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<tr>
<td>Author(s)</td>
<td>Ohno, Minoru; Oiwa, Toshihiko</td>
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Kyoto University
Determination of Natural Pyrethrins

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(Takei Laboratory)

Received October 19, 1953

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INTRODUCTION

Aiming at developing a method of direct quantitative determination of insecticidally active molecules through physico-chemical methods, and the consequent enhancement of accuracy and efficiency, the authors have long been engaged in the study on the possible methods of quantitative determination of all kinds of pyrethroids. As previously reported, their studies already resulted in the introduction of a new method of quantitative determination of allethrin° and allethroloneε based on the polarographic method. The present report concerns the polarographic and spectrophotometric determinations of natural pyrethrins, and the re-investigation of the Seil's method and the mercury-reduction method.

A remarkable progress and developmentε attained in these years in the chemistry of pyrethrum have revealed that the insecticidally active constituents in the pyrethrum are pyrethrin I and II and cinerin I and II, which are shown to be the esters of two cyclic keto-alcohols, pyrethrolone and cinerolone, with two acids, chrysanthemum-monocarboxylic acid and chrysanthemum-dicarboxylic acid monomethyl ester, respectively. The methods of quantitative determination for these active constituents practised are: the Seil'sε and the mercury-reduction methodsε which are used in foreign countries, and the modified Seil's methodε which was proposed by the staff of this laboratory and is now practised in Japan. It is to be pointed out that all these methods were developed at a time when the active constituents of pyrethrum were considered to be only pyrethrin I and II, with chrysanthemum-monocarboxylic acid and chrysanthemum-dicarboxylic acid as standard substances, and without the use of pure pyrethrins or cinerins, the insecticidally active ester forms. The oversight of these most important factors in the course of developing these methods has
resulted in many failures upon their practical application. The improvement of the procedure, and the over-all re-examination of these methods have, therefore, been strongly desired.

The existing methods being as above, ethylene diamine method based on a new idea is now being tentatively examined in the United States.* In neither of those methods, however, the ester form is determined directly, nor without organic-chemical change, and its principle is not entirely free from criticism.

In addition to such chemical methods as mentioned above, there have been several physico-chemical methods proposed for this purpose, such as the spectrophotometric method devised by Gillam and West(5) and proposed by Beckley(6) and Shulcis, et al.(7), and the polarographic method proposed by Yamada, et al.(8). These physico-chemical methods are advantageous over the chemical means in that (1) the ester form is determined directly, (2) the process is a simple and efficient one, and (3) the reproducibility is good. The purity of the standard substance used by the proposers of these methods, however, was that based on the analytical value of the Seil's method or the mercury-reduction method, and this fact justifies the argument that the drawbacks of the Seil's and the mercury-reduction methods still remain unremedied in these methods.

With these things and the present stage of chemistry of pyrethroids in consideration, the authors carried out experiments as described below:

The active components in the first place were separated from pyrethrum extracts and purified by column partition chromatographic method. As the result, 'pyrethrins' I (λ<sub>max</sub> = 224 m.μ., ε<sub>λmax</sub> = 34,250), which was either the mixture of pure pyrethrin I and cinerin I or the pure pyrethrin I or the pure cinerin I, and 'pyrethrins' II (λ<sub>max</sub> = 229 m.μ., ε<sub>λmax</sub> = 35,850), which likewise was either the mixture of pure pyrethrin II and cinerin II or the pure pyrethrin II or the cinerin II, were obtained. Theoretically, the standard substance should comprise pyrethrin I and II and cinerin I and II, each obtained in pure form. As this could not have been done, however, the standard substances used by the authors were 'pyrethrins' I and II, and α-dl-trans-allethrin, a crystalline isomer of allethrin. With these as standard substances, the Seil's method and the mercury-reduction method were examined. It was found that by the Seil's method, pyrethrins I could be estimated almost correctly, whereas the value of pyrethrins II estimated was far from being correct. By the mercury-reduction method, even the approximate value of pyrethrins I and II could not be obtained. If the total pyrethrins value is the sum of the values of pyrethrins I and II, these methods, which so specify, are but meaningless, since none of them is capable of accurately determining pyrethrins II, though the value of pyrethrins I may be measured with

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*According to a private communication (dd. Aug. 11, 53) from Dr. David Kelsey, Production and Marketing Administration, The United States Department of Agriculture.
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considerable accuracy by the Seil's method.

The authors, on the other hand, used as standard substances 'pyrethrins' I and II separated in pure forms, and developed two completely original methods of determination based on the polarographic and spectrophotometric procedures. By these methods, the exact value of total pyrethrins could be obtained, although theoretically the values of pyrethrins I and II could not be obtained separately. So long as pure 'pyrethrins' were used, these two methods proved equally successful. When pyrethrum flowers or extracts contaminated with other substances were treated, however, the spectrophotometric method was greatly affected by the presence of these other substances, and the value recorded was higher than the actual value. The polarographic method, however, proved entirely free from these influences, and showed exact values.

The experiment conducted by the authors' co-worker Nagasawa, on the knockdown effect on the housefly of 'pyrethrins' I and II kerosene solution gave a result quite contrary to the expectation: 'Pyrethrins' II was much more toxic than I. This fact leads to the assertion that the mere quantitative determination of pyrethrins I is without practical significance. At the present stage of pyrethrin usage, the determination of the value of total pyrethrins, which is the insecticidally active components in pyrethrum, is enough to meet the demands. The authors, therefore, wish to recommend a new method of inspecting pyrethrum products, the determination of total pyrethrins by polarographic method, the results of which should be indicated as "Total Pyrethrins Value (T. P. V.)".

**PART I**

**PREPARATION OF STANDARD 'PYRETHRINS' I AND II**

Methods of separation of pyrethrins used up to the present have sometimes involved alkaline hydrolysis, followed by separation of the acids and alcohols; the original constituents have been reformed by esterification for the purposes of toxicity testing. This procedure may result in isomerization of the compounds. It seems desirable, therefore, to investigate the possibility of separating the pyrethrins from one another, and from inactive material in the pyrethrum extracts by such process as would be considered to rid isomerization. The authors have succeeded in separating 'pyrethrins' I and II from each other and from other constituents of pyrethrum extracts by column partition chromatographic process, as the authors did in purifying the isomers of allethrin or the allethrolone.

It happened to come to the authors' attention that Lord, et al. recently published a report on the study on the chromatographic separation of the pyrethrins. They too have succeeded in separating the pyrethrins I and II from each other and from the inactive constituents of pyrethrum extracts. Theirs is the method in which the separation is attained by passing pyrethrum extract through alumina or silica column. The method, with the use of silica column much resembles that employed by the present authors. The seven methods, used by the authors to locate and iden-
tify the pyrethrins in the course of separation or after purification, are as follows:

a) Polarographic analysis.
b) Spectrophotometric analysis.
c) Elementary analysis.
d) Synthesis of the derivatives of pyrethrins.
e) Seil's method.
f) Mercury-reduction method.
g) Tests of knock-down effect on adults of the housefly.

The methods used by Lord, et al., on the other hand, include: (a) the reaction described by Lappin and Clark (1951) for the determination of carbonyl groups, (b) the spectrophotometric method, (c) the ester reaction applied to the pyrethrins by Lord (1950), (d) the mercury-reduction method, and (e) tests of biological activity. Pyrethrins I and II obtained by them and the authors are of like purity, where the same method of identification is commonly used by both.

**EXPERIMENTAL**

**Preparation of 'pyrethrins' I and II**

Commercial pyrethrum extracts were purified by the nitromethane and the liquid partition methods, and was divided into 'pyrethrins' I rich fraction, and 'pyrethrins' II rich fraction. Then the separation and purification of these fractions were undertaken by passing each of them several times through silica column under...

<table>
<thead>
<tr>
<th>µmax</th>
<th>εmax</th>
<th>Half-wave potential vs. N.C.E., v.</th>
<th>Elementary analysis</th>
<th>Seil's method</th>
<th>Mercury-reduction method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>C%</td>
<td>H%</td>
<td></td>
</tr>
<tr>
<td>Pyrethrins' I</td>
<td></td>
<td>224</td>
<td>34,250</td>
<td>-1.25</td>
<td>calcd.</td>
</tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Pyrethrins' II</td>
<td></td>
<td>229</td>
<td>35,860</td>
<td>-1.23</td>
<td>calcd.</td>
</tr>
<tr>
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</tbody>
</table>

a After the traces of solvents had been removed by means of high vacuum, the obtained 'pyrethrins' I was left standing, tightly covered, for about 21 hours under atmospheric pressure, before elementary analysis was undertaken.

b After the traces of solvents had been removed by means of high vacuum, the obtained 'pyrethrins' II was left standing under diminished pressure for about 3 hours, before elementary analysis was undertaken.

c Pys. : pyrethrins.
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different conditions, until the various physical constants and chemical characters no longer varied.

The characters of 'pyrethrins' I and II thus obtained are as shown in Table I, which can serve to testify that 'pyrethrins' I and II thus obtained are pure in that they are the esters of cyclic keto-alcohols components with 'chrysanthemum-monocarboxylic acid or -dicarboxylic acid monomethyl ester. However, doubt* remains as to whether 'pyrethrins' I is pyrethrin I, or cinerin I, or the mixture of both, and as to whether 'pyrethrins' II is pyrethrin II, or cinerin II, or the mixture of those two. These 'pyrethrins' I and II** were used as samples in the experiments detailed in Parts II, III, and IV.

Toxicity of 'pyrethrins' I and II

The biological assay of such samples as 'pyrethrins' I and II, pyrethrum extracts, from which 'pyrethrins' I and II were separated, a-dl-trans-allethrin and technical allethrin, from which a-dl-trans-allethrin was separated, was performed by the authors' co-worker Nagasawa*** to examine the relative knock-down effect of these samples on the common housefly. Its results are shown in Table 2. Remarkable are the two of the points clarified by this experiment: (1) 'pyrethrins' II, is more toxic than 'pyrethrins' I, and (2) the toxicity of pyrethrum extracts is shown as the sum of the toxicity of both 'pyrethrins' I and II. These points are quite contrary to the generally accepted concept.

Table 2. Relative knock-down effectiveness.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Relative effectivenessa</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Pyrethrins' I</td>
<td>1.47</td>
</tr>
<tr>
<td>'Pyrethrins' II</td>
<td>2.19</td>
</tr>
<tr>
<td>Pyrethrum extracts</td>
<td>1.82</td>
</tr>
<tr>
<td>(P. I. V., 6.1%, P. II. V., 5.6%, T. P. V., 11.7%)b</td>
<td></td>
</tr>
<tr>
<td>a-dl-trans-Allethrin</td>
<td>1.00</td>
</tr>
<tr>
<td>Technical allethrin</td>
<td>1.46</td>
</tr>
<tr>
<td>(Allethrin, 90.6%)b</td>
<td></td>
</tr>
</tbody>
</table>

a Relative effectiveness of the samples kerosene solution compared with a-dl-trans-allethrin kerosene solution calculated from the median knock-down time of adults of the common housefly, Musca domestica vicina Maqq. These solutions were prepared as the same concentrations by the Total Pyrethrins Value or the Allethrin Value.

b Obtained by the polarographic methods. (3.10)

* For organic-chemical clarification of this point, investigations are now being conducted. The results are to be published later.

** Thus, the authors' is different in meaning from the pyrethrins in its generally accepted sense. For this reason, pyrethrins, when used in this meaning, is marked with ' '.

In this report, for convenience's sake, mol of 'pyrethrins' I is decided at the mean value of mols of pure pyrethrin I and cinerin I, and mol of 'pyrethrins' II is the mean value of those of pyrethrin II and cinerin II.

*** Details in this connection will be reported by S. Nagasawa on the Botyu-Kagaku.
The reasons for this discrepancy may be as follows: (1) Purity of the samples used by the authors was much better than that of the samples in common use up to the moment. (2) As there was no possibility of isomerization in the processes of separation and purification, "pyrethrins" I and II could remain in the same molecular configuration as those contained in pyrethrum. (3) As the standard matter for the biological assay such a matter as \( \alpha \)-dl-trans-allethrin of good reproducibility and constancy was used and thus the exact relative value could be obtained.

PART II

RE-INVESTIGATION OF SEIL'S AND MERCURY-REDUCTION METHODS

In this part is given the result of the examination as to whether or not the Seil's method and the mercury-reduction method are theoretically correct.

(1) In case mono-acid is used: The value obtained by the Seil's method is 4~5% lower than the theoretical value, while that obtained by the mercury-reduction method well agrees to the theoretical value.

(2) Di-acid: Both the Seil's and the mercury-reduction methods give the values quite similar to the theoretical ones.

(3) \( \alpha \)-Allethrolone: In both the Seil's and the mercury-reduction methods, an acid very similar in character to mono-acid is produced in the process of determination, though only a trace in the former and in a small quantity in the latter. Also in both cases an acid similar in character to di-acid is produced in great quantities in the process of determination.

(4) \( \alpha \)-dl-trans-Allethrin: With the Seil's method, the value obtained is about 97% of the theoretical value, and in the process an acid similar in character to di-acid is produced. With the mercury-reduction method, the determined value is about 10% over the theoretical one, and an acid similar in character to di-acid is also produced.

(5) 'Pyrethrins' I: With the Seil's method, the value of pyrethrins I is 90% of the theoretical value, and an acid similar in character to di-acid is produced in the process. With the mercury-reduction method, the value of pyrethrins I obtained is about 10% over the theoretical value, and yet an acid similar in character to di-acid is also produced.

(6) 'Pyrethrins' II: The determined value of pyrethrins II is nearly 13% lower in the Seil's method than the theoretical value, and is almost the same in the mercury-reduction method with the theoretical one, though in both cases an acid similar in character to mono-acid is produced in the process.

From the result of the experiment described above, the following conclusion may be reached. By the Seil's method an approximate value of pyrethrins I may
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bo obtained, but no feasible value of pyrethrins II can be obtained. By the mercury-reduction method, an approximate value, too inaccurate to be put to use, of pyrethrins I may be obtained, while the value of pyrethrins II cannot be obtained. If the total pyrethrins value is to be obtained as the sum of the values of pyrethrins I and II, these methods, which so specify, are but meaningless, since none of them is capable of accurately determining pyrethrins II, though the value of pyrethrins I may be measured with considerable accuracy by the Seil’ method.

Apparently the cause for such results as above can be sought, among other things, in the decomposition products of alcohol component, an ester composit, to which no attention has been paid so far.

EXPERIMENTAL

The results obtained on various samples are shown in Table 3.

Table 3. Analytical results obtained on various samples by the Seil’s and the mercury-reduction methods.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Seil’ method</th>
<th>Mercury-reduction method</th>
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<tbody>
<tr>
<td></td>
<td>Mono-acid b or</td>
<td>Mono-acid d or</td>
</tr>
<tr>
<td></td>
<td>Di-acid b or</td>
<td>Di-acid d or</td>
</tr>
<tr>
<td></td>
<td>pyrethrins I, %</td>
<td>pyrethrins II, %</td>
</tr>
<tr>
<td></td>
<td>trace</td>
<td>trace</td>
</tr>
</tbody>
</table>

| Mono-acid | 96.8 a (7) | trace |
| Di-acid   | 99.3 a (2) | 100.3 a (2) |
| Mixture of| 95.0 a     | 99.1 a (4, HC1) |
| monoo- and di- | 100.3 a    | 99.1 a (2, HC1) |
| di-acid   | trace      | trace              |
| dl-Allethrolone| 77.6 a (2) | 65.8 a (2, H2SO4) |
| (λmax = 2295 A, |                         |
| ελmax = 11,049) |                         |
| a-dl-trans- | 96.9 a (2)   | 106.4 b (2, H2SO4) |
| Allethrin  | 10.0 a (2)   | 10.1 b (2, H2SO4)    |
| (mp. 50.5~51.0° C.) |              |                      |
| ‘Pyrethrins’ I | 90.1 a (2)   | 109.4 a (2, HCl)    |
| ‘Pyrethrins’ II | 1.1 c (2)    | 12.0 a (2, HCl)     |

1) Figures tagged a, b, c, d, e and f show the determination value (percentage) as calculated from the titration number, which is assumed, respectively, as resulting from the existence of mono-acid, allethrin, pyrethrins I (mixture of pyrethrin I and cinerin I (1:1)), di-acid, allethrin II (the ester of allethrolone with chrysanthemum-dicarboxylic acid monomethyl ester), and pyrethrins II (mixture of pyrethrin II and cinerin II (1:1)).

2) “HCl” and “H2SO4” mean the acidification of the solution by the respective chemicals.

3) * signifies that the 2 cc. of ethyl alcohol was not used in the case.

4) Italicized figures show the number of experiments repeated.
PART III
POLAROGRAPHIC DETERMINATION OF TOTAL PYRETHRINS

In this part is given the account of polarographic investigations made on the five substances, i.e., 'pyrethrins' I, 'pyrethrins' II, the mixture of 'pyrethrins' I and II, α-dl-trans-allethrin, and pyrethrum flowers and extracts. The chief points examined are: (1) a suitable composition of electrolytic solution for showing the typical reduction wave, (2) half-wave potentials, (3) the effect of pH, temperature, and time on reduction wave, (4) relations between the concentration and the wave height, (5) appropriateness of the system in which the four active constituents in pyrethrum are shown as a single active component, i.e., as total pyrethrins, and (6) the existence of substances in pyrethrum flowers or extracts which interfere with the typical reduction wave of the above-mentioned substances. As the result, an original method of polarographic determination of total pyrethrins was devised, in which α-dl-trans-allethrin was utilized as a standard substance. This method apparently is far superior in its accuracy and convenience to the Seil's, the mercury-reduction or the spectrophotometric method.

As shown in Fig. 1, 'pyrethrins' I and II, and the mixture of the two (1:1) show one-step wave, and the wave form of 'pyrethrins' I and that of α-dl-trans-allethrin are alike. The wave form of 'pyrethrins' II is slightly different from these. The reduction potential of 'pyrethrins' II, mixture of 'pyrethrins' I and II (1:1), 'pyrethrins' I and α-dl-trans-allethrin shifts to the negative in that order. The differences, however, are slight. Table 4, showing the relations between

![Fig. 1](image_url)

Polarograms of standard matters: I: α-dl-trans-allethrin (20×10⁻⁴M.), II: 'pyrethrins' II (20×10⁻⁴M.), III: mixture of 'pyrethrins' I and II (20×10⁻⁴M.), IV: 'pyrethrins' I (20×10⁻⁴M.). Each polarogram begins at -0.80 v.
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the concentration or the reduction temperature and the wave height, and other characters of these substances, can serve to testify that, as long at least as the polarographic characters specified therein are concerned, 'pyrethrins' I and a-dl-trans-

Table 4. Comparative polarographic characters obtained on standard matters.

<table>
<thead>
<tr>
<th></th>
<th>Half-wave potential vs. N.C.E., v.</th>
<th>Relations between concentration and wave height</th>
<th>Relations between temperature and wave height</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Pyrethrins' I</td>
<td>$-1.25 \text{id}_1 = 0.359 C + 0.003 \ldots (1)$</td>
<td>$\text{id}_1 = 0.0593 T + 3.605 (14.2 \times 10^{-4} \text{M.}) \ldots (5)$</td>
<td></td>
</tr>
<tr>
<td>'Pyrethrins' II</td>
<td>$-1.23 \text{id}_2 = 0.355 C - 0.009 \ldots (2)$</td>
<td>$\text{id}_2 = 0.0599 T + 2.322 (11.4 \times 10^{-4} \text{M.}) \ldots (6)$</td>
<td></td>
</tr>
<tr>
<td>Mixture of 'pyrethrins' I and II (1:1)</td>
<td>$-1.24 \text{id}_3 = 0.347 C - 0.002 \ldots (3)$</td>
<td>$\text{id}_3 = 0.0595 T + 2.952 (12.8 \times 10^{-4} \text{M.}) \ldots (7)$</td>
<td></td>
</tr>
<tr>
<td>a-dl-trans-Allethrin</td>
<td>$-1.27 \text{id}_4 = 0.360 C - 0.001 \ldots (4)$</td>
<td>$\text{id}_4 = 0.0595 T + 3.287 (13.3 \times 10^{-4} \text{M.}) \ldots (8)$</td>
<td></td>
</tr>
</tbody>
</table>

allelthrin are alike, while 'pyrethrins' II is slightly different. The characters of the mixture of 'pyrethrins' I and II (1:1) are about in the middle between those of its two constituents, and roughly agree to those of a-dl-trans-allelthrin.

As have already been pointed out in Part I, 'pyrethrins' I is either (a) pure pyrethrin I, or (b) pure cinerin I, or (c) the mixture of pure pyrethrin I and pure cinerin I. 'Pyrethrins' II is, likewise, either (a) pure pyrethrin II, or (b) pure cinerin II, or (c) the mixture of pure pyrethrin II and cinerin II. The above-mentioned experimental results can, therefore, be classified as below:

(a) The polarographic characters of either pyrethrin I or cinerin I agree well to those of a-dl-trans-allelthrin.
(b) The polarographic characters of the mixture of pyrethrin I and cinerin I agree well to those of a-dl-trans-allelthrin.
(c) The polarographic characters of either pyrethrin II or cinerin II are not much different from, and approximately the same with, those of a-dl-trans-allelthrin.
(d) The polarographic characters of the mixture of pyrethrin II and cinerin II are not much different from, and approximately the same with, those of a-dl-trans-allelthrin.
(e) The mixture (1:1) of either pyrethrin I or cinerin I or the mixture of pyrethrin I and cinerin I, with either pyrethrin II or cinerin II or the mixture of pyrethrin II and cinerin II, has a polarographic character resembling that of a-dl-trans-allelthrin.

The above-mentioned experimental results (a-e), plus such facts as that allethrin, pyrethrin and cinerin have like structures, and that 'step waves', which are supposed to result from the coexistence of pyrethrins and cinerins, are not seen in the polarograms of various pyrethrum flowers and extracts (as mentioned in later paragraphs), offer grounds for following suppositions:

(1) The polarographic characters of 'pyrethrins' I and those of the mixture of
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pyrethrin I and cinerin I are alike.

(2) The polarographic characters of 'pyrethrins' II and those of the mixture of pyrethrin II and cinerin II are alike.

(3) The polarographic characters of the mixture of 'pyrethrins' I and II (1:1) and those of the total pyrethrins in pyrethrum are alike.

The polarographic characters of the total pyrethrins in pyrethrum can be regarded as agreeing to those of α-dl-trans-allethrin. On the other hand, this conclusion is also supported by the fact that the polarograms of the all samples of pyrethrum flowers and extracts, from various sources, which were analysed by the authors were well resembled to that of α-dl-trans-allethrin.

Theoretically, the most desirable standard substance for determination is the mixture of pure pyrethrin I and II and cinerin I and II, each contained in equal molar. However, the practical phase is that the materialization of this procedure is not only extremely complex and almost impossible, but has little meaning, since they, if actually isolated, would be in liquid form, and would be so ready to change. On the other hand, α-dl-trans-allethrin, the first pyrethin homologue to be obtained as ester and in pure crystalline form, has many advantageous points for this purpose. It can be easily isolated from commercial allethrin products. Its purity can be ascertained simply by testing its melting point. It can, furthermore, be easily re-crystallized, when necessary, to improve purity. Those advantages of α-dl-trans-allethrin in ubiquity and constancy make it an excellent candidate for the standard substance in actual determination processes. Having fortunately reached the conclusion that the polarographic characters of the total pyrethrins and those of α-dl-trans-allethrin are approximately the same, the authors recommend for the practical purpose the use of α-dl-trans-allethrin as the standard substance for the polarographic determination of total pyrethrins. The method of determination is as follows:

The line of wave height vs. concentration of α-dl-trans-allethrin (Eq. 4) is first obtained, and from this and the modified equation (Eq. 9, as mentioned in later paragraphs) the line of wave height vs. concentration of total pyrethrins (Eq. 3) is obtained for calculating purposes. Then the molar concentration of total pyrethrins is obtained by placing measured wave height into the Eq. 3, followed by the calculation in milligram of total pyrethrins in the electrolytic solution. The total pyrethrins content will be indicated as "Total Pyrethrins Value (T. P. V.)."

Mean molecular weight of pyrethrins I and II, and total pyrethrins used for analysis: In the Seil's and the mercury-reduction methods, the calculation of milligram concentration from the titration value is based on the molecular weight of pyrethrin I or II alone, and is done without the consideration of cinerin I or II. Inasmuch as the insecticidally active constituents in pyrethrum consist of pyrethrin I and II and cinerin I and II, and as these methods aim at the deter-
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mination of pyrethrins I or II, both of which are the mixtures of pyrethrins and cinerins, some attention must of course be paid to the molecular weight of cinerins. Even in the polarographic and spectrophotometric methods, in which the total pyrethrins values are determined directly, the molecular weight for actual calculation must be obtained from the molecular weights of both pyrethrins and cinerins.

It is yet to be known in what proportion pyrethrin I and II and cinerin I and II exist in pyrethrum. The authors, therefore, suggest that they be assumed to exist in equal proportion, and that the molecular weight of pyrethrins I be decided at 322, the mean weight of pyrethrin I and cinerin I, that of pyrethrins II at 366, the mean weight of pyrethrin II and cinerin II, and that of total pyrethrins at 344, the mean weight of the four constituents. (The mean molecular weights used in this paper, of course, are these figures.)

Method of determination

The following is the method of determination for total pyrethrins devised after the fundamental investigations.

a. Electrolytic cell

The electrolytic cell is of the same type as used by Nakazima, et al. This cell can easily keep the temperature of electrolytic solution constant.

b. Standard o-dl-trans-allethrin and reagents

The reagents must be the ones that have undergone a blank test and shown no reduction waves. It is necessary that this blank test should be done each time before the reagent is used.

(1) Standard o-dl-trans-allethrin: It is obtained in crystalline form by cooling allethrin mixture and recrystallizing it from petroleum ether until at last the melting point becomes constant (50.5~51.0°C).

(2) Ethyl alcohol: Ethyl alcohol of bp. 78°C, from which aldehydes have been completely removed in the undermentioned way is used. Conc. sulphuric acid and water are added to alcohol (H₂SO₄ 5 cc., H₂O 20 cc., alcohol 1 litre), and distilled. To the distillate, silver nitrate and potassium hydroxide are added (AgNO₃ 10 g., KOH 1 g., the distillate 1 litre), and redistilled after several hours' boiling.

(3) Tetramethylammonium bromide solution: M/5 (CH₃)₄NBr is purified by recrystallization from alcohol, and dissolved into distilled water.

(4) Buffer solution: Sörensen's sodium citrate-hydrochloric acid buffer solution of pH about 3.0.

(5) Hydrogen: Oxygen is completely removed beforehand by passing it through at least five pyrogarol washing bottles (10 g. of pyrogarol is dissolved into 100 cc. of saturated KOH or NaOH solution).

(6) Mercury: Mercury used at cathode and anode has been purified by distillation, after being washed with nitric acid solution.
(7) Petroleum ether : bp. 30~50° C.

c. Procedure

(1) Pyrethrum flowers.

General procedure : Five grams of crushed sample (30 mesh per sq. cm.) are extracted in a Soxhlets extractor with petroleum ether for five hours. The circulation of petroleum ether solution must be over 15 times an hour. The solvent is removed under diminished pressure, in a 40° C. water bath, with the aid of nitrogen gas. Ethyl alcohol is added to the residual extracts. The solution is placed into a 10 cc. calibrated volumetric flask using analytical transfer technique, and is made to volume. After standing two hours, 1 cc. of the upper clear layer of this stock solution is taken into a test tube carrying a glass stopper, added with 4 cc. of ethyl alcohol and 1 cc. of M/5 (CH₃)₄NBr solution. To this solution, 4 cc. of buffer solution is added and shaken. This is poured into an electrolytic cell, which contains anode mercury, and whose temperature is kept at 25±0.5° C. When the procedure is over, dissolved oxygen is removed from the solution at 25±0.5° C. by a stream of hydrogen. Thirty minutes after the buffer solution is added, hydrogen is cut off, and the polarogram is taken at 25±0.5° C.

The wave height of the polarogram is measured by the construction method mentioned below. The concentration of total pyrethrins is calculated from the line of wave height vs. concentration of total pyrethrins (Eq. 3).

Single flower : A single flower is cut quantitatively and extracted for 5 hours by means of Soxhlets extractor with petroleum ether. Then the same procedure as that of the preceding experiment is followed until the 10 cc. ethyl alcohol stock solution is prepared. After standing two hours, 5 cc. of the upper clear layer of this stock solution is taken into a test tube carrying a glass stopper, and added with 1 cc. of M/5 (CH₃)₄NBr solution. The following procedure is altogether the same as that followed in the preceding experiment.

(2) Pyrethrum extracts.

Three hundred milligrams of sample are placed in a 10 cc. volumetric flask and is made to volume with ethyl alcohol. The further procedure is the same as that followed in the case of general flowers.

d. Method of measuring wave height

The method of construction is the same as in the case of allethrin. As indicated in Fig. 2, a slope line is drawn through the center of oscillations. A straight tangent line (AB) is drawn to the diffusion current part of the slope line, and another straight line (CD) is drawn at the bending at the foot in parallel with the line (AB). Then, a tangent line (EF) is drawn through the point of half-wave potential (M), and the points at which this line (EF) crosses the already drawn two lines (AB and CD) are marked G and H, respectively. The bisecting lines of
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Fig. 2. Method of measuring wave height.

the intersecting angles (\( \angle FGA \) and \( \angle DHE \)) are then drawn, and the points at which those lines intersect the slope line are marked I and J, respectively. The perpendicular distance between I and J, i.e., KL, is the wave height.

EXPERIMENTAL

1. Apparatus
A Heyrovsky-Shikata type polarograph (made by Yanagimoto Seisakusho Co.) was employed. The sensitivity of galvanometer employed was in all cases \( 3.09 \times 10^{-5} \) A per mm. per m. The capillary constants, measured at \(-1.0 \) v. in the electrolytic solution mentioned in the method of determination of part III were as follows:

\[
m = 0.725 \text{ mg/sec.}, \quad t = 4.20 \text{ sec./drop.}, \quad m^{1/3} t^{1/6} = 1.025
\]

The potential in this report is shown by N-Calomel Electrode Standard.

2. Preparing Electrolytic Solution
The composition of the electrolytic solution used was the same as in the case given in the first report:\( ^{(1)} \): Ethyl alcohol (50\%), \( M/5 (\text{CH}_3)_4 \text{NBr} \) solution (10\%), and buffer solution (40\%). As shown in Fig. 1, the typical reduction wave of pyrethrins can be obtained in this composition of the electrolytic solution.

The use of NaCl or KI as indifferent salts in place of \((\text{CH}_3)_4 \text{NBr}\), however, is not desirable, as it makes the diffusion current steeper as shown in Fig. 3.

3. Influence of pH on Reduction Wave and Half-Wave Potential
The polarograms of 'pyrethrins' I and II were taken, with the aid of various buffer solutions, by the method and under the conditions described in the method of determination of Part III. The result is shown in Figs. 4~9. The values of pH in the figures are of the electrolytic solution.

The authors, after carefully comparing those polarograms, and in order to make the wave height measurement as easy, and errors as little as possible, decided that the value of pH of the utilized buffer solution suitable for the analysis...
Fig. 3. Influence of indifferent salt on reduction wave of mixture of 'pyrethrins' I and II (1:1): I (M/5 KI), II (M/5 (CH₃)₄-NBr), III (M/5 NaCl). Each polarogram begins at —0.80 v.

Fig. 4. Polarograms of 12.4×10⁻¹ M. 'pyrethrins' I reduced at different pH values: I (pH=1.49), II (pH=2.56), III (pH=3.63), IV (pH=4.99). Each polarogram begins at —0.80 v.

Fig. 5. Polarograms of 12.4×10⁻⁴ M. 'pyrethrins' I reduced at different pH values: V (pH=5.67), VI (pH=6.75), VII (pH=7.63), VIII (pH=9.03). Each polarogram begins at —1.00 v.

Fig. 6. Polarograms of 12.4×10⁻¹ M. 'pyrethrins' I reduced at different pH values: IX (pH=10.27), X (pH=11.11), XI (pH=11.28), XII (pH=12.16). Each polarogram begins at —1.10 v.
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Fig. 7. Polarograms of $1.4 \times 10^{-1} M$ 'pyrethrins' II reduced at different pH values: I (pH=1.50), II (pH=2.54), III (pH=3.66), IV (pH=4.98). Each polarogram begins at -0.80 v.

Fig. 8. Polarograms of $1.4 \times 10^{-4} M$ 'pyrethrins' II reduced at different pH values: V (pH=5.67), VI (pH=6.73), VII (pH=7.64), VIII (pH=9.02). Each polarogram begins at -1.00 v.

Fig. 9. Polarograms of $1.4 \times 10^{-4} M$ 'pyrethrins' II reduced at different pH values: IX (pH=10.40), X (pH=10.97), XI (pH=11.58), XII (pH=12.44). Each polarogram begins at -1.10 v.

was about 3.0.

The half-wave potentials of 'pyrethrins' I and II and $\alpha$-dl-trans-allethrin under the conditions described in the method of determination of Part III are as in Table 4.

4. Effect of Temperature on Reduction Wave

The polarograms of 'pyrethrins' I, II, mixture of 'pyrethrins' I and II (1:1),

(403)
and \( \alpha \)-dl-trans-allethrin were taken at various degrees of temperature by the method shown in the method of determination of Part III, while the concentration and the composition of the electrolytic solution were kept constant. As temperature increased, the half-wave potential shifted slightly to the negative potential. The wave height increased linearly in proportion to the increase of temperature (See Fig. 10). Theoretical equations of the curves are as in Table 4. In these equations, \( \delta \) shows the wave height in centimeter and \( T \) signifies the temperature degree in Centigrade. Under these circumstances, therefore, the positive temperature coefficients of the wave height of mixture of ‘pyrethrins’ I and II (1:1) varied between about 1.8\% (at 5° C.) and about 1.3\% (at 30° C.).

![Graph showing wave height vs. temperature](image)

Consequently, from the analytical viewpoint, it is evident that the temperature should be controlled at least to the range of ±0.7° C., or better, in order that, when the temperature is about 25° C. (at which the procedure is comparatively simple), errors due to the temperature change be kept within ±1\%.

5. Relations between Concentration and Wave Height

By the method and under the conditions described in the method of determination of Part III, the relations between the concentration and the wave height of ‘pyrethrins’ I, ‘pyrethrins’ II, mixture of ‘pyrethrins’ I and II (1:1), and \( \alpha \)-dl-trans-allethrin were studied, and the results obtained are shown in Fig. 11.

![Graph showing wave height vs. concentration](image)
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The standard theoretical equations obtained cross the axis of coordinates at zero point, and are as in Table 4. In the equations, \( id \) is the wave height in centimeter, and \( C \) is the concentration shown in unit of \( 10^{-4}M \). Therefore, the wave height is proportional to the concentration, and the calculated and experimental values are almost the same, with possible experimental errors in consideration. Relations between Eqs. 3 and 4 are:

\[
id_3 = 0.964 \, id_1 \quad \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots 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No. 10, and samples No. 5~No. 7, agree well with those of the mixture of 'pyrethrins' I and II (1:1). The wave height of those substances, therefore, can be measured easily by the method mentioned above. Flowers of samples No. 1~No. 4 have lower Total Pyrethrins Values than the flowers of samples No. 5~No. 7, the former being approximately 60% of the latter. The second waves (→) of the samples No. 1~No. 4 and No. 8~No. 10 can be supposed to be of pyrethrolone or cinerolone, when the relations of allethrin with allethrolone\(^{10}\) are taken into consideration. The reduction potentials of these waves and of pyrethrins are fairly well apart to permit no interference with each other. Thus, in the flowers which have been stored long after production, the Total Pyrethrins Value is very low, while contaminating substances responsible for the second wave are many. It is supposed that, as one of these mechanisms, the ester conjugation of pyrethrins was hydrolysed to form pyrethrolone or cinerolone and chrysanthemum-monocarboxylic acid or -dicarboxylic acid.

With a view to ascertaining the existence, or nonexistence, of any substances which interfere with the reduction wave of pyrethrins in pyrethrum flowers or extracts, the samples No. 1~No. 10 were added with a given amount of \(\sigma\)-dl-trans-allethrin or of the mixture of 'pyrethrins' I and II (1:1), and were analysed. With all of these samples, the added value agreed with the determination value. It can be safely said, therefore, that the reduction wave of pyrethrins in pyrethrum flowers or extracts is not affected by other substances.

Investigation was also made on a few of the conditions of extraction by Soxhlets

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**Fig. 14.** Polarograms of pyrethrum extracts. Each polarogram begins at \(-0.80\) v.

**Fig. 15.** Polarograms of pyrethrum extracts (The solvent is removed by means of height vacuum). Each polarogram begins at \(-0.80\) v.
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extractor, which is one of the preliminary processes to be followed before the polarogram of pyrethrum flowers is taken. With the use of petroleum ether (bp. 30–50° C.) and with the circulation of 15–20 times an hour, total pyrethrins were extracted completely in four hours.

The waves of samples No. 8′—No. 10′ were those obtained after the removal of solvent kerosene at high vacuum from the samples No. 8—No. 10 (this procedure is the same as that described in the Experimental of Part IV). As can be seen in Figs. 14 and 15 and Table 6, no difference exists between the reduction waves of No. 8′—No. 10′ and those of the same samples taken without the removal of solvent, except that the residual current of the latter shows a slight confusion. The analyses of pyrethrum extracts, therefore, can be carried out correctly as long as the rules mentioned in the method of determination of Part III are observed, and the complex preliminary processes are unnecessary.

PART IV

SPECTROPHOTOMETRIC DETERMINATION OF TOTAL PYRETHRINS

In this part is given the account of spectrophotometric investigations made on the five substances, i. e., 'pyrethrins' I, 'pyrethrins' II, the mixture of 'pyrethrins' I and II, α-dl-trans-allethrin, and pyrethrum flowers and extracts. The chief points examined are: (1) the wave length of maximum of ultraviolet absorption, (2) the most suitable wave length for measuring the absorbency (optical density) of solutions containing pyrethrins, (3) relations between the concentration and the absorbency, and (4) the existence of substances in pyrethrum flowers or extracts, which interfere with the typical absorption of the above-mentioned substances.

As the result, a new method of spectrophotometric determination of total pyrethrins was devised, in which α-dl-trans-allethrin was utilized as a standard substance. This method gave exact value so long as pure pyrethrins were treated, but was greatly affected by the presence of other substances, the value recorded being higher than the actual value. This, however, is a defect common to all spectrophotometric methods including the one proposed by Shukis, et al. The spectrophotometric methods, on the whole, are of small value as the determination method for pyrethrum flowers and extracts. In order for this method to be put to practical use, an exceedingly complex preliminary process for removing impurities would be developed.

The ultraviolet absorption spectra of 'pyrethrins' I, 'pyrethrins' II, and the mixture of 'pyrethrins' I and II (1:1) taken with 95% ethyl alcohol as the solvent are shown in Fig. 16. The wave lengths of absorption maximum are:

- 'pyrethrins' I ..............................................224 mμ.
- 'pyrethrins' II ...........................................229 mμ.
the mixture of 'pyrethrins' I and II (1:1) ....................226 m\(\mu\).
\(\alpha\)-dl-trans-allethrin ..............................224 m\(\mu\).

Fig. 16. Absorption curves for 'pyrethrins' I and II, and mixture of 'pyrethrins' I and II (1:1), and \(\alpha\)-dl-trans-allethrin alcoholic solution. (\(2 \times 10^{-5}\)M., Cell: 1 cm.)

As was pointed out in Part I, 'pyrethrins' I is either (a) pure pyrethrin I, or (b) pure cinerin I, or (c) the mixture of pure pyrethrin I and pure cinerin I. From the facts that \(\alpha\)-dl-trans-allethrin and pyrethrin or cinerin have like structures, and that the wave length of absorption maximum of \(\alpha\)-dl-trans-allethrin agrees to that of 'pyrethrins' I, which in its composition is either of the above-mentioned three cases, it is safely assumed that the wave length of absorption maximum of the mixture of pure pyrethrin I and pure cinerin I (1:1), i.e., pyrethrins I, roughly agrees to that of 'pyrethrins' I.

'Pyrethrins' II, as mentioned earlier, is either (a) pure pyrethrin II, or (b) pure cinerin II, or (c) the mixture of both. It is safely assumed as in the case of 'pyrethrins' I that the wave length of absorption maximum of 'pyrethrins' II, which in its composition is either of the above-mentioned three cases, roughly agrees to that of the mixture of pure pyrethrin II and pure cinerin II (1:1), i.e., pyrethrins II.

It is probably safe to suppose that the wave length of absorption maximum of mixture of 'pyrethrins' I and II (1:1) roughly agrees to that of the total pyrethrins. The authors, in this light, concluded that 226 m\(\mu\) is the most suitable wave length for measuring the absorbency of solutions containing pyrethrins.

Data for the plot of concentration vs. absorbency were obtained by determining the absorbency at 226 m\(\mu\) of suitable concentrations of mixture of 'pyrethrins' I and II (1:1) or \(\alpha\)-dl-trans-allethrin. The results are as shown in Fig. 17. The standard theoretical equations obtained from these data cross the axis of
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coordinates at zero point, and are as follows:

- Mixture of 'pyrethrins' I and II (1:1)....\(A_1 = 0.337 \text{ C}\) ..................(10)
- \(\alpha\)-dl-trans-allethrin................. \(A_2 = 0.179 \text{ C}\) ..................(11)

The standard theoretical equations obtained at the maximum wavelength of 'pyrethrins' I and II are:

- 'pyrethrins' I .................................\(A_3 = 0.339 \text{ C}\) ..................(12)
- 'pyrethrins' II .................................\(A_4 = 0.337 \text{ C}\) ..................(13)

where, \(A\) is the absorbency, and \(C\) is the concentration shown in the unit of \(10^{-5}M\).

Eqs. 10 and 11 are related:
\[A_1 = 1.883 A_2\] ..................(14)

Theoretically, the best calibration curve for determining pyrethrum flowers or extracts is that obtained from the standard matter, in which pure pyrethrin I and II, pure cinerin I and II are contained in equal molar. As the second best means, however, the authors propose the use of Eq. 10. However, it is necessary that every experimenter, in order to ascertain that his procedure is the same as the authors', should obtain the equation (11') of concentration vs. absorbency of \(\alpha\)-dl-trans-allethrin, and compare the value thus obtained with that obtained by the authors (Eq. 11). Should the Eq. 11' thus obtained disagree to Eq. 11, the alternative may be to modify and correct Eq. 11', using the factor of Eq. 14, i.e., 1.883

Fig. 17. Absorbencies at 226 \(\mu\mu\), vs. concentrations of mixture of 'pyrethrins' I and II (1:1), and \(\alpha\)-dl-trans-allethrin.

\[\text{Concentration, } 10^{-5}M.\]

\[\log \frac{I_n}{I} \text{ vs. Concentration, } 10^{-5}M.\]

\[\text{Concentration, } 10^{-5}M.\]

\[\log \frac{I_n}{I} \text{ vs. Concentration, } 10^{-5}M.\]
and arrive at equation of concentration vs. absorbency of mixture of 'pyrethrins' I and II (total pyrethrins). The approximate value is obtainable by this method.

EXPERIMENTAL

1. Apparatus and solvent

A Beckman DU quartz spectrophotometer was used. The instrument was checked for the wave length setting using the various millimicron lines of the mercury arc.

As the solvent, 95% ethyl alcohol was used. An arbitrary standard for 95% ethyl alcohol was taken as not less than 50 percent of the transmittance of distilled water at 226 m.μ.

2. Procedure for pyrethrum flowers

The quantity (5 g.) of the sample containing 20 to 40 mg. of total pyrethrins was weighed. It was extracted in a Soxhlets extractor with petroleum ether (30~50° C.) for five hours. The solvent was removed by immersing the flask in a 40° C. water bath and applying a vacuum of about 3.0 mm. Hg. The residue was dissolved by adding 95% ethyl alcohol. It was then translated into a 10 cc. calibrated volumetric flask, with analytical transfer technique. It was made to volume at 20° C. with 95% ethyl alcohol (stock solution). The solution was allowed to stand 2 hours for precipitation of any wax and resinous matter. (The procedure described so far is identical with that used in the polarographic determination of flowers.)

One cc. of the upper clear layer of the solution was pipetted into a 10 cc. calibrated volumetric flask, and was made to volume at 20° C. with 95% ethyl alcohol. This process was repeated two more times in order to make the concentration of total pyrethrins about 2×10⁻⁵M.

The solution was then added to a calibrated silica spectrophotometer cell and the absorbency was determined at 226 m.μ., with the use of 95% ethyl alcohol.

![Absorption curves for the samples of pyrethrum extracts.](410)
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as the solvent blank in a similar calibrated silica cell. The value for the blank was subtracted from that for the solution. With this corrected value for absorbency, the pyrethrin concentration of the solution was determined from the Eq. 10, and Total Pyrethrins Value of the sample was calculated out.

The results obtained by this method on the samples of pyrethrum flowers from various sources are shown in Table 6, and the ultraviolet absorption spectra taken on the solution of samples are shown in Fig. 18.

3. Procedure for pyrethrum extracts

A quantity (300 mg.) of the sample containing 20 to 40 mg. of total pyrethrins was weighed into a 10 cc. calibrated volumetric flask. It was made to volume at 20° C. with 95% ethyl alcohol (stock solution). After a series of processes as described in the procedure for flowers, spectrum was taken and the determination was performed. The absorption spectra of the samples are shown in Fig. 19 (No. 8~No. 10). The wave lengths of maximum of ultraviolet absorption were 221 mμ in all cases, and were much shorter than that of the mixture of 'pyrethrins' I and II (1:1). These indicate that they were greatly affected by contamination, which, in return, account for the much greater Total Pyrethrins Values than had been expected.

![Absorption curves for the samples of pyrethrum extracts.](image)

Then, in accordance with the method of Shukis, et al.(9), kerosene solution, the solvents, had been removed from samples No. 8~No. 10, before spectrum was taken, and determination was performed.

The method of removing solvents : A quantity of the sample containing 20 to 40 mg. of total pyrethrins was weighed into a flask. A tube was attached to the cold trap of the Hy-Vac apparatus. The system was pumped with the Hy-Vac pump until a pressure of 0.1 mm. Hg. was reached (usually 20~30 minutes). Then
the diffusion pump was turned on, and pumping continued. After the system reached a pressure of $4.2 \times 10^{-4}$ mm. Hg., one hour was allowed at 40° C. (flask heated in 40° C. water bath) for complete removal of the solvent. The tube was disconnected, and 10 cc. of 95% ethyl alcohol was added (stock solution). The following procedure was the same as in the procedure for flowers.

The absorption spectra obtained were as shown in Fig. 19 (No. 8'~No. 10'), and the wave lengths of ultraviolet absorption maximum well agree to that of the mixture of 'pyrethrins' I and II (1:1). The types of absorption, however, are slightly different. The determined values are as shown in Table 6, and are noticeably lower than the corresponding values in No. 8~No. 10.

**CONCLUSION**

'Pyrethrins' I and II had been separated in pure form through application of column partition chromatography.

With these and a-dl-trans-allethrin as standard substances, a polarographic determination method of natural pyrethrins based on a completely original idea, was developed. The polarographic, the spectrophotometric, the Seil's and the mercury-reduction methods were compared in terms of accuracy and applicability. The conclusive results are shown in Table 5. As is evident from the Table, the accurate determination of total pyrethrins both in the pure pyrethrins and the commercial products is possible only by the polarographic method. By none of the four methods, pyrethrins I and II can be determined separately. Only the approximate value of pyrethrins I can be determined by the Seil's method.

The conclusion reached by the authors, therefore, is that, in determining the pyrethrum products, the Total Pyrethrins Value (T. P. V.) is to be sought for by the polarographic method, or, if necessary, after first obtaining Total Pyrethrins.
Determination of Natural Pyrethrins

Value by the polarographic method, the Pyrethrins I Value (P. I. V.), approximate as it may be, is to be obtained by the Seil's method, further to calculate out the Pyrethrins II Value (P. II. V.) by subtracting the Pyrethrins I Value from Total Pyrethrins Value.

The results of the determination by the four methods of total pyrethrins in the pyrethrum flowers or extracts, the same sample being prepared for each case, are shown in Table 6. The determination of the pyrethrins I and II in the same sample

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Produced in</th>
<th>Total Pyrethrins Value, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>1</td>
<td>June</td>
<td>0.56 0.56</td>
</tr>
<tr>
<td>2</td>
<td>July, 1952</td>
<td>0.53 0.56</td>
</tr>
<tr>
<td>3</td>
<td>July</td>
<td>1.03 1.05</td>
</tr>
<tr>
<td>4</td>
<td>July, 1952</td>
<td>0.95 0.89</td>
</tr>
<tr>
<td>5</td>
<td>June</td>
<td>11.7 11.7</td>
</tr>
<tr>
<td>6</td>
<td>July</td>
<td>15.9 16.2</td>
</tr>
<tr>
<td>7</td>
<td>July, 1952</td>
<td>11.8 10.6</td>
</tr>
<tr>
<td>8</td>
<td>Nov. 1952</td>
<td>16.4 15.6</td>
</tr>
<tr>
<td>9</td>
<td>Nov. 1953</td>
<td>11.2 15.6</td>
</tr>
</tbody>
</table>

* These determinations were performed during the period Sep. 1-15, 1953.

Table 7. Pyrethrins I and II Values: Comparative results obtained on samples of pyrethrum flowers and extracts*

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Produced in</th>
<th>Pyrethrins I and II Value, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Seil's method</td>
</tr>
<tr>
<td>1</td>
<td>June</td>
<td>0.30</td>
</tr>
<tr>
<td>2</td>
<td>July, 1952</td>
<td>0.26</td>
</tr>
<tr>
<td>3</td>
<td>July</td>
<td>0.53</td>
</tr>
<tr>
<td>4</td>
<td>July, 1952</td>
<td>0.48</td>
</tr>
<tr>
<td>5</td>
<td>Nov. 1952</td>
<td>5.9</td>
</tr>
</tbody>
</table>

* These determinations were performed during the period Sep. 1-15, 1953.
as above conducted by the authors', the Seil's and the mercury-reduction methods resulted in Table 7. From the results of the determination by the authors' method, the following points are clear:

1. In pyrethrum flowers, whether new or old, pyrethrins I and II exist nearly in equal composition ratio.

2. During the period of storage, both pyrethrins I and II undergo a marked decomposition. The decomposition percentage in both cases is the same.

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