

On the Insecticidal Principle of *Chrysanthemum cinerariifolium* Bocc. (Insect Powder).

Part 1.

By

Ryō Yamamoto.

[December 20, 1918]

Introduction

Since 1876 many authors have investigated the insecticidal principle of insect powder. Such authors as Rother¹⁾, Oscar Textor²⁾, H. Thorns³⁾, De Boise⁴⁾, Durrant⁵⁾, Gerard⁶⁾ and S. Sato⁷⁾ have ascribed the principle to the oily resinous substance in the powder. Schlagenhauffen and Reeb⁸⁾ isolated *Acidium pyrethrotoxicum* which was toxic to insects. They extracted the insect powder with petroleum ether and evaporated the extract into syrup, which was then shaken with alcohol.

The alcoholic solution was evaporated into syrup and treated with 3% caustic potash. The dissolved part was neutralized with tartaric acid and then extracted with ether. On evaporating the ethereal solution, they obtained an oily acid substance which was toxic to insects. K. Fujitani⁹⁾ isolated a neutral oily toxic syrup by the following method. He extracted at first the powdered flower of *Chrysanthemum cinerariifolium* with alcohol, evaporated the extract and dissolved it in ether. The ethereal solution was then thoroughly shaken with dilute caustic potash and the ether was evaporated off after being washed with acid and water.

The concentrated syrup was then dissolved in pet. ether. On evaporating the extract, he obtained a transparent yellowish oily syrup which was very toxic to insects to which he gave the name "Pyrethron."

Further, he saponified this pyrethron with alcoholic potash. In the saponification product, he obtained a crystalline alcohol which melts at 199°, having the formula $C_{21}H_{34}O$, and the following rotatory power $\alpha_D^{20} = +73.75$.

1) Rother: *Pharz. Jahresbericht.* 1876, 121.

2) Oscar Textor, *Pharz. Jahresbericht.* 1881, 146, 1883, 187.

3) H. Thorns: " " 1881, 61.

4) De Boise: " " 1895, 62.

5) Durrant: " " 1897, 90.

6) Gerard: " " 1897, 105.

7) S. Sato: *Yakugakuzasshi* (Japanese), 283, 304.

8) Schlagenhauffen: *Pharz. Jahresbericht.* 1891, 61, 1911, 177.

9) K. Fujitani: *Tokyo Igakukaishi* (Japanese), 21, and *Tokyo Igakuzasshi*, No. 5. (Japanese).

But he did not thoroughly investigate the acid of this saponification product. P. Siedler¹⁾ detected palmitic and other fatty acids, aldehyde, ketone and phenol, 'by steam distillation of the insect powder. Many authors have hitherto studied the principle of insecticides, but their investigation is not yet complete. My attempt is, therefore, to determine the chemical nature of the principle. For this purpose, I have isolated at first a strong insecticidal principle to which I gave the name "Pyrethron," according to Fujitani, and have studied its further application. Then I have investigated its chemical nature by saponification. And crystalline higher alcohols and fatty acids, liquid and solid, are isolated from the saponification product. From these results, I have ascertained that the principle is ester. But many points demand further investigation and I am intending to continue it.

Experimental.

I. Isolation of the Principle.

For the isolation of the insecticidal principle, I have used the flowers of the *Chrysanthemum cinerariifolium* which is widely cultivated in Japan. The fine powder of the flower was extracted with ether at the room temperature. The ethereal extract was then evaporated to a certain concentration, shaken with 2% caustic potash to remove resinous acid. After shaking the ethereal extract with acid and water, it was evaporated to dryness and extracted with 90% alcohol. By this treatment, a waxy substance of red colour was removed. On evaporating the alcoholic solution under diminished pressure avoiding high temperature, I obtained a strong toxic yellow syrup, which was sufficiently shaken with light petroleum (B. P. 50—60°). In this case, a white insoluble matter was precipitated, but it had no insecticidal power. Filtrating this precipitate, petroleum was evaporated off at below 60° under diminished pressure. I obtained a yellow transparent syrup which had the characteristic smell of insect powder with a strong insecticidal power. The yield was 0.8% of the powder.

The principle which I have isolated was not chemically pure and no further purification was successful in spite of all my efforts. The principle had a somewhat bitter taste and contained no nitrogen, was soluble in alcohol, ether, benzol and chloroform, but insoluble in water and showed the following chemical properties.

Acid value 1. (1 grm. principle was titrated, using phenolphthalein as indicator).

Saponification value 216. (1 grm. principle was saponified by 25 cc. of

1) P. Siedler, Chemical Abstract. 1915, 10, 663.

N/2 alcoholic Potash by cold method).

The fresh isolated principle was neutral, but its acidity increased after standing, especially in alcoholic solution. It is a notable characteristic that the principle was easily saponified by alcoholic potash at the room temperature, and instantly lost its insecticidal power, and further, its power was gradually reduced by heating, or after being exposed for a long time to the air, converting itself into a resinous substance. The principle is almost identical with Fujitani's "Pyrethron," and that is the reason why I have given the name "Pyrethron" to it.

II. Application of Insect Powder.

I. Insecticidal power of pyrethron.

5 grs. of pyrethron were dissolved in 100 cc. of 90% alcohol and 0.01% emulsion was prepared, diluting it by water. When house flies came into contact with this emulsion, they were soon seized with convulsions of the legs and died. By using a dilute soap solution instead of water, the emulsion could be more diluted and still killed all the flies. This was suspected to be due to the physical effect of soap solution in the emulsion as William Moor¹⁾ has written in his work. But in my case, the soap solution had no insecticidal power.

2. Controlling power of the germination of bacteria.

5 grs. of pyrethron were dissolved in 100 cc. of 90% alcohol and this alcoholic solution was dropped into 10 cc. sterilized meat extract agar culture medium and *Bac. Subtilis* was cultured on it for 14 days in three different ways.

1st, cultured on not heated culture medium.

2nd, cultured on heated culture medium.

3rd, cultured on alcoholic control.

The result was as follows:—

First case.

Concentration of pyrethron in culture medium	Result
0.015% (by volume)	germinates after 3 days.
0.031% "	" " 4 "
0.041% "	" " 5 "
0.061% "	" " 6 "
0.077% "	no germination.
0.092% "	" "

1) William Moor & Graham, Journal of Agr. Research. vol. 13, No. 11.

But in the second case, using the same medium containing pyrethron heated 30 minutes each day for 3 days, the same experiment was performed and germination occurred after 3 days in all concentrations.

In the third case, that is, in the case of the alcoholic control, the result was the same as in the second case. Considering these results, we see that, pyrethron, when to the extent of more than 0.077%, has a controlling power over the germination of bacteria and loses this power after heating at 100°C.

Upon the yeast of Shoyu (*Zygosaccharomyces salus*) pyrethron showed very weak antiseptic power.

3. The toxic action of pyrethron on the dog.

Three puppies were examined for this purpose, and 0.25 gr. of pyrethron per 1 kgr. body weight was introduced into the stomach of each of them through the mouth, but no external change in their body was recognized.

III. The Chemical Study of Pyrethron.

1. Saponification of pyrethron.

200 grs. of pyrethron was saponified with 4000 c.c. of N/2 alcoholic potash by the cold method. The large quantity of alcohol was now evaporated off in vacuum at below 40°C, the residual solution was diluted with a small quantity of water and repeatedly shaken with ether. On evaporating ethereal solution, needle crystal containing yellow oily substance was obtained.

The residual solution of the saponification product was neutralized with tartaric acid, extracted with ether, ether was evaporated and a resinous acid substance was obtained. In the saponification product, glycerine was not detected at all.

2. On higher alcohol.

Above needle crystal was purified by recrystallization in strong alcohol and by using different solubility in alcohol, I have separated the following alcohols having different melting points.

Melting point (not corrected)	yield.
199°C	7.5 grs.
182°—188°C	0.4
175°—179°C	1.3
163°—175°C	0.6
155°—158°C	0.3
120°—165°C	0.3
95°—112°C	1.0

The substance having the melting point 95° — 112°C was mingled with yellow oily substance and crystallized out when the alcoholic solution was slowly evaporated at room temperature, but if heated, it was converted into syrup. I did not ascertain the nature of this oily substance. These alcohols were readily soluble in ether, chloroform and benzol and hardly soluble in alcohol and petroleum ether. The reaction resembled that of cholesterol but the melting point is higher, and the chloroform layer tinted less by Sal-kowski's reaction. All of them colored pink to violet under Liverman's reaction.

The crystals were dried in a vacuum at 100°C . (the crystal which melts below 100°C . was dried at 78°C .) and when analysed the result was as follows:—

1. When the crystal melts at 95° — 112°C .
 - a. 0.1637 gr. subst. gave 0.4841 gr. CO_2 and 0.1786 gr. H_2O .
 - b. 0.1590 gr. subst. gave 0.4712 gr. CO_2 and 0.1704 gr. H_2O .

found	a.	80.65% C	12.12% H.
	b.	80.65% C	12.91% H.
2. When the crystal melts at 155° — 158°C .

0.1510 gr. subst. gave 0.4601 gr. CO_2 and 0.1617 gr. H_2O .			
found		83.10% C	11.90% H.
$\text{C}_{21}\text{H}_{24}\text{O}$	cal.	83.44	11.26
$\text{C}_{20}\text{H}_{22}\text{O}$	„	83.33	11.11
3. When the crystal melts at 163 — 175°C .
 - a. 0.1608 gr. subst. gave 0.4834 gr. CO_2 and 0.1729 gr. H_2O .
 - b. 0.1613 gr. subst. gave 0.4843 gr. CO_2 and 0.1746 gr. H_2O .

found	a.	81.99% C	11.95% H.
	b.	81.89 „	12.03 „
4. When the crystal melts at 175 — 179°C .
 - a. 0.1412 gr. subst. gave 0.4345 gr. CO_2 and 0.1544 gr. H_2O .
 - b. 0.1658 gr. subst. gave 0.5096 gr. CO_2 and 0.1800 gr. H_2O .

found	a.	83.92% C	12.15% H.
	b.	83.83 „	12.06 „
$\text{C}_{27}\text{H}_{40}\text{O}$	cal.	83.93 „	11.92 „
5. When the crystal melts at 182 — 188°C .

0.1523 gr. subst. gave 0.4642 gr. CO_2 and 0.1633 gr. H_2O .			
found		83.14% C	11.84% H.
$\text{C}_{21}\text{H}_{24}\text{O}$		83.44 „	11.26 „
$\text{C}_{20}\text{H}_{22}\text{O}$		83.33 „	11.11 „
6. When the crystal melts at 199°C .
 - a. 0.1558 gr. subst. gave 0.4764 gr. CO_2 and 0.1610 gr. H_2O .
 - b. 0.1541 gr. subst. gave 0.4703 gr. CO_2 and 0.1584 gr. H_2O .

found	a.	83.39% C	11.48% H.
	b.	83.23 „	11.42 „
$\text{C}_{21}\text{H}_{24}\text{O}$		83.44 „	11.26 „

Determination of molecular weight; using benzol as solvent, the molecular weight was determined by the depression of freezing point as follows.

1. When the crystal melts at 199°C.
0.1466 gr. subst gave 0.13°C. mol. depression.
found M. W. 326.
C₂₁H₃₄O requires 302.
2. When the crystal melts at 175—179°C.
0.1237 gr. subst. gave 0.09° mol. depression.
found M. W. 388.
C₂₇H₄₆O requires 386.

Optical character:—chloroform was used as a solvent.

1. When the crystal melts at 199°C.
1.33% substance gave 1.77 mean rotatory power.
found $[\alpha]_D^{19} = +85.72$
2. When the crystal melts at 195—179°C:
1.33% subst. gave 1.35 mean rotatory power.
found $[\alpha]_D^{19} = +67.67$

Iodine number;—the crystal melts at 199°C.

found 63.0 (by Hüble's method)

Calculated number as containing one double bond 84.7

Acetyl derivative;—Excepting the crystal which melts at 95—112°C, all other crystals produced scaly acetyl derivatives when heated with acetic anhydride. The acetyl derivatives changed again into original crystals by saponification and had following melting points.

Melting point of original crystal (not corrected)	Melting point of acetyl derivative.
199°C.	222—230°C.
182—188° „	204—214° „
175—179° „	188—198° „
155—158° „	178—185° „

The acetyl derivative which melts at 222—230°C. was dried at 100°C. and analysed with following result:—

0.1514 gr. subst. gave 0.4450 gr. CO ₂ and 0.1420 gr. H ₂ O.	
found	80.16% C 10.42% H.
(C ₂₁ H ₃₈ O)(OCH ₃ CO) cal.	80.23 „ 10.47 „

Considering these results, these crystals are higher alcohols having each probably one hydroxyl group and one double bond, except the lower number which melts at 95—112°C, and I have found two different higher alcohols which melt at 199°C. and 175—179°C, but as for the others it is uncertain whether they are isomer or mixtures.

Further, by the saponification of ripend flowers, I have isolated the alcohol which melts at 134—135°C, its acetyl derivative melts at 122—125°C,

and the reaction was the same as the phytosterol. Analysis gave the following results.

a. 0.1514 gr. subst. gave 0.4579 gr. CO₂ and 0.1623 gr. H₂O.

b. 0.1516 gr. " " 0.4553 gr. " and 0.1630 gr. "

found	a.	82.43% C	11.90% H.
	b.	82.00 " "	11.95 " "

C ₂₇ H ₄₆ O cal.	83.93 " "	11.92 " "
--	-----------	-----------

The above difference may be due to the presence of crystalline water.

The alcohol which melts at 199°C. is probably the same as Fujitani's *Pyrethrol*.

3. On the acid substance.

As mentioned already, I have obtained a resinous acid substance from the saponification product of the pyrethron. It was insoluble in water, but readily soluble in alcohol, ether and practically soluble in pet. ether. The alcoholic solution showed acid reaction to phenolphthalein, methyl orange and litmus and had slightly the characteristic odour of insect powder. The yield of the crude acid was 50% of pyrethrol. The crude acid was purified by the ester method. 50 grs. of the crude acid was dissolved in 100 grs. of absolute alcohol, passing dry hydrochloric acid gas through it until it became 3% of alcohol. The alcohol solution was now heated at 80° for 3 hours and then the alcohol was evaporated off. After neutralizing excess acid the solution was extracted with ether. The ethereal solution was repeatedly shaken with water and dried with fused sodium sulphate. On evaporating the ether, I obtained a neutral oily syrup, having a peculiar odour, the yield of which was 45 grs. The ester was distilled in vacuum at 1 mm pressure with following results.

Boiling point of the ester (not corrected)	yield.
---	--------

77—83°C.	7.0 grs.
----------	----------

The distillate was clear liquid having a peculiar odour.

85—115°C.	2.0 grs.
-----------	----------

The distillate was liquid at room temperature but partly solidified when cooled.

113—145°C.	3.0 grs.
------------	----------

The distillate was heavy oily liquid at room temperature but solidified when cooled and odourless.

173—179°C.	15 grs.
------------	---------

Same as the above.

A. Liquid acid.

The ethyl ester having the boiling point 77—83°C. was saponified with N-alcoholic potash. The saponification product was neutralized with tartaric

acid and extracted with ether. The ethereal solution was washed with water dehydrated with sodium sulphate and evaporated by passing the carbon dioxide gas. A heavy oily acid was obtained, which had a slightly yellow color with a peculiar odour. The acid was soluble in alcohol, ether and pet. ether, but insoluble in water. The acid was dried at 40—50°C. in vacuum and analysed with following results.

a. 0.1666 gr. substance gave 0.4302 gr. CO₂ and 0.1463 gr. H₂O.
 b. 0.1621 gr. " " 0.4196 gr. " and 0.1407 gr. "

found { a. 70.43% C 9.90% H.
 b. 70.60 " 9.67 " "

C₁₀H₁₈O₂ cal. 70.60 " 10.59 " "

Mol. weight, estimated by titration.

a. 0.3272 gr. subst. neutralized 19.0 cc. N/10 KOH.

b. 0.5239 " " 30.90 " "

found { a. M. W. 172.2
 b. " 172.7

C₁₀H₁₈O₂ cal. " 170.0

Iodine value, by Hüble's method, found 163.6

Calculated having one double bond 158.0

Ethyl ester, obtained by vacuum distillation as mentioned above.

a. 0.1530 gr. subst. gave 0.4067 gr. CO₂ and 0.1377 gr. H₂O.

b. 0.1642 " " 0.4367 " and 0.1500 " "

found { a. 72.50% C 10.00% H.
 b. 72.53 " 10.15 " "

(C₁₀H₁₇O)(C₂H₅O) cal. 72.23 " 11.11 " "

The acid has the formula C₁₀H₁₈O₂ and one double bond. The citronelllic acid has the same formula, but it is not certain whether they are the same or not.

The acid probably exists in large quantities in pyrethron, but the yield might be reduced by its decomposition by heating.

B. Solid acid, 1.

The ester having the boiling point 113—145°C. was saponified as mentioned above. As a saponification product, I have separated the crystal which melts at 62°C. after purification with alcohol. The crystal was soluble in alcohol, ether, chloroform and pet. ether and showed acid reaction. The acid was dried in a vacuum desiccator until it became of constant weight.

0.1532 gr. subst. gave 0.4197 gr. CO₂ and 0.1725 gr. H₂O.

found 74.72% C 11.52% H.

C₁₆H₃₂O cal. 75.00 " 11.58 " "

Mol. weight, determined by titration method.

0.4040 gr. substance neutralized 15.50 cc. N/10 KOH.

found M. W. 260.7

C₁₆H₃₂O₂ cal. 250.0

The melting point of the acid was not depressed by being mixed with pure palmitic acid, so I have concluded that the acid is palmitic.

C. *Solid acid, 2.*

I have separated the crystalline acid substance by the saponification of the ester which was distilled at 173—179°C. The acid recrystallized in alcohol melts at 56°C. and has almost the same property as palmitic acid. The analysis gave the following results.

a. 0.1541 gr. substance gave 0.4187 gr. CO₂ and 0.1682 gr. H₂O.

b. 0.1534 " " 0.4187 " and 0.1741 "

found { a. 74.10% C 12.14% H.
 b. 74.44 " 12.61 "

C₅H₃₀O₂ cal. 74.40 " 12.40 "

C₁₆H₃₂O₂ cal. 75.00 " 12.58 "

Mol. weight, determined by titration method.

a. 0.3677 gr. substance neutralized 13.9 cc. N/10 KOH.

b. 0.2843 gr. " " 10.7 " "

found M. W. a. 264.5

b. 265.6

C₁₅H₃₀O₂ cal. 242.0

C₁₆H₃₂O₂ cal. 256.0

Ethyle ester:—Analysis of the ethyl ester which was obtained by the vacuum distillation showed the following results.

a. 0.1641 gr. subst. gave 0.4527 gr. CO₂ and 0.1776 gr. H₂O.

b. 0.1583 gr. " " 0.4357 gr. " and 0.1689 gr. "

found { a. 75.23% C 12.03% H.
 b. 75.07 " 11.86 "

(C₁₅H₃₀O)(C₂H₅O) cal. 75.56 " 12.59 "

(C₁₆H₃₂O)(C₂H₅O) cal. 76.06 " 12.68 "

I have not ascertained whether the acid is isocetic acid, or a mixture of palmitic and other fatty acids.

Summary.

1. I have isolated a yellow, transparent neutral syrup named pyrethron as an insecticidal principle, which has the saponification value 216, and iodine value 116. Pyrethron is easily saponified with alcoholic potash and loses its insecticidal power after saponification. The power of this pyrethron is reduced by heating or when exposed to the air for a long time.

Pyrethron has the power to control the germination of bacteria in addition to a strong insecticidal power.

2. In the saponification product, I have isolated higher alcohols having the formulae $C_{27}H_{54}O$ (M. P. $199^{\circ}C.$) and $C_{27}H_{46}O$ (M. P. $175-179^{\circ}C.$) and fatty acids, liquid and solid, having the formulae $C_{10}H_{18}O_2$ and $C_{16}H_{32}O_2$ (palmitic). Judging from these results, it is probably true that the insecticidal principle of the insect powder is ester.

