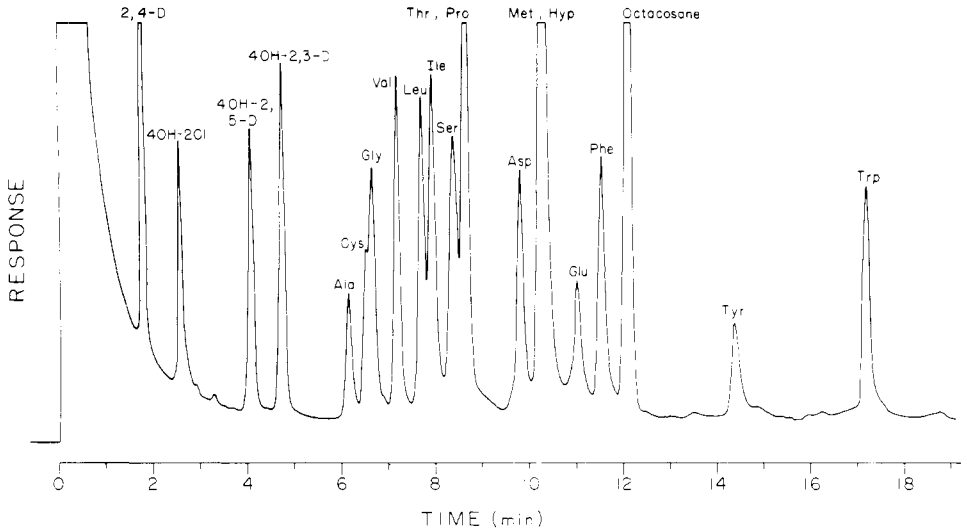

Figure 5. GLC separation of Me_3Si derivatives of 2,4-D-metabolites. Column: 2% OV-1 on 100/120 mesh Supelcoport, 6 ft × 4 mm i.d. glass. Temperature programmed at a rate of 5 °C/min up to 280 °C, initial temperature 170 °C. Each peak represents ca. 1.5 μg; flow rate, 60 ml/min.

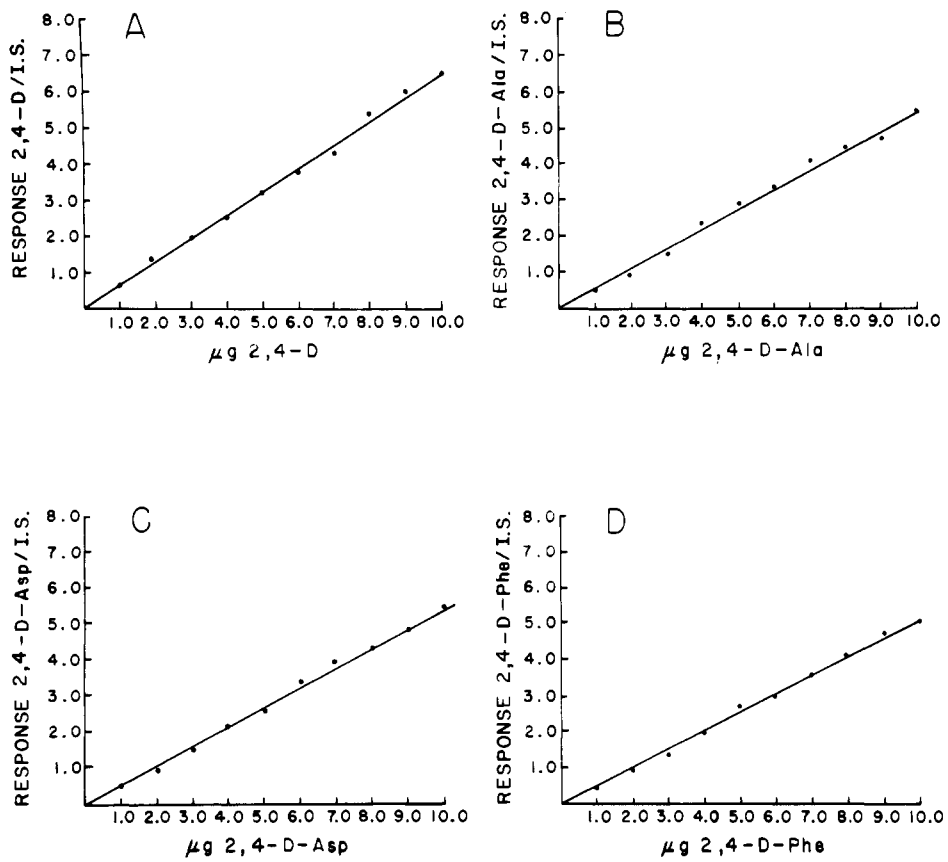

Figure 6. Relative response of Me_3Si derivatives vs. micrograms of derivatives injected: (A) 2,4-D-Me_3Si; (B) 2,4-D-Ala-Me_3Si; (C) 2,4-D-Asp-Me_3Si; and (D) 2,4-D-Phe-Me_3Si.

amino acid side chain), 328 (M − Cl), 246 (M − C(=O) − $OSi(CH_3)_3$), 187, and 158 (B).

The mass spectra of several Me_3Si derivatives of amino acid conjugates or 2,4-D and its hydroxylated metabolites are presented in Table I. It is apparent that the two carboxyl groups of 2,4-D-Asp are silylated and that 4-OH-2-Cl is also disilylated.

Conditions of Derivatization. Me_3Si derivatization with HMDS at reaction conditions of 60, 100, and 150 °C were investigated over a period of 4 h. The derivatization at 60 and 100 °C was very similar and did not appear to be complete until 2 h; however, derivatization at 100 °C did result in a slightly greater amount of product as is shown in Figure 3. Derivatization at 150 °C reached a maximum in 30 min and then continually decreased, reflecting products that were unstable at this reaction condition. Therefore, all derivatives for qualitative and quantitative analysis were prepared at 100 °C for 2 h. The metabolites were azeotropically dried with methylene chloride, prior to derivatization, to remove traces of moisture which interfere with the reaction. 2,4-D-His, 2,4-D-Arg, and 2,4-D-Lys were not soluble in acetonitrile and did not form Me_3Si derivatives. The Me_3Si derivatives of all tested compounds were stable at room temperature

Table I. Mass Spectra of Me_3Si Derivatives of 2,4-D and Its Metabolites

	Relative intensity of ions[a]														
m/e	I	II	III	IV	V	VI	VII	*m/e*	I	II	III	IV	V	VI	VII
70		4		8				170						7	
71		4						172						5	
72		32		10				173	4					6	
73	100	100	34	100	17	100	31	174	4		4				
74	52	84	4	20		12	6	175	5		8	12	4	7	4
75	65	100	21	100	35	100	98	176						3	4
76	6	36		32	4	26	9	177	4		5	10	3	6	9
77	7	40	3	26		17	10	179		24		8			
78	7	16				3	4	180						3	
79		16					3	182				4			
82						5		183	6			10		16	23
83		16		12		3		184				8	4	17	26
84				8	4	5		185	6			6		15	10
85				6				186				6	6	19	10
86					12			187			18	18		6	19
88		12						188			4	6			
89	5	12						189							4
90						3	4	197	6						
91		16				3	26	198	9						
92							5	199	4						
93	22	100	7	100	14	44	28	200	5				51	40	
94	4	12		30		6	6	201		20			11	8	6
95	11	36	3	126	5	16	13	202			4		4	5	3
96		8		10				203	23						3
97		8		6	8		4	204	5					7	
98			3	18	5	15	12	205	19					3	29
99				6		6	4	206	4						7
100				14		8	5	207		56		30	3	50	100B
101		12				5		209		12		8		9	15
103		12	4	20	3	100	5	210			7	68	3	6	
104						17	5	211				16		2	
105		12				8		212				24			
108				14		3		213	17	40		8			
110				8		3		214	4	8					
111				20		5		215	7	100B		12			
112						3		216		28					
114						3		217		40				26	
115			5	10		5		218		8	7	8	3	10	
116	5	24	11	16		8		219	21			24	5	27	79
117	19	32	4	22	3	9	34	220	5		5	8	4	5	28
118		8					5	221	16			20		21	10
119		8					4	222	4	24			3	3	13
120							7	223					6		
126				8	4	8	5	224	5						
128				10	3	16	9	229	5						
129		32		14		5	4	231	4						
130		16		6		6	4	232				8	10	7	
131		12				5	10	233	99					9	
132		16		12		7	12	234	16			14	8	6	12
133		16		6		5	6	235	66						4
135		12					6	236	11			8	3	14	
137		16						237	14	16				3	
140						5		238				16		6	
142						7		240				8			
143						6		241						3	
144				6		7	3	244						5	
145		12		10		6	3	245						3	
146				8		14	5	246			86	66	4	6	
147		40		76	3	24	12	247			11	10		5	
148		8		14		5	3	248			54	46	3	3	
149		20		16		10	9	249	23		7	6			
151		12						250	5		11	6			
153					3			251	17						
156				14		7		252	4	20			5		
157				8		8		253	5						
158			100B	92	5	9		254					3		
159	25		16	16		6	6	256	4						
160	4		7	6				257	64	16					
161	19					7	6	258	13						
162	14		21	100B	26	100B	72	259	24	8					
163	5			8	3	12	7	260	5				4	33	
164	9		13			63	44	261						9	
165		12		68	18	6	4	262					3	5	
166		4		6		10	10	267						3	6
167		16		14	3			268						3	
168				6				277	7						

Table I (*Continued*)

m/e	I	II	III	IV	V	VI	VII	*m/e*	I	II	III	IV	V	VI	VII
279	5							354							18
280				10	3			355						7	6
281		12		6		15	34	356							4
282				6		12	12	360						10	
283						6	7	362						8	
284						3		363			5P				
286							4	365			4				
287					5			369					4		4
288					100B		3	370					28		3
289					60	5		371					9		
290				8	66			372					12		
291				6	10	10		373					3		
292	100P				13			390					17		
293	17					8		391					4		
294	69						6	392					13		
302		24						393					3		
311				16				394					3		
312				8				400				10			
313				8				403							4
317				24	5	8		404							4
318				6				405					1.7P		
319				18	4	5		407					1.1		
321							15	415						6	
322							6	416						29	4
323							10	417						10	3
324							4	418						14	3
325						41	4	419						3	
326				40	3	12	3	420				10			
327				76	3	16	3	421						41	
328			83	30				422				8		14	
329			19	28				423						30	
330		8	33	8				424						9	10
333		6				27		425						9	4
334						7		426							7
335						21		429						6	
345				22		27		435				4P			
346		100P		8		7		436				1		19	
347		28		36		19		437				3		6	
348		48	37	10	9	6		438						15	
349			7	24	3	6		439							3P
350			23		6			441							2
351			5					451						1.5P	
352			5					453						1.0	

[a] All bases (B) were arbitrarily chosen above *m/e* 103; P = parent; I = 2,4-D-Me_3Si; II = 4-OH-2-Cl-diMe_3Si; III = 2,4-D-Ala-Me_3Si; IV = 2,4-D-Ala-diMe_3Si; V = 2,4-D-Leu-Me_3Si; VI = 2,4-D-Ser-diMe_3Si; and VII = 2,4-D-Phe-Me_3Si. All spectra were obtained at 20 eV except for II which was recorded at 70 eV.

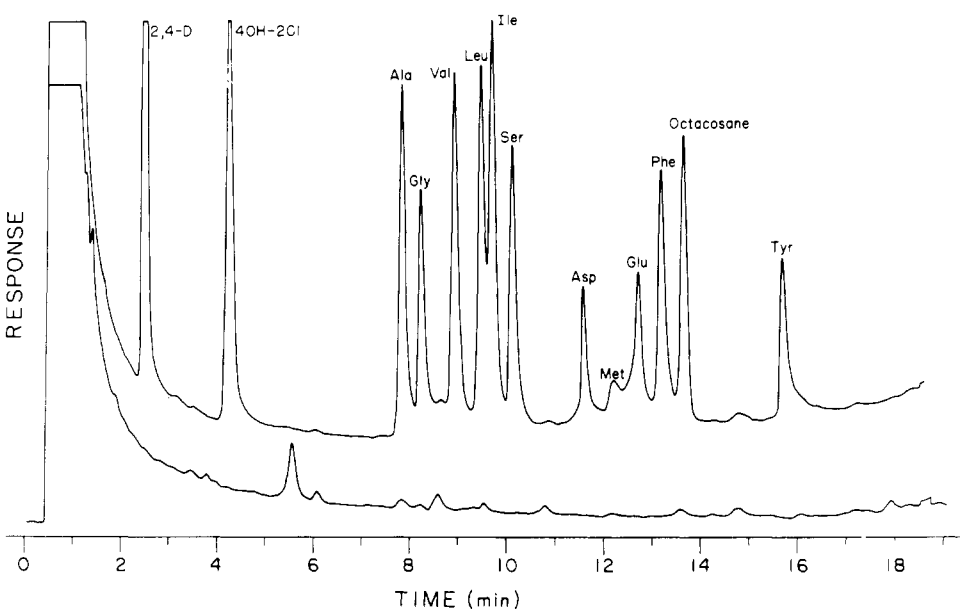

Figure 7. GLC of ethyl ether extract of soybean callus tissue (lower trace). Upper trace shows extract of tissue fortified with 30 ppm of 2,4-D, 4-OH-2-Cl, and amino acid conjugates of 2,4-D. Column: 2% OV-1 on 100/120 mesh Supelcoport, 6 ft × 4 mm i.d. glass. Temperature programmed at the rate of 5 °C/min up to 280 °C, initial temperature 170 °C; flow rate, 60 ml/min.

Table II. Percentage Recovery of 2,4-D-Amino Acid Conjugates Extracted from Soybean Callus Tissue Fortified with 30 ppm

Compound	% recovery
2,4-D	35.49
4-OH-2-Cl	54.72
2,4-D-Ala	72.33
2,4-D-Gly	60.48
2,4-D-Val	91.40
2,4-D-Leu	81.77
2,4-D-Ile	82.17
2,4-D-Ser	66.83
2,4-D-Asp	18.53
2,4-D-Met	8.28
2,4-D-Glu	32.04
2,4-D-Phe	71.88
2,4-D-Tyr	56.12

in closed vials over a period of 8 days.

Chromatographic Separations. The separations achieved by GLC on 1% OV-17 are illustrated in Figure 4. Eleven of the 19 Me_3Si derivatives (2,4-D, 4-OH-2-Cl, 4-OH-2,5-D, 4-OH-2,3-D, and 7 amino acid conjugates: Ala, Cys, Leu, Pro, Asp, Phe, and Tyr) were completely separated in less than 15 min. *n*-Octacosane proved to be a good internal standard; it did not interfere with the elution of any other compound and eluted at a time which is not far from other peaks. Unseparated Me_3Si derivatives were (1) 2,4-D-Val and 2,4-D-Gly, (2) 2,4-D-Ile, 2,4-D-Thr, and 2,4-D-Ser, and (3) 2,4-D-Met and 2,4-D-Glu. The Me_3Si derivative of 2,4-D-Trp did not elute on the OV-17 stationary phase under the conditions used.

The GLC separation achieved on 2% OV-1 is shown in Figure 5. Fourteen derivatives were separated completely. The Me_3Si derivatives which did not separate were (1) 2,4-D-Cys and 2,4-D-Gly, (2) 2,4-D-Thr and 2,4-D-Pro, and (3) 2,4-D-Met and 2,4-D-Hyp, none of which have yet been reported as metabolites of 2,4-D. 2,4-D-Trp-Me_3Si eluted from the OV-1 column in contrast to OV-17.

Comparatively, 2% OV-1 gave the more superior separations. Ten amino acid conjugates were completely separated in contrast to only seven conjugates when OV-17 was employed. Perhaps of greater importance is that when both columns were used all 16 amino acid conjugates can be completely separated and quantified since the overlapping compounds are different on each stationary phase.

Linearity Response of Flame Ionization Detector. The response of the flame ionization detector over a range of 1–10 μg of Me_3Si derivative was determined so that quantitative analysis could be performed. The response ratio of each compound to *n*-octacosane plotted against the microgram of that compound injected (1–10 μg) resulted in a straight line in all cases (Figure 6) except for 2,4-D-Trp.

Practical Application. Figure 7 shows the GLC analysis of a soybean callus extract fortified with 30 ppm of 2,4-D, 4-OH-2-Cl, and 11 amino acid conjugates of 2,4-D. No extraneous GLC peaks were observed which might cause interference. The percentage recovery of the derivatives was calculated and expressed in Table II. This investigation shows that the Me_3Si derivatives of amino acid conjugates of 2,4-D can be isolated, detected, and quantified by this method; however, a number of improvements are necessary before this method could be routinely used. As noted in Table II, a considerable variation occurs in the percentage recovery of each Me_3Si derivative. This probably results from losses in the extraction and cleanup techniques which need to be studied further. In this investigation, the tissue was fortified with 30 ppm of each derivative; however, a much lower concentration could have been used. Although electron capture detection is theoretically feasible, the presence of the silyl group renders the derivatives insensitive to detection in this manner.

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LITERATURE CITED

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., Hamilton, R. H., Mumma, R. O., *J. Agric. Food Chem.* **23**, 373 (1975).
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., Mumma, R. H., Hamilton, R. H., *J. Agric. Food Chem.* **22**, 307 (1974).
Gehrke, C. W., Leimer, K., *J. Chromatogr.* **57**, 219 (1971).
Gehrke, C. W., Roach, D., Zumwalt, R. W., Stalling, D. L., Wall, L. L., "Quantitative Gas–Liquid Chromatography of Amino Acids in Proteins and Biological Substances", 1968, 99 pp.
Hamilton, R. H., Hurter, J., Hall, J. K., Ercegovich, C. D., *J. Agric. Food Chem.* **19**, 480 (1971).
Pesticide Analytical Manual, Vol. 1, Food and Drug Administration, Section 221, 1969, p 13a.
Schultz, D. P., *J. Agric. Food Chem.* **21**, 186 (1973).
Yip, G., *J. Assoc. Off. Agric. Chem.* **45**, 367 (1962).
Yip, G., *J. Assoc. Off. Anal. Chem.* **54**, 966 (1971).
Yip, G., Ney, R. E., Jr., *Weeds* **14**, 167 (1966).

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