**The Conversion of an Insecticidal Compound, 1, 3-dithiocyanato-2-*N*, *N*-dimethyl-aminopropane, to Nereistoxin*.** Masayuki KATO (Experiment Department, Agricultural Chemicals Divsion, Takeda Chemical Industries, Ltd., Ichijoji, Sakyoku, Kyoto) Received June 20, 1967. *Botyu-Kagaku*, 32, 70, 1967.

## 9. イソメ毒 (Nereistoxin) の1誘導体, 1, 3-dithiocyanato-2-*N*, *N*-dimethylaminopropane の生体ホモジネートおよび SH-化合物との反応によるイソメ毒への変化

加藤正幸 (武田薬品工業株式会社 農薬事業部農薬試験部) 42. 6. 20 受理

昆虫, 哺乳動物の臓器のホモジネートあるいは植物汁液などを用いて 1, 3-dithiocyanato-2-*N*, *N*-dimethylaminopropane のチオシアナト基の代謝について検討した。その結果, 哺乳動物の肝臓に最も高い活性が見られ, その順序は哺乳動物の肝臓>脳>心臓>筋肉>昆虫>植物であった。活性の最も高いブタ肝臓のホモジネートについで検討した結果, この代謝を触媒する物質は酵素ではなく, 熱に安定な物質であると思われた。また反応液にSH阻害剤, SH化合物またはいわゆる代謝阻害剤を加えた実験, あるいは数種の昆虫および哺乳動物の肝臓ホモジネート中のNおよびSH基含量の定量の結果からこの反応にはSH基が関与しているものと思われた。この反応生成物はペーパークロマトグラフィー, ろ紙電気泳動, 試薬に対する呈色性あるいは抽出物の紫外部における吸光曲線などから, 本誘導体から脱シアンされたイソメ毒であろうと推定された。

一方SH基を有する化合物と本誘導体との反応について検討した結果, ホモジネートとの反応と同様にイソメ毒に変化し, またこの反応はSH基との混合比に応じて定量的に起るものと思われた。しかし本誘導体はSH基以外の還元性の化合物とは反応しなかった。これらの反応は殺虫試験の結果本誘導体の毒性を高めるものであろうと思われた。

1, 3-Dithiocyanato-2-*N*, *N*-dimethylaminopropane (abbreviated as TCMAP, a derivative of nereistoxin), was reported to have an insecticidal activity by Konishi *et al.*[1] It is effective in the practical control of rice stem borer, diamondback moth, common cabbage worm and citrus leafminer, and also highly toxic to house fly, azuki bean weevil, aphids and nematodes. However, the toxic action of TCMAP is reversible and the rate of the detoxication is considerably rapid when TCMAP is administered to insects in some application methods.[2]

The action of alkylthiocyanate and thiocyanoacetate insecticides on insects is known to cause reversible knockdown rather than death.[3] The toxic action of these thiocyanate insecticides on animals has been suggested to be due to the liberation of cyanide from thiocyanate in the body,[4,5,6] although the toxic action of hydrocyanic acid on insect is reversible only to some extent as reported by Broadbent *et al.*[7] In the previous report[2] it was shown that the poisonings in the insects treated with TCMAP were the depression of oxygen uptake and the recovery from anaesthesia, and these poisonings resemble to those with cyanide. The lethal dose of TCMAP to mice is approximately equal to that of potassium cyanide; that is, the LD-50 values of potassium cyanide and TCMAP, when administered to mouse orally, are quite close on the basis of molar concentration of the cyanide liberated. Therefore, it could be considered that the liberation of cyanide may play at least a part of the toxic action of TCMAP.

In this paper, the author wishes to report on the liberation of cyanide in the molecule of TCMAP by using homogenates of seveal species of animals and rice plant, and also on the degradative reaction by sulfhydryl compounds.

### Materials and Methods

The homogenates were prepared from the following materials. The fresh pig (*Sus scrofa* var. *domestica* Brisson) liver had been kept with solid carbon dioxide until it was homogenized. The organs of adult mouse (*Mus musculus* L., CF-1 strain) were used immediately after killing.

* A part of this report has been presented at the Annual Meeting of Japanese Society of Applied Entmology and Zoology (March, 1966, Kyoto).

The whole bodies of the final instar larvae of rice stem borer (*Chilo suppressalis* Walker) and the adults of azuki bean weevil (*Callosobruchus chinensis* L., 1- to 3-days-old) and the adult house flies (*Musca domestica* L., Lab-em-7-em strain, 3- to 5-days-old) were used. The mammalian organs and the insect whole bodies were homogenized with 2 m*l* of a phosphate buffer (1/15 M, pH 7.2) per one gram of the tissues in a waring blender and squeezed through two layers of gauze. The stems of rice plant (*Oryza sativa* L. var Manryo) were mashed in a mortar and squeezed through two layers of gauze, and the sap was used. The blood of the final instar larvae of tobacco cutworm (*Prodenia litura* Fabricius) was obtained by cutting off their legs.

TCMAP was supplied from synthetic laboratory of this Division. This sample showed a single spot on paper chromatography and paper electrophoresis. The substrate solution was prepared as follows ; 1 per cent solution of TCMAP in ethanol was diluted with the buffer solution until 1 mg of TCMAP was contained in 1 m*l*. Nitrates, metabolic inhibitors or other chemicals to be tested were added into the buffer solution.

The reaction mixture was prepared from 1 m*l* of the homogenate, blood or plant sap, 1 m*l* of the substrate solution and 1 m*l* of the additional solution, and the mixture was incubated in a test tube for 120 minutes at 30°C with shaking. Immediately after the incubation, 3 m*l* of chloroform was added to the mixture and shaked vigorously about 50 times and centrifuged. The experiment for the study of the reaction between TCMAP and SH-compounds was carried out as follows. The mixture of TCMAP and the SH-compound in 3 m*l* of the buffer solution was incubated and fractionated with chloroform in the same procedure as in the experiment with the homogenates.

The assay of TCMAP was done as follows. To 0.5 m*l* of the chloroform or the buffer layer, 1.0 m*l* of 5 per cent $K_2S$ aqueous solution was added, and the mixture was warmed in boiling water for 10 minutes (The chloroform was evaporated in this procedure.). By this procedure, thiocyanic ion is produced from both organic thiocyanates and inorganic cyanide.[8,9] Then the reaction mixture was acidified with 1.0 m*l* of 0.5 N HCl and warmed again in boiling water for 10 minutes. After the mixture was cooled, 1.0 m*l* of ferric chloride (0.37 M in 0.1 N HCl) was added and the resulting colored mixture was filtered through a glass filter of 5 to 10$\mu$ meshes. The filterate was made up to 10 m*l* with water and the absorbance at 460 m$\mu$ was measured with a Hitachi EPU-2A type spectrophotometer. The absorbance of this solution was proportional to the concentration of TCMAP in the range of 0 to 250 $\mu$g in 10m*l*. The spectrum was identical with that of the aqueous mixture of sodium thiocyanate and ferric chloride.

The chloroform fractions which were concentrated by the air-stream were fractionated by paper chromatography and paper electrophoresis. The paper chromatography was carried out on Toyo-Roshi No. 52 paper with the solvent system of *n*-butanol/ acetic acid/ water (4:1:2) at 25°C at the flow distance of 15 to 20 cm. The paper electrophoresis was done on Toyo-Roshi No. 50 paper in 2 N acetic acid for 100 minutes at 20 v/cm in a chamber kept at 3 to 5°C. The developed papers were dried in an atmosphere, and TCMAP and its metabolites were detected with Dragendorff, palladium chloride and sodium nitroprusside reagents.

The homogenate was digested with concentrated sulfuric acid and the total nitrogen was analyzed by the micro-Kjeldahl method. The sulfhydryl in the homogenate was titrated with silver nitrate in an ammonium buffer solution (pH about 9.5) with an amperometric titration apparatus.

The insecticidal assay shown in the experiment of Fig. 7 was done by a dry-film contact method; the chloroform layer of the pig liver homogenate was diluted to 5 times with chloroform, and one m*l* of the diluted solution was pipetted out onto a bottom of a petri dish (9 cm in diameter). After evaporation of the solvent, 20 female adults of azuki bean weevil were placed in the dish. Each insecticidal assay was replicated three times. The insecticidal assay with house fly was done as follows. A five-days-old adult female of house fly was injected with 0.72 $\mu$*l* of the

aqueous solution or soy bean oil solution of SH-compounds into the thorax. Then about 20 minutes after the injection, the injected fly was treated with 1.2$\mu l$ of acetone solution of TCMAP on the dosal side of the thorax by topical application method. Each treatment was done with 30 flies. The treated flies and weevils were kept at 25°C and the mortality counts were made after 24 hours.

### Results

Fig. 1 shows the result of the experiment in which TCMAP was incubated with the homogenates of the insects, the mammalian organs and the rice plant sap. The amount of inorganic thiocyanate recovered from the chloroform and buffer layer and that which was not recovered as much as added (designated as unknown) are shown in percentage. These amounts were interpretted from the reason explained in "Discussion" as follows: the part in the chloroform layer showed the amount of the undegradated TCMAP and the part in the buffer layer showed the amount of the degradated TCMAP. Fig. 1 shows that the degradation of TCMAP was observed in about 10 per cent even in buffer solution alone and to most extent with pig liver homogenate. Fig. 1 also shows the degradation of TCMAP by the mouse organs. The order of the degradation activity was liver, brain, heart and muscle. From the result of the experiment in Fig. 1, it is evident that the mammalian liver degradated TCMAP the most actively.

In order to know whether TCMAP was degradated by enzymatic reaction or not, the experiment shown in Table 1 was carried out. As shown in this table, the degradating activity on

Taale 1. Effects of heating and dialysis of pig liver homogenate on the degradation of TCMAP.

| Treatment | Per cent activity |
|---|---|
| untreated | 100 |
| heated (50°C, 30 min.) | 119 |
| heated (100°C, 30 min.) | 113 |
| dialysis | 51 |

The degradating activity was shown in percentage compared with that of untreated homogenate.

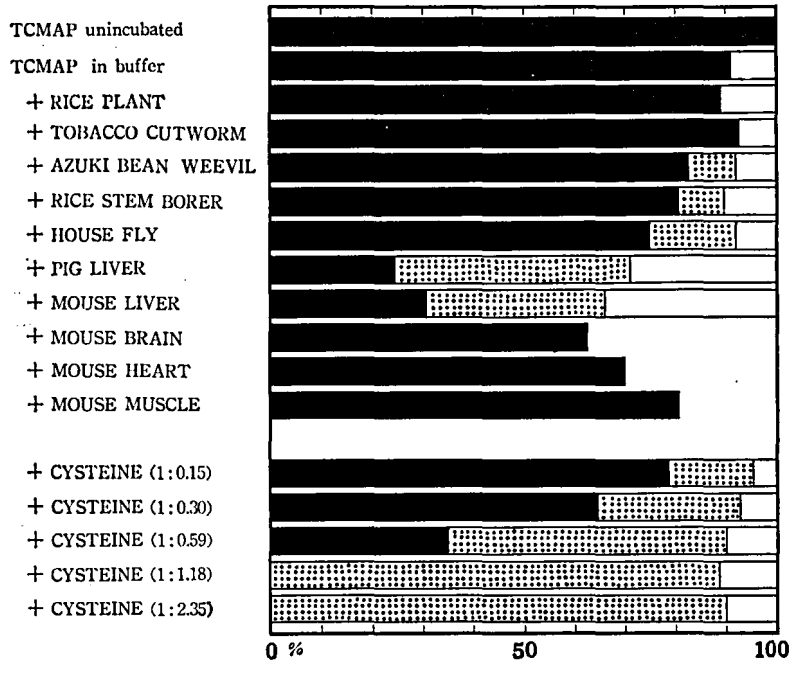


Fig. 1. The degradation of TCMAP incubated with homogenates or cysteine. Black bar : chloroform extractable ; white bar : water extractable ; dotted bar : unknown

TCMAP of the pig liver homogenate was not affected by heating, but it was decreased to about 50 per cent after the dialysis through the cellulose tube in the phosphate buffer solution containing 0.05 per cent of EDTA-disodium salt for 24 hours at 5 to 10°C. The effects of the hydrogen ion concentration on the degradation of TCMAP by the homogenate of the pig liver were studied in glycine-sodium hydroxide buffer solution, phosphate buffer solution and sodium acetate-hydrochloric acid buffer solution. The activity is not specific to the value of pH as shown in Fig. 2. The results given in Table 1 and Fig. 2 show that the degradation of TCMAP by pig liver homogenate is not due to enzymatic reaction.

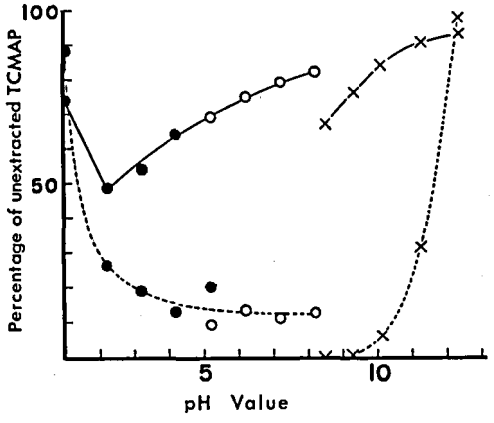


Fig. 2. Effect of pH on the degradation of TCMAP with or without pig liver homogenate. The degradation activity is indicated as the percentage unextractable TCMAP. Amount of TCMAP added is given as 100 per cent.
—— with homogenate, ····· in buffer,
× glycine-sodium hydroxide buffer,
○ phosphate buffer, ● sodium acetate-hydrochloric acid buffer

The effects of metalic ions, metabolic inhibitors and some other materials on the degradation of TCMAP were examined. The results are given in Table 2. The degradating activity of the pig liver homogenate was highly inhibited by the addition of "SH-inhibitor" such as $Hg^{++}$, $Cu^{++}$, $Ag^{+}$ phenylmercuric acetate and monoiodoacetate. On the contrary, the addition of "SH-compound" such as L-cysteine and sodium thioglycollate fairly increased. $Co^{++}$, $Ni^{++}$ and phenylhydrazine inhibited slightly and L-cystine, EDTA-disodium salt and glutathion (oxidized form) slightly increased the activity, although $Al^{+++}$, $Ca^{++}$, $Cr^{+++}$, $Fe^{++}$, $Fe^{+++}$, $Mg^{++}$, potassium cyanide, sodium fluoride, antimycin A, sodium azide, sodium arsenite, sodium fluoroacetate and L-methionine did not affect. These results suggest that sulfhydryl group plays an important role in the degradative reaction of TCMAP.

Table 2. Effects of cations and some chemicals added into pig liver homogenate on the degradation of TCMAP.

| Cations* or chemicals added | Final conc. (M) | Per cent activity** |
|---|---|---|
| $Ag^{+}$ | $3.3\times10^{-3}$ | 4 |
| $Al^{+++}$ | 〃 | 95 |
| $Ca^{++}$ | 〃 | 99 |
| $Co^{++}$ | 〃 | 76 |
| $Cr^{+++}$ | 〃 | 105 |
| $Cu^{++}$ | 〃 | 0 |
| $Fe^{++}$ | 〃 | 104 |
| $Fe^{+++}$ | 〃 | 103 |
| $Hg^{++}$ | 〃 | −9 |
| $Mg^{++}$ | 〃 | 102 |
| $Mn^{++}$ | 〃 | 107 |
| $Ni^{++}$ | 〃 | 73 |
| EDTA-disodium salt | 〃 | 115 |
| Potassium cyanide | 〃 | 100 |
| Glutathion (oxidized form) | $5.0\times10^{-3}$ | 122 |
| L-Cysteine HCl salt | 〃 | 165 |
| L-Cystine | 〃 | 110 |
| L-Methionine | 〃 | 101 |
| Monoiodoacetate | 〃 | 50 |
| Phenylhydrazine HCl salt | 〃 | 88 |
| Phenylmercuric acetate | 〃 | 9 |
| Sodium arsenite | 〃 | 98 |
| Sodium azide | 〃 | 104 |
| Sodium fluoroacetate | 〃 | 102 |
| Sodium thioglycollate | 〃 | 139 |
| Antimycin A | $1.2\times10^{-3}$ | 96 |
| Sodium fluoride | $1.1\times10^{-2}$ | 103 |

* These cations were supplied in the form of their nitrates with an exception of mercuric chloride.
** The activity was calculated as same way as given in Table 1.

Table 3. Nitrogen and sulfhydryl content in the homogenates.

| Homogenate | Amounts | |
|---|---|---|
| | N* | SH** |
| Rice stem borer | 9.96 | 0.66 |
| Azuki bean weevil | 7.28 | 2.88 |
| House fly | 11.1 | 2.40 |
| Mouse liver | 9.74 | 9.00 |
| Pig liver | 9.82 | 9.66 |

* Nitrogen contents, mg per m*l* of homogenate.
** The volume (m*l*) of $AgNO_3$ aq. ($10^{-3}$M) which is required to neutralize one m*l* of the homogenate.

Table 3 shows contents of nitrogen and sulfhydryl in the homogenates which were prepared just as those used in the experiment of Fig. 1. There were no noticeable differences of the nitrogen contents among insects and mammalian livers. This result reveals that the homogenates used in the experiment in Fig. 1 contain almost same amount of protein. On the contrary, the amounts of sufhydryl were markedly different among the homogenates of insects and mammalian livers. From these results, it seems likely that the degradating activity of the homogenates shown in Fig. 1 is related to the amount of sulfhydryl and not to protein content.

Therefore, in order to know whether TCMAP reacts with SH-compounds, the following experiments were performed. The chloroform layer of the reaction mixture of TCMAP ($1.7\times10^{-3}$) with SH-compounds ($3\times10^{-3}$M) was separated by the paper chromatography and the paper electrophoresis. Figs. 3 and 4 present the typical chromatograms of nereistoxin, TCMAP and its reaction products. TCMAP was detected only by Dragendorff reagent, while nereistoxin was detected by Dragendorff, palladium chloride and sodium nitroprusside reagents. On the chromatograms of the extracts of the reaction mixture with SH-compound, a spot which did not correspond to TCMAP but to nereistoxin was detected. TCMAP ($1.7\times10^{-3}$M) was also tested for the reaction with the compounds ($10^{-2}$M) below described, and the amount of thiocyanate which was produced from the chloroform layer of the reacted mixture was assayed by the previously described procedure. The result showed that sodium hypophosphite, sodium thiosulfate and sodium ascorbate did not catalyze the reaction, while TCMAP was degradated to large extent by cysteine, 1, 3-dimercaptopropanol, thiophenol, 2-mercapto-ethanol and *p*-nitrothiophenol. In Fig. 1, the result of the experiment in which TCMAP was reacted with cysteine at different ratio was shown. It is evident from these results

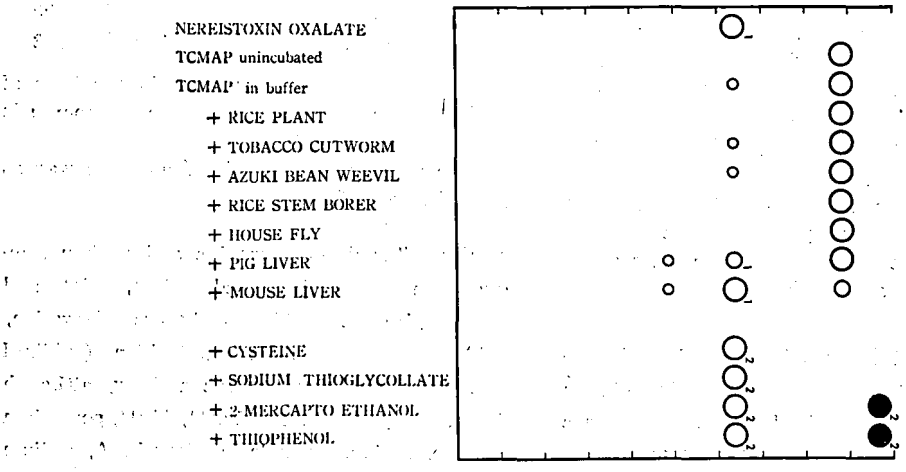


Fig. 3. Paper chromatogram of nereistoxin hydrogen oxalate, TCMAP and its metabolite (reaction products). TCMAP was incubated with homogenates and SH-compounds. Spots were detected by Dragendorff reagent except for black ones, by nitroprusside reagent (1) and by palladium chloride reagent (2).

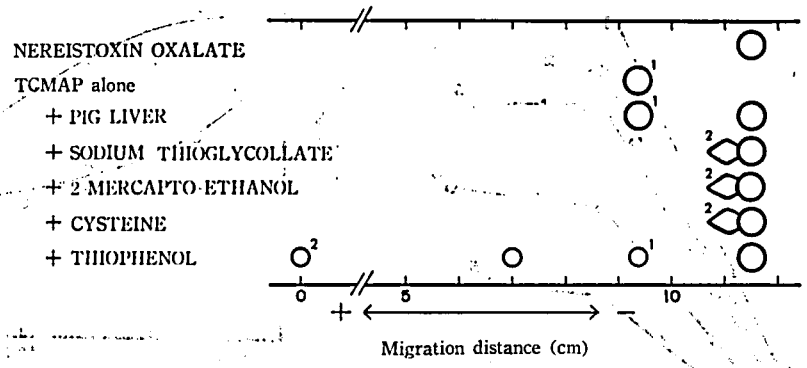


Fig. 4. Paper electrochromatogram of nereistoxin hydrogen oxalate, TCMAP and its reaction products when TCMAP was incubated with SH-compounds or pig liver homogenate. Spots except (2) were detected by Dragendorff reagent and spots except (1) were detected by palladium chloride reagent.

that TCMAP reacted with SH-compounds.

The chloroform layer of the experiment in Fig. 1 was separated by the paper chromatography and the paper electrophoresis. The results were shown in Figs. 3 and 4. The chloroform extract of the homogenate was separated to 3 spots (Rf values were 0.9, 0.65 and 0.5).

The aqueous solution of nereistoxin shows an absorption band around 320 mμ, however TCMAP shows no absorption in the range of 280 to 400 mμ (Fig. 5). Accordingly, the formation of nereistoxin from TCMAP can be detected by examining the optical absorption at the wave length around 320 mμ. The optical absorption of the reaction mixture of TCMAP with cysteine is shown in Fig. 5. The reaction mixture consisted of TCMAP and cysteine was incubated as done in the experiment of Fig. 3. A close similarity of the absorption spectrum of the reaction mixture to that of the solution of nereistoxin can be seen in Fig. 5.

The results, shown in Figs. 3, 4 and 5, show that the reaction product of TCMAP with SH-compounds may be assumed to be nereistoxin. The amount of the produced nereistoxin can be calculated by measuring the optical absorption at 320 mμ, since the aqueous solution of nereistoxin follows Beer's law as shown in Fig. 6. From this figure, it can be known that the absorbancy of the reaction mixture is linear to content of cysteine until the mole ratio of

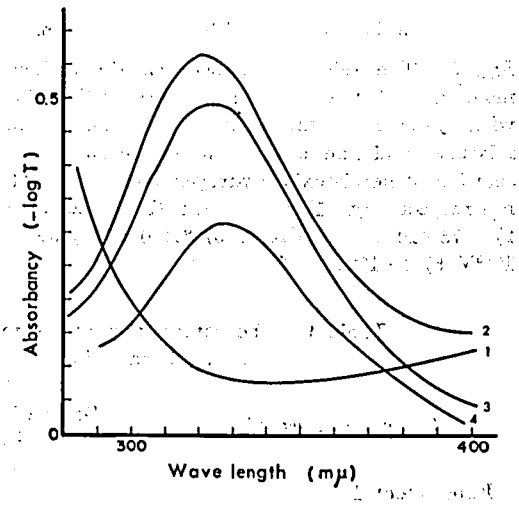


Fig. 5. The absorption spectra of nereistoxin, TCMAP and its reaction product with cysteine and pig liver homogenate. The spectrum of the reaction product with the homogenate was measured as following procedure: the chloroform extract of the homogenate incubated with or without TCMAP was separated by paper chromatography. The portion corresponding with nereistoxin was cut off and extracted with ethanol. 1) TCMAP ($8\times10^{-3}$M)/water 2) nereistoxin hydrogen oxalate ($5\times10^{-3}$M)/water 3) TCMAP ($3.75\times10^{-3}$M) + cysteine ($1.2\times10^{-2}$ M)/cysteine 4) extract of (TCMAP+homogenate)/extract of homogenate

cysteine/TCMAP reaches 1. This fact suggests that the reaction of TCMAP with cysteine is

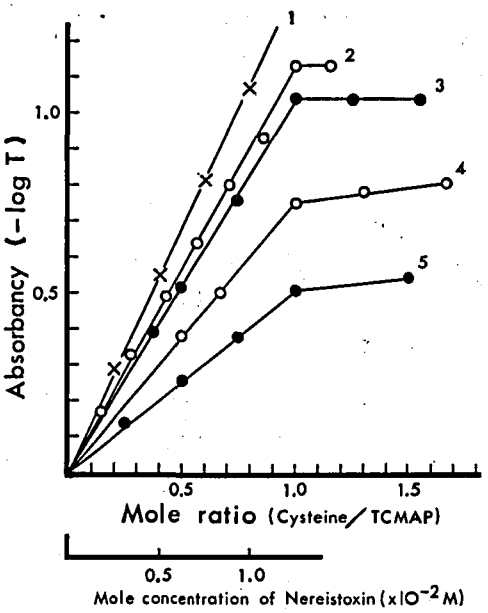


Fig. 6. The relationship between the various mole ratio of the reaction mixture of TCMAP with cysteine and the optical absorption of the mixture, and the absorption of the aqueous solution of nereistoxin hydrogen oxalate at 320 mμ respectively. 1) nereistoxin (below scale) 2) $8.75\times10^{-3}$M of TCMAP 3) $8\times10^{-3}$M 4) $6\times10^{-3}$M 5) $4\times10^{-3}$M

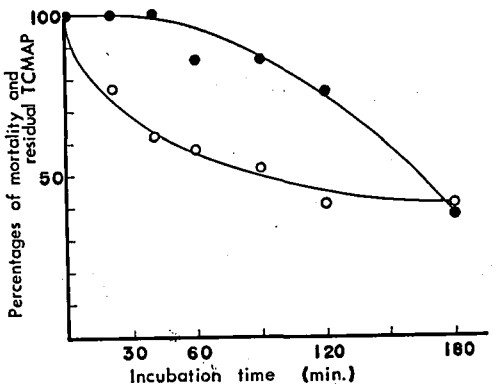


Fig. 7. Amount of TCMAP extracted from the incubation mixture with pig liver homogenate, and biological tests of the extract with the azuki bean weevils under various incubating times.
● per cent mortality, ○ amount of TCMAP (in ratio)

quantitative and that the mole ratio of the reaction is 1 : 1.

Fig. 7 shows the time course of the degradation of TCMAP by pig liver homogenate when it was assayed chemically and biologically. Both the insecticidal activity of the chloroform layer and the residual TCMAP decreased with the lapse of the incubation time. The chloroform layer without TCMAP was not toxic by this procedure.

Table 4. The potentiation of the toxicity of TCMAP against house flies by the interthoracic injection of SH-compounds.

| Chemicals injected | Dosage injected per fly | | Percent mortality | |
|---|---|---|---|---|
| | | | injection alone | injection and topical application* |
| Experiment 1 | | | | |
| Water | — | 0.72 μl | 0 | 43.3 |
| Cysteine | $2\times10^{-2}$M | 〃 | 0 | 56.7 |
| Sodium thioglycollate | 〃 | 〃 | 3.3 | 70.0 |
| 2-Mercapto-ethanol | 〃 | 〃 | 6.7 | 76.7 |
| Sodium thiosulfate | 〃 | 〃 | 6.7 | 76.7 |
| Thiophenol | 〃 | 〃 | 0 | 60.0 |
| Experiment 2 | | | | |
| Soy bean oil | — | 〃 | 3.3 | 60.0 |
| Cysteine | 1 μg | 〃 | 0 | 80.0 |
| Sodium thioglycollate | 〃 | 〃 | 0 | 80.0 |
| 2-Mercapto-ethanol | 〃 | 〃 | 0 | 90.0 |
| Thiophenol | 〃 | 〃 | 6.7 | 93.3 |
| 1,3-Dimercapto-propanol | 〃 | 〃 | 0 | 80.0 |

* TCMAP was topically applied at the dose of 1.6 μg/fly in the experiment 1 and 2.0 μg/fly in the experiment 2.

The effect of interthoracic injection of SH-compounds on the toxicity of TCMAP is shown in Table 4. SH-Compounds slightly potentiated the toxicity.

## Discussion

In this paper it was shown that the homogenates of insects and mammalian organs degradate the thiocyanate groups of TCMAP and that this degradation proceeds through not enzymatic but chemical reaction with sulfhydryl groups in the homogenates.

Fig. 1 shows the amount of inorganic thiocyanate which is obtained from either the chloroform or buffer layer ; and this chloroform layer was separated by paper chromatography as shown in Fig. 3. The same chromatographed strip which was not sprayed with the chromogenic reagent was cut into a certain length in order to know the distribution of the thiocyanate-obtainable-metabolite(s)* on the strip. A piece of the strip was put into a test tube with a small amount of water and colorized by the same procedure as used in the experiment of Fig. 1. According to this procedure there were detected no thiocyanate-obtainable metabolite(s)* except the part which was of the same Rf value (0.9) with the undegradated TCMAP. This part was detectable by Dragendorff reagent and not by sodium nitroprusside reagent. In addition to these coincidences, the thiocyanate-obtainable metabolite* in the chloroform layer decreased in the accordance with the lapse of the incubation time (Fig. 7). These results show that the chloroform layer in the experiment of Fig. 1 contained only one metabolite from which the inorganic thiocyanate was liberated, and that this metabolite must be identical with TCMAP. Therefore, the thiocyanate-obtainable metabolite* in chloroform layer is considered to be TCMAP and the amount of the thiocyanate from chloroform layer was interpretted to be the amount of the undegradated TCMAP (Figs. 1, 2 and 7, Tables 1 and 2). On the other hand, the amount of the thiocyanate from the the buffer layer is thought to be that of the liberated cyanide (Fig. 1), that is, it is considered from the following reasons that TCMAP liberated cyanide and changed to nereistoxin. In the chloroform layer, a metabolite (reaction product) which was considered to be nereistoxin was detected in the paper chromatography and the paper electrophoresis (Figs. 3 and 4). The absorption spectrum of the reaction mixtures of TCMAP with cysteine and pig liver homogenate showed a close simirality to that of nereistoxin (Fig. 5). Moreover, the buffer layer in the experiment of Fig. 1 contained the thiocyanate-obtainable metabolite*, and this layer was not colorized when the ferric chloride was directly added.

Basing on the above identifications, it is revealed that the highest degradation activity exists in the liver of mammals, but the other organs, insects and rice plant bear slight connection with the degradation (Fig. 1). The substance connected with the degradation in the liver is thought to be a low molecular and heat stable substance (Table 1). The amount of nitrogen in the homogenate had no connection with the activity (Table 3 and Fig. 1). The addition of the SH-inhibitors was inhibitory, while the addition of the SH-compounds was promotive on this degradation (Table 2). Moreover, the amount of the sulfhydryl in the homogenates was much more in livers than in insects. This difference in the amount of sulfhydryl between the livers and insects is related to the degradating activity (Table 3 and Fig. 1). From these results it is considered that the catalyzing substance of the TCMAP degradation is not enzyme, but low molecular substances having sulfhydryl group.

Tan *et al*[10] studied the degradation of TCMAP with polarography, and suggested that TCMAP decomposed to nereistoxin and cyanide in the buffer solution at the pH value of above 4.0. The same result was obtained in the present study ; that is, about 10, 32 and 98 per cent of TCMAP were not recovered from the buffer solutions of the pH values of 7.2, 11.2 and 12.3 respectively (Figs. 1 and 2). However, it is conceivable that sulfhydryl plays a more important role than an alkaline condition in the incubation mixture of the pH value of 7.2, since the homogenates and SH-compounds degra-

* Inorganic thiocyanate could be produced by the procedure given in Fig. 1.

dated TCMAP much more than in the buffer solution alone (Figs. 1, 2 and 3, Table 2).

It is reported by Oetting *et al*[4] that the effect of alkylthiocyanates administered to rats and mice orally or by subcutaneous injection on their respiration simulated very closely that observed in cyanide poisoning and that the pulped rabbit liver liberated hydrogen cyanide from lower alkylthiocyanates. On aliphatic thiocyanate Gustafson *et al*[5] and on thiocyanoketone and thiocyanoacetate Grove *et al*[6] suggested that the liberation of hydrogen cyanide in the body would be the cause of poisoning. Therefore, it is reasonable to consider that hydrogen cyanide would be liberated from TCMAP as described above, though this degradative reaction of TCMAP was thought to be non-enzymatic. Nevertheless, Goldstein *et al*[11] has found a new enzyme in erythrocytes of man, dog, rabbit and rat, which oxidizes thiocyanate to cyanide. They also reported that this enzyme contained sulfhydryls and that its reaction optimum was 40°C and a pH of 7.4.

Sakai[12] has reported that nereistoxin antagonizes the action of acetylcholine which contracts the frog rectus abdominis muscle. However, the dithiocyanate derivative (TCMAP) does not antagonize the action of acetylcholine, unless it is incubated with cysteine[13]. The effect of the interthoracic injection of SH-compounds on the toxicity of TCMAP is shown in Table 4. As shown in this table, the toxicity was slightly potentiated by the injection of SH-compound. These phenomena seem to suggest to the author that the conversion is an "activation" of the derivative.

In animals or plants, there are many kinds of SH-compounds, such as protein, glutathion, cysteine, Co A, dihydroxylipoic acid, and these sulfhydryls react with carbonyl group, double bond, metal cations, arsenical, mercury compounds, alkylating agents and oxidizing agents.[14] Such pesticides as captan[15], methylbromide[16] or organic mercuric compounds[14,17,18] are known to react with sulfhydryl. It was revealed in this report that TCMAP also reacted with sulfhydryl and changed to nereistoxin and that the mole ratio of the reaction would be 1 to 1.

**Summary**

The degradation of thiocyanate groups of 1,3-dithiocyanato-2-*N*, *N*-dimethylaminopropane (TCMAP) by the homogenate of insects, mammalian organs and rice plant was investigated. This degradation was catalyzed most actively by the mammalian liver homogenate. The degradative reaction is considered not to be enzymatic, since the activity was not decreased by heating of the homogenate and also by varying the pH values of the incubation mixture. The addition of SH-inhibitors to the incubation mixture decreased the activity, while SH-compound increased. There were no difference in the amount of nitrogen between the homogenates of the different species of insects and mammalian livers. However, the amount of sulfhydryl was much larger in the liver homogenate than in the insects. The amount of sulfhydryl was thought to have a close relation to the degradative activity. From these result it is considered that the sulfhydryl in the homogenate degradated the thiocyanate radicals of TCMAP through the non-enzymatic reaction.

Therefore, the degradative reaction of TCMAP with sulfhydryl compound was investigated. SH-compounds, such as cysteine, thiophenol, sodium thioglycollate, 2-mercapto-ethanol, 1, 3-dimercaptopropanol and *p*-nitrophenol degradated TCMAP. But reductive compounds such as sodium hypophosphite, sodium thiosulfate and sodium ascorbate did not catalyze. From the results experiment on the reaction of TCMAP with cysteine, it was revealed that TCMAP reacted with sulfhydryl as well as with the homogenates and changed to nereistoxin, and that this reaction proceeded quantitatively.

The conversion of TCMAP to nereistoxin seemed to be the cause of the potentiation of the toxic action.

**Acknowledgements** : The author wishes to express his sincere thanks to Dr. C. Harukawa and Mr. M. Sakai of the Takeda Chemical Industries Ltd. for their useful suggestions and kindly readings of the original manuscript. He is also greatful to Dr. M. Goto of the company for his direction and Dr. T. Harukawa and Mr. K. Konishi

of the company for supplying the compounds.

### References


1) Konishi, K., Okutani, T., Sakai, M., Kato, M. and Sato, Y.: *Abstracts of the Annual Meeting of Japanese Soc. Appl. Ent. Zool.* 20 (April 1964, Tokyo)
2) Kato, M.: *Ann. Rept. Takeda Res. Lab.* 25, 154 (1966).
3) Negherbon, W. O.: *Handbook of Toxicology*, 3 (The National Academy of Sciences, Philadelphia and London, 1959).
4) Von Oetting, W. F., Huepner, W. C. and Deickmann-Gruebber, W.: *J. industr. Hyg.* 18, 310 (1936).
5) Gustafson, C. Jr., Lies, T. and Wagner-Jauregg, T.: *J. Econ, Entmol.* 46, 620 (1953).
6) Grove, J. F. and Bovington, H. H. S.: *Ann. Appl. Biol.* 34, 113 (1947).
7) Broadbent, B. M. and Bliss, C. I.: *J. Econ. Entmol.* 29, 143 (1936).
8) Takiura, K. and Takino, Y.: *J. Pharm. Soc. Japan*, 74, 839 (1954).
9) Snell, F. D. and Snell, C. T.: *Colorimetric Methods of Analysis*, 2, 856 (D. Van Nostrand Company, Inc., New York, 1949).
10) Tan, N., Nishi, K., Ôwaki, R. and Asahi, Y.: *Pesticide and Technique*, 16, 20 (1967).
11) Goldstein, F. and Reiders, F.: *Am. J. Physiol.* 173, 287 (1953).
12) Sakai, M.: *Botyu-Kagaku*, 31, 61 (1966).
13) Sakai, M.: Unpublished data (1966).
14) Boyer P. D.: *The Enzymes*, 1, 511 (Academic Press, New York, 1959).
15) Lukens, R. J. and Sisler, H. D.: *Phytopathology*, 48, 235 (1958).
16) Stewart, C. P. and Stolman, A.: *Toxicology*, 1, 45 (Academic Press, New York, 1960).
17) Yamada, T.: *Annal. Phytopathol. Soc. Jap.* 29, 6 (1964).
18) Boyer, P. D.: *J. Am. Chem. Soc.* 76, 4331 (1954).


# 綜　　説

**Recent Advances and Graphic Analysis of Joint Action of Insecticides.** Seiroku Sakai (Daito Bunka University, Takasaka, Saitama, Japan) *Botyu-Kagaku*, 32, 79, 1967.

**殺虫剤の連合作用の最近の進歩と図解法**　酒井清六（大東文化大学）

Isobole の問題，図解による交互検定法，最近の発展および抵抗性理論との関係について綜説する．数量的なプロビット評価は酒井[37]に記した．

### 図解法

連合作用の交互検定法　Alternative method は Horsfall (1945)[23], Reilley *et al.* (1951)[35], Sakai *et al.* (1951)[39,40], Storrs & Burchfield (1954)[42], Sakai (1960)[36], Hewlett (1960)[16], Turner (1958)[49], de Jongh (1961)[11] などによっていろいろの方法が提唱され，その分析が簡単なので実際に利用されている．

**図解法**　Bologram は死亡率を直接作図するものと等毒量 Equivalent dose を利用するものとがある．Loewe (1926)[27] は等反応単位を Isobole と呼んだ．たとえば，Aの 50mg と B の 70mg とが同じ反応を示すなら，Isobole の概念から A の50＝Bの70＝1単位という．Isobole は Quantitatively identical effect の点である．

図解法は Isobole の概念を利用して作図することが多いので，つぎのような条件のときは利用できないかまたは結論を検討すべきである．

1. 実験時間または投与時間が違っている両薬剤，2. 両薬剤の作用直線の勾配 $b_1 \neq b_2$ が違うとき，両薬剤の比較毒力，すなわち，何倍効くかということが濃度によって変化するとき，3. Aの薬剤が呼吸量をふやし，Bの薬剤が減らすように相殺作用を呈するとき，4. Isobole 法は一見簡単なようであるが，薬剤試験の場合，ある一定薬量の実験で任意の反応を観察する方がある一定の反応を得るための限定された薬量の実験をするより容易である．Isobole 法は薬量より一定の反応に重点を置いているので実験がやりにくい．5. 混合実験で，50%の反応の Isobole と25%や75%の反応の Isobole とは違う．従って A bundle of Isoboles が必要になってくる．

第1図-1は Isobole を使った作図で，Y軸はAの薬量，X軸はBの薬量を示している．P点は A 薬剤が50%の致死率を示す点で，同様に Q点は B 薬剤が50%の