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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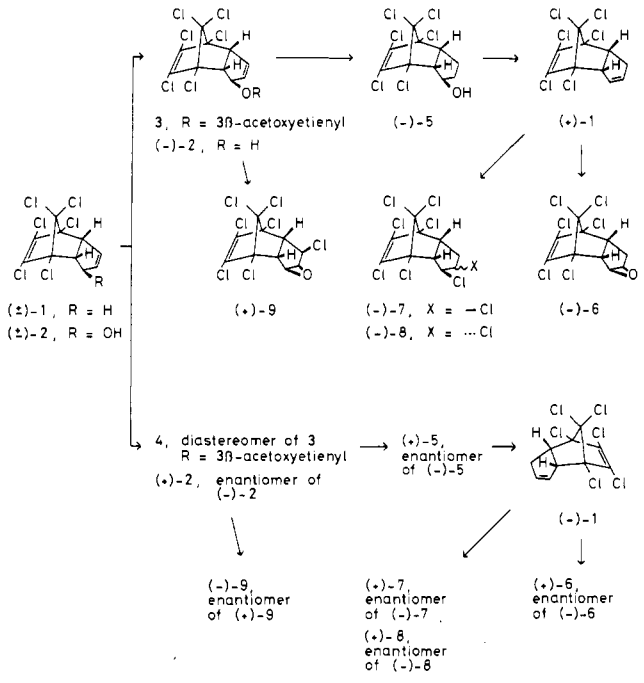
Scheme I



dehydrated ($SOCl_2$ in pyridine) to (+)-chlordene [(+)-1] [81% from (-)-2]: mp 153 °C; $[\alpha]_D$ +35.0° (*c* 0.96, EtOH). (+)-2 was similarly converted via (+)-5, $[\alpha]_D$ +6.2° (*c* 1.68, EtOH), into (-)-chlordene [(-)-1] [85% from (+)-2]: mp 153 °C; $[\alpha]_D$ -35.8° (*c* 0.93, EtOH).

Optically active forms of chlordene epoxide and *cis*- and *trans*-chlordanes was synthesized starting from the enantiomers of 1. Namely, (+)-1 was converted upon epoxidation (*m*-chloroperbenzoic acid in $CHCl_3$) into (-)-**6** (quantitative): mp 243 °C; $[\alpha]_D$ -2.5° (*c* 1.83, MeOH), and (-)-1 into (+)-6, (quantitative): mp 243 °C; $[\alpha]_D$ +2.4° (*c* 1.72, MeOH). With successive three-step reactions, i.e., *tert*-butyl hypochlorite in AcOH (Brooks et al., 1970), hydrolysis (N/10 H_2SO_4 in MeOH), and chlorination ($SOCl_2$ in pyridine), (+)- and (-)-1 were converted into (-)-**7** [65% from (+)-1]: mp 100 °C; $[\alpha]_D$ -84.3° (*c* 0.25, $CHCl_3$), and (+)-7 [65% from (-)-1]: mp 100 °C; $[\alpha]_D$ +80.2° (*c* 0.26, $CHCl_3$), respectively. Chlorination [SO_2Cl_2 with a trace of $AlCl_3$ (Kleiman, 1952)] of (+)-1 produced (-)-**8** (40%): mp 103 °C; $[\alpha]_D$ -9.6° (*c* 1.44, $CHCl_3$) and that of (-)-1 gave (+)-8 (40%): mp 103 °C; $[\alpha]_D$ +9.3° (*c* 1.43, $CHCl_3$). Optically active heptachlor epoxides, i.e., (+)-**9** [mp 166 °C; $[\alpha]_D$ +92.8° (*c* 0.21, $CHCl_3$)], and (-)-9 [mp 166 °C; $[\alpha]_D$ -94.1° (*c* 0.22, $CHCl_3$)], were prepared from (-)- and (+)-2, (76 and 61%), respectively, through chlorination (Cl_2 in $CHCl_3$–CCl_4) and subsequent epoxide formation (N/5 KOH in aqueous dioxane) (Carlson, 1964).

Both (-)- and (+)-**2** were converted into dechlorinated tricyclic ketones **10** and **10′**, respectively, by successive treatment with an excess of $LiAlH_4$ in THF, Pd/C in MeOH under hydrogen, and then CrO_3 in AcOH. All compounds synthesized in this communication showed satisfactory spectral (IR, NMR, and mass) data, which were identical with those of authentic specimen of the racemates, except optical rotation.

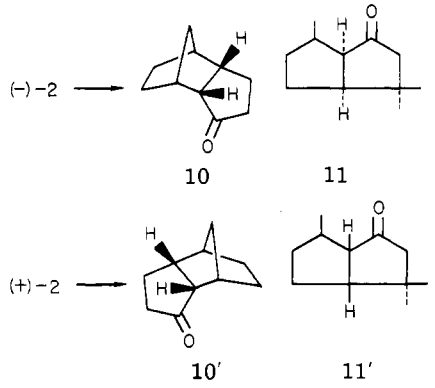
RESULTS AND DISCUSSION

The absolute stereochemistry of optically active cyclodiene insecticides synthesized above was determined by CD of dechlorinated tricyclic ketones, **10** and **10′**. The former CD Cotton effect, observed at 302 nm ($[\theta]_{302}$ -8650), and the latter one at 303 nm ($[\theta]_{303}$ +9310), when compared with those of reference compounds such as **11** ($[\theta]_{302}$ -11520) and **11′** ($[\theta]_{301}$ +11350) (Crabbe, 1972), established that the cis fused bicyclo[3.3.0]octanone system in **10** has the same absolute stereochemistry as that of **11**, and the one in **10′** as that of **11′**. Thus, (+)-chlordene was concluded to have a structure as (+)-**1**, and the (-) one as (-)-**1**. The absolute stereochemistry of the other compounds derived from enantiomers of **1** and **2** was determined as shown in Scheme I.

Table I. Insecticidal Activity of the Enantiomers of Chlordene, Chlordene Epoxide, and Heptachlor Epoxide[a]

	Dose, μg/g				
	300	233	178	126	94.4
(+)-Chlordene, (+)-1	100	94.4	72.2	33.3	11.1
Racemic chlordene, (±)-1	28.6				
(-)-Chlordene, (-)-1	0.0				
	Dose, μg/g				
	200	133.3	88.9	59.3	
(-)-Chlordene epoxide, (-)-6	86.7	73.3	66.7	33.3	
Racemic chlordene epoxide, (±)-6	66.7	40.0	13.3	0.0	
(+)-Chlordene epoxide, (+)-6	0.0				
	Dose, μg/g				
	6.0	3.0	1.5	0.75	0.375
(+)-Heptachlor epoxide, (+)-9	100	86.7	46.7	33.3	6.7
Racemic heptachlor epoxide, (±)-9	93.3	80.0	33.3	13.3	0.0
(-)-Heptachlor epoxide, (-)-9	93.3	46.7	13.3	0.0	0.0

[a] A sample dissolved in 1.25 μL of acetone was applied topically to 10 to 20 numbers of insects per dose, and the bioassay was replicated twice. The toxicity was expressed as percentage mortality at 24 h after application of the sample, and none of the control insects treated only with acetone showed mortality under these experimental conditions.



Insecticidal activity of the enantiomers of cyclodiene compounds thus prepared was measured, except chlordanes, on male adults of German cockroach. The results are shown in Table I. (+)-Chlordene, (-)-chlordene epoxide, and (+)-heptachlor epoxide, all of which have the same absolute stereochemistry, exhibited stronger activity than the corresponding racemates and antipodes. Among them, the remarkable difference was found between the enantiomers of chlordene as well as chlordene epoxide. $LD_{28.6}$ of (+)-chlordene was calculated as 129 μg/g, and LD_{50} of (-)-chlordene epoxide and its racemate, to be 76 and 157 μg/g, respectively. These data suggested that the insecticidal activity of these racemates is attributed mainly to the active enantiomer contained in the racemate, unrelated with another inactive enantiomer contained in the same racemate. LD_{50} of (+)-, racemic and (-)-heptachlor epoxide was calculated as 1.29, 1.82, and 2.98 μg/g, re-

spectively. In the case of heptachlor epoxide, the difference between the enantiomers was not so remarkable (about 2.3 times) as compared with the above two compounds. Recently, cyclodiene insecticides have been limited by their use as agricultural chemicals, because of their carcinogenic and chronic toxicity. Our present findings may suggest that optically active forms of these insecticides should be further investigated on their mode of action, biochemical metabolism in animals, and possible use as agricultural chemicals.

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Modification of the Mojonnier Fat-Testing Method for Soy-Protein-Lipid Concentrate

Application of acid hydrolysis to the Mojonnier (modified Roese-Gottlieb) procedure has been studied for the determination of the lipid content of soy-protein-lipid concentrate. The time-saving new method was found to give significantly greater test values than the conventional ether-extraction method.

The standard ether-extraction (EE) procedure employing the Soxhlet apparatus for the determination of the fat content of soybean meal, soybean flour, and other similar products is known to give low values and, therefore it has been sometimes used to estimate free-fat content (Hand et al., 1964). Also, it is time-consuming. Further, acid treatment of the sample has been reported to give more accurate results (Genotti, 1968; Zhukov and Vereschagin, 1976). So, it was proposed to suitably modify the Mojonnier method (Milk Industry Foundation, 1959), widely used for dairy products, with incorporation of acid hydrolysis for soy-protein-lipid concentrate (precipitate of soymilk prepared by the method of Nelson et al., 1975).

EXPERIMENTAL SECTION

Materials. The Mojonnier equipment used was the Model D (Mojonnier Bros. Co., Chicago). The chemicals were as follows: hydrochloric acid (BDH), analytical grade, specific gravity, 1.18; 95% ethyl alcohol, specific gravity, 0.817 at 15.6 °C; petroleum ether (BDH), bp 40–60 °C and ethyl ether (Sarabhai M. Chemicals), laboratory grade.

Methods. The following variables were studied: (a) the amount of sample, (b) addition of warm water, (c) acid hydrolysis with different time–temperature combinations, (d) addition of alcohol to avoid gelling, and (e) addition of ethyl and petroleum ethers.

The alcohol levels for different extractions with different treatments were decided through preliminary trials which also included varying levels of samples as well as water. Those combinations giving quick and satisfactory separation of the two phases in the extraction flask were adopted for complete test. The experimental tests were compared with the classical EE procedure (AOAC, 1975).

RESULTS AND DISCUSSION

The results of the Mojonnier procedure with various acid treatments and those of the EE procedure have been presented in Table I. The percent fat test after first extraction with some treatments was very low, and, so, they were discarded. Among the remaining treatments, some gave a fat test fairly close to that given by EE method whereas others showed appreciably higher test values. The results with seven treatments showing higher test values were subjected to statistical analysis.

As indicated in Table II, the percent fat obtained with the EE procedure was significantly higher than that obtained after the first extraction with the Mojonnier method with or without acid treatment. However, all the seven treatments yielded appreciably higher fat values when the extraction was extended to second and third stages. Two treatments (nos. 9 and 12) giving the highest test values did not vary appreciably from each other. In the case of treatment no. 12 involving sample hydrolysis with 5 mL of acid, while the second extraction resulted in significantly higher test values, compared to the first extraction, the third extraction showed no further rise in the fat test

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