Metabolism of Endosulfan* Isomers by *Aspergillus niger*

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Endosulfan is being widely used in the Sudan for the control of cotton insect pests. Consequently there is a growing interest in its fate in the environment. Although the role of micro-organisms in the breakdown of chlorinated cyclodiene insecticides has already been established (BROOKS 1969), little information exists about endosulfan in this respect. OESER et al. (1971) reported the degradation of endosulfan by two algae, Chlorella and Scenedesmus, and in sewage sludge. The nontoxic endosulfan alcohol** was identified as the only metabolite in both cases.

The present investigation is concerned with the metabolism of the two isomeric endosulfans, which are the main constituents of the technical material, in cultures of the mould A. niger Van Tiegh.

Materials and Methods

α-endosulfan (1 fig.1) m.p. 108-110°C, β-endosulfan (11 fig.1) m.p. 208-210°C and endosulfan alcohol (111 fig.1) were a gift from Farbwerke Hoechst AG, Frankfurt.

The reagent Tri-Sil (a mixture of hexamethyldisilazane and trimethylchlorosilane in pyridine) was obtained from Pierce Chemical Company, Rockford, Ill.

The mould used was isolated from the Gezira soil. The liquid medium consisted of dextrose 10g, asparagin 5g, potassium dihydrogen phosphate 1g and hydrated magnesium sulphate 0.5g dissolved in 1 litre of distilled water.

The medium (250 ml) was placed in 500 ml Erlenmeyer flasks and inoculated with spores of A. niger. The substrates (250 µg) were delivered dissolved in 95% ethanol (50 µl), achieving a final concentration of 1 ppm. Ethanolic solutions of the substrates were freshly prepared. Control flasks containing fungus-free medium but with the substrates added were included. Flasks were left to stand at room temperature for 10 days during which

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* 1,2,3,4,7,7-hexachlorobicyclo (2,2,1) - 2 - hepten 5, 6 - bis (hydroxymethylen) - sulphite.

** 1,4,5,6,7,7-hexachloro-2,3 - bis (hydroxymethyl) - bicyclo (2,2,1) heptene - 5.

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period the fungus attained maximum growth. Flask contents were then filtered through muslin to separate the mycelia from the medium prior to extraction.

The mycelia (2g) were mixed with anhydrous sodium sulphate (5g) and sand (2.5g) in a mortar and ground to powder which was extracted in n-hexane using a Soxhlet apparatus. The medium (50 ml) was extracted in 3 x 10 ml volumes of a mixture of 20% diethyl ether in n-hexane by shaking in a 50 ml mixing cylinder. The organic phase was removed, dried over anhydrous sodium sulphate and made up to 50 ml using n-hexane.

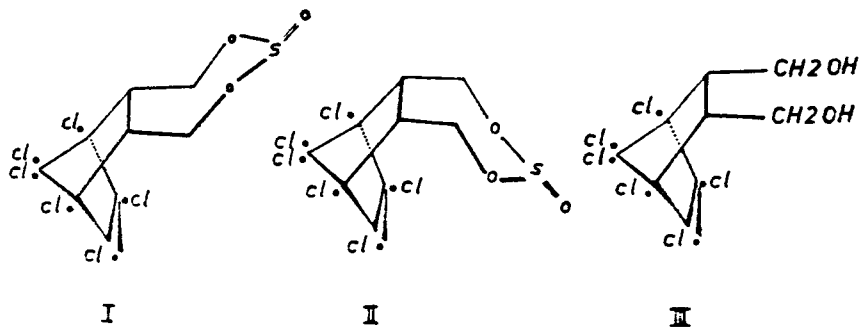

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Figure 1
1 - endosulfan, 11 - endosulfan, 111 - endosulfan alcohol.

For preparing the trimethylsilyl ether of the metabolite, 2 ml aliquots of each extract were treated with Tri-Sil reagent (25 µl) in a 10 ml glass stoppered tube and kept at 60°C for 30 minutes. Excess reagent was then destroyed by the addition of water (1 ml).

Analysis was carried out by electron-capture gas chromatography using a Perkin Elmer F 11 instrument, and thin layer chromatography, under the conditions detailed in Table 1.

Results and Discussion

The gas-liquid and thin-layer chromatographic properties of endosulfan isomers and endosulfan alcohol are shown in Table 1. Trimethylsilylation which was shown to be useful in the gas chromatographic analysis of numerous hydroxylated derivatives of cyclodiene compounds (KORTE & ARENT 1965; BROOKS & HARRISON 1966; EL-ZORGANI 1970) has similarly proved useful in the determination of endosulfan alcohol.

TABLE 1

GLC and TLC Constants of endosulfan Isomers and endosulfan alcohol.

Substance	*RRT(a)	*RRT(b)	R_f(c)
α-endosulfan	100	100	0.80
β-endosulfan	183	214	0.78
Endosulfan alcohol	67	-	0.20
Endosulfan alcohol TMS	84	91	0.30

* Relative to α-endosulfan.

(a) All glass column 6 ft. 1.5 mm i-d, packed with SE 52 2.5%, 0.5% epikote on chromosorb W (DMCS) 80-100. Column temp. 220°C, detector 225°C, injection block 280°C and nitrogen flow at 80 ml/min.

(b) Stainless steel column, 2 ft., 4.6 mm i-d., packed with SE 30 3%, 0.5% epikote on chromosorb W (DMCS) 80-100. Other conditions similar to those of (a).

(c) Adsorbent, kieselgel G 250 μ thick, mobile phase chloroform.

Considerable metabolism took place in the active incubations while the substrates were recovered unchanged from control treatments. The same metabolite was formed from both the α- and β- isomers and was identified as endosulfan alcohol by comparing its chromatographic properties with those of the authentic material. In Table II the quantities of the substrates recovered and the metabolite formed are expressed as a percentage of the total recovery after correcting for the quantity of the alcohol by multiplying by a factor of 1.127 to account for the decrease in molecular weight.

TABLE II

Hydrolysis and Distribution of Endosulfan Isomers in Cultures of A. niger.

Substrate	% recovered unchanged		% as endosulfan alcohol	
	Mycelia	Medium	Mycelia	Medium
α-endosulfan	37.50	28.30	-	34.20
β-endosulfan	37.40	31.90	-	30.70

The unchanged substrates are nearly equally distributed between the mycelia and the medium, indicating extensive mycelial uptake of the substrates. All the alcohol detected was present in the medium. It is interesting that both isomers were hydrolysed to the same extent in spite of their stereochemical differences. Should a difference in the rate of hydrolysis exist, it could not have been observed in this experiment because of the fairly long incubation period.

GORBACH et al. (1971) demonstrated the rapid disappearance of endosulfan residues in the water system of East Java, Indonesia, following large scale application of Thiodan (endosulfan) in rice. The maximum residue level detected was below 0.01 ppm. The results with A. niger strongly suggest that fungi are likely to play an important role in the decontamination of the environment from this insecticide.

Addendum

It was brought to our notice that Professor Domsch and coworkers in Germany have recently carried out some trials on the metabolism of endosulfan by microbes and fungi. Beside the diol as a main metabolite they also detected the oxidation product endosulfan sulfate.

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