

## RAPID COMMUNICATIONS

### Structure and Asymmetry in the Isomeric Conversion of $\beta$ - to $\alpha$ -Endosulfan

**Keywords:**  $\alpha$ - and  $\beta$ -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  $\alpha$  and  $\beta$  forms (Brooks 1974), the fate of the  $\alpha$  and  $\beta$  forms varies, and the observed ratio of the isomers is dependent upon the physical state of environmental compartments. The  $\alpha$ -isomer is predominant in air samples (Hoff *et al.*, 1992; Burgoyne and Hites, 1993; Bidleman *et al.*, 1995; Wallace and Hites, 1996), the  $\beta$  form is favored in rain samples (Strachan and Huneault, 1979; Chan and Perkins, 1989; Chan *et al.*, 1994), and the  $\alpha$ -isomer is greater in snow (Gregor and Gummer, 1989). Furthermore, some researchers have observed substantial conversion of the  $\beta$  isomer to the  $\alpha$  isomer and little concomitant conversion of  $\alpha$  to  $\beta$  (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

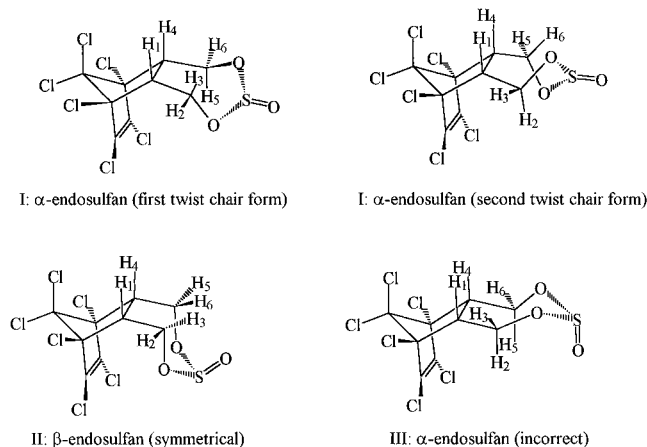
**NMR Spectroscopy.**  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra and HETCOR, COSY, NOESY, and APT experiments were conducted on a Bruker QE Plus 300 MHz spectrometer (Billerica, MA). Spectra were collected at 25, 50, 75, 100, 125, 150, and 175  $\pm$  0.3  $^\circ\text{C}$  in  $\text{DMSO}-d_6$  (Aldrich, Milwaukee, WI). Chemical shifts were referenced to  $\text{DMSO}-d_5$ .  $^1\text{H}$ -NMR spectra (including

PSDQF COSY for  $\alpha$ -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  $\alpha$ - and  $\beta$ -endosulfan were purchased from Supelco (Bellefonte, PA):  $^1\text{H}$  NMR  $\alpha$ -endosulfan  $\delta$  4.61 (1H, m), 4.58 (1H, m), 4.17 (1H, m), 4.16 (1H, m), 3.54 (1H, m), 3.53 (1H, m);  $\beta$ -endosulfan  $\delta$  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 [ $\text{Mo K}\alpha = 0.71073 \text{ \AA}$ ] at  $T = 153(2) \text{ K}$  with  $\omega/2\theta$  scans to  $27.5^\circ$  in  $\theta$ . 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  $55^\circ$  in  $2\theta$ , 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:  $\alpha$ -endosulfan,  $R(F) = 3.96\%$ ,  $wR(F^2) = 9.24\%$ , and  $\text{GOF} = 1.104$  for all 6516 unique reflections;  $\beta$ -endosulfan,  $R(F) = 3.01\%$ ,  $wR(F^2) = 6.59\%$ , and  $\text{GOF} = 1.080$  for all 1748 unique reflections.

#### RESULTS AND DISCUSSION

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



**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  $\beta$ -form ( $\nu_A = \nu_B$ , and  $J_{AB} = 0$ ), but not for the  $\alpha$ -isomer.

In the previous study, a computer deconvolution of the NMR frequencies at 60 MHz of the  $\alpha$  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  $\alpha$ -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  $\alpha$ -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,  $\alpha$ -endosulfan is asymmetrical.

The symmetry of the  $\beta$ -isomer and the asymmetry of the  $\alpha$ -isomer were confirmed by X-ray crystallographic studies.  $\alpha$ -Endosulfan was found to crystallize in the noncentrosymmetric space group  $P2_1$  with two, basically identical, molecules within the asymmetric unit.  $\beta$ -En-

dosulfan 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.

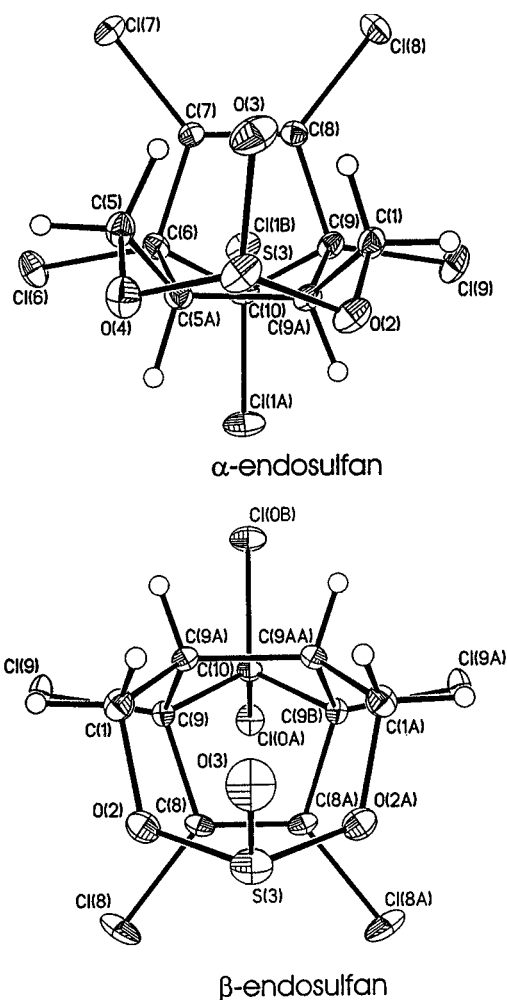
The two isomers are essentially identical over the 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano portions, while they differ significantly over the remaining 2,4,3-benzodioxathiepin 3-oxide portion. Bond distance comparisons between the two molecules were basically equivalent, with only 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  $\alpha$  and  $\beta$  forms of endosulfan. These dramatic differences are shown in Figure 2.

NMR temperature studies of the  $\beta$ - and  $\alpha$ -isomers support this symmetry/asymmetry difference. Unlike the  $\beta$ -isomer for which no effect was observed, increasing the temperature for the  $\alpha$ -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  $\beta$ -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  $\beta$ -isomer has been shown in some studies to be shorter than that of the  $\alpha$ -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  $\alpha$  to  $\beta$  requires an increase in order; that is, an asymmetrical molecule must be converted to a symmetrical one. The conversion of symmetrical  $\beta$  molecules to  $\alpha$ , on the other hand, results in asymmetry, which is a net decrease in entropy.

**Table 1.** Selected Bond Angles of  $\alpha$ - and  $\beta$ -Endosulfan

$\alpha$ -endosulfan	molecule 1	molecule 2	$\beta$ -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)



**Figure 2.** ORTEP drawings of  $\alpha$ - and  $\beta$ -endosulfan, end-on view. The C(6)–C(7)–C(8)–C(9) atoms define the plane of the paper.

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

The twisted chair state is structurally identical between the  $\alpha$ - and  $\beta$ -isomers. The mechanism of  $\beta$  conversion to  $\alpha$  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  $\beta$ -isomer is reached, the molecule becomes asymmetrical and stays asymmetrical; that is, it becomes  $\alpha$ -endosulfan.

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

The knowledge that  $\beta$  readily converts to  $\alpha$  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  $\alpha$  and  $\beta$  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.

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