state according to the Tschesche method and characterized, respectively, as tigogenin- and gitogenin-tetraglycosides both of which equally have one mole each of D-galactose and D-xylose and two moles of D-glucose as the sugar moieties. I-1, m.p. 284~286° (decomp.), $[\alpha]_D^{28}$ −64.0°, was named desgalactotigonin and I-2, m.p. 252~255° (decomp.), $[\alpha]_D^{25}$ −58.5°, F-gitonin. Three pairs of saponins in II were assumed to be penta-, hexa- and hepta-glycosides and each pair was presumed to consist of tigogenin- and gitogenin-glycosides both of which might have the same sugar composition.

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## 183. Masaaki Horiguchi, Mitsuo Ishida, and Nobuyuki Higosaki : Determination of Some Organophosphorous Insecticides by Gas-Liquid Partition Chromatography.

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Recently, many reports on gas chromatography of insecticides have been published with advances in gas chromatographic techniques. The electron capture type detector[1] was preferably used for the determination of insecticide residue in these papers.[2~6]

Organochlorine insecticides have been chiefly investigated, while only a few studies on organophosphorous insecticide have been reported.[3,7~11]

Many kinds of organophosphorous insecticides have been widely used in agriculture and in the other fields. The qualitative and quantitative analysis of these compounds required very complicated procedures, so the analysis of mixtures was quite difficult. Therefore, the application of gas chromatographic technique to a rapid and simplified determination of these organophosphorous compounds was carried out. Nine organophosphorous compounds were selected from various kinds of insecticides, and good analytical results were obtained from the following investigation.

The insecticides selected were as follows :

(1) O,O-Dimethyl O-(*p*-nitrophenyl)phosphorothioate. (Methyl Parathion)
(2) O,O-Diethyl O-(*p*-nitrophenyl)phosphorothioate. (Parathion)
(3) O,O-Dimethyl S-[1,2-bis(ethoxycarbonyl)ethyl]phosphorodithioate. (Malathion)
(4) O,O-Diethyl O-(2-isopropyl-4-methyl-6-pyrimidinyl)phosphorothioate. (Diazinon)
(5) O,O-Dimethyl S-(N-methylcarbamoylmethyl)phosphorodithioate. (Dimethoate)
(6) O,O-Dimethyl-O-(3-methyl-4-nitrophenyl)phosphorothioate. (Sumithion)

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(7) 1,2-Dibromo-2,2-dichloroethyl dimethyl phosphate. (Dibrom)
(8) O,O-Dimethyl O-(3,4,6-trichlorophenyl)phosphorothioate. (Nankor)
(9) O-Ethyl O-*p*-nitrophenyl phenyl phosphorothioate. (E.P.N.)

Column packings used in this study were i) 1% SE-30 on 80~100 mesh Chromosorb-W (SE-30), ii) 1% FS-1265 (QF-1) on 80~100 mesh Chromosorb-W (FS-1265), iii) 1% polydiethyleneglycol succinate on 80~100 mesh Chromosorb-W (DEGS). Each sample, 3~6 μl. of 0.1% acetone solution, was injected by microsyringe.

Each organophosphorous insecticide used was purified by recrystallization or redistillation, until it gave a single peak on gas chromatogram.

In the first step the gas chromatography of each compound was studied, and the optimum conditions for the separation were selected. Under these selected conditions the gas chromatogram of a mixture of eight insecticides (except E. P. N.) was measured.

The chromatogram on SE-30 is shown in Fig. 1.

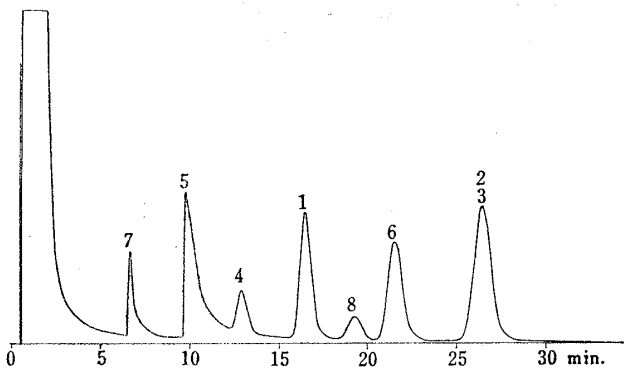


Fig. 1. 1% SE-30 on 80~100 mesh Chromosorb-W
column temp. 125°, argon flow rate 62.5 ml./min.

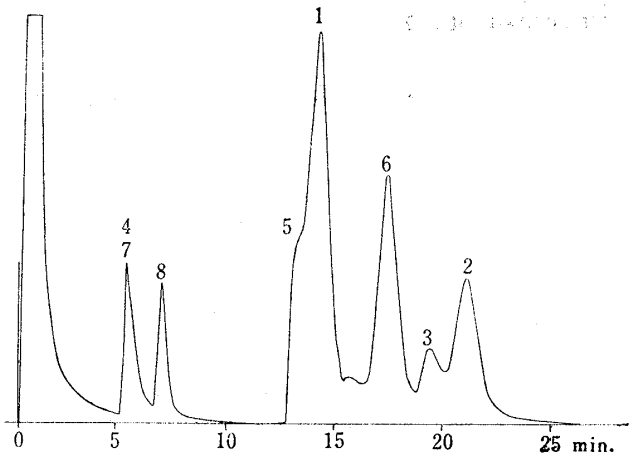


Fig. 2. 1% FS-1265 on 80~100 mesh Chromosorb-W
column temp. 130°, argon flow rate 108 ml./min.

E.P.N. had a longer retention time than those of other eight insecticides. Under these conditions, its retention time was about 8.2 times longer than that of Methyl parathion and showed a broad peak. Therefore, the chromatogram of E.P.N. was obtained under other conditions: column temp. 180°, argon flow rate 80 ml./min.

The chromatogram on FS-1265 is shown in Fig. 2.

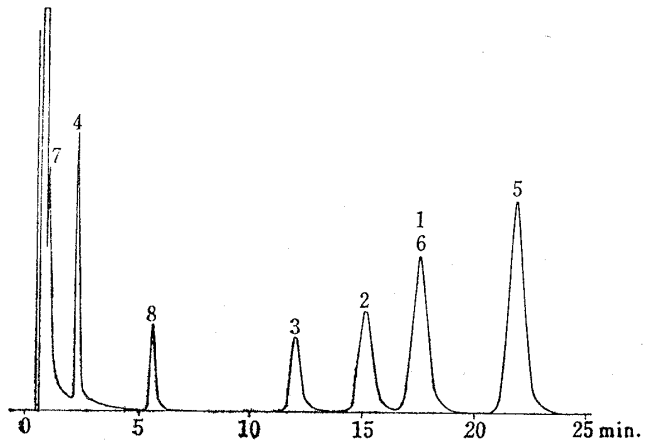


Fig. 3. 1% DEGS on 80~100 mesh Chromosorb-W
column temp. 145°, argon flow rate 104.5 ml./min.

Under these conditions the retention time of E.P.N. was about 6.4 times longer than Methyl parathion.

Fig. 3 shows the chromatogram on DEGS.

The peaks of Dibrom and Diazinon appeared too fast under these conditions. Therefore the column temperature was decreased to 130° and good result were obtained. Under the conditions giving Fig. 3, E.P.N. has also longer retention time similar to the case of SE-30 and FS-1265. The retention time of E.P.N. was about 5.3 times longer than that of Methyl parathion.

In order to obtain a more suitable chromatogram of E.P.N., the column temperature was maintained at 190° and carrier gas flow rate was kept at 82 ml./min. A symmetrical single sharp peak could be obtained at the retention time of 13 minutes.

Relative retention times of each insecticide on three column packings were listed in Table I.

Table I. Relative Retention Times[a,b] of Nine Insecticides

| Compound | SE-30 | FS-1265 | DEGS |
|---|---|---|---|
| Methyl parathion | 1.00[c] | 1.00[d] | 1.00[e] |
| Parathion | 1.62 | 1.51 | 0.86 |
| Malathion | 1.62 | 1.38 | 0.68 |
| Diazinon | 0.78 | 0.37 | 0.11 |
| Dimethoate | 0.58 | 0.94 | 1.26 |
| Sumithion | 1.31 | 1.24 | 1.00 |
| Dibrom | 0.39 | 0.37 | 0.03 |
| Nankor | 1.17 | 0.48 | 0.30 |
| E.P.N. | 8.20 | 6.40 | 5.30 |

*a*) Gas hold up adjusted.
*b*) Methyl parathion=1.00
*c*) Time, 15.9 min.; column temp., 125°; argon flow rate, 62.5 ml./min.
*d*) Time, 13.7 min.; column temp., 130°; argon flow rate, 108 ml./min.
*e*) Time, 16.9 min.; column temp., 145°; argon flow rate, 104.5 ml./min.

From the experimental results described above, the retention time of Dimethoate was found to increase with the increase of the polarity of column packings, but that of Malathion decreased. The peaks of the following insecticides could not be separated completely, because of the same or almost the same retention times, Parathion and Malathion on SE-30 column, Dibrom and Diazinon, Dimethoate and Methyl parathion on FS-1265 column; Sumithion and Methyl parathion on DEGS column. As shown in Figs. 1~3, DEGS column gave the best peaks, and SE-30 and FS-1265 gave better peaks. From these experimental results, a nine insecticide mixture could not be separated completely on only one kind of column, but using two column packings, *i.e.* DEGS and SE-30, complete separation was attained.

The most suitable conditions which were obtained in this study for the separation of each compound as well as the detectable limits of nine insecticides are listed in Table II.

Table II. Suitable Conditions and Detectable Limits

| Compound | Column | Column temp. (°C) | Detectable limit (μg.) |
|---|---|---|---|
| Methyl parathion | SE-30 | 125 | 0.4 |
| Parathion | DEGS | 145 | 0.6 |
| Malathion | 〃 | 145 | 0.6 |
| Diazinon | 〃 | 130 | 0.2 |
| Dimethoate | 〃 | 145 | 0.8 |
| Sumithion | SE-30 | 125 | 0.8 |
| Dibrom | 〃 | 125 | 0.8 |
| Nankor | 〃 | 125 | 0.3 |
| E.P.N. | 〃 | 180 | 0.2 |

These results were determined with the maximum sensitivity, because the sensitivity of a detector used was lower than that of the electron capture detector.[1)]

When the sample injection part temperature was over 200°, Dimethoate decomposed, so it could not give a single peak. The results of the experiments on the separation

were applied to the quantitative determination of insecticides. Commercially available 1.5% Malathion dusts were chosen as the sample. Nankor was chosen as internal standard, because it was easily purified and had a suitable retention time relative to Malathion.

Gas chromatographic conditions adopted were as follows: 1% DEGS column and the temperatures of the column, flash heater and detector were 145°, 210°, and 170° respectively; the detector voltage was 1400 v. and electrometer full scale sensitivity range at $3\times10^{-7}$ amp. Argon flow rate was 60 ml./min. Under these conditions, the gain control was unnecessary to obtain the chromatogram of a mixture of 10~20 γ Malathion and 5 γ of Nankor.

Five solutions of suitable concentrations were prepared for obtaining a calibration curve. The procedure was as follows; standard Malathion was weighed and dissolved in about 0.1% acetone solution of Nankor which was accurately weighed. About 5 μl. of each solution was injected and a calibration curve was drawn from the ratio of peak area and of weight between Malathion and Nankor.

The sample extracted from Malathion dusts was dissolved in Nankor solution and made up to a suitable concentration. Malathion content was estimated from the calibration curve. The experiment was repeated four times and the Malathion contents in the dusts were as follows: 1) 1.645%, 2) 1.655%, 3) 1.743%, 4) 1.748%.

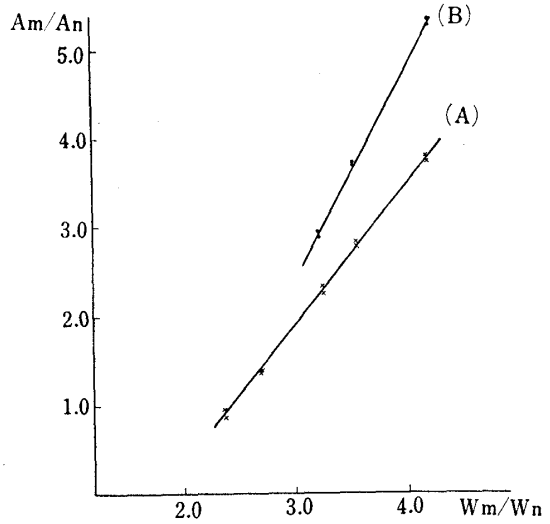


Fig. 4. Calibration Curve of Malathion

Am/An=peak area of Malathion/peak area of Nankor
Wm/Wn=weight of Malathion/weight of Nankor

On the other hand, the colorimetric determination of Malathion (O,O-dimethyl dithiophosphate cupric salt method) was carried out and repeated three times.

The results were as follows: 1) 1.65%, 2) 1.68%, 3) 1.68%.

The reproducibility of gas chromatographic method was not superior to that of colorimetric method, but the mean values of two methods were almost equivalent. The calibration curve remained unchanged over the period of a few days, when the instruments operated continuously under constants conditions. As shown in Fig. 4, however, once the instruments were switched off, it was difficult to obtain the same calibration curve even though the conditions were reset carefully and internal standard methods were employed. Curves A and B in Fig. 4 were obtained by two measurements on other days, and the differences indicate the influence of switching off the instruments.

## Experimental

**Apparatus and Column Packings**——A Barber Column Model 10 gas chromatograph equipped with a tritium argon ionization detector was used in this investigation. The column was a U-shaped Pyrex glass tube, 6 mm. i.d. and 2 m. length, filled with a solid support of acid washed Chromosorb-W, treated with dimethyldichlorosilane and coated with a liquid phase. The liquid phases used are as follows; 1% SE-30 (G.E. Methyl substituted type silicone gum), 1% FS-1265 (QF-1. Dow Corning, fluoro alkyl silicone polymer), 1% Polydiethyleneglycol succinate (Craig's polyester succinate).

**Extraction of Malathion from 1.5% Malathion Dusts (commercially available)**——In a 100 ml. measuring flask, place 0.4 g. of an accurately weighed Malathion dusts, add about 70 ml. of abs. EtOH and shake well for 15 min. Then make up exactly to 100 ml. with abs. EtOH and filter this solution through a filter paper. Apply the filtrate to gas chromatographic and colorimetric determinations.

**Sample of Gas Chromatographic Determination**——Take 50 ml. from extracted (100 ml.) sample solution accurately, remove the solvent by distillation and dissolve in a solution containing suitable amounts of the internal standard.

**Colorimetric Determination of Malathion**——In a separatory funnel place 20 ml. of abs. EtOH, then add 5 ml. of sample extract, add 2 ml. of 0.5*N* NaOH solution. Shake to mix them for 15~20 seconds and let the solution stand for 3 min. To this solution add 75 ml. of ferric reagent (dissolve 0.2 g. of $FeCl_3$ in a small quantity of distilled water, add 8 ml. conc. HCl and adjust the volume to 1000 ml. with distilled water), shake throughly and let the solution stand for 5 min. Then add 25 ml. of carbon tetrachloride (freshly redistilled) and 2 ml. of $CuSO_4$ solution (dissolve 1 g. of $CuSO_4 \cdot 5H_2O$ in distilled water and dilute to 100 ml.), immediately shake vigorously for 1 min., let the solution stand for 3 min. to permit the layers to separate, draw off the $CCl_4$ layer and filter the separated $CCl_4$ layer through a piece of dried filter paper. Determine the absorbance of the filtrate at 418 mμ within 10~25 min. after addition of $CuSO_4$ solution. The absorbance determined is defined as $E_1$. On the other hand, weigh about 0.22 g. of standard Malathion accurately, dissolve in abs. EtOH and make up to 100 ml. Treat a 5 ml. portion of this solution in the same manner as that for sample solution. The absorbance obtained by this standard is defined as $E_2$. The quantity of Malathion is obtained by the following equation :

The percentage of Malathion in sample

$$\frac{E_1}{E_2} \times \frac{\text{wt. of Malathion in standard (mg.)}}{\text{wt. of sample (mg.)}} \times 0.1 \times 100$$

The authors wish to express their deep gratitude to Dr. M. Matsui, Director of this laboratory, and Dr. A. Ito, group leader, for their warm encouragement during the course of this work.

## Summary

Gas chromatography was successfully applied to the separation of a mixture of nine organophosphorous insecticides using three different column under varying conditions. This method was applicable to the quantitative analysis of commercial Malathion dusts and provided results comparable to the colorimetric estimation.

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## 184. Kazukichi Kato : A New Color Reaction of Steroid with Anhydrous Aluminum Chloride and Anisaldehyde. IV.*1 Studies on the Reaction Mechanism.

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The new color reaction of steroid with anhydrous aluminum chloride and anisaldehyde was proposed by the present author at first for the colorimetric determination of ethylestrenol,[1] and was employed in the determination of allylestrenol and cholesterol.[2]

As reported in the second paper of this series,[2] a conclusion drawn from the results of an investigation on the selectivity of this color reaction was that a double bond in steroidal molecule may be responsible for coloration; the steroid having an isolated double bond or conjugated double bonds between four carbon atoms gave a positive

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