

residual 1-hexanal-forming activity of chloroplasts treated with potato LAHase at the same inhibition rate as the enzyme activity of untreated chloroplasts.

During harvesting, storage, and processing of vegetables including green leaves, LAHase may attack membrane lipids, as LAHase involved in green leaves hydrolyzes both galactolipids and phospholipids (Matsuda et al., 1979; Matsuda and Hirayama, 1979). Free C₁₈ fatty acids thus released by hydrolysis of lipids may be better substrates than esters of fatty acids (e.g., glycerolipids) for C₆ aldehyde formation in tea leaves (Sekiya et al., 1976) and cucumber fruits (Galliard and Phillips, 1976). However, degradation of membrane lipids during harvesting, storage, and processing of vegetables may cause inactivation of the enzyme system responsible for C₆ aldehyde formation from C₁₈ fatty acids, even though degradation of membrane lipids provides free fatty acids. Therefore, it is important to know rates of acyl hydrolysis of lipids and inactivation of C₆ aldehyde forming activity by leaf LAHase to evaluate flavor (C₆ aldehyde) formation.

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Convenient Preparation of Chlordecone Alcohol (Kepone Alcohol) and Its Deuterated, Tritiated, and Dechlorinated Derivatives

Successful synthesis of chlordecone alcohol and its deuterated, tritiated, and dechlorinated analogues is described. Borohydride reduction of chlordecone or of one of its dechlorinated derivatives resulted in the formation of the corresponding alcohol in greater than 60% yield. Characterization of reduction products was established by chromatographic (GLC; TLC) and spectral (IR; NMR; MS) analyses.

Chlordecone (CD; 1,1a,3,3a,4,5,5,5a,5b,6-decachlorooctahydro-1,3,4-metheno-2H-cyclobuta[cd]pentalen-2-one), an organochlorine pesticide reported to be a liver carcinogen in rats and mice (Reuber, 1978), was manufactured in Hopewell, VA, from 1966 to 1975. Due to inappropriate industrial disposal procedures, primarily by a small, independent company, this pesticide now contaminates the rivers and marine life in the Tidewater region of Virginia. It is anticipated that area residents will be exposed to small amounts of this chemical for the foreseeable future since CD undergoes minimal degradation in the environment.

The only alteration products of CD detected in environmental samples (water, soil, and fish) are low levels of dechlorinated derivatives presumably formed by photolysis (Harless et al., 1978; Carver and Griffith, 1979). In contrast, a major pathway for CD (1) metabolism in humans has recently been discovered. The first step in the biotransformation of CD is its reduction to chlordecone alcohol (2) (CDOH; 1,1a,3,3a,4,5,5,5a,5b,6-decachlorooctahydro-1,3,4-metheno-2H-cyclobuta[cd]pentalen-2-ol). In chemical workers poisoned with large quantities of CD, reduction appears to be the major pathway for CD inas-

much as 75% of the total pesticide excreted in bile is CDOH (Fariss et al., 1980). Because it was previously believed that CD is not subject to metabolism, little information is available regarding the measurement or the toxicology of CDOH. In order to acquire needed information regarding the pharmacokinetics and the toxicity of this reduced form of CD, synthetic procedures were developed to provide pure standards of CDOH and related derivatives.

EXPERIMENTAL SECTION

Materials. CD (1) (99% pure), monohydrochlordecone (5) (monohydro-CD; 1a,3,3a,4,5,5,5a,5b,6-nonachlorooctahydro-1,3,4-metheno-2H-cyclobuta[cd]pentalen-2-one) (95% pure), and dihydrochlordecone (6) (dihydro-CD; 1a,3,4,5,5,5a,5b,6-octachlorooctahydro-1,3,4-metheno-2H-cyclobuta[cd]pentalen-2-one) (98% pure) were provided by the Environmental Protection Agency (Research Triangle Park, NC). Sodium borodeuteride (99% deuterated) was purchased from Stohler Chemicals (Waltham, MA) and tritiated sodium borohydride (260.2 mCi/mM) from New England Nuclear (Boston, MA).

Gas-Liquid Chromatography (GLC). A Hewlett-Packard Model 5840 gas chromatograph equipped with a ^{63}Ni electron capture detector and a 1.8 m \times 2 mm glass column packed with 3% OV-101 on Supelcoport (80–100 mesh) was used. The column temperature was held at 190 °C for 5 min and then programmed from 190 to 240 °C at 5 °C/min with an argon-methane (95:5 v/v) flow rate of 25 mL/min.

Thin-Layer Chromatography (TLC). A precoated (0.25 mm) silica gel 60 F-254 aluminum sheet was developed at room temperature with benzene-ethyl acetate (80:20 v/v) until the solvent front was 15 cm above the origin. A Packard radiochromatogram scanner was used for visualization.

Spectral Information. Chemical ionization (CI) and electron impact (EI) mass spectra were obtained with a Hewlett-Packard Model 5985A gas-liquid chromatograph-mass spectrometer data system. The electron energy and emission current was 150 eV and 155 μA , respectively, in the CI mode and 70 eV and 300 μA in the EI mode. Nuclear magnetic resonance (NMR) spectra were run on a Hitachi Perkin-Elmer R-24 high-resolution instrument using deuterated chloroform (with 1% tetramethylsilane). Infrared absorption (IR) spectra were run on a Beckman Acculab 8 instrument with a Nujol mull and NaCl windows.

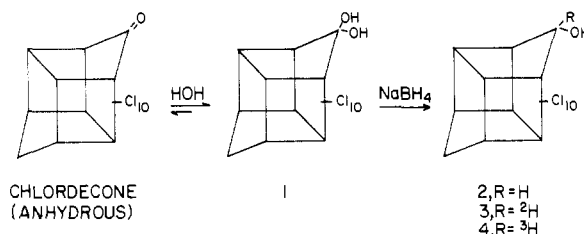
Synthesis of CDOH (2), Deuterated CDOH (3), and Tritiated CDOH (4). A solution of CD (1) (0.5 g, 1.0 mM) in 10 mL of 2-propanol was added to a test tube containing a 3-fold excess of sodium borohydride (NaBH_4) (0.1 g, 2.6 mM). After the solution was stirred for 24 h, the 2-propanol was evaporated under reduced pressure and 10 mL of 5% H_2SO_4 was added. The acidic solution was extracted with benzene and the organic extract was washed several times with 0.5 N NaOH to eliminate unreacted CD. The benzene was then evaporated and the product was recrystallized from hexane, giving 0.3 g (62% yield) of CDOH (2). Substitution of deuterated NaBH_4 or tritiated NaBH_4 for NaBH_4 in the preceding procedure resulted in the formation of deuterated CDOH (3) and tritiated CDOH (4), respectively. Because reduction with tritiated NaBH_4 generates tritiated hydrogen gas, a gas buret was used to trap the radioactive gas and permit proper disposal.

Synthesis of Monohydrochlordecone Alcohol (7) and Dihydrochlordecone Alcohol (8). The reducing agent, zinc borohydride [$\text{Zn}(\text{BH}_4)_2$], was prepared by adding NaBH_4 (80 mg, 2.1 mM) to a solution of zinc chloride (157 mg, 1.2 mM) in 2-propanol. After the mixture was stirred for 30 min, the pH was measured to confirm neutrality. The $\text{Zn}(\text{BH}_4)_2$ solution was then combined with a solution of monohydro-CD (5) (24 mg, 0.05 mM) or dihydro-CD (6) (15 mg, 0.04 mM) in 2 mL of 2-propanol and stirred for 24 h in an ice bath. The final product, monohydrochlordecone alcohol (7) (monohydro-CDOH; 1a,3,3a,4,5,5a,5b,6-nonachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta[*cd*]pentalen-2-ol) (14.5 mg, 62% yield) or dihydrochlordecone alcohol (8) (dihydro-CDOH; 1a,3,4,5,5a,5b,6-octachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta[*cd*]pentalen-2-ol) (9 mg, 60% yield), was then isolated by using the procedure described for CDOH synthesis.

RESULTS AND DISCUSSION

Successful synthesis of CDOH has been reported by several investigators using lithium aluminum hydride (LiAlH_4) as the reducing agent (Gilbert et al., 1966; Walle, 1965). Our attempts to reduce CD with this metal hydride resulted in the formation of not only CDOH but also dechlorinated derivatives of CDOH (unpublished data).

Scheme I



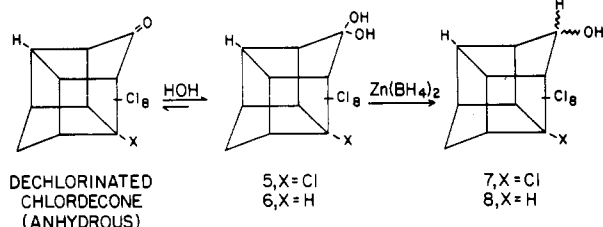
Because separation of the latter products from CDOH requires tedious chromatographic procedures, a new synthetic method was developed. One explanation for the dechlorination of CDOH could be due to the strong alkaline nature of LiAlH_4 . Accordingly, a much weaker base, NaBH_4 , was used in the present study and has proven to be an ideal reducing agent for CD. The NaBH_4 reduction procedure is rapid and safe. The conversion forms essentially only one product, CDOH (2), in a 62% yield. GLC analysis of CDOH showed only one peak at a retention time (RT) of 10.5 min. The CI and EI mass spectra, the IR spectrum and the NMR spectrum were identical with those previously reported for CDOH (Gilbert et al., 1966; Harless et al., 1978; Dilling et al., 1967; Dilling and Dilling, 1967).

The reduction of CD with deuterated NaBH_4 also forms a single product, deuterated CDOH (3). Analysis of this compound by GLC demonstrated one peak at an RT identical with that of CDOH. The NMR spectrum displayed a broad band centered around 2.6 ppm ($-\text{OH}$), but unlike CDOH a singlet was not observed at 4.4 ppm ($-\text{CH}$). The CI mass spectrum was characterized by ion clusters at m/e 490 ($\text{M} + \text{H}$) $^+$, m/e 472 [$(\text{M} + \text{H}) - \text{HOH}$] $^+$, and m/e 454, ($\text{M} - \text{Cl}$) $^+$. Except for a 1 mass unit increase resulting from the substitution of a deuterium for a hydrogen, these ions are identical with those observed for CDOH. The EI mass spectrum demonstrated major fragments at m/e 184, 219, and 235. The base peak fragment at m/e 219 ($\text{C}_5\text{Cl}_4\text{DOH}$) $^+$ resulted from the splitting of the pentacyclodecane nucleus in half, like CDOH but with the addition of 1 mass unit. Though experimental use of deuterated CDOH is limited to analytical procedures, its synthesis and spectral analysis validated the proposed structures of compounds prepared by NaBH_4 reduction of CD (Scheme I).

Following each borohydride reduction, unreacted CD was eliminated from the final product with a NaOH wash. Unfortunately, in the case of tritiated CDOH, purification was not achieved by alkali extraction. We have no explanation for this finding. Since complete reduction of CD was essential in preparing this tritiated compound, initial reduction of CD with tritiated NaBH_4 was followed by complete reduction with NaBH_4 . This resulted in a CD free product, tritiated CDOH (4) (21.1 mCi/mM). The chemical and radiochemical purity of this compound was verified by GLC and TLC analyses. Both chromatographic techniques demonstrated one peak (99%) at an RT or R_f value (0.52) identical with that of CDOH.

An unexpected finding was that reaction of NaBH_4 with dechlorinated derivatives of CD resulted in additional dechlorination as well as reduction. Mass spectral analysis confirmed the products of monohydro-CD reduction as monohydro-CDOH (33%) and dihydro-CDOH (67%) while the reduction of dihydro-CD formed dihydro-CDOH (33%) and trihydrochlordecone alcohol (67%). As previously suggested for CDOH formation, dehalogenation may be related to the alkaline nature of the reducing agent. So that this idea could be tested, a neutral reducing agent,

Scheme II



$\text{Zn(BH}_4)_2$ (Yoon et al., 1976) was used to reduce monohydro-CD (5) and dihydro-CD (6). Analysis of the products by GLC demonstrated only monohydro-CDOH (7) (RT = 8.3 min) or dihydro-CDOH (8) (RT = 5.7 min) and no other dechlorinated derivatives. The stereochemistry of monohydro-CDOH (7) is unknown. The CI mass spectra for these reduction products were characterized by the ion clusters; $(M + H)^+$, $[(M + H) - \text{HOH}]^+$, and $(M - \text{Cl})^+$. These ions were found at m/e 455, 437, and 419 for monohydro-CDOH and m/e 421, 403, and 385 for dihydro-CDOH. The EI mass spectra demonstrated major fragments at m/e 149, 184 (base peak), and 218 for monohydro-CDOH and m/e 149 and 184 (base peak) for dihydro-CDOH. These data suggest that reduction of monohydro- and dihydro-CD with $\text{Zn(BH}_4)_2$ forms pure standards of monohydro- and dihydro-CDOH while avoiding further dehalogenation (Scheme II). The dechlorinated derivatives of CD appear more sensitive to the alkalinity of the reducing agent than CD itself. Consequently, the structure of the reactant in addition to the base strength of the reducing agent must be considered in the preparation of CDOH and its analogues.

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Distribution of Chlorinated Pesticides in Animal Feed Components and Finished Feeds

Frequency of occurrence and levels of selected organohalogens were monitored in animal feed components and animal feed over a 7-year period. Random samples collected for quality assurance were screened for lindane, β -BHC, aldrin, dieldrin, p,p' -DDE, o,p' -DDT, p,p' -DDD, p,p' -DDT, heptachlor epoxide, and heptachlor. Gas chromatographic analysis revealed average organochlorine contamination levels of less than 10 ppb.

Numerous examples of contamination of animals through animal feeds have been reported in the literature (Buck, 1970, 1975; Van Houweling et al., 1977). However, public awareness of this did not become acute until 1974 when polybrominated biphenyls (PBB) were associated with an animal feed accident in Michigan. The direct impact of this incident resulted in the destruction of 30 000 cattle and 6000 swine, as well as sheep, poultry, and dairy products. In addition, hundreds of millions of dollars in lawsuits remain to be settled.

The total indirect impact of this and other incidents has yet to be measured. However, one of the outgrowths has been the difficulty of feed companies in obtaining and/or continuing to maintain liability insurance.

In an attempt to circumvent this problem, the University of Iowa, supported by a local feed company, developed a

program for the monitoring of selected organohalogens, organophosphorus, and inorganic contaminants in the production of animal feeds. This paper presents the protocol and results of this program for organohalogen compounds which has been in existence for the last 7 years and represents a sale of more than 100 million dollars worth of feed products.

EXPERIMENTAL SECTION

General. A list of animal feed components, which represent the input into this company, is presented in Table I. The components are divided into four major categories: grains, meals, byproducts, and miscellaneous. The grain category comprises the largest portion of the feed. Although pesticides are widely used in grain production, residue levels in the harvested grain are relatively