SYNTHESIS OF ^{14}C-DESMETHYLENE ALDRIN, DESMETHYLENE DIELDRIN* AND DESMETHYLENE ALDRIN CIS AND TRANS-DIHYDRODIOLS

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Summary

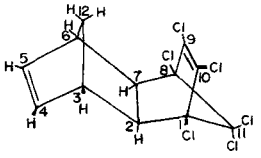
The synthesis of ^{14}C-desmethylene aldrin (I) from ^{14}C-U1-hexachloro cyclopentadiene and cyclohexa-1,4-diene via a Diels-Alder reaction and its conversion to desmethylene dieldrin (II) and desmethylene cis (III) and trans (IV)-dihydrodiols are described.

Introduction

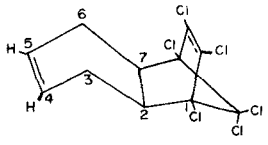
Polycyclodiene pesticides such as aldrin and dieldrin have been used extensively as chemical models for the study of the mechanism of oxidative (epoxidative, hydroxylative, etc.) and hydrolytic reactions which occur in the metabolism and detoxification of these chlorinated compounds. Although the metabolisms of both aldrin and dieldrin have been extensively elaborated[3],

*Desmethylene aldrin (DMA) and desmethylene dieldrin (DMD) refer to 1,2,3,4,9,9-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-methanonaphthalene and 1,2,3,4,9,9-hexachloro-1,4,4a,5,6,7,8,8a-octahydro-6,7-epoxy-1,4-methanonaphthalene respectively as described by Brooks & Harrison (1). DMD is also known as HEOM (II) (2).

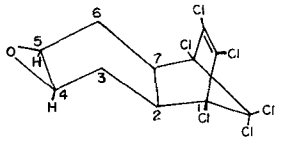
work at this location indicated that these chlorinated polycyclodiene insecticides also constitute good models for investigations into the stereospecificities of their enzyme reactions[4-6], since both are quite stable and have a rigid structure. This structural rigidity results in part from the methylene bridge joining C3 to C6 (Fig 1) preventing movement at these carbon atoms‡. The isolated double bond at



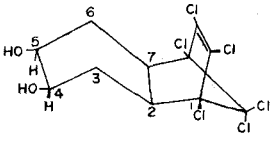
Aldrin



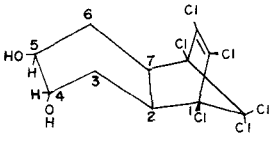
I. Desmethylene Aldrin



II. Desmethylene Dieldrin



III. Cis-dihydrodiol



IV. Trans-dihydrodiol

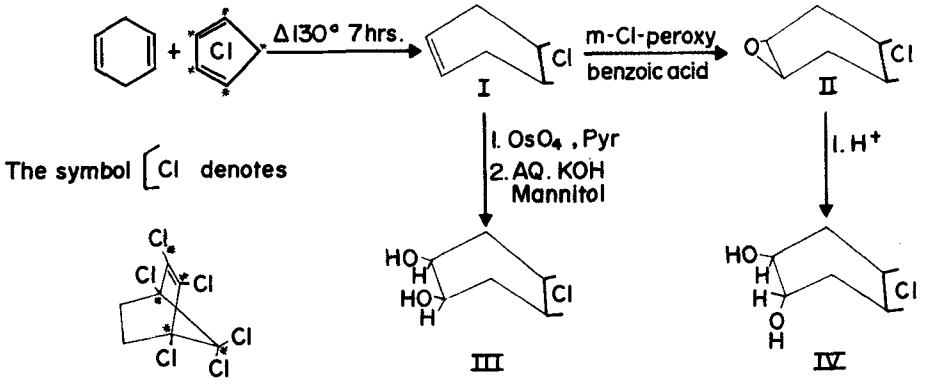
C4 in the case of aldrin and the epoxide oxygen linking C4 and C5 in dieldrin are of primary importance in biochemical pathways leading to the metabolism and detoxification of both these compounds; therefore, the possible relationship between structural rigidity and enzymatic stereospecificity involving reactions at these sites was of particular interest. It was postulated that desmethylene aldrin (I), desmethylene dieldrin (II), and their derivatives would provide less sterically

‡The numbering system is that used throughout our published work with these compounds.

specificities in enzyme reactions and rates of reaction involving the double bond at C4 (DMA) or the epoxide oxygen (DMD). In order to facilitate these studies the synthesis of ^{14}C-desmethylene aldrin, ^{14}C-desmethylene dieldrin, and ^{14}C-desmethylene aldrin cis and trans-dihydrodiols was undertaken.

Discussion

The sequence of reactions used to synthesize DMA and its derivatives is outlined in Scheme 1. Uniformly labeled ^{14}C-hexachlorocyclopentadiene, specific activity 5.31 mCi/mM, was obtained from Mallinckrodt Nuclear (now California Bionuclear, Sun Valley, Ca.) and diluted with unlabeled hexachlorocyclopentadiene to specific activity of 1.06 mCi/mM. Cyclohexa-1,4-diene was obtained from Aldrich Chemical Co. (Milwaukee, Wisc.). The synthesis of DMA and DMD



has been previously reported by Brooks and Harrison (1). Desmethylene aldrin trans-dihydrodiol (IV) was prepared according to the method described previously by McKinney et al. (7) for aldrin dihydrodiol. This involves refluxing ^{14}C-DMD in a 1,4-dioxane-water solution with sulfuric acid. ^{14}C-DMA cis-dihydrodiol was also prepared according to the previous procedure of McKinney et al. which was used to prepare aldrin cis-dihydrodiol. This method requires oxidation of the double bond (C4) with osmium tetroxide in the presence of pyridine followed by reductive hydrolysis of the osmate ester with alkaline D-mannitol. Desmethylene

aldrin and its derivatives were purified by preparative thin layer chromatography on Silica Gel GF plates (250 micron, Analtech) utilizing several different mobile phases.

The stereochemical structure for DMD (II) was determined since it was not known if the Diels Alder reaction used would afford the desired endo-exo skeletal ring fusion found in dieldrin or possibly the endo-endo ring fusion found in endrin. In addition the configuration of the epoxide (DMD) was in question since the conformational equilibrium of DMA could yield both exo and endo (in relation to the dichloromethylene bridge) epoxides.

It was not possible to make first order proton assignments for DMA and its derivatives due to complex coupling patterns with much overlapping of proton signals. The stereochemical structure was studied by ^{1}H nuclear magnetic resonance (NMR) using chemical shift reagents in conjunction with homonuclear decoupling experiments as in previous studies of chlorinated polycyclodiene pesticides done at this laboratory[4,8]. Additional data to support the stereochemical structure given for desmethylenedieldrin in Figure 1 was obtained from ^{13}C-NMR studies involving both the use of shift reagents and heteronuclear decoupling experiments using dieldrin as a comparative model. The ^{13}C chemical shifts observed for carbons 2, 3, and 4 in DMD were C2-42.356 PPM, C3-22.284 PPM, and C4-49.204 PPM.

Labeled compounds were detected by autoradiography, thin layer chromatography using a radioscanner, and UV light when sufficient material was present (with silica gel GF). Quantitation of radioactivity was done by removing radioactive spots or bands and counting in a liquid scintillation system. Non-radioactive spots were visualized with UV light and exposure to iodine vapor. The crude ^{14}C-DMA and DMD which had been retained and the dihydro diols (III, IV) were characterized and their radiochemical purity determined by using gas chromatography (GC), thin layer chromatography (TLC), mass spectroscopy (MS), and both ^{1}H and ^{13}C-NMR spectroscopy. Gas chromatography was done on a Varian model 1520B gas chromatograph utilizing a flame ionization detector with a 10% OV-101 column on gas chrom Q (Applied Science Laboratories, Inc.) at

220°C. Thin layer chromatography was done on Silica Gel, GF-250 μ (Analtech, Inc.) utilizing the solvent systems in Table 1. Finnigan model 1015D quadrupole chemical ionization and electron impact mass spectrometers were used to obtain the low resolution mass spectra of the various products. The NMR studies were made with Varian XL-100 Fourier transform and T-60 spectrometers. Radiopurity was determined by scanning the thin layer chromatograms with a Varian Aerograph radioscanner and by exposure to "Kodak No Screen" medical X-ray film (autoradiography). Radioactive bands were removed and quantitated by counting in a Packard Tri-carb liquid scintillation system.

The labeled products thus purified had both a chemical and radiochemical purity of >99% except DMD (96%). All products were indistinguishable from unlabeled standards by GC and TLC. Yields of purified products based on their crude precursors, specific activity, total activity and radiochemical purity are given in Table 2.

Table 1

THIN LAYER CHROMATOGRAPHIC DATA

	$R_{dieldrin}$[1] in Solvent Systems (Silica Gel GF-250μ)		
Compound	Hexane	Hexane: Acetone (9:1)	Hexane: Acetone (2:1)
I	7.6	1.2	1.1
II	1.0	0.93	0.96
III	-[2]	0.12	0.64
IV	-[2]	0.10	0.55

1. $R_{dieldrin} = R_f$ compound/R_f dieldrin
2. These compounds remained at origin in hexane

Table 2

YIELDS AND RADIOACTIVITY

Compound	Yield[1] in mg (%)	Specific Activity (mCi/mM)	Total Activity (μci)	Radiochemical Purity
I	10.4 (42%)	1.08	31.8	>99%
II	12.3 (38%)	1.00	33.2	∿96%
III	34.6 (41%)	1.09	97.2	>99
IV	18.1 (31%)	0.91	42.3	>99

1. Yields (%) of II, III, and IV are based on crude precursor.

Experimental

Desmethylene Aldrin (I)-^{14}C at Chlorinated Carbons

^{14}C-Ul-Hexachlorocyclopentadiene (41.2 mg, 0.15 mmole, 0.80 mCi) was diluted to a specific activity of 1.06 mCi/mmole with 164.8 mg unlabeled hexachlorocyclopentadiene and placed in a thick wall sealable tube along with 0.150 ml cyclohexa-1,4-diene and 10 mg quinol. The tube was evacuated, sealed with a torch and the reaction mixture heated at 130°C for 7 hours in an oil bath. Upon cooling to room temperature the contents were transferred to a Büchi Kugel-Rohr distillation apparatus with several washings of hot chloroform. The product distilled at 140-160°C 2 mmHg leaving the bis-adduct as a high boiling residue. Yield crude I: 188 mg (70% based on hexachlorocyclopentadiene). A portion (17.5 mg) of this product was retained for later purification by thin layer chromatography.

Desmethylene Dieldrin (II)-^{14}C

Crude desmethylene aldrin (I), 94 mg (0.27 mmole) and 95 mg (0.55 mmole) m-chloroperoxybenzoic acid were reacted in 12 ml chloroform at room temperature for 60 hours. The reaction mixture was washed once with a 10% sodium carbonate solution and twice with water (until neutral pH), dried over anhydrous sodium sulfate and evaporated under nitrogen to yield 87 mg (88% theory based on I) of crude desmethylene dieldrin. A portion of this product (29 mg) was retained for later purification by thin layer chromatography.

Desmethylene Aldrin-cis-dihydrodiol (III)-^{14}C

Osmium tetroxide (46 mg - 0.18 mmoles) in 4.6 ml absolute ether was added to 77 mg (0.22 mmoles) of crude I in 2.0 ml absolute ether along with 0.15 ml pyridine. The mixture was stirred magnetically for 2 hours and allowed to stand overnight unexposed to any light. The ether was then evaporated under nitrogen, and the brown residue was dissolved in 10 ml methylene chloride. The DMA-osmium tetroxide-pyridine adduct was hydrolyzed by stirring vigorously with 6.5 ml water containing 67 mg (1.2 mmoles) potassium hydroxide and 638 mg (3.5 mmole) mannitol until the dark methylene chloride layer became almost colorless while the aqueous phase became a dark red-brown. The methylene chloride layer was removed, washied once with 10 ml water and dried over anhydrous sodium sulfate. Evaporation of the methylene chloride extract yielded 71 mg (85% theory base on crude I) of crude cis-dihydrodiol (III).

Desmethylene Aldrin-Trans-Dihydrodiol (IV)-^{14}C

Crude desmethylene dieldrin (II, 58 mg - 0.157 mmoles), 10 ml water, 5 ml 1,4-dioxane, and 0.8 ml concentrated sulfuric acid were refluxed for 72 hours. On cooling to room temperature, the reaction mixture was diluted with 20 ml water and extracted twice with 40 ml chloroform. The combined chloroform extracts were washed once with a saturated sodium bicarbonate solution and twice with distilled water before drying over anhydrous sodium sulfate. The chloroform was evaporated to yield 46 mg (78% theory based on crude II) of crude trans-dihydrodiol (IV).

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