An Adsorption Chromatography of Dieldrin with a Colour Indicator. Etsurō Ōta (Kaken-Kogyo Co., Ltd.) Received Jan. 26. 1959. *Botyu-Kagaku* **24**, 26, 1959.

5. 指示色素を用いた Dieldrin の吸着クロマトグラフ

太田悦郎（化研工業株式会社）

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Dieldrin を定量分析するに当っては，現在用い得るどの定量法によるにしても，まず試料中から dieldrin の有効殺虫成分である HEOD を定量的に分離，精製することが必要である．この目的にこれまでクロマトグラフ法がしばしば用いられてきたが，目的成分が無色であるため簡単で適確な分離が困難であり，いずれも満足できる分離法ではなかった．著者は，*p*-methoxy-azobenzene（黄色々素）を指示色素として，やや活性度の低いアルミナ柱上に HEOD をクロマトグラフすると，色素と HEOD とが全く一致した挙動を示すことを知った．この方法で色素部分の流下液を採ると HEOD は定量的に回収される．またこのクロマトグラフ法により，HEOD は *p*, *p*′-DDT, *γ*-BHC, 及び aldrin から明確に分離される．この方法で，ある Technical Dieldrin 中の HEOD を定量したところ 89—90% であった．

For the determination of HEOD, the total chlorine method is recommended on account of the simplicity. The method is, however, not specific for HEOD but for every halogen compounds. It is accordingly required to separate HEOD from contaminants in the sample before the determination. The present investigation was undertaken to devise a clear-cut chromatographic separation of HEOD, in which *p*-methoxy-azobenzene, a yellow dye, was used as an indicator. The indicator can follow up so closely the movement of HEOD in the course of the procedure that only the coloured fraction in the effluent is collected and subjected to the determination of HEOD. The separation of HEOD from its mixture with each of *p*, *p*′-DDT, *γ*-BHC, and aldrin were also described.

During the cource of an investigation of dieldrin it became desirable to have a method of separating a micro quantity of the toxicant from contaminants.

By literature three typical methods are available for determining dieldrin chemically or physically; that is, total chlorine[1,2,3,4], infra-red spectrophotometric[5,6], and colourimetric[7] method. Although the infra-red spectrophotometric and the colourimetric method are specific for HEOD (1, 2, 3, 4, 10, 10-hexachloro-6, 7-epoxy-1, 4, 4a, 5, 6, 7, 8, 8a-octahydro-1, 4, 5, 8-dimethanonaphthalene), the main insecticidally active ingredient in dieldrin, they require a fairly purified sample. On the other hand, the total chlorine method does not require such purification of the sample, but it is non-specific for HEOD, because it cannot distinguish HEOD from other organic halogen-compounds. However, when there is no halogen-compound except HEOD in the sample, the total chlorine method is applicable to determination of HEOD with advantages of simplicity[1] or high sensibility[2]. Therefore it is necessary to devise a method of quantitative separation of HEOD from contaminants before the determination.

To isolate small quantities of HEOD, chromatograpraphy has been often employed[7,8], which may be one of the most suitable for that purpose. The toxicant, however, being colourless, it was difficult in any cases to follow up HEOD on chromatogram or in effluent. To eliminate this difficulty, it seems convenient to use some colouring matters as indicator, which makes the operation very easy. Beckmann[9] presented a partition chromatography of dieldrin with two indicator dyes, but the procedure failed to detect the HEOD front in the effluent. Regarding this disadvantage, an another dye, DuPont Anthraquinone Green G Base Dye, was presented[10] as an adequate one, but details were not given.

This paper proposes an adsorption chromatography of dieldrin with *p*-methoxy-azobenzene (MAB), a yellow dye, as an indicator, which makes it possible to trace the movement of HEOD during the course of the procedure; the HEOD front is effluted quite together with that

of MAB, collecting all amounts of the yellow-coloured effluent gives a quantitative recovery of HEOD, and, if necessary, the MAB is easily removed from the HEOD thus separated.

Reagents

Technical Dieldrin: Tan flakes, mp 125-150°, supplied by Shell Sekiyu Kabushiki Kaisha, Japan.

HEOD: purified from Technical Dieldrin by recrystallization from ligroin, mp 178.5-9°.

p-Methoxy-azobene (MAB): Prepared by methylation of *p*-hydroxy-azobenzene with dimethylsulfate in methanol in presence of an excess of potassium carbonate, recrystallized from petroleum ether, mp 57-8°.

Aldrin: Recrystallized from ethanol, mp 99-102°, slightly impure.

γ-BHC: Recrystallized from benzene, mp 113.5-4°.

p,p'-DDT: Recrystallized from ligroin, mp 109-9.5°.

Active alumina: "Aluminium oxide standardized for chromatographic adsorption analysis acc. to Brockmann", so-called "Brockmann's alumina", a product of E. Merck A. G., Germany (Brockmann's alumina), or "Alumina, activated for chromatographic adsorption analysis, 200 mesh", a product of Wako Pure Chemical Industries, Ltd. Japan. (Wako's alumina). The active alumina commercially available is tested for its activity according to Brockmann's method[11]. When the alumina shows an activity of grade II, it can be used without any more treatments; when an activity of grade I, its activity is reduced to grade II by standing overnight in wet air.

Petroleum ether: Purified by treatment with conc. sulfuric acid and subsequent distillation, bp 40-70°, dried over metallic sodium.

Benzene: Purfied by the similar way to petroleum ether, bp 80°, dried over metallic sodium.

Mixed solvent: Prepared by mixing benzene (1 vol.) with petroleum ether (4 vol.).

Stannous chloride solution: Prepared by dissolving stannous chloride, $SnCl_2 \cdot 2H_2O$, (5g) in conc. hydrochloric acid (20cc) and subsequent diluting with distilled water to 100cc, stored in a tightly stoppered bottle.

Dil. hydrochloric acid: Prepared by diluting conc. hydrochloric acid (1 vol.) with distilled water (9 vol.).

Apparatus and Procedure

Here is described the procedure applicable to samples containing more or less than 10mg of HEOD in a rather pure state.

Chromatography: An adsorption column is prepared of the active alumina (7g) using a burette-shaped glass tube of about 10mm in diameter according to either dry- or wet-process.

A solution of sample with MAB (1mg) in petroleum ether (about 10cc) is poured on the column, and as the solution passed into the column, a yellow adsorption band is formed on the upper end of the alumina layer. Added with the mixed solvent, the yellow band, which is not developed with petroleum ether, begins to move downward, and is effluted at the lower end of the column. The yellow-coloured effluent is collected, the solvent is carefully evaporated to almost dryness on a boiling water bath, and the last trace of the solvent is removed by gentle aeration at a room temperature. The yellow residue thus obtained contains all amounts of HEOD in the sample.

Decolourization: The yellow residue is dissolved in 95% ethanol (10cc), added with the stannous chloride solution (5cc), and heated slowly just to boiling; then the decolourization is performed. The colourless ethanolic solution is transferred into a 500cc-separatory funnel, rinsing the container with 95% ethanol (3×5cc) and distilled water successively, and added with the dil. hydrochloric acid (50cc) and a sufficient amount of distilled water to make up the whole volume to about 200cc. The solution is shaken with petroleum ether (100cc) for 5 minutes, and allowed to stand until separation. The aqueous layer is discarded, and the remaining solvent layer is washed with the dil. hydrochloric acid (2×50cc) and distilled water (3×100cc) successively. The solvent layer is transferred into a

weighed flask, and evaporated carefully as described above. A colourless residue is obtained, which usually consists of crystalline HEOD.

The decolourization process could be omitted for the purpose of prepurification in the total chlorine method.

Relationship between HEOD and MAB in their chromatographic behavior.

To prove the adequate property of MAB as the indicator, a series of chromatographic experiments, over twenty, was carried out under such various conditions as shown in Fig. 1.

Procedure: A sample solution containing HEOD (10mg) and MAB (1mg) was chromatographed according to the procedure described above except that the effluent was fractionated in 1cc. After removal of the solvent the fractions were determined for HEOD by the total chlorine method, which is the slightly modified Stepanow's method, of recovery of 98%, and accurate to within 1%. The HEOD concentration thus determined was plotted against the volume (cc) of the effluent fractionated. Some of the results are given in Fig. 1., in which the broken curves represent pualitative intensity of the yellow colour in the fractions.

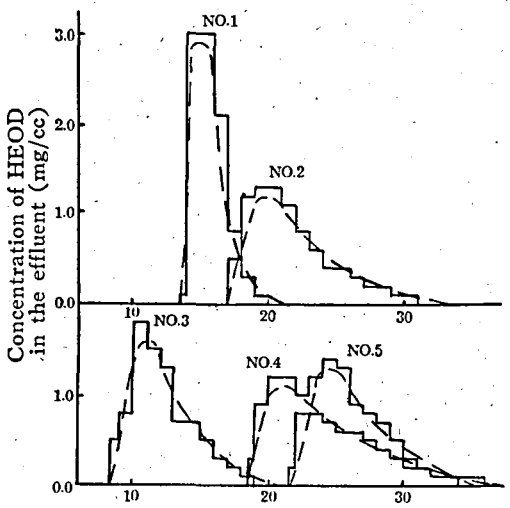

Fig. 1. Relationship between HEOD and MAB in their chromatographic behavior.

Conditions applied:

No. of the graph	Kind of the alumina	Activity of the alumina	Process of packing column	Temperature (°C)
1	Wako	Medium	dry	29
2	Wako	High	dry	33
3	Brockmann	Low	dry	26
4	Brockmann	Medium	wet	16
5	Brockmann	Medium	dry	10

In Fig. 1. it is shown that MAB always follow up HEOD on any conditions, although the volume of the fore-running solvent and sharpness of the separation of HEOD are influenced. This shows that MAB proves to be adequate as the indicator.

Recovery of hemimicro quantity of HEOD.

Procedure: The same chromatography, as that in the above experiment except the fractination was carried out on a column of Brockmann's alumina (7g) prepared by dry-process. The first 15cc of the effluent after the yellow front reached the lower end of the column was collected as the main fraction. After removal of the solvent the fraction was subjected to determination of HEOD by the total chlorine method. No HEOD was found in other fractions than the main fraction. The results are given in Table 1.

Table 1. Recovery of hemimicro quantity of HEOD.

Added(mg)	Recovered (mg)		
	Fore fraction	Main fraction	Back fraction
10.0	0.0	9.6	0.1
10.0	0.0	9.7	0.0
10.0	0.0	9.6	0.0
10.0	0.0	9.7	0.0
10.0	0.0	9.8	0.0

From the results given in Table 1, it is concluded that 10mg of HEOD was recovered quantitatively.

To confirm complete elution of HEOD, the columns were eluted with methanol after the elution with the mixed solvent, and the methanolic eluates were determined by the same method; thay gave usually 0.7-0.9mg of apparent HEOD, while the same values were given also by blank test. Therefore the substance which

gave the apparent HEOD should not be HEOD, but, judged from its water-solubility, it might be some inorganic salts, contained in the alumina used.

Recovery of macro quantity of HEOD and determination of HEOD in Technical Dieldrin.

Procedure: A column was prepared of Brockmann's alumina (20g) using a glass tube of 20mm in diameter. A solution of HEOD (100.0 mg) or Technical Dieldrin (100.0mg) and MAB (10mg) in petroleum ether (20cc) was chromatographed on the column similarly to the procedure described above. When about 30-33cc (at 30°) of the mixed solvent had passed into the column, the yellow front appeared in effluent, and the yellow effluent, usually 60-70cc, was collected as the main fraction. Both 10cc of the effluent just before and after the main fraction were collected and determined for HEOD by the total chlorine method. After removal of the solvent, the main fraction was decolourized according to the procedure described above; then it gave a colourless crystalline residue, which was then weighed and measured its melting point.

The residue gained from either HEOD or Technical Dieldrin gave the identical infra-red spectra with the authentic sample of HEOD, and the mixed melting point was not depressed. These facts indicate that the residues consisted of pure HEOD. Then, from the results given in Table 2., it is concluded that 100mg of HEOD was recovered quantitatively, and that the HEOD content in the Technical Dieldrin used should be 89-90%.

Separation of HEOD from other insecticides.

Procedure: A solution of HEOD (10mg), each (10mg) of other insecticides, *p,p'*-DDT, γ-BHC, and aldrin, and MAB(1mg) was chromatographed on a column of Wako's alumina (7g) according to the same procedure described above except that the fractionation of effluent in 1cc started from the addition of the sample solution, because some of the insectides had been eluted readily with petroleum ether before the mixed solvent was added. The fractions were determined for each insecticide by the total chlorine method.

As shown in Fig. 2. it is evident that HEOD could be separated clearly from other insecticides by this method.

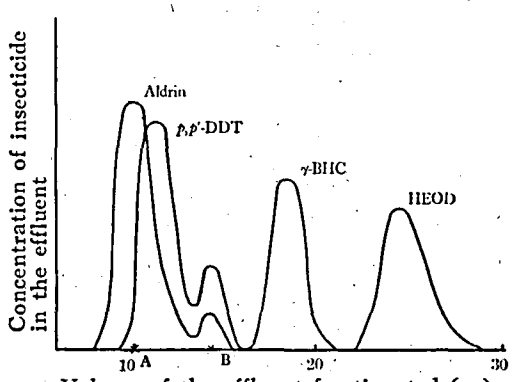

Fig. 2. Separation of HEOD from other insecticides.
A: The addition of the mixed solvent.
B: The front of the mixed solvent.

Table 2. Determination of HEOD in Technical Dieldrin.

Sample (mp °C)	Added (mg)	Recovered (mg)		
		Fore fraction	Main fraction (mp °C)	Back fraction
HEOD (As standard) (178.5-9)	100.0	0.0	100.0 (177.5-8.5)	0.1
	100.0	0.0	99.0 (177 -8)	0.0
	100.0	0.0	99.6 (177 -8)	0.0
	100.0	0.0	99.0 (177 -8)	0.0
Technical Dieldrin (125-50)	100.0	0.0	89.9 (177 -8)	0.1
	100.0	0.0	89.3 (177 -8)	0.0
	100.0	0.2	89.9 (177 -8)	0.0
	100.0	0.0	89.0 (177 -8)	0.0

Summary

An adsorption chromatography of HEOD with *p*-methoxy-azobenzene as indicator was investigated. The indicator makes it possible to trace the movement of HEOD during the procedure, and HEOD is recovered quantitatively.

By this method, the HEOD content in a Techical Dieldrin was detemined to be 89-90%, and also, HEOD was separated successfully from its mixtures with each of *p,p'*-DDT, *γ*-BHC, and aldrin.

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Parathion Residue in Rice Grains. Chemical Studies on Organophosphorous Insecticides. I. Sinko Goto, Itiro Muta, Rokuro Sato (Agricultural Chemicals Inspection Station, Ministry of Agriculture and Forestry Kodaira-mati, Tokyo.). Received Jan. 27. 1959. *Botyu-Kagaku*, **24**, 30, 1959 (with English résumé, 34).

6. 玄米中の Parathion 残留量

有機燐殺虫剤の化学的研究 (第1報) 後藤真康・牟田一郎・佐藤六郎 (農林省農薬検査所) 34. 1. 27 受理

Parathion の微量化学分析法の Averell-Norris 法を玄米中の parathion の定量に適するように改変した. 本法の回収率は 100%, 誤差 4γ, 検出限界は 100g の試料に対し 0.04p.p.m. である. 本法を用いて parathion 撒布歴の明かな32年度産米について分析した所, parathion は検出されなかった. 又玄米中での parathion の経時変化について研究した.

Parathionは我が国において水稲のめい虫防除をはじめ, 果樹・そ菜等に最も多量に使われている農薬であるが, その毒性がかなり大きいため, 海外においては早くから作物体中の残留量が問題とされ, 多くの定量が行われている[1,2]. 我が国においても, 果実・茶・煙草等については既に若干の定量が行われているが[3,4] 米については, 稲体上の残留量・浸透移行量等の研究は行われたが[5], 玄米については, 撒布時期が大体出穂以前であり, 稲体上での消滅もかなり速かで, 浸透移行量もあまり大でないので, 玄米中に parathion が残存する危険については従来ほとんど考えられず, 定量も行われなかった. しかるに32年7月, 石川県において, 同県産の玄米・糠等よりかなり多量の parathion が検出された事が新聞紙上に報道され, 一般のつよい関心をよんだ. 我々は直ちに同県において検出試験を行った試料の分与をうけ, 追試を行ったが, その結果は既報の如く[6], parathion は検出されなかった. その後更に分析法に検討を加え, 又農林省植物

昭和33年3月, 日本農芸化学会関東支部講演会にて講演.