Ring Contraction Products from 2,3-Dihydro-3,5-dihydroxy-6-methyl-4(*H*)-pyran-4-one: 4-Hydroxy-5-hydroxymethyl-2-methyl-3(2*H*)-furanone and 2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone

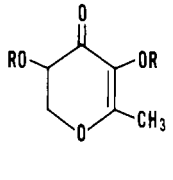
Frank D. Mills

Evidence is presented that establishes the structure of two ring contraction products isolated from an aqueous, alkaline treatment of 2,3-dihydro-3,5-dihydroxy-6-methyl-(4*H*)-pyran-4-one, a nonenzymic product found in dehydrated and cooked foods. The structure of the hydroxymethyl-3(2*H*)-furanone was assigned from mass spectral and proton magnetic resonance data obtained on the unsubstituted compound and its diacetate, along with carbon-13 analysis of the diacetate. Ultraviolet and infrared spectra coupled with a positive Tillmans reaction support the assignment. The other 3(2*H*)-furanone was identified by combination gas–liquid chromatography–mass spectral analysis.

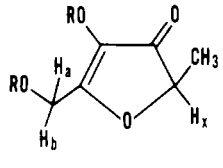
Because the title dihydropyranone has been identified as an early sugar degradation product in Maillard-type browning reactions (Mills et al., 1970b; Mills and Hodge, 1976) and has been found in dehydrated orange juice (Tatum et al., 1967) and many cooked foods (Ledl et al., 1976), the further conversions of 2,3-dihydro-3,5-dihydroxy-6-methyl-(4*H*)-pyran-4-one (DHDHMP) (**1**), under various reaction conditions, are of practical import and interest. When DHDHMP was reacted with L-proline (Mills and Hodge, 1976) or was subjected to acidic media (Severin and Seilmier, 1968; Shaw et al., 1971), various ring contraction and dehydration reactions occurred that produced dihydrofuranones, methylfurfurylamines, hydroxmethylfurfurylamines, and pyranones.

Isolation and identification of the title hydroxymethylfuranone (HHMMF) (**3**) is considered important because, although HHMMF has been proposed as a nonenzymic browning product, this compound has not been identified in previous investigations. Also, characterization of HHMMF would add information to the structure–aroma relationship of α-hydroxy-β-methylenones that is identified (Hodge, 1967) and verified (Pittet et al., 1970).

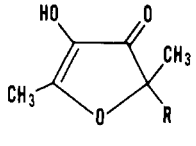
The title 4(*H*)-pyran-4-one (**1**), its homogeneity confirmed by gas–liquid chromatography (GLC), was treated with hot 1 N aqueous alkali, and during this decomposition study, the nonenzymic browning intermediate, 4-hydroxy-5-hydroxymethyl-2-methyl-3(2*H*)-furanone (**3**) was isolated. Compound **3** is now characterized as a nearly odorless, crystalline solid that produces a lightly charred aroma when its effluent, resulting from GLC, is analyzed. The trace, fragrant, burnt aroma of the solid isolate is shown by combination GLC–mass spectrometry to originate from a slight impurity attributed to furanone **5** (Table I). The latter 3(2*H*)-furanone had been previously prepared (Hodge and Fisher, 1963b) and identified in earlier pyrolysis studies of Amadori compounds (Mills et al., 1970a; Mills and Hodge, 1976).



1. R = H
2. R = Ac



3. R = H
4. R = Ac



5. R = H
6. R = OH

The high-resolution mass spectrum of **3** (Table II) establishes the compositional formula as $C_6H_8O_4$ and reveals a fragmentation pattern substantially different from those of either **1** or **6**. The high mass fragments (Table II) indicate a stepwise cleavage from m/e 144 → 129 → 100, and the most likely fragmentation paths (Scheme I) are compatible with the proposed structure of **3**. Additionally, the mass spectral analysis confirms that **3** forms a diacetate upon treatment with sodium acetate and acetic anhydride in chloroform (Tables I and II). Further, ultraviolet (UV) and infrared (IR) spectral analyses of the crystalline isolate **3** show the ring contraction product to contain a five-membered, heterocyclic ring with an α-hydroxyenone moiety; λ_{max} (EtOH) 289 nm, which is similar to the calculated value for **3** (Scott, 1964) and identical with that for another 3(2*H*)-furanone (Nunomura et al., 1976); and IR bands at 1685 (s) and 1630 (vs), which are similar to other α-hydroxyenones (Mills et al., 1970b; Goto and Miyagi, 1964). The reaction of isolate **3** with 2,6-dichloroindolphenol (Tillmans reagent) supports the UV and IR data; in dilute acetic acid, **3** produces an instant reduction of the reagent that suggests opening of the ring and formation of a dihydroxyenone grouping.

Proton magnetic resonance (^{1}H NMR) examination of the isolate **3** in $CDCl_3$ showed only that the compound contained a methyl doublet at δ 1.43 (3 H) and a broadened singlet at δ 4.32 (3 H); irradiation of the doublet sharpened the downfield resonance, and downfield irradiation collapsed the methyl doublet to a singlet. Two hydroxyl protons shown as a broadened singlet, which can be removed with D_2O, were located at δ 6.85. Analysis in pyridine-d_5 indicated a single methyl doublet at δ 1.57 and a far better resolved, complex, three-proton multiplet at δ 4.46. From these spectra, it was apparent that a C-methyl group was present rather than a vinyl-methyl group, and the isomeric 4-hydroxy-2-hydroxymethyl-5-

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Table I. Mass Spectra of 1-6

Compd	*m/e* (rel %)[a]						
1	144 (20)	115 (15)	101 (20)	85 (2)	73 (8)	72 (15)	55 (20)
2	228 (1)	186 (12)	144 (5)	126 (15)	101 (7)	84 (4)	55 (18)
	43 (100)						
3	144 (3)	142 (1)	129 (1)	102 (4)	100 (1)	87 (1)	85 (1)
	59 (0.1)	58 (41)	54 (6)	43 (100)			
4	228 (0.4)	187 (1)	186 (10)	144 (13)	142 (1)	102 (10)	
	100 (2)	85 (1)	74 (22)	59 (3)	58 (41)	54 (6)	
	43 (100)						
5	128 (32)	85 (17)	57 (63)	45 (11)	43 (100)		
6	144 (42)	101 (62)	73 (51)	55 (57)	43 (100)		

[a] Rel % = % of the base peak.

Table II. High-Resolution Mass Spectra of 3 and 4

Compd	Obsd ion mass	Calcd ion mass	Ion formula
3	144.0423	144.0422	$C_6H_8O_4$
	129.0188	129.0187	$C_5H_5O_4$
	102.0318	102.0316	$C_4H_6O_3$
	100.0159	100.0160	$C_4H_4O_3$
	58.0416	58.0418	C_3H_6O
	54.0106	54.0105	C_3H_2O
4	228.0645	228.0634	$C_{10}H_{12}O_6$
	186.0527	186.0528	$C_8H_{10}O_5$
	144.0417	144.0422	$C_6H_8O_4$
	102.0294	102.0317	$C_4H_6O_3$

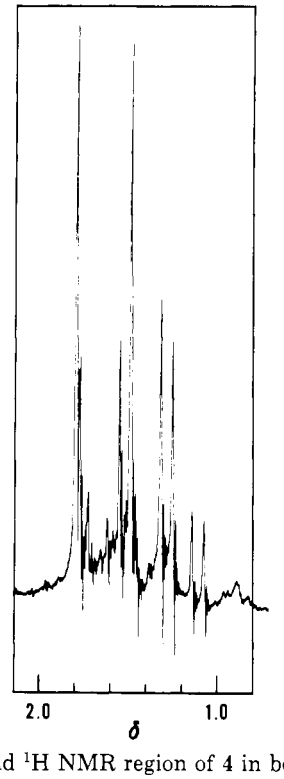


Figure 1. Upfield ^1H NMR region of 4 in benzene-d_6.

methyl-3(2*H*)-furanone structure could be excluded. A further examination of **3** in benzene-d_6 or acetone-d_6–benzene-d_6 did not resolve the downfield protons sufficiently for analysis. Consequently, **3** was acetylated and the diacetate **4** was examined in $CDCl_3$ and benzene-d_6 (Table III). Analysis in $CDCl_3$ (Table III) showed two multiplets, representing one proton; another multiplet, comprised of two protons, was further upfield at δ 4.32. Additionally, a C-methyl doublet as well as a single peak made of the six acetoxymethyl protons were observed. Because the resolution of the downfield protons was incomplete, the diacetate was studied in benzene-d_6. A well-resolved spectrum was finally achieved, and additional

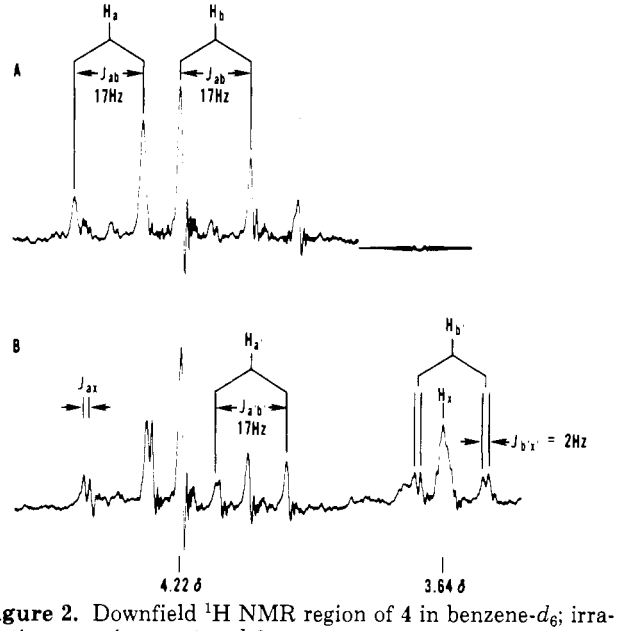


Figure 2. Downfield ^1H NMR region of 4 in benzene-d_6; irradiation experiments 1 and 2.

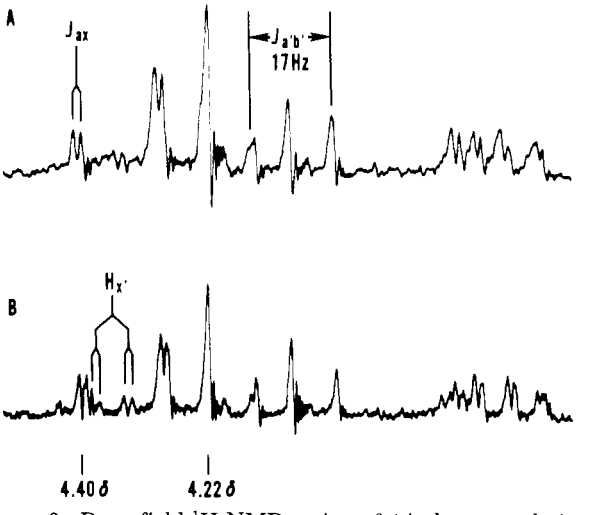


Figure 3. Downfield ^1H NMR region of 4 in benzene-d_6; irradiation experiments 3 and 4.

irradiation experiments made analysis possible for the three protons in each of the two separate ABX subgroups in the six-spin system of the isomeric compounds. Two sets of two acetyl methyl groups and C-methyl protons, each appearing as a doublet, can be identified (Figure 1). When the resonance at δ 3.64 is irradiated (Figure 2A), the four-line pattern of the major component becomes clear, and the calculation of the shifts for H_a and H_b can be made (Bible, 1965). In the next decoupling experiment (Figure 2B), a broadened H_x singlet and the isolated $H_{b'}$ pattern

Scheme I

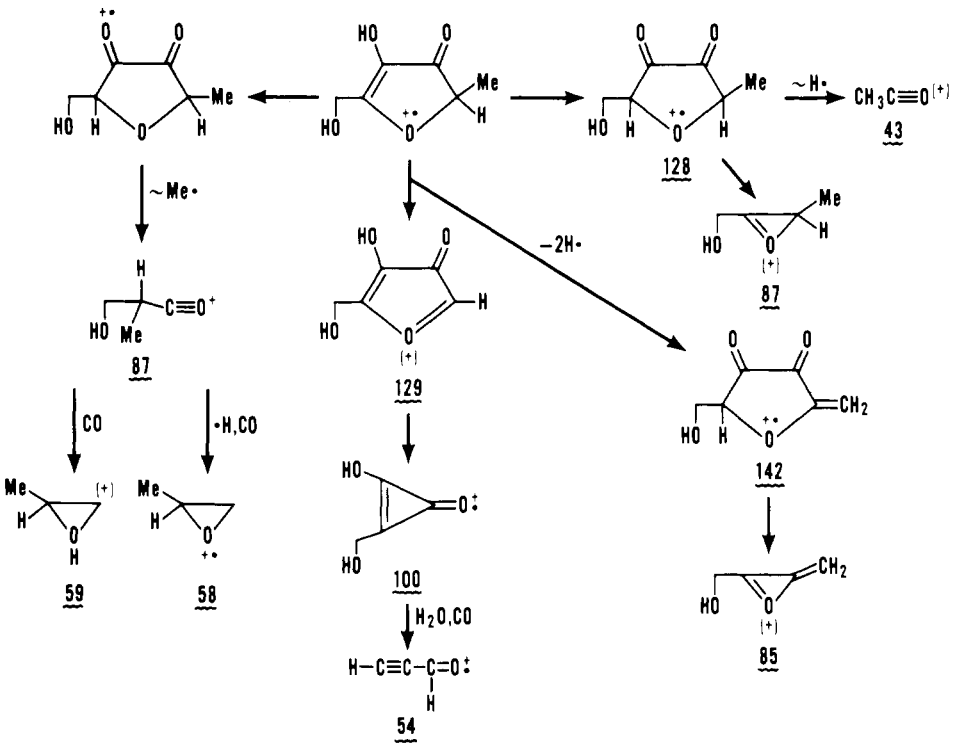


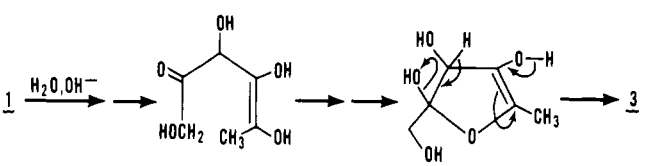
Table III. ^{1}H NMR Spectra of 4-Acetoxy-5-acetoxymethyl-2-methyl-3(2*H*)-furanone (4)

Chemical shift (H)[a]	Coupling constant (J) Hz		Proton assignment
		In $CDCl_3$	
4.83 } (1)[b]			H_x
4.72 }			$H_{x'}$
4.32 (2)[b]			H_a, H_b, $H_{a'}$, $H_{b'}$
2.22 (6)			Acetoxymethyls
1.43 (3)	J_{x,CH_3}	6.5	C-methyl
		In C_6D_6	
4.38 (1)	J_{ab}	−17	H_a
4.14 (1)			H_b
3.64 (1)	J_{ax}	2	H_x
	J_{bx}	0	
1.79 (3)[c]			Vinyl acetoxymethyl
1.48 (3)			Methylene acetoxymethyl
1.28 (3)	J_{x,CH_3}	6.5	C-methyl, major isomer (74%)
4.40 (1)	$J_{a'x'}$	0	$H_{x'}$
	$J_{b'x'}$	2	
4.03 (1)	$J_{a'b'}$	−17	$H_{a'}$
3.70 (1)			$H_{b'}$
1.77 (3)[c]			Vinyl acetoxymethyl
1.54 (3)			Methylene acetoxymethyl
1.12 (3)	J_{x',CH_3}	6.5	C-methyl, minor isomer (26%)

[a] (H) is the number of protons, arrived at by taking the peak areas for a particular isomer. [b] Figures represent the center of a multiplet. [c] Lowerfield resonance is assigned to the enol/acetate because of the proximity of the double bond (Bhacca and Williams, 1964).

were produced when the spectrum was irradiated at δ 1.28. Also, the $H_{a'}$ assignment is apparent as are the coupling constants $J_{a'b'}$ and $J_{b'x'}$. A third irradiation at δ 1.10 (Figure 3A) dissolved the $H_{x'}$ proton into the baseline, but the chemical shift could be evaluated for the proton from Figure 3B which involved irradiation at δ 1.12. The results from a carbon-13 analysis of 4 also aided the amassed structural information. A broad-band, proton-decoupled spectrum of 4, using chloroform-*d* as the solvent and tetramethylsilane as an internal standard, contained a twelve-line pattern. The two peaks at 15.34 and 17.09 ppm are assigned to the 2-methyl carbons (Hicks et al., 1974), each representing one stereoisomer. The resonance at 64.60 ppm arises from the derivatized hydroxymethylene carbon, while the peaks at 71.4 and 70.8 ppm are due to

Scheme II



the enantiomeric, methine carbons. Four other resonances at 154.4, 157.1, 165.9, and 167.7 ppm overlap the ester and olefinic regions of the spectrum (Levy and Nelson, 1972), and, as a result, these are assigned without specific designation to the acetyl carbonyls and to the 4 and 5 carbons of the 3(2*H*)-furanone. The ring carbonyl produces the final peak in the spectrum at 190.5 ppm.

From the above spectral data, structure **4** is affirmed. Compound **3** is 4-hydroxy-5-hydroxymethyl-2-methyl-3(2*H*)-furanone. Formation of **3** can be best explained (Scheme II) through nucleophilic attack at the 2 position of the dihydropyranone (**1**) (1,4 addition of a water unit) with concurrent ring opening. Following this addition, ketonization α to the hydroxymethyl and methyl groups produces the necessary intermediate. The dione then enolizes with concomitant recyclization, and the newly formed dihydrofuranone ketonizes and dehydrates to produce the hydroxymethyl-3(2*H*)-furanone. This mechanism would also account for the nonstereospecificity at the 2 position. The isolation of **3** completes the identification of the possible dihydrofuranones that are expected from a reducing hexose via nonenzymic browning reactions.

The lack of caramel-like aroma in **3** is attributed to the presence of a hydroxymethyl group instead of a methyl or ethyl moiety in the β position of the enolone. Although the hydroxymethyl group normally confers a lower volatility than an alkyl substituent, as in pyromeconic acid and allomaltol, it may also result in the loss of the caramel-like odor (Hodge, 1967). Finally, **3** may be synthesized in 3–6% overall yields by using D-glucose and piperidine to yield **1** (van den Ouweland and Peer, 1970; Hodge and Fisher, 1963a) and the herein described isomerization to conclude the preparation.

EXPERIMENTAL SECTION

General. All melting points were determined on a Thomas-Hoover Unimelt apparatus and are uncorrected. The mass spectra were determined with a Nuclide 90G double-focusing spectrometer, and a direct or heated inlet or a Packard gas chromatograph, Model 846, was used. IR spectra were obtained with a Perkin-Elmer Model 621 spectrophotometer and from solutions in chloroform. The UV spectrum was recorded from an ethanol solution using a Beckman Model DB spectrophotometer. The ^{1}H NMR data were generated with a Varian Model HA-100 instrument from chloroform-*d* or benzene-d_6 solutions using tetramethylsilane as an internal standard. The C-13 spectrum was obtained with a Brucker Model WH-90 spectrometer (26 MHz) and from 9.01×10^4 scans of a chloroform-*d* solution. GLC was performed on a Varian Model 1848 chromatograph, equipped with effluent splitters and with columns (a) 182 cm × 3.2 mm, 3% Silar 5CP on 80–100 Chromosorb Q and (b) 122 cm × 3.2 mm 10% 8BP on 80–100 Chromosorb W.

4-Hydroxy-5-hydroxymethyl-2-methyl-3(2*H*)-furanone (3). Dihydropyranone 1 (van den Ouweland and Peer, 1970), 1.0 g, was added to 150 mL of 1 N sodium hydroxide. The resulting solution became lightly yellow. After blanketing the solution with nitrogen, the reaction mixture was heated in a water bath at 60–70 °C. After 1 h, the solution was cooled, acidified with 3 N hydrochloric acid, saturated with sodium chloride, and then extracted three times with chloroform (100 mL total volume). The combined organic extract was dried (sodium sulfate) and filtered. The solvent was removed in vacuo and a viscous oil resulted. The oil was initially treated with 1 mL of ethanol and then dissolved in ether–hexane. Upon cooling, crystals separated, mp 90 °C. The yields of several preparations ranged from 16 to 33%. The isolate was stored at −15 °C and could also be kept in anhydrous methanol at −15 °C.

Anal. Calcd for $C_6H_8O_4 \cdot C_2H_5OH$: C, 50.50; H, 7.37. Found: C, 49.78; H, 7.34.

^{1}H NMR analysis also indicated ethanol to be present. The solvent could be removed from **3** by storing the sample for 16 h under reduced pressure and over phosphorus pentoxide.

GLC analyses of **1** and **3**, employing 2-methyl-3-hydroxy-4(*H*)-pyran-4-one (maltol) as an internal reference, produced the following retention parameters relative to maltol (R_{maltol}): column a, **1** (1.56), **3** (2.25); column b, **1** (1.31), **3** (1.79). The former program was 100 °C for 4 min and then 4 °C/min to 200 °C, and the latter was 120 °C for 4 min and then 4 °C/min to 200 °C.

4-Acetoxy-5-acetoxymethyl-2-methyl-3(2*H*)-furanone (4). Dihydrofuranone **3**, 50 mg, was added to 40 mL of chloroform containing 5 mg of anhydrous sodium acetate (freshly fused) and 2 mL of acetic anhydride. The resulting solution was refluxed for 3 h and, after cooling, poured onto ice and stirred for 1 h. The chloroform was separated and the remaining aqueous phase was extracted with 20 mL of chloroform. The combined organic phase was dried (sodium sulfate), filtered, and concentrated to a light oil. The oil formed a semi-solid mass (35 mg). Although the product did not fully crystallize on standing, TLC on silica gel, using 15% ethyl acetate in benzene, showed the product to be homogeneous. The diacetate was stored for several weeks at −15 °C.

2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone (5). Combination GLC–MS, using column a, of the crude hydrolysis extract, allowed identification of **5**. The retention time and mass fragmentation pattern obtained were identical with those for an authentic sample of **5** (Hodge and Fisher, 1963b).

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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

Masood Arjmand, Robert H. Hamilton, and Ralph O. Mumma*

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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