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X-ray crystal structure of endosulfan sulfate

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Abstract

X-ray crystallography is an important method used to confirm the three-dimensional structure of a chemical compound. In this study, the crystal structure of endosulfan sulfate was investigated. Endosulfan sulfate is the major metabolite of the insecticide endosulfan, which is composed of two stereoisomers (α and β). From GC–MS analysis, α- and β-endosulfan each gave a single peak in the endosulfan sample, but only one peak was observed for endosulfan sulfate. Interestingly, in X-ray crystallography, two conformers of endosulfan sulfate (A and B) were observed at a ratio of 2(A):1(B). A heterocyclic seven-membered ring of conformer B assumed a horizontal-chair form, differing from two twisted forms of α-endosulfan while a vertical-chair form was observed for conformer A, showing the very similar structure to β-endosulfan; this difference in conformation is caused by differing bond angles at O(1)–C(8)–C(3) and O(2)–C(9)–C(4). In space packing, two asymmetric units were obtained, and three molecules were aligned in the order of A–A–B conformers in each unit. The total potential energy of A was slightly lower (approximately 4 kcal/mol) than B, possibly resulting in the two molecules of A that exist in a rigid crystal state. However, A and B conformers should not exist at room temperature in a solution state for GC–MS analysis, likely due to the small energy difference.

Keywords: X-ray crystallography, Endosulfan sulfate, Crystal, Structural analysis

Introduction

X-ray crystallography is an important approach used to identify the exact structure of a chemical compound; the results of crystallographic analysis provide highly reliable and accurate 3D-structure information, which is an essential element in structure-based research [1]. In general, many nonmolecular compounds, such as microporous materials and ionic compounds, or relatively large-sized materials, for example, oligopeptides and multinuclear arrays, were studied with X-ray crystallography since its results are powerful and detailed at the 3D level [2]. However, as crystallographic techniques have evolved over the past three decades, the speed of analysis, application coverage and reliability have improved dramatically so that crystallography plays an important role in modern chemistry as one of the primary structural identification techniques for chemical and biochemical molecules, accompanied by NMR and mass spectrometry [2].

Endosulfan sulfate (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,3,4-benzodioxathiepin-3,3-dioxide) is the oxidative metabolite of endosulfan, an organochlorine insecticide, which has been widely used for over 30 years on various crops [3]. Because of its widespread use, high persistency, and potential for movement in the environment, environmental contamination of endosulfan has begun to emerge as a problem [4]. Among the several metabolites produced from endosulfan in a bioenvironmental system, endosulfan sulfate is more toxic and persistent than endosulfan, while others, such as endosulfan diol, lactone, ether, and hydroxy ether, are less toxic [5–10].

Endosulfan is composed of two stereoisomers, α- and β-endosulfan, that were found at a ratio of about 7(α):3(β) in standard, technical and commercial formulations [9], while no stereoisomers were observed for endosulfan sulfate from analysis [11, 12]. In the biological oxidative reaction of endosulfan with human liver microsomes, only one form of endosulfan sulfate was observed from two endosulfan isomers [7]. These observations are interesting because endosulfan sulfate is a metabolite

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produced by the oxidation of endosulfan; this simple degradation reaction occurs without breaking a molecular bond, and thus, two stereoisomers would be expected. To date, only one review paper has referred to the structure of endosulfan sulfate [13], but an actual crystal structure was not provided. Therefore, this study was conducted to investigate the accurate molecular 3D structure of endosulfan sulfate by X-ray crystallography.

Materials and methods

Chemicals and reagents

Endosulfan and endosulfan sulfate were purchased from Chem Service Inc. (West Chester, PA). Acetone was provided by Fisher Scientific (Pittsburgh, PA). All chemicals were used at the highest available commercial grade.

Mass spectrometry

Individual standard solutions of endosulfan and endosulfan sulfate were prepared at 10 mg/L in acetone before GC–MS analysis [GCMS-TQ8040 (Shimadzu Corporation, Kyoto, Japan)] with a BPX-5 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) [TRAJAN (Victoria, Australia)]. The column flow rate of helium was 1.5 mL/min, and 2 μL of sample was injected on splitless mode. The temperature of the GC injector was set at 280 °C. The temperature of the column oven was maintained at 80 °C for 2 min. It was subsequently increased at a rate of 22 °C/min until 300 °C was reached and then maintained for 3 min. MS analysis was performed in full scan mode (m/z 60–500) by electron ionization at 70 eV.

X-ray crystallography

Small clear crystals of endosulfan sulfate were obtained from standard commercial material. Mo $K\alpha_1$ on a RIGAKU R-AXIS RAPID diffractometer was used for collecting data ($a = 12.661(4)$ Å, $b = 13.966(5)$ Å, $c = 15.172(5)$ Å, $\alpha = 66.465(14)°$, $\beta = 69.490(13)°$, $\gamma = 67.307(13)°$ and 2207.2(13) Å^3). Intensity data were obtained by the ω-2θ scanning technique. The final cycle of refinement performed on Fo^2 with all 21,953 unique reflections afforded residuals $wR_2 = 0.0987$, and the conventional R index based on the reflections having $Fo > 2\sigma(Fo^2)$ is 0.0314. A summary of crystal and structural refinement data for endosulfan sulfate is shown in Table 1.

Table 1 Crystal data and structure refinement for endosulfan sulfate

Empirical formula	$C_9H_6Cl_6O_4S$
Formula weight	422.92
Temperature (K)	293(2)
Wavelength (Å)	0.71073
Crystal system	Triclinic
Space group	$P\bar{1}$
Unit cell dimensions [*a, b, c* (Å), α, β, γ (°))]	$a = 12.661(4)$, $b = 13.966(5)$, $c = 15.172(5)$, $\alpha = 66.465(14)$, $\beta = 69.490(13)$, $\gamma = 67.307(13)$
Volume (Å^3)	2207.2(13)
Z	2
Calculated density (Mg/m^3)	0.954
Absorption coefficient (mm^{-1})	0.657
F (000)	630
θ range for data collection (°)	3.01 to 27.48
Limiting indices	$-14 \le h \le 16$, $-18 \le k \le 18$, $-19 \le l \le 19$
Reflections collected/unique	21,953/10,077 [R(int) = 0.0119]
Completeness to θ = 27.48 (%)	99.4
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	10,077/0/613
Goodness-of-fit on F^2	1.117
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0314$, $wR_2 = 0.0952$
R indices (all data)	$R_1 = 0.0359$, $wR_2 = 0.0987$
Largest diff. peak and hole (e Å^3)	0.688 and −0.497

Total potential energy calculation of conformers

Total potential energy calculations were performed on an Intel Core 2 Quad Q6600 (2.4 GHz) Linux PC with Sybyl 7.3 software (Tripos, St. Louis, MO, USA).

Results and discussion

GC–MS analysis was optimized for the separation of endosulfan and endosulfan sulfate. Total ion chromatograms of endosulfan and endosulfan sulfate by GC–MS analysis (Fig. 1) showed that α-endosulfan and β-endosulfan were clearly separated, with a ratio of approximately 7(α):3(β) (Fig. 1a), while endosulfan sulfate was represented by only one peak, as reported previously (Fig. 1b) [9, 11]. The GC–MS scan spectrum and fragmentation pattern of endosulfan sulfate confirms its structure (Fig. 2).

The structures of α- and β-endosulfan were identified by NMR spectroscopy and X-ray crystallography [14], reporting that β-endosulfan is a symmetrical compound, whereas α–endosulfan exists as two asymmetrical isomers. Those results possibly explain why two stereoisomers of α- and β-endosulfan gave peaks with the ratio of about 7(α):3(β), as observed in other reports and this study. Although endosulfan sulfate was detected by only one peak in GC–MS analysis, it is interesting that two conformers of endosulfan sulfate (A and B) were identified in the crystal state in this study.

ORTEP diagrams of two conformers, A and B, are shown in Fig. 3. In addition, bond lengths and angles are given in Tables 2 and 3, respectively. Bond lengths of the two conformers are almost equivalent (Table 2); however, the bond angles at O(1)–C(8)–C(3) and O(2)–C(9)–C(4) were significantly different (Table 3 and Fig. 3c). As shown in Fig. 3, the stereochemical structure of A adopts a vertical-chair form of the seven membered ring, but the B conformer assumes a horizontal-chair form. Endosulfan sulfate A shows very similar structure to β-endosulfan [14]. It seems that just one oxygen atom was attached to sulfur atom axially. However, endosulfan sulfate B adopted one chair from compared to the two twisted forms of α-endosulfan [14]. Figure 4 shows the packing diagram of the molecules in the unit cell. Six molecules of endosulfan sulfate were packed as two asymmetrical units, with each unit containing two molecules of A (A-1 and A-2) and one molecule of B. To understand the 2:1 ratio of A:B

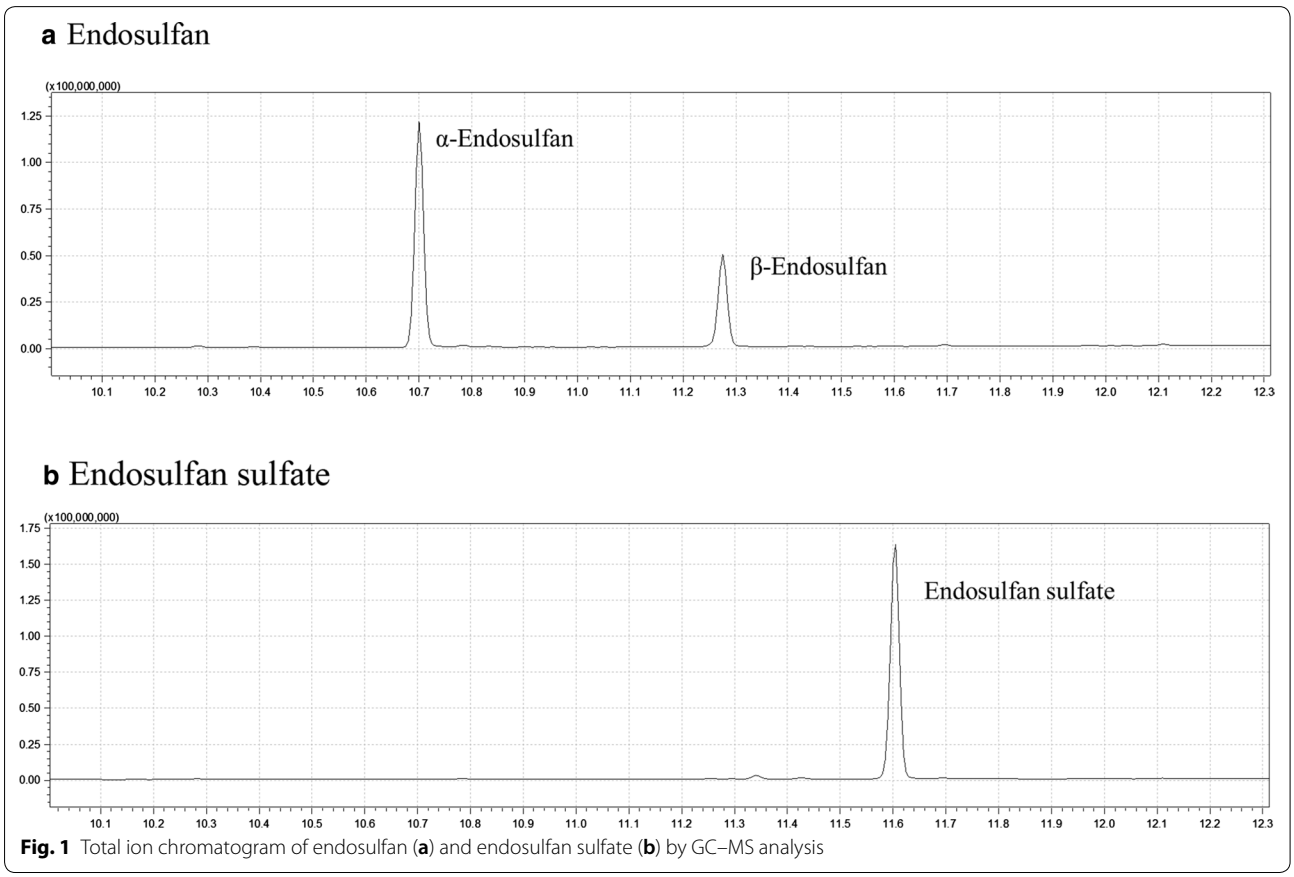


Fig. 1 Total ion chromatogram of endosulfan (**a**) and endosulfan sulfate (**b**) by GC–MS analysis

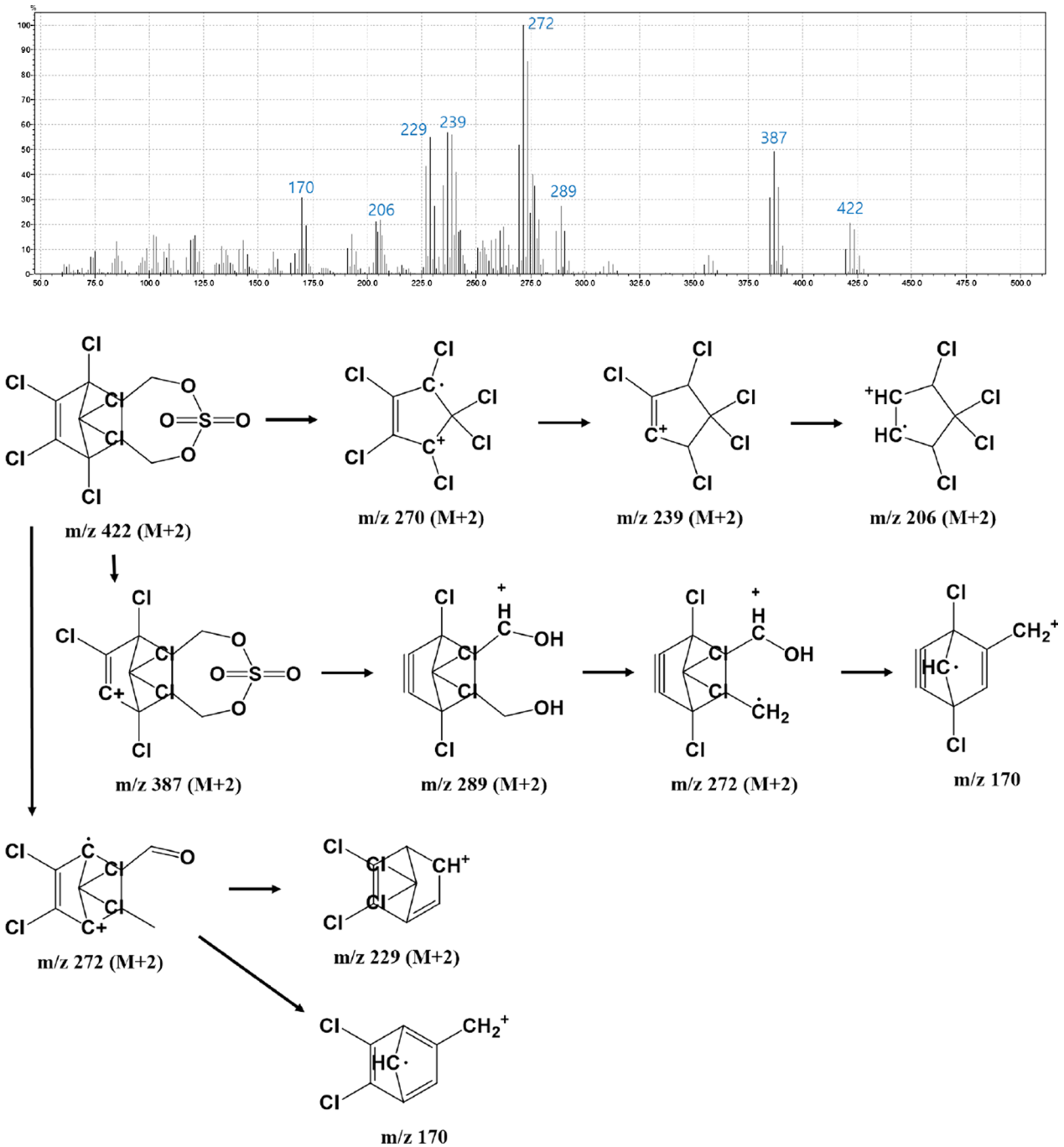
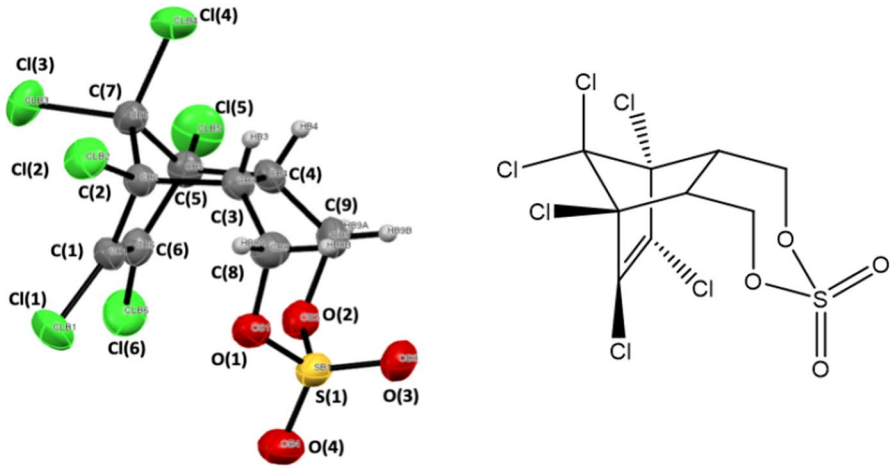
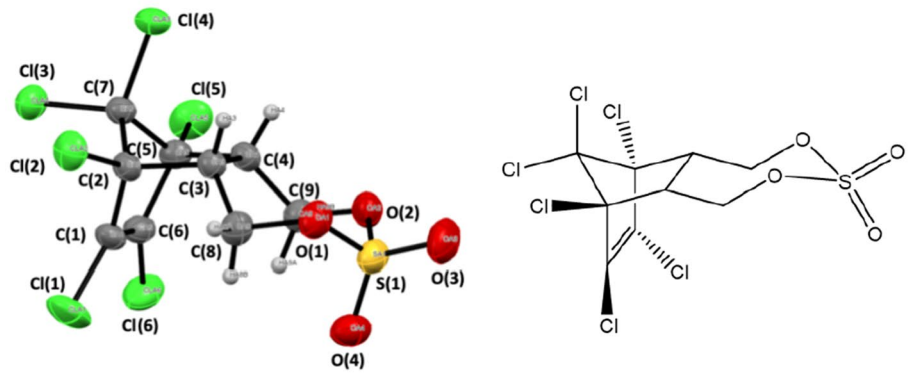


Fig. 2 GC–MS scan spectrum and fragmentation pattern of endosulfan sulfate

a Endosulfan sulfate A



b Endosulfan sulfate B



c Side view of endosulfan sulfate A and B

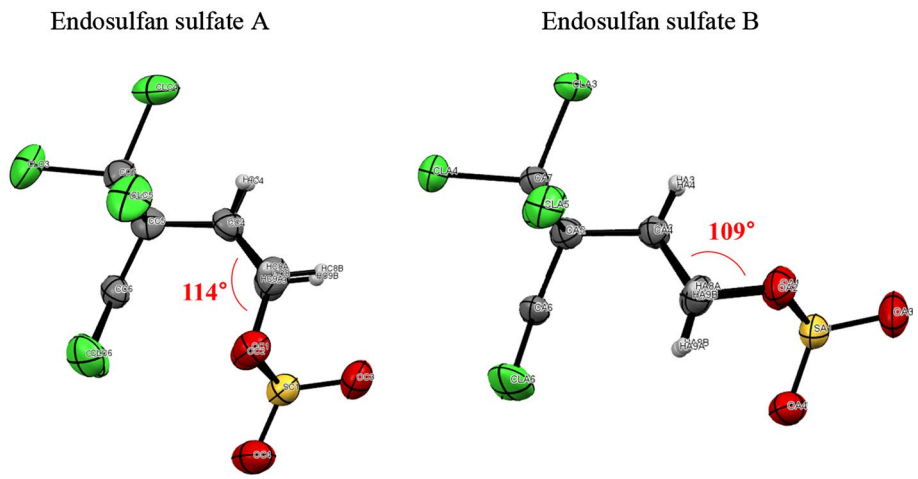


Fig. 3 ORTEP diagrams and numbering scheme of endosulfan sulfate. **a** Endosulfan sulfate A, **b** endosulfan sulfate B and **c** side view of endosulfan sulfate A and B

Table 2 Bond lengths (Å) of endosulfan sulfate

Bond	A-1	A-2	B
C(1)–C(2)	1.521(3)	1.522(3)	1.523(3)
C(1)–C(6)	1.321(3)	1.324(3)	1.322(3)
C(2)–C(3)	1.556(3)	1.554(3)	1.557(3)
C(2)–C(7)	1.552(3)	1.553(3)	1.553(3)
C(3)–C(4)	1.551(3)	1.563(3)	1.566(3)
C(3)–C(8)	1.512(3)	1.512(3)	1.515(3)
C(4)–C(5)	1.557(3)	1.561(3)	1.557(3)
C(4)–C(9)	1.511(3)	1.510(3)	1.514(3)
C(5)–C(6)	1.519(3)	1.508(3)	1.519(3)
C(5)–C(7)	1.557(3)	1.552(3)	1.549(3)
O(1)–C(8)	1.462(3)	1.454(3)	1.462(3)
O(1)–S(1)	1.5606(18)	1.5686(18)	1.5636(18)
O(2)–C(9)	1.462(3)	1.460(3)	1.462(3)
O(2)–S(1)	1.5553(18)	1.5584(17)	1.5599(18)
O(3)–S(1)	1.4212(17)	1.4148(19)	1.4100(18)
O(4)–S(1)	1.4099(18)	1.407(2)	1.4171(17)
Cl(1)–C(1)	1.696(2)	1.692(2)	1.690(2)
Cl(2)–C(2)	1.750(2)	1.746(2)	1.747(2)
Cl(3)–C(7)	1.761(2)	1.765(3)	1.7668(19)
Cl(4)–C(7)	1.759(2)	1.765(3)	1.762(2)
Cl(5)–C(5)	1.741(2)	1.747(2)	1.746(2)
Cl(6)–C(6)	1.694(2)	1.689(2)	1.689(2)

conformers, the total potential energy was calculated as 30.632 kcal/mol for A and 34.524 kcal/mol for B; these results indicate that the total energy of A is slightly lower than that of B, possibly leading to the generation of two molecules of A in the rigid crystal state. However, the two conformers only produced one peak in GC–MS analysis because two conformers should not exist in solution at room temperature for GC–MS analysis, which is likely due to the small energy difference of approximately 4 kcal/mol [15, 16].

Table 3 Bond angles (°) of endosulfan sulfate

Bond	A-1	A-2	B
C(8)–O(1)–S(1)	118.42(15)	118.90(16)	117.89(14)
C(9)–O(2)–S(1)	119.23(15)	118.37(15)	117.63(14)
O(4)–S(1)–O(3)	120.26(11)	121.54(12)	120.97(12)
O(4)–S(1)–O(2)	106.41(11)	106.54(12)	109.88(10)
O(3)–S(1)–O(2)	110.06(11)	109.33(10)	106.09(12)
O(4)–S(1)–O(1)	105.95(12)	104.84(11)	108.93(11)
O(3)–S(1)–O(1)	109.49(11)	110.10(12)	105.91(11)
O(2)–S(1)–O(1)	103.31(9)	102.86(9)	103.61(9)
C(6)–C(1)–C(2)	107.32(17)	107.11(19)	108.08(16)
C(6)–C(1)–Cl(1)	127.83(17)	128.07(18)	128.21(16)
C(2)–C(1)–Cl(1)	124.83(15)	124.80(16)	123.54(16)
C(1)–C(2)–C(7)	98.74(16)	98.49(18)	98.84(15)
C(1)–C(2)–C(3)	109.53(16)	109.78(17)	108.24(16)
C(7)–C(2)–C(3)	101.07(15)	100.93(18)	100.85(14)
C(1)–C(2)–Cl(2)	116.10(14)	115.83(16)	115.66(14)
C(7)–C(2)–Cl(2)	116.14(14)	116.37(16)	116.04(14)
C(3)–C(2)–Cl(2)	113.41(14)	113.56(16)	115.10(13)
C(8)–C(3)–C(4)	119.87(18)	118.40(19)	117.55(17)
C(8)–C(3)–C(2)	115.06(18)	116.8(2)	111.97(16)
C(4)–C(3)–C(2)	102.62(15)	102.66(17)	102.60(15)
C(9)–C(4)–C(3)	118.44(18)	118.55(18)	117.36(17)
C(9)–C(4)–C(5)	115.66(18)	115.51(19)	111.52(16)
C(3)–C(4)–C(5)	102.85(16)	102.32(17)	102.41(15)
C(6)–C(5)–C(7)	98.64(16)	98.62(17)	99.60(16)
C(6)–C(5)–C(4)	108.76(16)	108.43(17)	107.70(15)
C(7)–C(5)–C(4)	101.00(17)	101.74(17)	101.55(15)
C(6)–C(5)–Cl(5)	116.10(16)	116.37(16)	115.46(14)
C(7)–C(5)–Cl(5)	115.93(14)	116.09(16)	116.31(14)
C(4)–C(5)–Cl(5)	114.37(14)	113.68(15)	114.33(14)
C(1)–C(6)–C(5)	107.78(18)	107.99(18)	107.08(17)
C(1)–C(6)–Cl(6)	128.12(17)	128.07(18)	129.01(16)
C(5)–C(6)–Cl(6)	124.08(16)	123.80(16)	123.74(16)
C(2)–C(7)–C(5)	92.04(15)	92.23(16)	92.47(14)
C(2)–C(7)–Cl(4)	114.38(15)	114.81(17)	114.26(13)
C(5)–C(7)–Cl(4)	114.52(15)	113.88(17)	113.97(14)
C(2)–C(7)–Cl(3)	113.95(14)	113.91(18)	113.96(13)
C(5)–C(7)–Cl(3)	113.94(15)	113.77(17)	113.49(13)
Cl(4)–C(7)–Cl(3)	107.64(11)	107.84(13)	108.23(11)
O(1)–C(8)–C(3)	**114.19(17)**	**113.50(19)**	**109.12(17)**
O(2)–C(9)–C(4)	**113.86(17)**	**113.70(17)**	**108.94(17)**

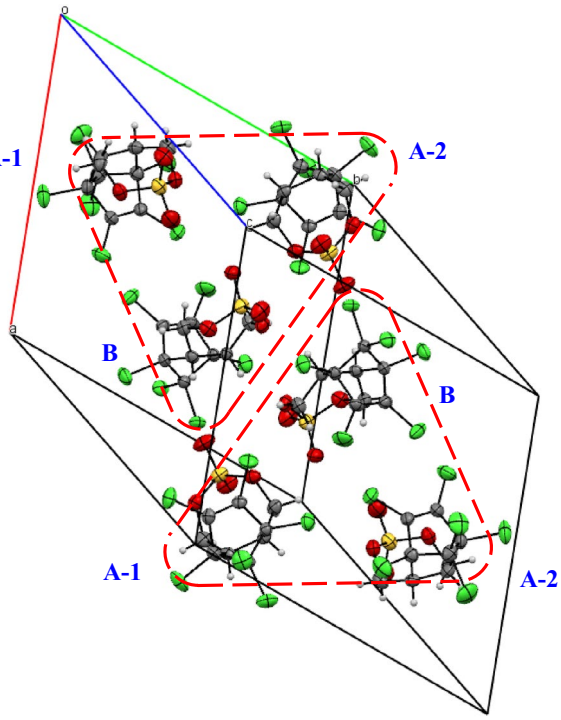


Fig. 4 Space packing diagram of endosulfan sulfate

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Authors' contributions
Author HKL performed the data analysis, interpretation, and wrote final manuscript. JL and JL contributed to design the experimental conditions of instrumental analysis. JKM assisted with the design of experiment. JHK supervised the project and revised the final manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Competing interests
The authors declare that they have no competing interests.

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