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DETERMINATION OF OPTICAL PURITY AND PROCHIRALITY OF CHLORINATED POLYCYCLODIENE PESTICIDE METABOLITES

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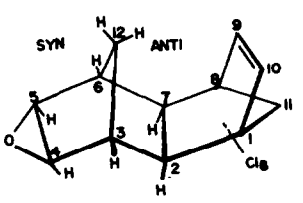
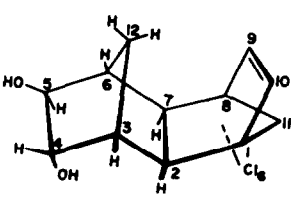
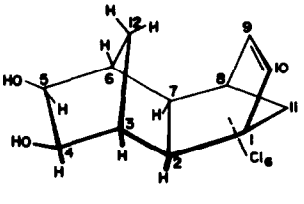
For some time it has been known (1) that dieldrin (I), one of the most extensively used chlorinated polycyclodiene pesticides, is metabolized in a variety of systems to trans-4,5-dihydroxy-4,5-dihydroaldrin (II). There is at least one report (2) that the trans-diol metabolite is optically active suggesting stereospecific opening of the epoxide ring. Dieldrin is a rather stable epoxide whose steric constraints may play a role in the ring opening process in an enzymatic reaction. In this regard, there is chemical evidence (3) that under conditions favoring intimate ion pair formation, possibly simulating the enzyme reaction, the epoxide is opened to an appreciable extent to form the cis-4,5-dihydroxy-4,5-dihydroaldrin (III) in preference to the trans product.



I



II



III

Metabolism studies (in vitro with rat liver tissue) in our laboratory (4) designed to detect the formation of the cis-diol have shown that it is indeed a metabolite of dieldrin and appears to reach a steady state concentration while the trans-diol production increases constantly. These results suggested that the cis-diol might be an intermediate in the formation of the trans diol and that optical activity observed in this product could result from stereospecific epimerization of III. Metabolism studies designed to determine the feasibility of this

epimerization process were carried out, and the results indicated that the cis-diol could be rapidly converted to trans. Approximately 4 mg of metabolite II was isolated from the epimerization studies and was then subjected to nmr analysis in the presence of a chiral shift reagent [tris (3-heptafluoropropylhydroxymethylene)-d-camphorato)europium (III)], $Eu(HFC)_3$ (5), to measure its optical purity and thereby possibly confirm the stereospecificity of this biological epimerization reaction. The nmr studies were done on a Varian XL-100 Fourier transform (FT) system. The results of these studies involving optically inactive racemic trans-diol and meso-cis-aldrin diol, prepared as previously reported (6), and the biological trans-diol metabolite are the subject of this report.

The quantity available and the solubility of standard synthetic II were sufficient to allow spectra to be obtained in a single scan in the CW mode. The limited solubility of cis-diol III in chloroform-d and the limited amount of isolable metabolite, however, made it necessary to average 40 to 200 transients in the FT mode for each spectrum. The proton assignments and stereochemistry of the diols have been previously (6) determined by nmr utilizing the achiral shift reagent, tris(dipivalomethanato)europium [$Eu(DPM)_3$]. The nmr spectra of trans diol-II in the presence of the achiral shift reagent suggested that the C-2 proton, which is in close proximity (2.2A°) to the endo-hydroxyl group (a potential binding site of the shift reagent), would give the most discernible information with the chiral reagent since its resonance is a doublet with a fairly large coupling constant ($J_{2,7}$ = 8.0Hz) which is well separated from most interfering proton signals. Spectra of II at a molar ratio of chiral shift reagent to II, (SR/II), between 0.1 and 0.2 clearly evidence two overlapping doublets, as shown in Figure IA for the C-2 proton. Further addition of shift reagent effected coalescence of the two inner components which finally pass one another, so that separate doublets are again apparent; however, resolution is poor in the latter stages of shift reagent addition. As expected, the peak areas correspond to an optical purity near zero for this racemic material.

The biological trans-diol, whose identity had been previously confirmed by gas chromatography and chemical ionization mass spectrometry, was subjected to nmr analysis in the same manner as was the standard. The molar ratio SR/II required to give overlapping doublets for the biological material was greater than that for the standard, 0.38 compared to 0.13. The increase is probably due to the uptake of some shift reagent by impurities which came through the purification procedures. Nevertheless, Figure IB shows that one enantiomer is present in substantially larger amounts. The peak areas correspond to an optical purity of 27-29%. Further study of the

spectra from both the standard and biological trans-diol show that other proton patterns occur in duplicate reflecting the same enantiomer distribution.

The cis-diol III is a symmetrical compound possessing a reflective plane of symmetry with no rotational symmetry operations. It was suspected that since the two reflective halves of the molecule bear an enantiotopic relationship to each other, each half of the molecule could interact with a dissymmetric environment such as an enzyme surface to produce an optically active product. In a similar fashion, the halves might interact differently with a chiral chemical reagent such as the chiral nmr shift reagent.

III possesses no endo-hydroxyl group and therefore the C2-C7 protons are affected very little by the presence of an nmr shift reagent (6). The C3-C6 protons, although their resonances are complex multiplets, experience relatively small coupling (<0.5Hz), and their signal appears as a broad singlet. These protons are in fairly close proximity (2.6A^{0}) to the exo-hydroxyl groups. Addition of 0.21M $Eu(HFC)_3$ to a saturated chloroform-d solution of III in one microliter increments shows (Figure II) that the C3-C6 signal is transformed from the broad singlet to a pattern which is clearly doublet in character although each component remains broad. This study confirms that each half of the molecule (III) can interact differently with a chiral shift reagent, as suspected since the achiral reagent fails to resolve the C3-C6 signal into a doublet (6).

Our prediction of the stereospecific epimerization (7) of the cis-diol III has been proved by these experimental results. In addition, it seems likely that chiral nmr shift reagents may find further application in establishing enantiotopic as well as enantiomeric properties in a variety of biologically significant compounds.

REFERENCES

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4. H. Matthews and J. D. McKinney, (manuscript in preparation), 1973.
5. See G. M. Whitesides, and D. W. Lewis, J. Am. Chem. Soc. 93(22), 5913 (1971) for the utility of such systems in separating the resonances of enantiomeric alcohols, etc.
6. J. D. McKinney, L. H. Keith, A. Alford, and C. E. Fletcher, Can. J. Chem. 49(12), 1993 (1971).
7. Evidence for an α-hydroxy ketone intermediate will be reported elsewhere.

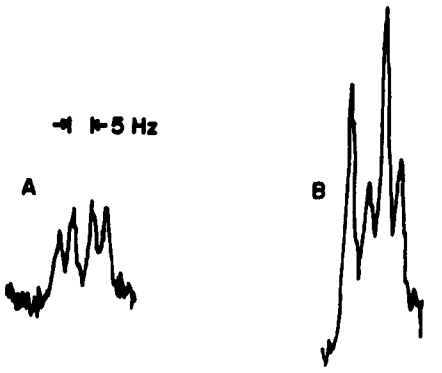

Figure I

(A) C-2 proton signals of racemic trans-aldrindiol-II (0.23M) with 0.03M $Eu(HFC)_3$ in $CDCl_3$ at 100MHz, SR/II=0.13; (B) C-2 proton signals of biological trans-aldrindiol-II (0.035M) with 0.013M $Eu(HFC)_3$ in $CDCl_3$ at 100 MHz, SR/II=0.38.

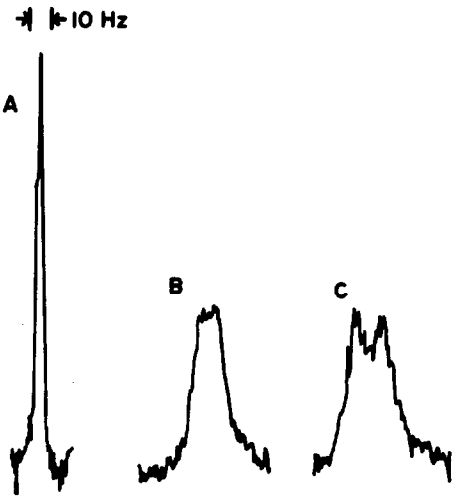

Figure II

C3-C6 proton signals of standard meso-cis-aldrindiol-III (sat. sol.) with (A) no $Eu(HFC)_3$; (B) with 0.008M $Eu(HFC)_3$; (C) with 0.013M $Eu(HFC)_3$ in $CDCl_3$ at 100MHz.