Comparative Metabolism of Enantiomers of Chlordene and Chlordene Epoxide in German Cockroaches, in Relation to Their Remarkably Different Insecticidal Activity

Both enantiomers (optically pure) of chlordene and chlordene epoxide were metabolized in German cockroaches in order to clarify the remarkable difference of toxicity between both enantiomers of these insecticides. Of four metabolic products of (+)- and (-)-chlordenes, the amount of chlordene epoxide produced from (+)-chlordene was about sevenfold larger than that from its (-) enantiomer, and this amount was comparable to the unmetabolized (-)-chlordene epoxide remaining after application of an amount comparable in toxicity to that of (+)-chlordene. These results, combined with our previous findings that (+)-chlordene produced chemically its (-) epoxide, and both (-)-chlordene and its oxidized (+) epoxide had no activity, unambiguously indicated that (-)-chlordene epoxide was insecticidal, per se, while (+)-chlordene was insecticidal after metabolic conversion to the corresponding (-) epoxide.

The remarkable difference of insecticidal activity between the enantiomers of some cyclodiene insecticides has been recently reported by us (Miyazaki et al., 1978). The (+) enantiomer of chlordene and the (-) one of chlordene epoxide, both of which have the same absolute stereochemistry, were shown to exhibit toxicity in German cockroaches, whereas their optical antipodes did not exhibit appreciable toxicity. Other cyclodiene insecticides we prepared, such as (+)-heptachlor epoxide and the (-) enantiomers of both cis- and trans-chlordanes, whose absolute stereochemistries were also the same as those of the toxic enantiomers of the above two chemicals, showed similarly the stronger activity to that of the corresponding optical antipodes; however, the difference in toxicity between the enantiomers was less (ca. 2.3 times) in these chemicals. These results clearly indicate that the absolute stereochemistry plays an important role for induction of toxicity in chiral cyclodiene insecticides. We wish now to describe the comparative metabolism of enantiomers of chlordene and chlordene epoxide in German cockroaches to show that their remarkable difference in toxicity is caused by their different metabolic pathways or by their different absolute stereochemistry.

EXPERIMENTAL SECTION

Chemicals. Both enantiomers (optically pure) of chlordene and chlordene epoxide used as substrates in the present metabolic experiments were synthesized by the methods previously described (Miyazaki et al., 1978). As reference compounds for identification of metabolic products by gas–liquid chromatography, racemic 1-hydroxychlordene, mp 213 °C; mass m/e 352 (M^+), was prepared from (±)-chlordene upon oxidation with SeO_2 in acetic acid, racemic 1-hydroxy-2,3-epoxychlordene, mp 225 °C; mass m/e 368 (M^+), from (±)-1-hydroxychlordene upon oxidation with perbenzoic acid in chloroform (Brooks and Harrison, 1965), and racemic 1,2-trans-chlordenediol, mp 130 °C; mass m/e 370 (M^+), from (±)-chlordene epoxide by acid hydrolysis.

Metabolism of Enantiomers of Chlordene and Chlordene Epoxide by German Cockroaches. Eight groups of 20 adult male cockroaches, reared for 20–25 days after emergence, were anesthetized with CO_2 gas, and the pesticide dissolved in 0.51 μL of acetone was topically applied on the abdominal region of insects. The dosages applied were approximately two-thirds of LD_{50} of the toxic enantiomer, that is, 100 μg/g (70% of LD_{50}) of chlordene and 50 μg/g (66% of LD_{50}) of chlordene epoxide.

Insects dosed with chlordene or chlordene epoxide were killed 24 and 48 h after application, respectively, by washing them with hexane three times to remove the pesticide remaining on a surface of the insect body, and then homogenized in acetone with Biotron homogenizer (Biotrona in Switzerland). The homogenized insects were extracted with diethyl ether three times, and the extracts gave after evaporation the crude metabolic products. The glass vessels for insects dosed with the pesticides were washed with acetone, and the acetone solution was evaporated to the dried residue, from which the pesticide was recovered by extracting with diethyl ether.

Purification and Identification of Metabolic Products. The diethyl ether extracts of the homogenized insects were subjected to column chromatography on Florisil (Floridine Co.). The metabolic products as well as the unchanged chemical were recovered by successive elution with 15% diethyl ether in hexane and diethyl ether. The metabolic products that eluted from the column were analyzed by gas–liquid chromatography with Varian Aerograph Model 2100, equipped with an electron-capture detector. Two kinds of glass columns (6 ft × 2 mm i.d.) contained 2% OV-17 or 2% OV-210 on Gas-Chrom Q (100–200 mesh) were operated isothermally at 180 °C. The identification and quantitative estimation of each product were carried out by comparison of their retention times with those of the authentic specimen. The optical rotation of metabolic products could not be measured owing to a small experimental scale. However, that of chlordene epoxide obtained from (+)-chlordene should be minus, based on a similarity in biological and chemical epoxidation of double bond. The ambiguity in the chiral nature of the other three products was not important because of their nontoxic properties.

RESULTS AND DISCUSSION

First, the metabolic experiments with the enantiomers of chlordene were carried out. The results obtained are shown in Table I. Four kinds of metabolic products, i.e., chlordene epoxide, 1-hydroxychlordene, 1-hydroxy-2,3-epoxychlordene, and 1,2-trans-chlordenediol, were identified from both enantiomers of chlordene. A similar amount of 1-hydroxychlordene and 1,2-trans-chlordenediol was produced from both enantiomers, but a larger amount of 1-hydroxy-2,3-epoxychlordene was produced from the (+) enantiomer. All of these products, however, have been shown nontoxic to houseflies (Brooks and Harrison, 1965). The most noticeable difference between the enantiomers was observed with chlordene epoxide, whose production from toxic (+)-chlordene was about seven times larger than that of the nontoxic (-) enantiomer. Although the optical rotation was not measured, the epoxide from (+)-chlordene

Table I. Metabolic Products of (+)- and (-)-Chlordenes by German Cockroaches[a]

applied enantiomers	total amounts of metabolic products	chlordene	chlordene epoxide	1-hydroxy-chlordene	1-hydroxy-2,3-epoxy-chlordene	1,2-*trans*-chlordenediol
			recovered amounts, μg			
			After 24 h			
(+)-chlordene	30.67[b]	7.32	2.27	7.60	10.73	2.75
	(100)[c]	(23.8)	(7.4)	(24.8)	(35.0)	(9.0)
(-)-chlordene	21.32	5.13	0.34	10.40	2.61	2.84
	(100)	(24.1)	(1.6)	(48.8)	(12.2)	(13.3)
			After 48 h			
(+)-chlordene	17.09	3.70	1.13	4.81	6.16	1.29
	(100)	(21.7)	(6.6)	(28.2)	(36.0)	(7.5)
(-)-chlordene	23.66	1.55	0.17	16.21	3.37	2.36
	(100)	(6.6)	(0.7)	(68.5)	(14.2)	(10.0)

[a] Total recovery of chemicals from all fractions, i.e., homogenized insects, the acetone solution washing insect bodies, and glass vessels rearing insects, was calculated as 52% from (+)-chlordene and 62% from (-)-chlordene applied. [b] Amounts (micrograms) of chemicals extracted from the homogenized 20 cockroaches dosed with 148 μg of (+)-chlordene or (-)-chlordene, 24 h after application. [c] Values in parentheses are percentage yields of each products to b.

Table II. Metabolic Products of (-)- and (+)-Chlordene Epoxides by German Cockroaches[a]

applied enantiomers	total amounts of metabolic products	chlordene epoxide	1-hydroxy-chlordene	1-hydroxy-2,3-epoxy-chlordene	1,2-*trans*-chlordenediol
		recovered amounts, μg			
		After 24 h			
(-)-chlordene epoxide	9.56[b]	1.76	0.68	4.18	2.94
	(100)[c]	(18.4)	(7.1)	(43.7)	(30.8)
(+)-chlordene epoxide	9.38	1.55	0.30	2.39	5.14
	(100)	(16.5)	(3.2)	(25.5)	(54.8)
		After 48 h			
(-)-chlordene epoxide	6.93	1.31	0.93	2.59	2.10
	(100)	(18.9)	(13.4)	(37.4)	(30.3)
(+)-chlordene epoxide	9.57	1.23	0.38	1.82	6.14
	(100)	(12.9)	(4.0)	(19.0)	(64.1)

[a] Total recovery of chemicals from all fractions, i.e., homogenized insects, the acetone solution washing insect bodies, and glass vessels rearing insects, was calculated as 72% from (-)-chlordene epoxide and 75% from (+)-chlordene epoxide applied. [b] Amounts (micrograms) of chemicals extracted from the homogenized 20 cockroaches dosed with 74 μg of (-)-chlorodene epoxide or (+)-chlordene epoxide, 24 h after application. [c] Values in parentheses are percentage yields of each product to b.

should be optically minus, based on a similar chemical epoxidation reaction. Thus, the amount of (-)-chlordene epoxide (2.27 and 1.13 μg/20 cockroaches 24 and 48 h after application, respectively) produced from the (+)-chlordene was almost comparable to (1.76 and 1.31 μg, Table II) that of unchanged (-)-chlordene epoxide that remained in the insects when the latter compound was applied at a rate that yielded comparable toxicity (ca. 70% of LD_{50}). These results strongly indicate that the insecticidal activity of (+)-chlordene should become active after its metabolic conversion via epoxidation to (-)-chlordene epoxide and that (+)-chlordene itself is nontoxic.

Next, the metabolic products of toxic (-)-chlordene epoxide and nontoxic (+)-chlordene epoxide were investigated. The results obtained are shown in Table II. Three kinds of metabolic products, 1-hydroxychlordene, 1-hydroxy-2,3-epoxychlordene, and 1,2-*trans*-chlordenediol, were identified from the both enantiomers, besides unchanged chlordene epoxide. In this case, no significant difference was observed between the enantiomers in metabolic pathways and amounts. The in vivo hydroxylation at the α position, hydration, or deoxygenation of an epoxy ring occurred similarly on the both enantiomers. These results clearly indicate that the different toxicity between the enantiomers of chlordene epoxide was not ascribed to the difference of the metabolic pathways, but to their absolute stereochemistry. Only the (-) enantiomer is capable of inducing toxicity when the both enantiomers (that is, racemic mixture) contact with a chiral receptor site of the nervous system of insects. Figure 1 shows structures
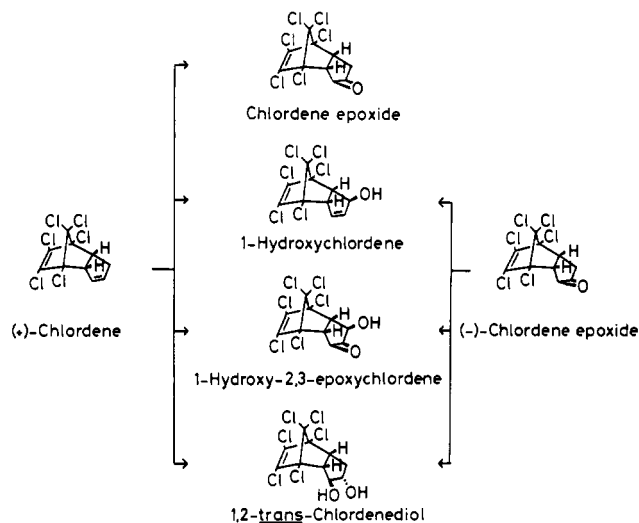

Figure 1. Structures of four and three metabolic products obtained from (+)-chlordene and (-)-chlordene epoxide, respectively.

of four and three metabolic products obtained from (+)-chlordene and (-)-chlordene epoxide, respectively.

The present studies, combined with our previous investigation (Miyazaki et al., 1978), showed that chlordene epoxide is insecticidal because of its (-) enantiomer. This toxicity is due to a specific absolute stereochemistry and is not due to bioactivation. The (+) enantiomer of chlordene is only active as a result of biochemical trans-

formation into its epoxide derivative, (–)-chlordene epoxide. Although several investigations to rationalize bioactivation of some cyclodiene insecticides have been reported on racemic heptachlor and achiral aldrin (Perry, 1960; Brooks and Harrison, 1963; Hamilton, 1971), our research with optically pure form of insecticides unambiguously established that (–)-chlordene epoxide is toxic, per se, while (+)-chlordene is insecticidal after metabolic conversion to the corresponding (–) epoxide.

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Effect of Differential Heat Treatments on the Protein Quality of Casein and Lactalbumin

The effect of autoclaving (121 °C for 30 or 90 min) in the presence and absence of lactose on the protein quality of casein and lactalbumin was examined. Methods of evaluation were amino acid profile (a.a.p.), 1-fluoro-2,4-dinitrobenzene-available lysine, protein efficiency ratio, modified PER, net protein ratio, and net protein utilization. The protein quality of casein was less prone to heat damage than that of lactalbumin. The a.a.p. was the least discriminating method of protein evaluation; all of the four different rat assays detected the heat damage to the proteins. Care must be exercised when choosing a method for protein quality evaluation of milk proteins since some procedures will not demonstrate heat-induced changes.

The detrimental effects of heat and of reducing sugars on the protein quality of lactalbumin and casein have been demonstrated previously (Eldred et al., 1946; Davis et al., 1949; Lea and Hannan, 1950; Schroeder et al., 1951; Osner and Johnson, 1975). However, neither Mabee and Morgan (1951) nor Ford and Shorrock (1971) could detect a significant change in the protein quality of casein after air-oven heating (140 °C) or autoclaving (121 °C). Many of the studies on the effect of heat on these two milk proteins were performed in the 1940's and 1950's. Some of these studies used unrealistic temperatures and times, and the methods of evaluating protein quality changes have been replaced by more reproducible tests.

The object of the present study is to determine chemically and biologically the effect of practical heating times and temperatures on the protein value of lactalbumin and casein. The use of amino acid profiles is being advocated for the prediction of protein quality (Happich et al., 1975). Both Bujard et al. (1967) and Boctor and Harper (1968) state that the acid hydrolysis of the protein into its constituent amino acids is a poor measure of protein quality in a heated protein because there is no distinction between biologically available and unavailable amino acids. Furthermore, protein efficiency ratio (PER) is the biological assay required by regulatory agencies in the United States if claims are made concerning the protein quality of a food. Large discrepancies are reported in the literature for the PER values of the casein control diet (Hegarty, 1975). It seemed imperative, therefore, that the effect of a reducing sugar, lactose, and of realistic heating temperatures and

times be determined on casein and lactalbumin by measuring amino acid profiles, chemically determined available lysine, and the comparison of four different biological tests for protein quality.

MATERIALS AND METHODS

Casein (Humko Sheffield, Norwich, NY) or lactalbumin (Sigma Co., St. Louis, MO) was placed in shallow aluminum pans to a depth of 3 cm and autoclaved (American Sterilizer Co., Erie, PA; Model 57-CR) at 121 °C using the automatic fast exhaust and dry cycle. Washed lactalbumin was prepared by adding 10 L of distilled water to 1 kg of lactalbumin. The mixture was stirred for 1 min, and then the powder was allowed to settle at room temperature. Most of the water was siphoned off, and the wash procedure was repeated 15 times. The final two washings were with doubly distilled water. The washed lactalbumin was freeze-dried and then either autoclaved for 30 min (see above) or left untreated. The casein plus lactose mixture was prepared by mixing 950 g of casein and 50 g of β-D-lactose in 2.5 L of doubly distilled water. This mixture was freeze-dried, yielding a casein mixture containing approximately 5% lactose. This amount of lactose is similar to the lactose concentration in commercial grade lactalbumin. The material was then autoclaved for 30 min as described above.

The following biological and chemical tests were performed: protein efficiency ratio (PER) (AOAC, 1965), modified PER (McLaughlan, 1976), net protein utilization (NPU) (Miller and Bender, 1955), net protein ratio (NPR)