Rapid Identification Method for Endosulfan from Glc Peak Shifts under the Influence of Aykali

P. A. Greve* and S. L. Wit

A simple method is described for the identification of endosulfan by observing the glc peak shifts brought about by diluted alcoholic alkali. The two isomers of endosulfan yield upon this treatment one new product, the retention time of which is half the sum of the retention times of the original isomers. The method is valid for amounts down to 0.05 ng α- plus β-endosulfan. The structure of the new compound could be derived from spectroscopical and chemical data.

The proper assignment of the glc peaks encountered in pesticide analysis can offer problems if the history of the sample under investigation is unknown. One method which can be useful in such cases is observation of the changes in the gas chromatogram after some (preferably simple) chemical or physical treatment (Asai *et al.*, 1967; Banks and Bills, 1968; Chau and Cochrane, 1968, 1969a, b; Chau, 1969; Cochrane, 1969; Hamence *et al.*, 1965; Kawano *et al.*, 1969; McCully, 1969; Pionke *et al.*, 1969; Wiencke and Burke, 1969). In the course of our investigations on the occurrence of endosulfan in surface waters (notably the river Rhine) we found that observation of the glc peak shifts under the influence of diluted ethanolic alkali can provide useful additional evidence for the presence of that insecticide. For practical reasons, the experimental conditions (which are given in detail below) were chosen according to those used by Pionke *et al.* (1969) for the identification of DDT-complex and methoxychlor; however, these conditions are not critical.

The glc peak shifts which can be observed are illustrated in Figures 1 and 2. Before alkali treatment two peaks, corresponding to α- and β-endosulfan, are present (A); after alkali treatment these peaks have merged into one new peak (B). The height of this new peak is about one sixth of the sum of the heights of the two original peaks. [The concentration after alkali treatment is chosen two times as high as before (Experimental).] The retention time of the new peak is about half the sum of the retention times of the two original peaks. Incomplete disappearance of one or both original peaks, or other deviations from the shift pattern described, indicates the presence of interfering substances (*e.g.*, endrin). This has been observed in a limited number of practical cases, notably for the β-endosulfan peak. The values found in quantitative analysis must be corrected in those cases.

The appearance of the new peak is still well observable if in the original injection 0.05 ng α- plus β-endosulfan is present. For our standard analytical method (Wit and Greve, 1971), in which a 500 ml water sample is taken into analysis, this corresponds to 0.1 ppb α- plus β-endosulfan, whose sensitivity is quite satisfactory for practical purposes. The simplicity of the method described here is an advantage over the method used by Chau (1969), in which reduction with $LiAlH_4$ and subsequent silylation have to be carried out prior to gas chromatographical analysis.

Laboratory for Toxicology, National Institute of Public Health, Sterrenbos 1, Utrecht, The Netherlands.

The compound formed by the treatment of endosulfan with ethanolic alkali has been synthesized on a semi-micro-scale (see Experimental) in order to establish its identity.

The mass spectrum of this compound (compound I) showed a parent peak-pattern with 5 Cl-atoms at *m/e* 368. Fragmentation patterns were present *inter alia* at *m/e* 351 (Cl_5, $-OH$), 333 (Cl_4, $-Cl$), and 323 (Cl_5, $-OC_2H_5$). The exact mass of the 333 peak was 332.9627, calculated for $C_{11}H_{13}O_3{}^{35}Cl_4$:332.9619.

The 100 MHz pmr spectrum is summarized in Table I.

A different compound is obtained if, instead of ethanol,

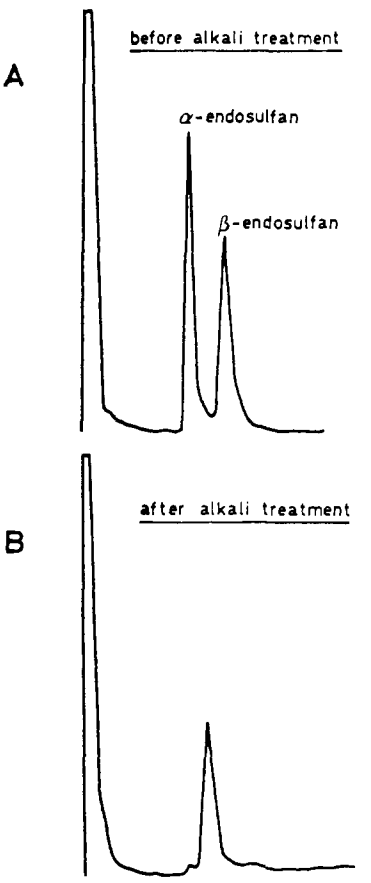


Figure 1. Gas chromatogram of an endosulfan standard solution before (A) and after (B) treatment with ethanolic alkali. Gas chromatographic conditions: Pyrex column, 5 ft 1/8 in. filled with 5% DC-200 on Aeropak 30. Carrier gas: nitrogen, 40 ml/min. Temperature: 200° C. Electron capture detection (H^3). The peaks in the upper chromatogram (A) correspond with 0.15 ng α- and 0.14 ng β-endosulfan

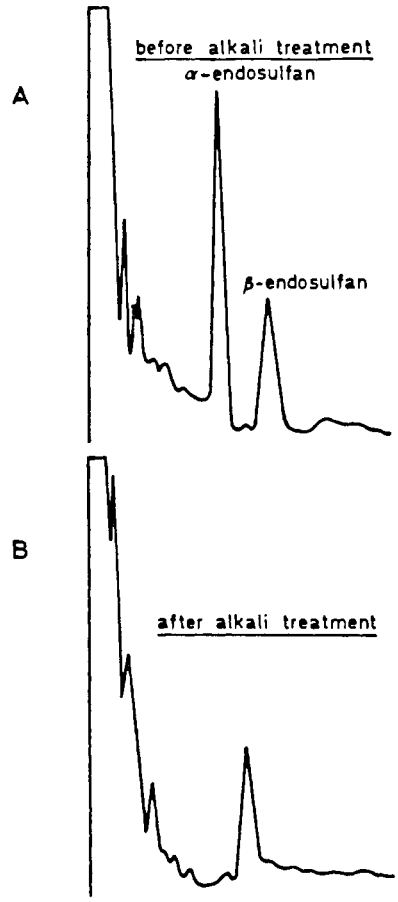


Figure 2. Gas chromatogram of a Rhine water extract before (A) and after (B) treatment with ethanolic alkali. Gas chromatographic conditions as indicated under Figure 1. The Rhine water extract corresponds to 500 ml water

methanol is used as the solvent. The 100 MHz pmr spectrum of this compound (compound II) is also summarized in Table I.

Typical differences in the ir spectra of endosulfan on one hand, and of compounds I and II on the other hand, are illustrated in Table II.

Both compound I and compound II are unchanged after 1 hr boiling with ethanolic and methanolic alkali, respec-

Table I. 100 MHz Pmr Spectrum of Compounds I and II in $CDCl_3$

δ (ppm)	Nature of the Pattern	J (Hz)	Coupled with Protons at δ =
	Compound I		
1.21	3 H, triplet X_3-part of ABX_3-pattern	7.1	3.82
2.02	1 H, broad (OH)	...	...
2.87	1 H, quartet	10.2[a] (broadened)	3.19
		3.1	4.30
3.19	1 H, multiplet	10.2[a]	2.87
		3.3	4.04
		10.0	4.33
		2.7[a]	4.73
3.82	2 H, center of AB-part of ABX_3-pattern	7.1	1.21
		10.0	
4.04	1 H, quartet	3.3	3.19
		10.5	4.33
4.11	1 H, doublet	9.5 (broadened)	4.30
4.30	1 H, quartet	3.1	2.87
		9.5	4.11
4.33	1 H, quartet	10.0	3.19
		10.5	4.04
4.73	1 H, doublet	2.7[a]	3.19
	Compound II		
2.02	1 H, broad (OH)	...	...
2.89	1 H, quartet	10.1 (broadened)	3.20
		3.0	4.33
3.20	1 H, multiplet	10.1	2.89
		3.4	4.03
		10.2	4.33
		2.7	4.74
3.53	3 H, singlet	...	...
4.03	1 H, quartet	3.4	3.20
		11	4.33
4.15	1 H, doublet	9.5 (broadened)	4.33
4.33	1 H, quartet	10.2	3.20
		11	4.03
	1 H, quartet	3.0	2.89
		9.2	4.15
4.74	1 H, doublet	2.7	3.20

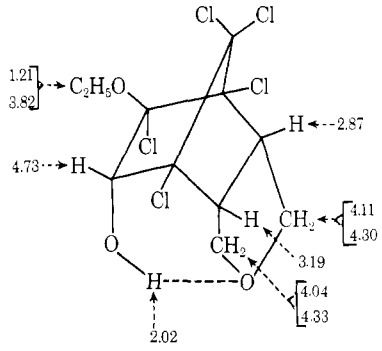
[a] Confirmed with double resonance.

Table II. Typical Features in the Ir Spectra of Endosulfan, Compound I, and Compound II (3‰ in KBr)

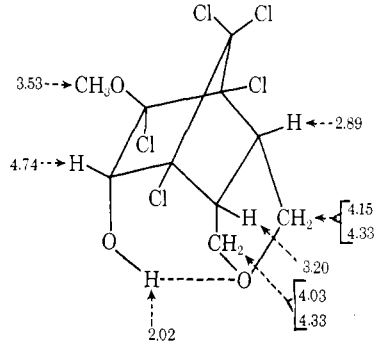
Endosulfan		Compound I		Compound II	
Wavelength (μ)	Structure-element[a]	Wavelength (μ)	Structure-element[a]	Wavelength (μ)	Structure-element[a]
...	...	2.85, 2.91	OH[b]	2.85, 2.91	OH[b]
6.27	C=C	...	...	...	...
...	...	7.87	OH	7.90	OH
8.41	S=O	...	...	...	...
...	...	8.43	C—O—C[c]	8.38	C—O—C[c]
...	...	9.39	C—O—C[c]	9.43	C—O—C[c]
...	...	9.62	C—O—C[c]	9.71	C—O—C[c]
9.98	S—O—CH_2	...	...	...	...
10.22	S—O—CH_2	...	...	...	...
...	...	11.10	C—O—C[c]	11.13	C—O—C[c]
11.61	S—O—CH_2	...	...	...	...
13.26	S—O	...	...	...	...
14.30	S—O	...	...	...	...

[a] Colthrup *et al.*, 1964; IRSCOT, 1967. [b] With H-bonding (intramolecular). [c] Part of a tetrahydrofuran ring.

tively, and after 1 hr boiling with 10% hydrochloric acid (acetone-water 1:1 medium). On behalf of these properties we propose the following structures for compounds I and II (chemical shifts indicated).



Compound I



Compound II

The mass spectra were recorded on an AEI MS-9 double-focusing mass spectrometer, the pmr spectra on a Varian HA-100 apparatus, and the ir spectra on a Perkin-Elmer Infracord 137 spectrophotometer.

EXPERIMENTAL

Analytical Procedure. One microliter of a standard solution containing 0.15 ng α-endosulfan and 0.14 ng β-endosulfan per μl was injected in a gas chromatograph under the following conditions. Column: 5 ft $^1/_8$ in. (o.d.), all-glass (Pyrex), filled with 5% DC-200 on Aeropak 30, 80-100 mesh, activated at 225° C during about 24 hr. Carrier gas: nitrogen, 40 ml/min. Detector: electron capture detector with H^3-foil. Temperatures; column and detector: 200° C; injection port: 230° C. The chromatogram obtained is given in Figure 1A.

One milliliter of the same solution was evaporated to dryness with a gentle nitrogen flow; the residue was dissolved in 10 ml of a 2% solution of KOH in 96% ethanol. The mixture was refluxed 12 min and after dilution with 100 ml of water the organic material was extracted with 20 ml hexane. The organic layer was dried with Na_2SO_4 and evaporated to dryness at room temperature with a gentle nitrogen flow. The residue was dissolved in 0.5 ml ethylacetate, and 1 μl of this solution was injected in the gas chromatograph under the same conditions as above.

The gas chromatogram subsequently obtained is shown in Figure 1B.

The same procedure applied to a typical field sample (Rhine water extract) yielded the chromatograms shown in Figures 2A and B. (NOTE: The same shift pattern is obtained if the refluxing is carried out with 100 ml of a 5% KOH solution for 30 min. This indicates that the experimental conditions for the derivatization are not very critical.)

Synthesis Procedure. Four grams of technical endosulfan (2:1 mixture of α- and β-endosulfan) was dissolved in 100 ml of a 5% solution of KOH in 96% ethanol. The mixture was refluxed for 30 min, diluted with water, neutralized with 10% hydrochloric acid, and extracted twice with methylene chloride. The organic layers were combined, washed, dried, and evaporated to dryness. The residue was dissolved in a few milliliters of chloroform, and *n*-hexane (about 150 ml) was added until the solution became cloudy. The oil was dissolved by gentle heating and the mixture was allowed to cool slowly to room temperature during the night. The next day the mixture was placed in the refrigerator (about 4° C) and kept another day. Well-shaped white crystals appeared which were collected, washed with hexane, and dried. The yield was 2.0 g (52%), m.p. 135–6° C. Compound II is obtained if the ethanol is replaced by methanol, but the refluxing time must then be extended to 1 hr. The melting point of compound II is 145–6° C.

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