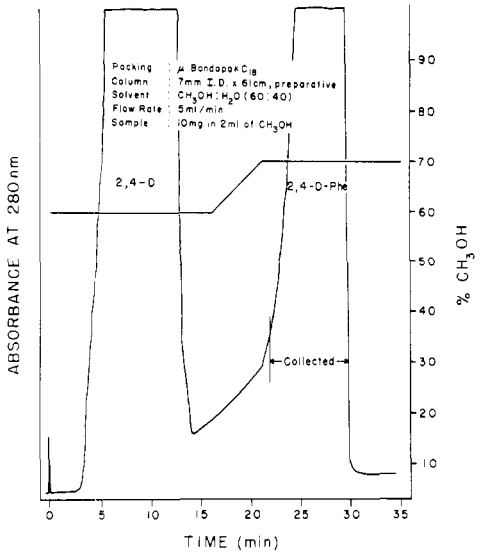

Figure 2. The purification of 2,4-D-Phe by LC employing a preparative μBondapak C_{18} column.

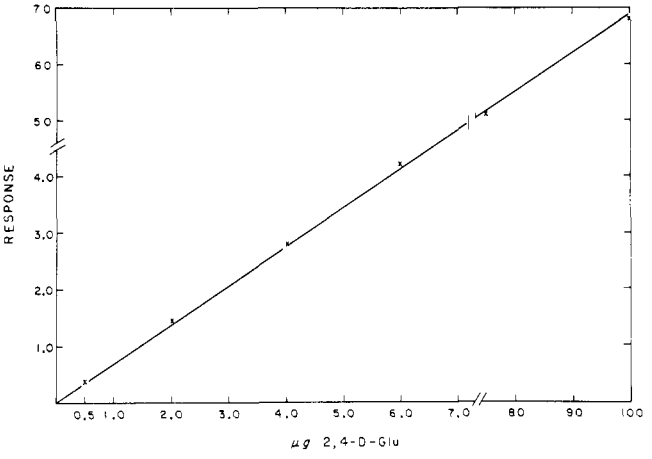

Figure 3. Response of the UV detector to 2,4-D-Glu.

separation achieved to purify 100-mg quantities of 2,4-D-Phe using a preparative μBondapak C_{18} column.

The UV detector gave a linear response over the entire range analyzed from 100 ng to 100 μg (Figure 3). This is a much wider linear response than that achieved with gas chromatographic procedures (Arjmand and Mumma, 1976a,b). The lower sensitivity limit for the conjugates was ca. 50 ng which is nearly 20 times better than what was achieved by gas chromatography methods employing flame detection (Arjmand and Mumma, 1976b).

LC offers a good alternative method of analysis for the amino acid conjugates of 2,4-D. This technique is more sensitive than reported procedures; it provides good separations, allows for ease of collection of samples, can be used in the purification of synthetic conjugates, and can assist in the quantification of metabolites. The importance of the use of ion-pair chromatography must be emphasized for good resolution of the mixture of the conjugates. These procedures have potential applicability, not only to amino acid conjugates of 2,4-D, but to amino acid conjugates of other xenobiotics and perhaps to other ionic metabolites.

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COMMUNICATIONS

A Simple and Rapid Quantitative Method for Total Phenols

A simple and rapid quantitative procedure for determining total phenols is described based on the colored complex formed with titanium. Determination of the chlorogenic acid content of defatted sunflower meal by the titanium reagent demonstrates the applicability of this method to agricultural products.

Phenolic compounds are widely distributed in plants contributing to flavor and color problems associated with flours, grains, and oilseeds (Cater et al., 1972; Sabir et al., 1974; Maga and Lorenz, 1973). A number of methods are

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Table I. Spectrophotometric Data for Phenolic-Titanium Systems

Phenolic Compd	Max, nm	Molar absorptivity	Measured at nm	Beer's law plot (r^2)
Phenol	410	2.4×10^2	410	0.97
Catechol	430	1.9×10^3	430	1.00
Resorcinol	410	1.6×10^2	410	1.00
Quinol	440	4.1×10^3	440	1.00
Phloroglucinol	410	1.1×10^3	410	0.96
p-Hydroxybenzoic acid	410	1.0×10^2	410	1.00
4-Hydroxy-3-methoxy-benzoic acid	405	1.2×10^2	405	0.99
p-Hydroxycinnamic acid	410	4.6×10^2	410	0.99
3,4-Dihydroxycinnamic acid	450	2.0×10^3	450	1.00
3,5-Dimethoxy-4-hydroxycinnamic acid	405	9.0×10^2	405	0.94
Chlorogenic acid	450	2.1×10^3	450	1.00
Catechin	430	3.5×10^3	430	1.00
Apigenin	410	9.6×10^3	410	0.99
Naringenin	410	8.8×10^3	410	0.99

available for measuring phenols (Swain and Hill, 1959; Pomenta and Burns, 1971); however, this paper reports a new and simple quantitative procedure based on the formation of a colored complex between phenols and titanium ions.

EXPERIMENTAL SECTION

Reagents. The phenolic compounds were of reagent grade and included *p*-hydroxybenzoic acid and *p*-hydroxycinnamic acid (Eastman Kodak Co., Rochester, N.Y.); 3,5-dimethoxy-4-hydroxycinnamic acid, 4-hydroxy-3-methoxybenzoic acid, and apigenin (Aldrich Chemical Co., Phillipsburg, N.J.); phloroglucinol, catechol, and resorcinol (Analar, British Drug Houses Ltd., Poole, England); naringenin (Mann Research Laboratories Inc., N.Y.); catechin (Nutritional Biochemicals Corp., Cleveland, Ohio). Titanium tetrachloride and Folin reagent were purchased from British Drug Houses (Toronto, Canada).

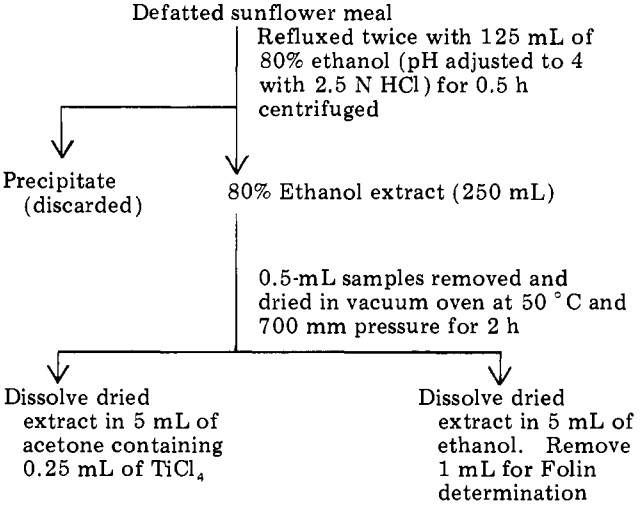
Procedure. *Preparation of Phenolic Standards.* A series of standard solutions were prepared for each phenolic compound (0–200 μg/mL) in 10 mL of acetone in 15-mL screw-top glass tubes. To each tube was added 0.5 mL of titanium reagent (20% $TiCl_4$ in concentrated HCl), and the solutions were thoroughly mixed on a Vortex for 15 s. A colored complex between the phenolic compounds and titanium formed immediately, and the absorbance spectra were examined using an SP 800 Unicam Spectrophotometer over 390–500 nm. Individual absorbances were read on a Pye Unicam SP6-300 Spectrophotometer against an equivalent blank.

Extraction of Chlorogenic Acid from Defatted Sunflower Meal. The applicability of this procedure for determination of total phenols in agricultural products was examined using defatted sunflower meal. Phenols were extracted from sunflower meal according to the procedure described by Dorrell (1976) outlined in Scheme I. It was necessary to extract with 80% ethanol, as acetone only partially extracted polyphenols, such as chlorogenic acid from sunflower meal. In order to ensure no losses occurred during the drying process, a known sample of chlorogenic acid was dried under the same conditions. The total phenols were determined by both Titanium and Folin methods (Swain and Hill, 1959).

RESULTS AND DISCUSSION

The spectrophotometric data for the phenolic–titanium systems are summarized in Table I. Molar absorptivities, however, are only approximate. All systems yielded Linear Beer's law plots as indicated by the coefficient of determination (r^2) values in Table I. The colored complex was

Scheme I. Extraction of Phenols from Defatted Sunflower Meal and Their Determination by Titanium and Folin Methods



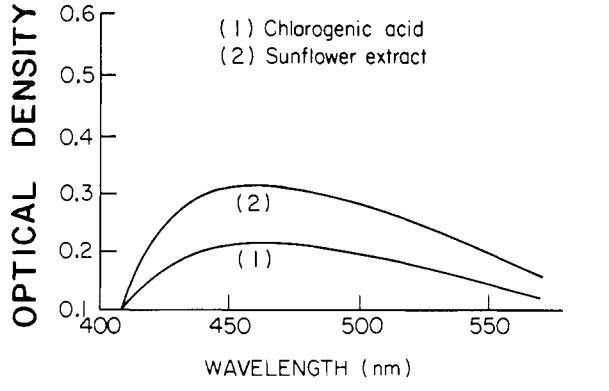


Figure 1. Absorbance spectra of titanium–tetrachloride complexes with chlorogenic acid and sunflower seed extract.

stable with simple phenolics for several hours, while some degradation occurred, however, with the complex polyphenols, resulting in a slight shift in the absorbance spectra. Nevertheless consistent readings could still be obtained for up to 1–2 h. An advantage of the titanium reagent is that it is stable over an extended period of time.

The chlorogenic acid content of the defatted sunflower meal as determined by Folin and Titanium methods was 3.36 ± 0.023 and 3.32 ± 0.063 g/100 g of defatted meal, respectively. These results were not significantly different as determined by the *t* test. The absorbance spectrum of

the extracted sample with titanium was characteristic of chlorogenic acid as shown in Figure 1. The presence of other ethanol-soluble components such as tyrosine and phenylalanine did not interfere with the titanium method. A 100% recovery of chlorogenic acid was obtained following drying which indicated no losses occurred during the drying procedure. While the Folin reagent can be added directly to the 80% ethanol extract, the time involved in preparing the reagent, adjusting the dilution of the extract, plus the hour required for the development of the Folin reagent would be equivalent to the 2-h period for the ethanol samples to be dried. This is required using the Titanium reagent since the reaction proceeds in acetone and not in ethanol or water. This study thus establishes the titanium method as a new and simple procedure for the determination of total phenols.

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Synthesis, Absolute Stereochemistry, and Biological Activity of Optically Active Cyclodiene Insecticides

Both enantiomers, optically pure, of cyclodiene insecticides such as chlordene (**1**), chlordene epoxide (**6**), *cis*- and *trans*-chlordanes (**7** and **8**), and heptachlor epoxide (**9**) were synthesized via optical resolution of racemic 1-hydroxychlordene (**2**) as a diastereomeric mixture of 3β-acetoxyetienyl esters. The absolute stereochemistry of these synthetic insecticides was determined by circular dichroism of dechlorinated tricyclic ketone derivatives of (+)- and (−)-**2** to be shown as (+)-**1**, (−)-**6**, (−)-**7**, (−)-**8**, and (+)-**9**, respectively. Insecticidal activity of the enantiomers of these compounds, except chlordanes, was measured on male adults of German cockroach. (+)-**1** and (−)-**6** exhibited much stronger activity than the corresponding antipodes, while a slight difference (about 2.3 times) was observed between the enantiomers of **9**.

Relationship between absolute stereochemistry and biological activity has been intensively investigated with juvenile hormone (Loew and Johnson, 1971; Imai et al., 1976) and pheromones (Iwaki et al., 1974; Riley et al., 1974; Mori, 1974, 1975, 1976; Mori et al., 1976a,b) of insects. These studies revealed the chiral nature of receptor organs of insects by showing that a specific absolute stereochemistry was required to exhibit the biological activity. In our research of this line, we took synthetic insecticidal chemicals with chiral nature in the molecule and now wish to describe the synthesis, determination of the absolute stereochemistry, and also the biological activity of both enantiomers of cyclodiene insecticides such as chlordene, chlordene epoxide, *cis*- and *trans*-chlordanes, and heptachlor epoxide. This is the first preparation of optically active synthetic insecticides whose carbon skeleton is chiral, and we wish to emphasize that a considerable difference was found in the activity between the enantiomers of these insecticides.

EXPERIMENTAL SECTION

Optically active chlordenes [(+)- and (−)-**1**] were first synthesized via optical resolution of racemic 1-hydroxychlordene [(±)-**2**]. Racemic chlordene [(±)-**1**], prepared by Bluestone's (1951) method, was oxidized (SeO_2 in AcOH) to racemic **2** (Kleiman and Goldman, 1954), yield 75%, mp 213 °C. (±)-**2** was converted (3β-acetoxyetienyl chloride and pyridine; Woodward and Katz, 1959) into a diastereomeric mixture of 3β-acetoxyetienyl esters (**3** and **4**) (49%). The resulting mixture was separated through column chromatography on silicic acid (5% ethyl acetate in *n*-hexane) and by subsequent recrystallization (acetone), into diastereomerically pure **3** and **4**. **3** was obtained as colorless needles: mp 218.5 °C; mass *m/e* 659 (M^+ − Cl); $[\alpha]_D$ −121° (*c* 0.68, $CHCl_3$); IR ($CHCl_3$) 1740 (shoulder) and 1725 cm^{-1}; NMR ($CDCl_3$) δ 0.66 (s, 3 H), 1.01 (s, 3 H), 2.02 (s, 3 H), 3.32 (dd, J = 7.5 and 2.5 Hz, 1 H), 4.05 (dd, J = 7.5 and 2 Hz, 1 H), 4.60 (m, 1 H), 5.40 (broad d, J = 4 Hz, 1 H), 5.68 (q, J = 2.5 and 2 Hz, 1 H), 6.01 (s, 2 H). **4** was obtained as colorless plates: mp 201 °C; mass *m/e* 659 (M^+ − Cl); $[\alpha]_D$ +85.5° (*c* 0.67, $CHCl_3$); IR ($CHCl_3$) 1735 (shoulder) and 1725 cm^{-1}; the NMR spectrum was quite similar to that of **3**. Reductive cleavage ($LiAlH_4$ in diethyl ether, 0 °C) of the ester bond of these diastereomers afforded optically pure alcohols; (−)-1-hydroxychlordene [(−)-**2**] from **3** (69%): mp 213 °C; $[\alpha]_D$ −85.8° (*c* 0.53, $CHCl_3$) and (+)-**2** from **4** (73%): mp 213 °C; $[\alpha]_D$ +86.4° (*c* 0.46, $CHCl_3$). (−)-**2** afforded upon catalytic hydrogenation (Pd/C in MeOH) dihydroalcohol [(−)-**5**]: $[\alpha]_D$ −6.5° (*c* 1.67, EtOH); mass *m/e* 354 (M^+), which was

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