Structure and Asymmetry in the Isomeric Conversion of β- to α-Endosulfan

ARTICLE *in* JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · APRIL 1997

Impact Factor: 2.91 · DOI: 10.1021/jf970020t · Source: OAI

5 AUTHORS, INCLUDING:

Walter Schmidt
United States Department of Agriculture
182 PUBLICATIONS **2,214** CITATIONS
SEE PROFILE

James C Fettinger
University of California, Davis
436 PUBLICATIONS **10,010** CITATIONS
SEE PROFILE

Clifford P Rice
Agricultural Research Service, Beltsville, M…
130 PUBLICATIONS **2,842** CITATIONS
SEE PROFILE

Journal of Agricultural and Food Chemistry

APRIL 1997
VOLUME 45, NUMBER 4

RAPID COMMUNICATIONS

Structure and Asymmetry in the Isomeric Conversion of β- to α-Endosulfan

Keywords: *α- and β-endosulfan; conformation; X-ray crystallography; intramolecular conversion; NMR spectroscopy*

INTRODUCTION

Endosulfan is a broad spectrum organochlorine insecticide used on a variety of cereals, fruits, vegetables, and cotton. Recently, endosulfan has been shown to have estrogenic effects (Soto *et al.*, 1994). Synergistic effects with environmentally relevant organochlorine pesticides have also been observed (Arnold *et al.*, 1996). Although the commercial product is applied as a 7:3 isomeric mixture of α and β forms (Brooks 1974), the fate of the α and β forms varies, and the observed ratio of the isomers is dependent upon the physical state of environmental compartments. The α-isomer is predominant in air samples (Hoff *et al.*, 1992; Burgoyne and Hites, 1993; Bidleman *et al.*, 1995; Wallace and Hites, 1996), the β form is favored in rain samples (Strachan and Huneault, 1979; Chan and Perkins, 1989; Chan *et al.*, 1994), and the α-isomer is greater in snow (Gregor and Gummer, 1989). Furthermore, some researchers have observed substantial conversion of the β isomer to the α isomer and little concomitant conversion of α to β (Cotham and Bidleman, 1989; Singh *et al.*, 1991; Guerin and Kennedy, 1992; Rice *et al.*, 1997). Previous molecular structure and physical attributes of the endosulfan isomers cannot adequately explain this unequal distribution of isomers or a mechanism for conversion between the isomers. In addition, structural differences and conversion between the isomers are of fundamental significance in relation to endocrine mimic activity/binding.

MATERIALS AND METHODS

NMR Spectroscopy. ^{1}H- and ^{13}C-NMR spectra and HETCOR, COSY, NOESY, and APT experiments were conducted on a Bruker QE Plus 300 MHz spectrometer (Billerica, MA). Spectra were collected at 25, 50, 75, 100, 125, 150, and 175 ± 0.3 °C in DMSO-d_6 (Aldrich, Milwaukee, WI). Chemical shifts were referenced to DMSO-d_5. ^{1}H-NMR spectra (including PSDQF COSY for α-endosulfan) were also obtained on a Bruker AMX 500 MHz spectrometer courtesy of Dr. Yui-Fai Lam, Department of Chemistry and Biochemistry, University of Maryland, College Park, MD. The following certified analytical samples of α- and β-endosulfan were purchased from Supelco (Bellefonte, PA): ^{1}H NMR α-endosulfan δ 4.61 (1H, m), 4.58 (1H, m), 4.17 (1H, m), 4.16 (1H, m), 3.54 (1H, m), 3.53 (1H, m); β-endosulfan δ 5.08 (2H, d, J = 15 Hz), 4.10 (2H, d, J = 15 Hz), 3.32 (2H, s).

X-ray Crystallography. X-ray crystallographic data were acquired on a Enraf-Nonius CAD-4 diffractometer controlled with a Digital Equipment Corp. MicroVAX II (MVII) computer and the Enraf-Nonius VAX\VMS CAD4 Express control program. Crystals were optically centered with each crystal's final cell parameters and crystal orientation matrix determined from 25 reflections well dispersed in reciprocal space and confirmed with axial photographs. Data forms were collected [Mo Kα = 0.71073 Å] at T = 153(2) K with $\omega/2\theta$ scans to 27.5° in θ. Data profiles were recorded in 96 steps with the outermost 16 steps on each end of the scan being used for background determination. Both structures, multiple forms of data collected to 55° in 2θ, were refined to convergence [$\Delta/\sigma \leq 0.001$] with all atoms positional parameters refining freely; all non-hydrogen atoms were refined to convergence with anisotropic thermal parameters, while hydrogen atoms were refined isotropically. Final difference−Fourier maps were featureless, indicating the structures to be both correct and complete. Crystal chirality was also determined, when appropriate, using the Flack(x) absolute structure parameter. Additionally, a correction for extinction was applied when appropriate. Final residuals: α-endosulfan, $R(F)$ = 3.96%, $wR(F^2)$ = 9.24%, and GOF = 1.104 for all 6516 unique reflections; β-endosulfan, $R(F)$ = 3.01%, $wR(F^2)$ = 6.59%, and GOF = 1.080 for all 1748 unique reflections.

RESULTS AND DISCUSSION

The conformation of the α- and β-isomers is defined by the shape of the flexible seven-member ring in endosulfan as determined from the dihedral angles among the corresponding protons. α- and β-endosulfan (Figure 1, III and II, respectively) have been considered

S0021-8561(97)00020-4 CCC: $14.00

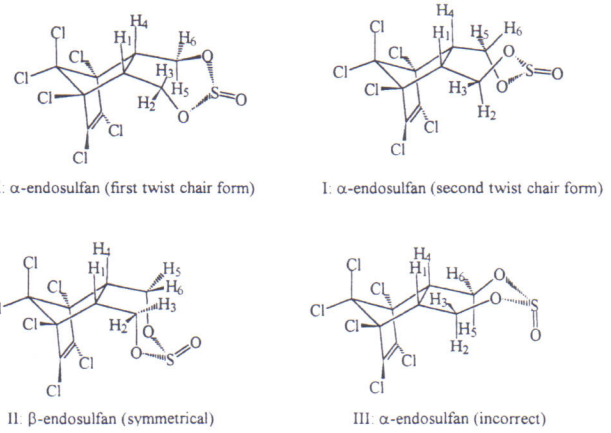


Figure 1. Endosulfan conformers.

to be symmetrical (Forman *et al.*, 1965; Smith *et al.*, 1977). In both of these conformers, the methine protons (H_1 and H_4) were assigned as the furthest upfield signal and as magnetically equivalent. By definition, two protons that are magnetically equivalent cannot have J coupling with each other. When two adjacent methine protons are not magnetically equivalent, $\nu_A \neq \nu_B$, and $J_{AB} \neq 0$, a complex non-first-order spectrum results (Jackman and Sternshell, 1969). Analysis of the data obtained from 300- and 500-MHz NMR spectrometers supports the symmetry of the β-form ($\nu_A = \nu_B$, and $J_{AB} = 0$), but not for the α-isomer.

In the previous study, a computer deconvolution of the NMR frequencies at 60 MHz of the α form incorrectly assumed the absence of chemical shift differences (methine $\nu_A = \nu_B$) and assigned J coupling differences that do not exist (Forman *et al.*, 1965). Structural assignments established by APT and HETCOR experiments of the α-isomer unambiguously proved that the methine proton H_1 occurs at 4.6 ppm, which contrasts with the previous study in which H_1 and H_4 were assigned to 3.5 ppm (Forman *et al.*, 1965). The signal at 3.5 ppm actually corresponds to two of the four methylene protons. H_2 and H_3 cannot couple with H_5 and H_6 because they are nonadjacent methylene protons. A phase sensitive double quantum filtered (PS-DQF) COSY spectrum on a 500-MHz NMR spectrometer of α-endosulfan verified that none of the frequencies of the six protons are magnetically equivalent, i.e., that the three protons on the left half of the molecule are not identical with the three on the right half of the molecule. Thus, α-endosulfan is asymmetrical.

The symmetry of the β-isomer and the asymmetry of the α-isomer were confirmed by X-ray crystallographic studies. α-Endosulfan was found to crystallize in the noncentrosymmetric space group $P2_1$ with two, basically identical, molecules within the asymmetric unit. β-Endosulfan crystallized in the hexagonal centrosymmetric space group $P6_3/m$ with the molecule lying on the mirror plane, thereby requiring only half of the molecule to be located, the remainder being generated by symmetry.

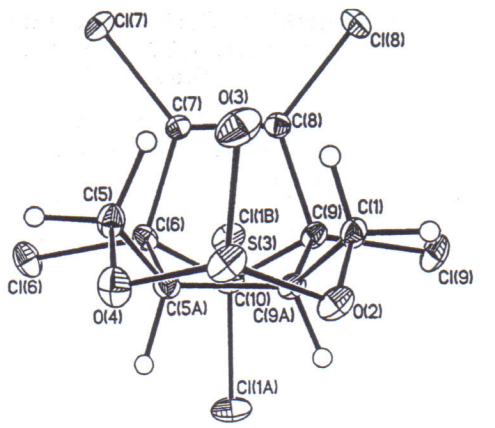
The two isomers are essentially identical over the 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano portions, while they differ significantly over the remaining 2,4,3-benzodioxathiepin 3-oxide portion. Bond distance comparisons between the two molecules were basically equivalent, with only very minor variations (data not shown). A series of selected bond angles for the 2,4,3-benzodioxathiepin 3-oxide portions of the two molecules is shown in Table 1 (those for the 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano portion have been omitted). The angles at C(1), C(5A), and C(9A) vary markedly between the α and β forms of endosulfan. These dramatic differences are shown in Figure 2.

NMR temperature studies of the β- and α-isomers support this symmetry/asymmetry difference. Unlike the β-isomer for which no effect was observed, increasing the temperature for the α-isomer unexpectedly increased the resolution of all the multiplets. Structurally, this can be explained by pseudorotation of the seven-member ring, for which the twisted chair is the most stable conformer (Hendrickson *et al.*, 1973; Bocian *et al.*, 1975). A set of enantiomers, I and I′ (Figure 1), would be expected to have relative energies <2.7 kcal/mol apart (Bocian *et al.*, 1975). With increasing temperature pseudorotation is so fast that only I and I′ (and not the intermediates) are observed in the NMR time frame. Thus, line broadening was observed when the temperature was decreased. The β-isomer does not engage in pseudorotation because it is symmetrical and, with increasing thermal energy (temperature), reversible symmetrical motion remains symmetrical.

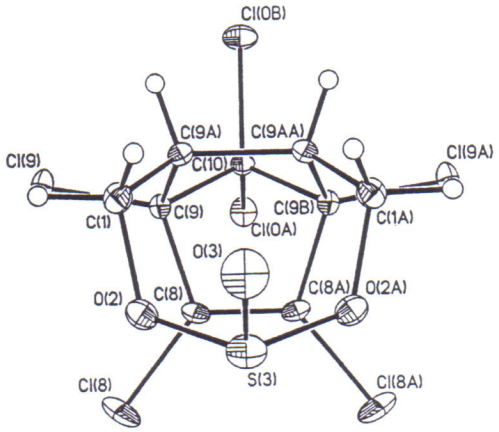
The environmental half-life of the β-isomer has been shown in some studies to be shorter than that of the α-isomer (Brooks 1974; Chopra and Moahfouz, 1977; Miles and Moy, 1979; Cotham and Bidleman, 1989; Singh *et al.*, 1991; Guerin and Kennedy, 1992; Rice *et al.*, 1997). The conversion between the two isomers has been postulated to be the result of hydrolysis or that assistance from water is necessary for the "equilibrium" to occur (Chopra and Moahfouz, 1977; Miles and Moy, 1979). However, these observations can readily be explained by examining the structural relationship between the isomers. Conversion of α to β requires an increase in order; that is, an asymmetrical molecule must be converted to a symmetrical one. The conversion of symmetrical β molecules to α, on the other hand, results in asymmetry, which is a net decrease in entropy.

Table 1. Selected Bond Angles of α- and β-Endosulfan

α-endosulfan	molecule 1	molecule 2	β-endosulfan	
O(2)–C(1)–C(9A)	107.9 (3)	107.6 (3)	O(2)–C(1)–C(9A)	113.87 (13)
C(1)–O(2)–S(3)	118.6 (2)	118.7 (2)	C(1)–O(2)–S(3)	119.06 (11)
O(3)–S(3)–O(4)	107.5 (2)	106.6 (2)	O(3)–S(3)–O(2A)	107.66 (7)
O(3)–S(3)–O(2)	106.7 (2)	106.7 (2)	O(3)–S(3)–O(2)	107.66 (7)
O(4)–S(3)–O(2)	101.5 (2)	101.17 (14)	O(2)–S(3)–O(2A)	100.16 (9)
C(5)–O(4)–S(3)	118.6 (2)	118.7 (2)	C(1)–O(2)–S(3)	119.06 (11)
C(5)–C(5A)–C(6)	111.9 (3)	113.2 (3)	C(1)–C(9A)–C(9)	115.81 (13)
C(5)–C(5A)–C(9A)	116.2 (3)	115.8 (3)	C(1)–C(9A)–C(9AA)	118.85 (9)
O(4)–C(5)–C(5A)	109.1 (3)	108.1 (3)	O(2)–C(1)–C(9A)	113.87 (13)
C(1)–C(9A)–C(9)	114.3 (3)	112.8 (3)	C(1)–C(9A)–C(9)	115.81 (13)
C(1)–C(9A)–C(5A)	116.2 (3)	116.2 (3)	C(1)–C(9A)–C(9AA)	118.85 (9)
C(9)–C(9A)–C(5A)	102.9 (3)	102.2 (3)	C(9)–C(9A)–C(9AA)	102.36 (8)



α-endosulfan



β-endosulfan

Figure 2. ORTEP drawings of α- and β-endosulfan, end-on view. The C(6)–C(7)–C(8)–C(9) atoms define the plane of the paper.

Predictably, β conversion to α is much more favorable, as was demonstrated in the following experiment. A small portion of β (containing 1% α-isomer) was heated to 160 °C, and with no net loss of mass, 9 ± 1% of β was converted to α. However, no conversion was observed when α (containing 0.65% β) was heated to temperatures up to 280 °C.

The twisted chair state is structurally identical between the α- and β-isomers. The mechanism of β conversion to α can be envisioned as a "twisting" of one of the equatorial hydrogen atoms by 90°, so that this hydrogen becomes axial. Once the energy barrier to asymmetry for the β-isomer is reached, the molecule becomes asymmetrical and stays asymmetrical; that is, it becomes α-endosulfan.

In conclusion, the environmental fate of endosulfan inherently depends on the structural relationship of α and β. Previous studies concerning endosulfan were based on incorrect NMR structural assignments. The results presented here provide unequivocal proof that the β-isomer is symmetrical, whereas the α-isomer is a mixture of two structurally indistinguishable asymmetrical molecules. Physical state transitions (e.g., volatilization) which cause asymmetry in the β-isomer can readily cause transformation to the α-isomer. For the energetically unfavorable reverse process, the asymmetrical α-isomers must be made symmetrical.

The knowledge that β readily converts to α must be considered when one is discerning the loss processes of endosulfan. The findings of this investigation strongly suggest that previous studies in which the half-life of endosulfan was described are now suspect if quantitation of both α and β was not considered. Conformational changes between the isomers will also occur within a chiral environment and may result in enhancement or reduction in biological activity. Further, any one of the three (not two) isomers in principle could be responsible for the estrogenic effects. This information is absolutely necessary to delineate the mechanisms and interactions of endosulfan with endocrine receptor sites.

LITERATURE CITED

Arnold, S. F.; Klotz, D. M.; Collins, B. M.; Vonier, P. M.; Guillette, L. J., Jr.; McLachlen, J. A. Synergistic Activation of Estrogen Receptor with Combinations of Environmental Chemicals. *Science* **1996**, *272*, 1489–1492.

Bidleman, T. F.; Falconer, R. L.; Walla, M. D. Toxaphene and other Organochlorine Compounds in Air and Water at Resolute Bay, N. W. T. Canada. *Sci. Total Environ.* **1995**, *160/161*, 55–63.

Bocian, D. F.; Pickett, H. M.; Rounds, T. C.; Strauss, H. L. Conformations of Cycloheptane. *J. Am. Chem. Soc.* **1975**, *97*, 687–695.

Brooks, G. T. *Chlorinated Insecticides*; CRC Press: Cleveland, OH, 1974; Vol. I.

Burgoyne, T. W.; Hites, R. A. Effects of Temperature and Wind Direction on Atmospheric Concentrations of α-Endosulfan. *Environ. Sci. Technol.* **1993**, *27*, 910–914.

Chan, C. H.; Perkins, L. H. Monitoring of Trace Organic Contaminants in Atmospheric Precipitation. *J. Great Lakes Res.* **1989**, *15*, 465–475.

Chan, C. H.; Bruce, G.; Harrison, B. Wet Deposition of Organochlorine Pesticides and Polychlorinated Biphenyls to the Great Lakes. *J. Great Lakes Res.* **1994**, *20*, 546–560.

Chopra, N. M.; Mahfouz, A. M. Metabolism of Endosulfan I, Endosulfan II, and Endosulfan Sulfate in Tobacco Leaf. *J. Agric. Food Chem.* **1977**, *25*, 32–36.

Cotham, W. E., Jr.; Bidleman, T. F. Degradation of Malathion, Endosulfan and Fenvalerate in Seawater and Seawater/Sediment Microcosms. *J. Agric. Food Chem.* **1989**, *37*, 824–828.

Forman, S. E.; Durbetaki, A. J.; Cohen, M. V.; Olofson, R. A. Conformational Equilibria in Cyclic Sulfites and Sulfates. The Configurations and Conformations of the Two Isomeric Thiodans. *J. Org. Chem.* **1965**, *30*, 169–175.

Gregor, D. J.; Gummer, W. D. Evidence of Atmospheric Transport and Deposition of Organochlorine Pesticides and Polychlorinated Biphenyls in Canadian Arctic Snow. *Environ. Sci. Technol.* **1989**, *23*, 561–565.

Guerin, T. F.; Kennedy, I. R. Distribution and Dissipation of Endosulfan and Related Cyclodienes in Sterile Aqueous Systems: Implications for Studies on Biodegradation. *J. Agric. Food Chem.* **1992**, *40*, 2315–2323.

Hendrickson, J. B.; Boeckman, R. K., Jr.; Glickson, J. D.; Grunwald, E. Molecular Geometry. VIII. Proton Magnetic Resonance Studies of Cycloheptane Conformations. *J. Am. Chem. Soc.* **1973**, *95*, 494–505.

Hoff, R. M.; Muir, D. C. G.; Grift, N. P. Annual Cycle of Polychlorinated Biphenyls and Organohalogen Pesticides in Air in Southern Ontario. 1. Air Concentration Data. *Environ. Sci. Technol.* **1992**, *26*, 266–275.

Jackman, L. M.; Sternshell, S. *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*; Pergamon Press: New York, 1969.

Miles, J. R. W.; Moy, P. Degradation of Endosulfan and its Metabolites by a Mixed Culture of Soil Microorganisms. *Bull. Environm. Contam. Toxicol.* **1979**, *23*, 13–19.

Rice, C. P.; Chernyak, S. M.; Hapeman, C. J.; Bilboulian, S. Air-Water Distribution of the Endosulfan Isomers. *J. Environ. Qual.* **1997**, in press.

Singh, N. C.; Dasgupta, T. P.; Roberts, E. V.; Mansingh, A. Dynamics of Pesticides in Tropical Conditions. 1. Kinetic Studies of Volatilization, Hydrolysis, and Photolysis of

Dieldrin and α- and β-Endosulfan. *J. Agric. Food Chem.* **1991**, *39*, 575−579.

Smith, G.; Kennard, C. H. L.; Shields, K. G. Insecticides. XI. Crystal Structure of Endosulfan, α-6,7,8,9,10,10-Hexachloro-1,5,5a,6,9,9a-hexahydro-*endo*-6,9-methano-2,4,3-benzodioxathiepin 3-Oxide. *Aust. J. Chem.* **1977**, *30*, 911−916.

Soto, A. M.; Chung, K. L.; Sonnenschein, C. The Pesticides Endosulfan, Toxaphene and Dieldrin Have Estrogenic Effects on Human Estrogen-Sensitive Cells. *Environ. Health Perspect.* **1994**, *102*, 380−383.

Strachan, W. M. J.; Huneault, H. Polychlorinated Biphenyls and Organochlorine Pesticides in Great Lakes Precipitation. *J. Great Lakes Res.* **1979**, *5*, 61−68.

Wallace, J. F.; Hites, R. A. Diural Variations in Atmospheric Concentrations in Polychlorinated Biphenyls and Endosuflan: Implications for Sampling Protocols. *Environ. Sci. Technol.* **1996**, *30*, 444−452.

Received for review January 7, 1997. Accepted January 29, 1997. Mention of specific products or suppliers is for identification and does not imply endorsement by the U.S. Department of Agriculture to the exclusion of other suitable products or suppliers.

Walter F. Schmidt,*,† Cathleen J. Hapeman,† James C. Fettinger,‡ Clifford P. Rice,† and Susanna Bilboulian†,‡

Environmental Chemistry Laboratory, Natural Resources Institute, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705, and Department of Chemistry and Biochemistry, University of Maryland, College Park, Maryland 20742

JF970020T

* Author to whom correspondence should be addressed [fax (301) 504-6922; e-mail wschmidt@clunker.arsusda.gov].

† U.S. Department of Agriculture.

‡ University of Maryland.