recognized maximum amount in U. S. A. of vaporized lindane[2] to be used in the room for insect control is 1 gram per 15,000—20,000 cubic feet per 24 hours. The maximum amount to be allowed in the Japanese rooms are shown in Table 6.

In the above experiments, that is, in cases of 0.2, 0.4 and 0.8% lindane solutions 4.5mg., 8.7mg. and 15.2mg. of lindane respectively were vaporized per hour under proper conditions. Therefore the desired amount of lindane can be vaporized continuously for a long time, if the capacity of bottle, the condition of the platinum-asbestos and the concentration of lindane are controlled. In this way, it becomes possible to control insects in the room without any ill-effects on human being. This apparatus can be used also in green house for fumigation of plant pests

In practical use, it was found convenient to employ the bottles with capacities of two times as large as those employed above. With such bottle necessary and sufficient amount of lindane can be vaporized during about 8 to 10 hours.

The author is indebted to Prof. Sankichi Takei and Assist. Prof. Minoru Ohno for their kind guidances and encouragements. He also wishes to express his appreciation to Mr. Takashi Yamaguchi, the President of Osaka Kasei Co. who invented this apparatus.

This invention was applied to patents of Japan, U. S. A., Great Britain, Germany and Brazil.

Polarographic Determination of Natural Pyrethrins. (Studies on Determination of Pyrethroids. III.) Toshihiko OIWA, Terumi SHINOHARA, Yasuhiko TAKESHITA and Minoru OHNO (Takei Laboratory, Institute for Chemical Research, Kyoto University)
Received Sept. 21, 1953. *Botyu-Kagaku*, 18, 142 (1953). (with English text 143)

26. 天然ピレトリン類のポーラログラフ法による定量（ピレトリン類緑物質の定量に関する研究 第3報） 大岩俊彦，篠原照巳，竹下康彦，大野 稔（京都大学 化学研究所 武居研究室） 28. 9. 21. 受理

'Pyrethrins'-I 及び-II を分別クロマトグラフ法で純粋に分離し，このものを標準としてポーラログラフ法によつて全 pyrethrins の正確な定量を行ふ事に成功した。本定量法は従來の Seil's 法や水銀還元法に較べてその正確度がはるかに勝つている。これら純粋な 'pyrethrins' のイエバエに対する落下效果は意外にもIよりもIIの方が大であつた。

吾々はかねてから，次に述べる様な観点に立つて，pyrethroids 全般の定量法の確立を意図し，研究を進めている。即ち物理化学的手段を用いて，殺虫力を示す分子を直接定量することによつて，分析の正確度を増し操作の簡易迅速化を計ることである。この様な立場で行つた研究成果の一つとして，先にポーラログラフ法による allethrin[1] 及び allethrolone[5] の定量法を報告した。今回は天然 pyrethrins のポーラログラフ法及び分光光度計法による新定量法並びに従来の Seil's 法及び水銀還元法による定量の再検討の結果を報告する。

近年除虫菊の化学が長足の進歩を遂げ，除虫菊の有効成分は，pyrethrin I 及び II 並びに cinerin I 及び II の四物質であることが発見され，次で夫々の化学構造も決定された[3]。現在これらの有効成分の定量法として，諸外国では Seil's 法[4] 或は水銀還元法[5] 等が，又吾が国では吾々の研究室の提案になる Seil's 法の変法[6] が広く使用されている。これらの諸方法は除虫菊の有効成分が pyrethrin I 及び II の二つであるとされた頃，第1及び第2菊酸を標準として考案されたものであつて，殺虫力をもつエステル態即ち純 pyrethrins 或は cinerins を使用して定量法の可否の検討がなされていない，斯様に此等の方法は当然行わなくてはならない根本的な検討を省いているばかりか，他方実際の操作上にも多くの矛盾があることが指摘せられ，操作の改良，更には定量法の再検討が強く要望されている実状である。

現行法が斯様な状態にあるので，米国* ではこれら

* United States Department of Agricultural, Production and Marketing Administration の David Kelsey 氏からの私信 (1953. 8. 11) による。

と異なつた新しい考えになる ethylene diamine 法等が試案として現在検討されつつあるが，これらも殺虫力を持つエステル態を直接定量するものではなく，又原理的にも疑点がある方法である。

以上の化学的な方法の外に，物理化学的方法として Gillam 及び West [7] の創案になり Beckley [8]，Shukis等[9] の提案する分光光度計法並びに山田等[10] の提案するポーラログラフ法等がある。これらの様に物理化学的手段による方法は上述の化学的手段に比べエステル態を直接定量しうる点，操作が簡易迅速である点，或は再現性が高いと云う点で優れている。然し乍ら以上の人々がこれらの物理化学的方法に用いた標準物質の純度は何れも Seil's 法及び水銀還元法による分析値に基礎を置いたものであつて，結局その中に Seil's 法及び水銀還元法のもつ欠陥を包蔵することになる。

吾々は以上のことや，pyrethroids の化学の現状を考慮して，次の様な順序で研究を行つた。即ち先づクロマトグラフ法により除虫菊エキス中から有効成分の分離精製をした。その結果 'pyrethrins' Ⅰ (λ_{max} =224 Mμ., $\varepsilon_{\lambda max}$=34250；pyrethrin Ⅰ と cinerin Ⅰ の純品の混合物か，或は pyrethrin Ⅰ の純品か，或は cinerin Ⅰ の純品かの何れかである）と，'pyrethrins' Ⅱ (λ_{max}= 229 Mμ., $\varepsilon_{\lambda max}$=35850；pyrethrin Ⅱ と cinerin Ⅱ の純品の混合物か，或は pyrethrin Ⅱ の純品であるか，或は又 cinerin Ⅱ の純品かの何れかである）とを得ることに成功した。理論的には勿論 pyrethrin Ⅰ 及び Ⅱ 並びに cinerin Ⅰ 及び Ⅱ の四成分を純粋に得て標準物質とするのが正しいが，今回はその目的を達し得なかつたので，次善の策として，'pyrethrins' Ⅰ と 'pyrethrins' Ⅱ 及びそれらの近縁化合物である allethrin の結晶状一異性体 α-dl-trans-allethrin を標準物質として使用することにした。

これらの物質を標準として Seil's 法及び水銀還元法を検討した処，Seil's 法では pyrethrins Ⅰ 値は近似的に定量されるが，pyrethrins Ⅱ 値は近似値さえも求められない。又水銀還元法では pyrethrins Ⅰ 及び pyrethrins Ⅱ の近似値さえも共に求められないことを知つた。従つて両値の和を全 pyrethrins 値とするこれらの方法は，全 pyrethrins の定量法として意味がないわけである。

こゝに於て吾々は 'pyrethrins' Ⅰ 及び Ⅱ を標準として，ポーラログラフ法及び分光光度計法による定量に就いて研究し，独自の方法を考案した。これら両方法は純品の pyrethrins Ⅰ と pyrethrins Ⅱ でも現在原理的に両者の分離定量は不可能であるが，全 pyrethrins 値は正確に求められる。尚純品に関する限り両方法の間に優劣は認められないが，除虫菊乾花及びエキスの様に他の物質が混在する時は，分光光度計法の方は混在する pyrethrins 以外の物質の影響を大きく受け，結果として非常に高い値を示した。然るにポーラログラフ法はこの様な影響を受けず正しい値を示し，非常に優れたものであることを知つた。

協同研究者 長沢によつて求められた，'pyrethrins' Ⅰ と 'pyrethrins' Ⅱ の kerosene 溶液の家蝿に対する knock-down 効果は第2表の様で，従来の結果[11] とは異なつて，意外にも 'pyrethrins' Ⅱ の方が 'pyrethrins' Ⅰ に比べて著しく強力であることを知つた。従つて単に pyrethrins Ⅰ 値を求める定量法は実際的に意味が少ない。又除虫菊利用の現状では，実際には除虫菊中の全殺虫成分即ち全 pyrethrins の値を知れば事足りる。従つて吾々は除虫菊製品の検定をするのにもポーラログラフ法により全 pyrethrins を定量し，その表示は全ピレトリン値 "(*Total Pyrethrins Value*; *T. P. V.*)" とすることを提案する。茲に提案する方法は尚多くの検討を要すると思うが一応発表して大方の識者の御批判を仰ぐ次第である。

'Pyrethrins' I and II have been separated in pure forms through application of column partition chromatography. With these as standard substances, the accurate determination of total pyrethrins by polarographic method has been attained successfully. This method apparently is far superior in its accuracy and convenience to the commonly practised methods such as the Seil's or the mercury-reduction methods. The experiment on the knock-down effect of these pure 'pyrethrins' on housefly gave an unexpected result: 'Pyrethrins' II is more toxic than I.

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[1] and allethrolone[2] 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 development[3] 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's[4] and the mercury-reduction methods[5] which are used in foreign countries, and the modified Seil's method[6] 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[7] and proposed by Beckley[8] and Shukis et al.[9], and the polarographic method proposed by Yamada et al.[10] 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_{max}=224 Mμ., $\varepsilon_{\lambda max}$ = 34250), 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_{max} =229 Mμ., $\varepsilon_{\lambda max}$=35850), 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 substances 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

* According to a private communication (dd. Aug. 11, 53) from Dr. David Kelsey, Production and Marketing Administration, The United States Department of Agriculture.

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 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 knock-down effect on adults of the housfly of 'pyrethrins' I and II kerosene solution gave a result quite contrary to the expectation[11]: '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 component 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 PURE '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[1] or the allethrolone[2].

It happened to come to the authors' attention that Lord et al.[12] 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 mentioned in experimental part were used by the authors to locate and identify the pyrethrins in the course of separation or after purification.

The methods used by Lord et al., on the other hand, iuclude: (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.

Table 1

Comparative results of various identifications on 'pyrethrins' I and II.

	λ_{max} Mμ	$\varepsilon_{\lambda max}$	Half-wave potential vs. N.C.E., v.		Elementary analysis	C%	H%	Seil's method Pys. I	Seil's method Pys. II	Mercury-reduction method Pys. I	Mercury-reduction method Pys. II
'Pyrethrins' I	224	34250	−1.25	calcd.	for $C_{21}H_{28}O_3$ (pyrethrin I)	76.83	8.53				
					for $C_{20}H_{28}O_3$ (cinerin I)	75.94	8.86	90.1	8.8	109.4	7.4
				found[1]		76.09	8.86				
						76.10	8.65				
'Pyrethrins' II	229	35850	−1.23	calcd.	for $C_{22}H_{28}O_5$ (pyrethrin II)	70.96	7.53				
					for $C_{21}H_{28}O_5$ (cinerin II)	70.00	7.77	1.1	86.5	12.0	96.9
				found[2]		70.59	7.63				
						70.68	8.01				

1. 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.
2. 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.
3. Pys.: pyrethrins.

Preparation of 'pyrethrins' I and II

The separation procedures followed by the authors were as follows:

Commercial pyrethrum extracts were purified by nitromethane method. Its *n*-hexane solution was then cooled with the mixture of dry ice and ethyl alcohol, and, in accordance with the difference in solubility, 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 different conditions, until the various physical constants and chemical characters no longer varied.

In this procedure, each fraction can be obtained in great quantities and with ease. The characters of 'pyrethrins' I and II thus obtained are as shown in Table 1, 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, α-dl-trans-allethrin, and technical allethrin, from which α-dl-trans-allethrin was separated, was conducted by the authors' co-worker Nagasawa *** to examine the relative knock-down effect of these samples on adults of the common housefly, *Musca domestica vicina* Macq. Its results

* 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.*

are shown in Table 2. Remarkable are the two of the points clarified by this experiment: (1) 'pyrethrins' II, is more toxic to adults of the common housefly 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[11].

Table 2

Relative effectiveness of 'pyrethrins' I, II, pyrethrum extracts and technical allethrin kerosene solution compared with α-dl-trans-allethrin kerosene solution calculated from the median knock-down time of adults of the common housefly, *Musca domestica vicina* Macq.

Sample		The authors' method	Seil's method	Mercury-reduction method	Relative effectiveness
'Pyrethrins' I					1.47
'Pyrethrins' II					2.19
Pyrethrum extracts	P. I. V., %	6.1	6.1	6.7	1.82
	P. II. V., %	5.6	8.3	8.3	
	T. P. V., %	11.7	14.4	15.0	
α-dl-trans-Allethrin					1.00
Technical allethrin	(Allethrin %, 90.6)				1.46

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 process 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 α-dl-trans-allethrin of good reproducibility and constancy was used and thus the exact relative value could be obtained.

EXPERIMENTAL

1. Location and identification of pyrethrins and the column partition chromatography.

Various tests(*a*~*g*)were conducted in locating and identifying pyrethrins.

a) *Polarographic analysis*: Pyrethrins I shows half-wave potential of about −1.25 v., and pyrethrins II about −1.23 v.

b) *Spectrophotometric analysis*: Pyrethrins I shows maximum ultraviolet absorption, which is about 5 Mμ. lower than that of pyrethrins II.

Pure samples of pyrethrins I and II can be distinguished by these two methods, but the potentials or the peaks are too close for the methods to give a reliable check on contamination. Nevertheless, the methods have proved useful for ascertaining the presence and determining the quantity of pyrethrins.

c) *Elementary analysis*: This is a very useful method, because it can distinguish between pyrethrins I and II, although the result of the elementary analysis alone cannot serve to distinguish between cinerin and pyrethrin.

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.*

Experimental conditions for *a*, *b*, and *e*, *f* were such as shown in the Experimentals of Parts III, IV, and II, respectively.

Column partition chromatographic column used was the same as in the case of allethrin and allethrolone[1,2], and was as follows:

To 100 g. of silicic acid (size of the particle was about 20~30 Mμ.) in a large mixing vessel, 10 g. of nitromethane was added and mixed thoroughly. Then, some amount of immobile solvent was added and mixed thoroughly until it became a slurry. The resultant slurry was poured into an absorption tube (pyrex pipe, 70 cm. long × 2.4 cm. inside diameter)and pressure(nitrogen gas) was applied on to it. When there was sufficient space in the tube, the rest of the slurry was poured in, and pressure was applied again. When the gel became so firm that it retained its shape on tipping, the pressure was released.

2. Preparation of 'pyrethrins' I and II.

Two hundred grams of pyrethrum extracts

(T. P. V. =11.7%, P. I. V. =6.1%, P. II. V. =5.6%) were purified by the process of nitromethane method[13], and 43 g. of oil (Oil-1, T. P. V. = 48.1%) was obtained. Oil-1 was dissolved[14] in 187 cc. of glacial acetic acid and 130 cc. of *n*-hexane in a separatory funnel, and 20 cc. of water was added in small portions with vigorous shaking. The addition of water caused the solution to separate into two layers, an *n*-hexane fraction (S-1) and an acetic acid fraction. The latter, when washed six times with 19 cc. portions of *n*-hexane, yielded an acid fraction (S-2). The *n*-hexane solutions were combined (S-3). Solution S-1 and S-3 were combined and the solvent was removed by immersing the flask in 40°C. water bath and applying a vacuum of about 10 mm. Hg, with the aid of nitrogen gas. (In this part, unless otherwise stated, the removal of solvent was performed in the similar conditions.) Fifteen point one grams of oil (Oil-2, T. P. V. = 60.1%) was obtained, and was set aside for the isolation of 'pyrethrins' I.

The dissolved material of solution S-2 was isolated by dilution with water and extracted with *n*-hexane. (During this process, brownish resinous matter was produced in the middle layer. This matter was separated.) The solvent was removed, and 14.4 g. of oil (Oil-3, T. P. V. =75.6%) was obtained. This was set aside for the isolation of 'pyrethrins' II.

Preparation of 'pyrethrins' I: Nine point two grams of Oil-2 was dissolved in 50 cc. of *n*-hexane, and the solution was added to the column. Compressed nitrogen gas was applied to the column until all the solution containing Oil-2 had just entered the absorbent. *n*-Hexane was added to the column and the pressure was applied again. Twenty five milliliters of the solutions was collected in each tared flask from the bottom of the column and the solvent was removed. The fractions, in which the content of pyrethrins I was supposed to be the greatest, were combined, and were purified three times in the same procedure. When the procedure was over, the wave height, the half-wave potential, the wave length of maximum of ultraviolet absorption, and the absorbency were all constant, and some amount of oil (Oil-4) was obtained. The last traces of solvents contaminating Oil-4 were removed by means of high vacuum (4.4×10^{-4}mm. Hg) for 30 minutes in a 40°C. water bath. One point two one grams of light-yellow viscus oil ('pyrethrins' I) was obtained, and was immediately located and identified by the various tests mentioned in **I**. The results of these tests are shown in Tables 1 and 2. The polarogram and the spectrum of 'pyrethrins' I are shown in Figs. 1 and 16, respectively.

Preparation of 2,4-dinitrophenylhydrazone of 'pyrethrins' I: Ninety-eight milligrams of 'pyrethrins' I was dissolved in small amount of 95% ethyl alcohol. To 58.6 mg. of 2,4-dinitrophenylhydrazine, 3 drops of conc. H_2SO_4 were added, and mixed thoroughly, and 7 cc. of 95% ethyl alcohol was added to it. This solution was added to the above 'pyrethrins' I alcohol solution. After standing one day at room temperature, 112 mg. of crystals, of which the melting point was about 105°C., were obtained. The crystals were dissolved in 50 cc. of the mixture of ethyl ether and *n*-hexane (3:1), and the solution was added to the column (mixture of ethyl ether and *n*-hexane (3:1) was used as immobil solvent). Compressed nitrogen gas was applied to the column until all the solution containing the crystals had just entered the absorbent.

Mtxture of ethyl ether and *n*-hexane (3:1) was added to the column and the pressure was applied again. Twenty-five milliliters of the solutions was collected in each tared flask from the bottom of the column, and the solvent was removed. Six fractions [No. 1 (14.2 mg.), No. 2 (34.4 mg.), No. 3 (28.4 mg.), No. 4 (7.5 mg.), No. 5 (2.2 mg.), and No. 6 (1.4 mg.)], were obtained. The crystals of fractions No. 1, No. 2, and No. 3 were recrystallized with 95% ethyl alcohol, respectively. Five mg. of crystals I of melting point of 124.5~125.5°C. (from No. 1), 26 mg. of crystals II of melting point of 125.5~126.5°C. (from No. 2), and 13 mg. of crystals III of melting point of 125~126°C. (from No. 3) were obtained. When mixed with 2,4-dinitrophenylhydrazone of α-dl-trans-allethrin of melting

point 127~128°C., these crystals were depressed of melting point by about 9~10°C. The results of elementary analysis of these three crystals were as follows:

		C%	H%
Calcd.	for $C_{27}H_{32}O_6N_4$ (2, 4-dinitrophenylhydrazone of pyrethrin I)	63.77	6.34
	for $C_{26}H_{32}O_6N_4$ (2, 4-dinitrophenylhydrazone of cinerin I)	62.95	6.50
Found	crystals I	64.00	6.42
	crystals II	63.47 63.43	6.40 6.41
	crystals III	63.64 63.73	6.39 6.39

Preparation of 'pyrethrins' II: One hundred and forty milliliters of *n*-hexane was added to 14.4 g. of Oil-3, and was cooled at −70~−80°C. by means of the mixture of dry ice and ethyl alcohol. Separated oil was taken by decantation. One hundred and sixty milliliters of *n*-hexane was again added to 8.2 g. of this oil, and was cooled at −70~−80°C., by means of the mixture of dry ice and ethyl alcohol, and the separated oil was taken by decantation. The solvent contaminating this oil was removed, and 4.66 g. of oil (Oil-5, T. P. V. = 71.6%; T. P. V. is less in this case than in Oil-3, as pyrethrins II become richer) was obtained. Oil-5 was dissolved in 50 cc. of *n*-hexane, and the solution was added to the column. Compressed nitrogen gas was applied to the column until all the solution containing Oil-5 had just entered the absorbent. One hundred milliliters of *n*-hexane was added to the column and the pressure was applied again. After *n*-hexane had entered the absorbent, mixture of *n*-hexane and ethyl ether (1:1) was added to the column, and pressure was applied again. Twenty-five milliliters of the solution was collected in each tared flask from the bottom of the column and the solvent was removed. The fractions, in which the content of pyrethrins II was supposed to be the greatest, were combined and were purified four times in the same procedure. After the procedure was over, the wave height, half-wave potential, the wave length of maximum of ultraviolet absorption, and the absorbency became constant, and some amount of oil was obtained. The last traces of solvents contaminating this oil were removed by means of high vacuum (9.4×10^{-4} mm. Hg) for 30 minutes in an about 40°C. water bath. One point one three grams of light-yellow viscus oil ('pyrethrins' II) was obtained, and was immediately located and identified by the various tests mentioned in I. The results of these tests are shown in Tables 1 and 2. The polarogram and the spectrum of 'pyrethrins' II are shown in Figs. 1 and 16, respectively.

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[4] and the mercury-reduction[5] method are theoretically correct. For the purpose, (1) chrysanthemum-monocarboxylic acid (mono-acid), (2) chrysanthemum-dicarboxylic acid (di-acid), (3) dl-allethrolone, (4) α-dl-trans-allethrin, (5) 'pyrethrins' I, and (6) 'pyrethrins' II were used.

Table 3 shows the values, indicated according to the rules set for the respective cases, of these matters determined by means of the analytical method described in the Experimental.

This table makes the following points clear.

(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) *dl-Allethrolone*: In both the Seil's and the mercury-reduction methods, an acid very simlar 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) *α-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 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 be 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's 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

1. Preparation of standard pure matters.

The standard pure matters, other than 'pyrethrins' I and II, used in Parts II~IV are just as follows:

(1) *Chrysanthemum-monocarboxylic acid*: bp. 115~116°C./4 mm. Its purity as titrated with *N*/50 NaOH was 99.9%.

(2) *Chrysanthemum-dicarboxylic acid*: mp. 164°C. Its purity as titrated with *N*/50 NaOH was 99.9%.

(3) *α-dl-trans-Allethrin*: mp. 50.5~51°C.

(4) *dl-Allethrolone*: The crude matter was distilled and was thoroughly purified by column partition chromatography until at last the wave height of the polarogram of the dl-allethrolone became constant. The wave lenght of maximum of ultraviolet absorption of this substance in the ethyl alcohol solution is 2295Å, and the molecular extinction coefficient in this case is 11049.

2. Procedure.

Seil's method: The method[4] proposed by Seil in 1947 was adapted, although the apparatus for steam distillation was the one devised in the present laboratory[6]. It was first confirmed with mono-acid and di-acid that the method could give values nearly the same as the theoretical ones. Analyses, as shown in Table 3 were completed upon this confirmation.

Mercury-reduction method[5].

3. Results.

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

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

Table 3

Analytical results obtained on various samples by the Seil's and the mercury-reduction methods

Sample	Seil's method		Mercury-reduction method	
	Mono-acid[1] or allethrin[2] or pyrethrins I[3], %	Di-acid[4] or allethrin II[5] or pyrethrins II[6], %	Mono-acid[1] or allethrin[2] or pyrethrins I[3], %	Di-acid[4] or allethrin II[5] or pyrethrins II[6], %
Mono-acid	96.8[1] (7)	trace	99.6[1] (4, HCl) 100.2[1] (2, H_2SO_4)	trace trace
Di-acid	trace	99.3[4] (2)	trace	100.3[4]* (HCl)
Mixture of mono- and di-acid	95.0[1]	100.3[4]	99.9[1] (4, HCl)	99.1[4]* (HCl)
dl-Allethrolone	trace	77.6[5] (2)	3.1[2] (2, HCl)	65.8[5]* (2, HCl)
α-dl-trans-Allethrin	96.9[2] (2) —	10.0[5] (2) —	111.3[2] (4, HCl) 106.4[2] (2, H_2SO_4)	6.5[5]* (4, HCl) 10.1[5]* (2, H_2SO_4)
Mixture of pyrethrum extracts (0.7256 g.) and α-dl-trans-allethrin (30 mg.)	98.6[1]**	104.7[4]**	—	—
Mixture of pyrethrum extracts (0.7124 g.) and α-dl-trans-allethrin (40 mg.)	96.6[1]**	112.2[4]**	—	—
'Pyrethrins' I	90.1[3] (2)	8.8[6] (2)	109.4[3] (2, HCl)	7.4[6]* (2, HCl)
'Pyrethrins' II	1.1[3] (2)	86.5[6] (2)	12.0[3] (2, HCl)	96.9[6] (2, HCl)

1) Figures tagged 1, 2, 3, 4, 5 and 6 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 "H_2SO_4" mean the acidification of the solution by the respective chemicals[5].

3) * signifies that the 2 cc. of ethyl alcohol was not used in the case[5].

4) Italycized figures show the number of experiments repeated.

5) ** These are the percentage of mono- and di-acids determined to the theoretical values of the respective acids in the mixture of pyrethrum extracts (No. 8 of Table 14, the determination of pyrethrins I and II was performed by Seil's method.) and α-dl-trans-allethrin.

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.

A. POLAROGRAMS OF 'PYRETHRINS' I AND II

1. Preparing Electrolytic Solution.

The composition of the electrolytic solution used was the same as in the case given in the first report : Ethyl alcohol (50%), $M/5$ $(CH_3)_4$-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 $(CH_3)_4$ NBr, however, is not desirable, as it makes the diffusion current steeper as shown in Fig. 2.

2. 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 C of Part III. The result is shown in Figs. 3~8. The values of pH in the

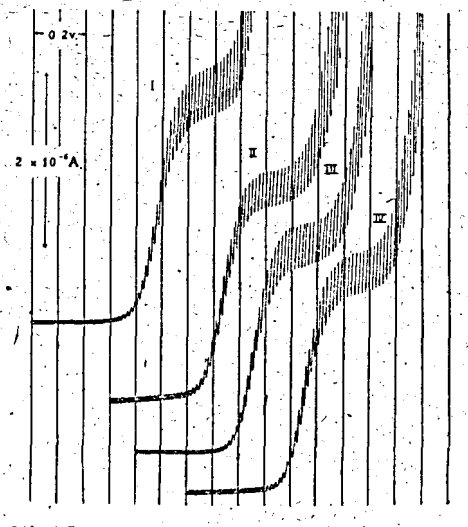

Fig. 1—Polarograms of standard matters: I α-dl-trans-allethrin ($20\times10^{-4}M.$), II 'pyrethrins' II ($20\times10^{-4}M.$), III mixture of 'pyrethrins' I and II ($20\times10^{-4}M.$), IV 'pyrethrins' I ($20\times10^{-4}M.$). Each polarogram begins at −0.80 v.

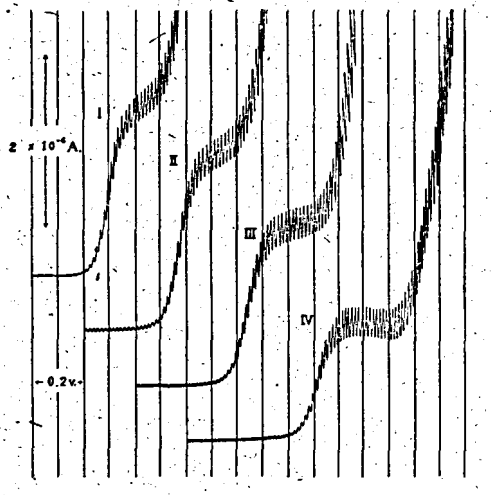

Fig. 3—Polarograms of $12.4\times10^{-4}M.$ 'pyrethrins' I reduced at different pH values: I (pH=1.49), II (pH=2.56), III (pH=3.63), IV (pH=4.99). Each polarogram begins at −0.80 v.

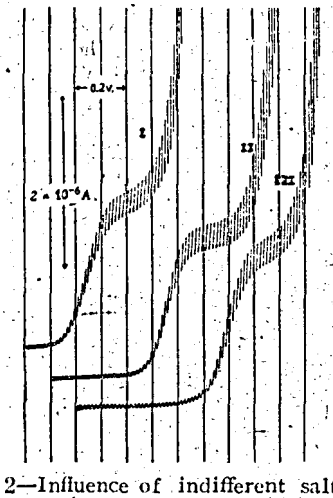

Fig. 2—Influence of indifferent salt on reduction wave of mixture of 'pyrethrins' I and II (1:1): I ($M/5$ KI), II ($M/5$ $(CH_3)_4$NBr), III ($M/5$ NaCl). Each polarogram begins at −0.80 v.

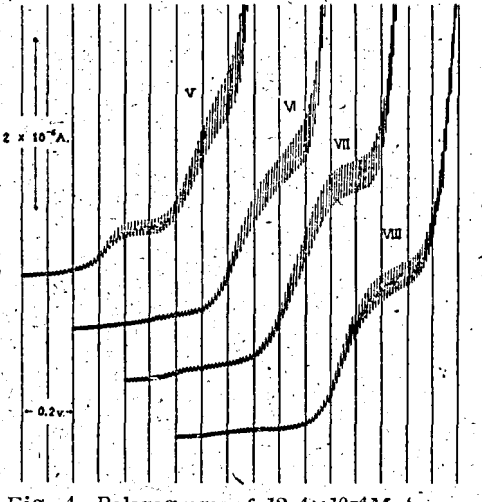

Fig. 4—Polarograms of $12.4\times10^{-4}M.$ 'pyrethrins' I reduced at different pH values: V (pH=5.67), VI (pH=6.75), VII (pH=7.63), VIII (pH=9.03). Each polarogram begins at −1.00 v.

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 was about 3.0.

The half-wave potentials of 'pyrethrins' I and II and α-dl-trans-allethrin under the conditions described in C of Part III are as in Table 4.

3. Effect of Temperature on Reduction Wave.

The polarograms of 'pyrethrins' I, II, mixture of 'pyrethrins' I and II (1:1), and α-dl-trans-

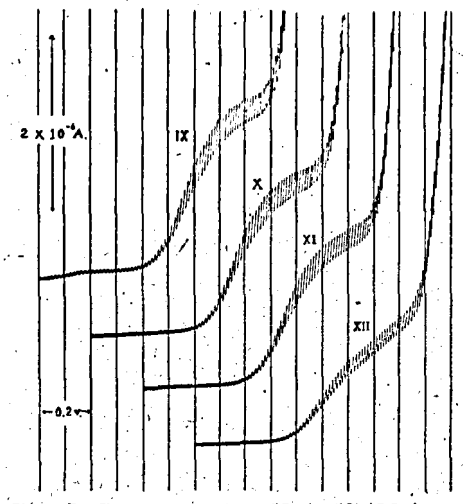

Fig. 5—Polarograms of $12.4\times10^{-4}M.$ 'pyrethrins' I reduced at different pH values: **IX** (pH=10.27), **X** (pH=11.11), **XI** (pH=11.28), **XII** (pH=12.16). Each polarogram begins at −1.10 v.

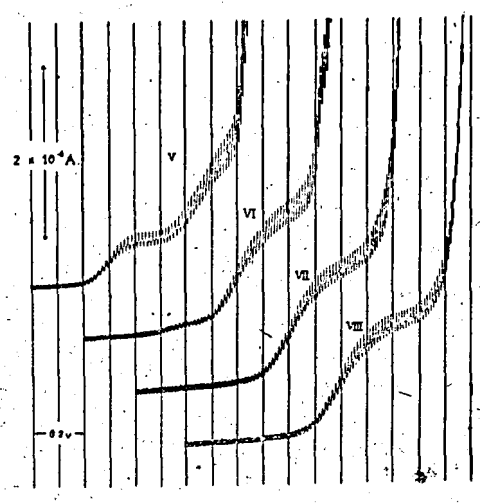

Fig. 7—Polarograms of 11.4×10^{-4} *M.* 'pyrethrins' II reduced at different pH values: **V** (pH=5.67), **VI** (pH=6.73), **VII** (pH=7.64), **VIII** (pH=9.02). Each polarogram begins at −1.00 v.

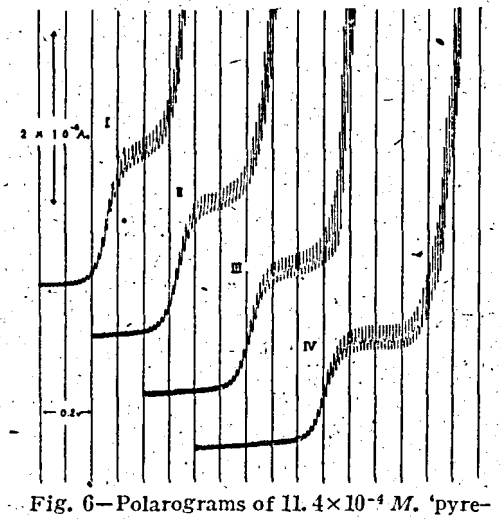

Fig. 6—Polarograms of 11.4×10^{-4} *M.* 'pyrethrins' II reduced at different pH values: **I** (pH=1.50), **II** (pH=2.54), **III** (pH=3.66), **IV** (pH=4.98). Each polarogram begins at −0.80 v.

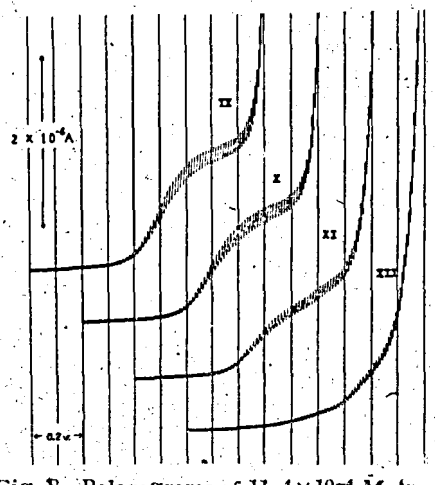

Fig. 8—Polarograms of 11.4×10^{-4} *M.* 'pyrethrins' II reduced at different pH values: **IX** (pH=10.40), **X** (pH=10.97), **XI** (pH=11.58), **XII** (pH=12.44). Each polarogram begins at −1.10 v.

Table 4

Comparative polarographic characters obtained on standard matters

	Half-wave potential *vs.* N. C. E., v.	Relations between concentration and wave height	Relations between temperature and wave height
'Pyrethrins' I	−1.25	$id_1=0.359\,C+0.003$ ··(1)	$id_1=0.0593\,T+3.605(14.2\times10^{-4}M.)$ ··(5)
'Pyrethrins' II	−1.23	$id_2=0.335\,C-0.009$ ··(2)	$id_2=0.0599\,T+2.322(11.4\times10^{-4}M.)$ ··(6)
Mixture of 'pyrethrins' I and II (1:1)	−1.24	$id_3=0.347\,C-0.002$ ··(3)	$id_3=0.0595\,T+2.952(12.8\times10^{-4}M.)$ ··(7)
α-dl-trans-Allethrin	−1.27	$id_4=0.360\,C-0.001$ ··(4)	$id_4=0.0605\,T+3.287(13.3\times10^{-4}M.)$ ··(8)

allethrin were taken at various degrees of temperature by the method shown in C 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. 9). The theoretical equations of the curves are as in Table 4. In these equations, id 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.).

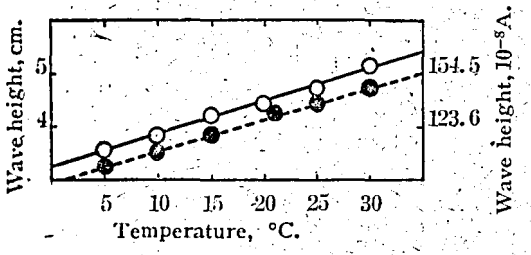

Fig. 9—Wave height of 13.3×10^{-4} *M.* α-dl-trans-allethrin and 12.8×10^{-4} *M.* mixture of 'pyrethrins' I and II *vs.* temperature. ○, indicates of α-dl-trans-allethrin. ●, indicates of mixture of 'pyrethrins' I and II (1:1).

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%.

4. Relations between Concentration and Wave Height.

By the method and under the conditions described in C 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 α-dl-trans-allethrin were studied, and the results obtained are shown in Fig. 10.

The standard theoretical equations obtained cross the axis of coordinates at zero point,

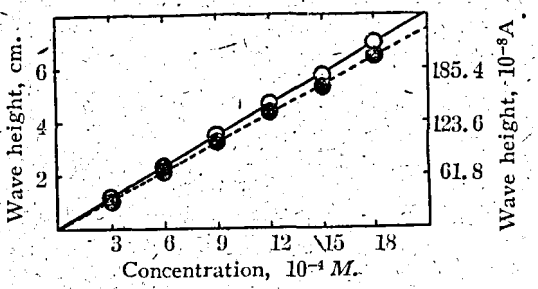

Fig. 10—Wave height of α-dl-trans-allethrin and mixture of 'pyrethrins' I and II (1:1) *vs.* concentration. ○, indicates of α-dl-trans-allethrin. ●, indicates of mixture of 'pyrethrins' I and II (1:1).

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. 7 and 8 are:

$$id_3 = 0.964\ id_4 \quad \cdots\cdots\cdots\cdots (9)$$

Study was made on whether or not the reduction wave, under the conditions described in C of part III, showed any change after the lapse of time. Neither the wave form nor the wave height showed any change after 3 hours at 25 ±0.2°C.

B. THE AUTHORS' IDEA OF POLAROGRAPHIC DETERMINATION OF TOTAL PYRETHRINS

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 the reduction temperature or the concentration and the wave height, and other characters of those substances, can serve to testify that, as long at least as the polarographic characters specified therein are

concerned, 'pyrethrins' I and α-dl-trans-allethrin 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 α-dl-trans-allethrin.

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 α-dl-trans-allethrin.

(b) The polarographic characters of the mixture of pyrethrin I and cinerin I agree well to those of α-dl-trans-allethrin.

(c) The polarographic characters of either pyrethrin II or cinerin II are not much different from, and approximately the same with, those of α-dl-trans-allethrin.

(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 α-dl-trans-allethrin.

(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 α-dl-trans-allethrin.

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 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 pyrethrin 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 charachters of the total pyrethrins and those of α-dl-trans-allethrin are aproximately 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)

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. For the reasons mentioned below, *the mean molecular weight of the total pyrethrins to be used here is determined at* 344. *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 pyrethrins 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 determination 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 weight 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 pyrethris at 344, the mean weight of the four constituents. (The mean moluecular weights used in this paper, of course, are these figures.)

What, then, is the range of error when the mean molecular weights are decided as above? If the active constituents of pyrethrum consisted solely of pyrethrin II, whose weight is the largest (372) of the four constituents, calculation of total pyrethrins value through application of the mean molecular weight (344) involves the error of −8.14%. Should, on the other hand, cinerin I, with the smallest weight (316), be the sole active constituent of the sample, the error involved in calculation is +8.86%. In other words, the theoretical error range involved in the calculation of total pyrethrins value with mean molecular weight at 344 is −8.14%～+8.86%. In like manner, the determination of pyrethrins I with the mean molecular weight at 322 involves the theoretical error range of −1.83%～+1.90%, and that of pyrethrins II with the mean molecular weight at 366 involves the theoretical error range of −1.61%～+1.67%.

C. QUANTITATIVE DETERMINATION OF TOTAL PYRETHRINS

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

1. Method of determination.

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 α-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 α-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 (H_2SO_4 5 cc., H_2O 20 cc., alcohol 1 litre), and distilled. To the distillate, silver nitrate and potassium hydroxide are added ($AgNO_3$ 10 g.,

KOH 1 g., the distillate 1 litre), and redistilled after several hours' boiling.

(*3*) *Tetramethylammonium bromide solution* : *M*/5 $(CH_3)_4$ NBr 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 diminshed 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 $(CH_3)_4$ NBr 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). (See **B** of Part III).

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 $(CH_3)_4$ NBr 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.

e. Method of measuring wave height.

The method of construction is the same as in the case of allethrin[6]. As indicated in Fig. 11, 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

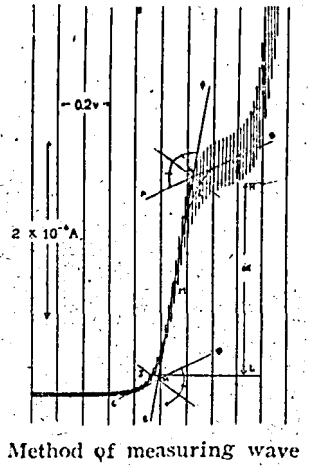

Fig. 11 Method of measuring wave height.

Table 5
Buffer solutions used and pH values of electrolytic solutions

Buffer solution used		Actual pH value 25±0.2°C.	pH Value of electrolytic solution, 25±0.2°C.	
Classification			'Pyrethrins' I	'Pyrethrins' II
Sörensen's HCl-Na-citrate	1.04	1.04	1.49	1.50
	2.27	2.00	2.56	2.54
	2.97	2.94	3.63	3.66
	3.95	4.01	4.99	4.98
	4.96	5.05	5.67	5.67
Kolthoff's KH_2PO_4-borax	6.00 (18°C.)	5.93	6.75	6.73
	7.00 (18°C.)	6.90	7.63	7.64
	8.00 (18°C.)	8.17	9.03	9.02
Sörensen's NaOH-borax	9.18 (26°C.)	9.22	10.27	—
	9.86 (26°C.)	10.04	11.11	—
	10.91 (26°C.)	10.98	11.28 (26°C.)	—
	12.13 (26°C.)	11.99	12.16 (26°C.)	—
	9.24 (18°C.)	9.15	—	10.40
	9.94 (18°C.)	9.89	—	10.97
	11.08 (18°C.)	10.83	—	11.58
	12.38 (18°C.)	12.08	—	12.44

CD) are marked G and H, respectively. The bisecting lines of the intersecting angles (∠FGA and ∠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 3.09×10^{-8} A. per mm. per m. The capillary constants, measured at −1.0 v. in the electrolytic solution mentinned in C of part III were as follows:

$$m=0.725\ \text{mg./sec.},\quad t=4.20\ \text{sec./drop.},$$
$$m^{2/3}\,t^{1/6}=1.025$$

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

2. Influence of pH on reduction wave of 'pyrethrins' I and II.

Buffer solutions of various pH's as shown in Table 5 were used, and the polarograms of 'pyrethrins' I and II were taken by the methed described in C of part III. The measured values of pH's of these electrolytic solutions are shown in the third and fouth columns of Table 5, and the polarograms are shown in Figs. 3~8.

3. Effect of temperature on reduction wave of 'pyrethrins' I and II, and mixture of 'pyrethrins' I and II (1:1), and α-dl-trans-allethrin.

The polarograms of 'pyrethrins' I and II, the mixture of 'pyrethrins' I and II (1:1), and α-dl-trans-allethrin were taken at various degrees of temperature by the method described in C of part III, while the concentration and the composition of the electrolytic solution were kept constant. The half-wave potentials and the wave heights are shown in Tables 6~9.

Table 6
Wave hights of 14.2×10^{-4} *M.* 'pyrethrins' I at different temperatures

Temperature	Wave height		Half-wave potential
°C.	cm.	10^{-8}A.	v.
5.3±0.2	3.92	121.13	−1.24
9.8±0.2	4.19	129.47	−1.24
15.0±0.2	4.49	138.74	−1.24
20.7±0.2	4.83	149.25	−1.24
25.1±0.2	5.09	152.81	−1.25
29.7±0.2	5.37	165.93	−1.25

Table 7
Wave heights of 11.4×10^{-4} *M.* 'pyrethrins' II at different temperatures

Temperature	Wave height		Half-wave potentical
°C.	cm.	10^{-8}A.	v.
4.2±0.2	2.58	79.72	−1.22
10.0±0.2	2.92	90.22	−1.22
14.8±0.2	3.21	99.19	−1.22
20.0±0.2	3.52	108.77	−1.23
24.8±0.2	3.81	117.73	−1.23
29.5±0.2	4.09	126.38	−1.23

Table 8

Wave heights of 12.8×10^{-4} *M.* mixture of 'pyrethrins' I and II (1:1) at different temperatures

Temperature	Wave height		Half-wave potential
°C.	cm.	10^{-8}A.	v.
3.8±0.2	3.18	98.26	−1.23
10.2±0.2	3.56	110.00	−1.23
16.2±0.2	3.91	120.82	−1.23
22.1±0.2	4.27	131.94	−1.23
25.4±0.2	4.46	137.81	−1.24
30.2±0.2	4.75	146.78	−1.24

Table 9

Wave heights of 13.3×10^{-4} *M.* α-dl-trans-allethrin at different temperatures

Temperature	Wave height.		Half-wave potential
°C.	cm.	10^{-8}A.	v.
5.3±0.2	3.61	111.55	−1.25
9.8±0.2	3.88	119.89	−1.26
15.3±0.2	4.21	130.01	−1.26
19.2±0.2	4.44	137.19	−1.26
24.8±0.2	4.78	147.70	−1.27
29.7±0.2	5.10	157.59	−1.27

4. Relations between the concentration and the wave height of 'pyrethrins' I and II, and mixture of 'pyrethrins' I and II (1:1), and α-dl-trans-allethrin.

The wave heights of 'pyrethrins' I and II, and the mixture of 'pyrethrins' I and II (1:1), and α-dl-trans-allethrin at various concentrations were determined by the method and under the conditions described in **C** of part **III.** The results are shown in Table 10.

5. Change by the lapse of time of reduction wave of mixture of 'pyrethrins' I and II (1:1).

The electrolytic solution of the mixture of 'pyrethrins' I and II (1:1), whose composition was as indicated in **C** of part **III,** was left at 25±0.2°C. for a certain period of time. Then the polarograms were taken, and the half-wave potentials and the wave heights were measured. The results are shown in Table 11.

Table 10

Wave heights of 'pyrethrins' I, and II, and mixture of 'pyrethrins' I and II (1:1), and α-dl-trans-allethrin at different concentrations

Concentration	Wave height, found							
	'Pys.' I		'Pys.' II		Mixture of 'Pys.' I and II		α-dl-trans-Allethrin	
10^{-4}*M.*	cm.	10^{-8}A.	cm.	10^{-8}A.	cm.	10^{-8}A.	cm.	10^{-8}A.
1	0.36	11.12	0.33	10.19	0.35	10.82	0.35	10.82
3	1.05	32.44	1.00	30.90	1.04	32.14	1.09	33.68
6	2.19	67.67	2.00	61.80	2.08	64.27	2.15	66.43
9	3.26	100.73	3.02	93.32	3.13	97.72	3.26	100.73
12	4.34	134.11	4.02	124.22	4.16	128.54	4.31	133.18
15	5.36	165.62	5.03	155.42	5.21	160.99	5.39	166.55
18	6.50	200.85	6.00	185.40	6.25	193.13	6.49	200.54
20	7.18	221.86	6.73	207.96	6.96	215.06	7.20	222.48

* Pys: Pyrethrins.

Table 11

Wave heights and half-wave potentials of α-dl-trans-allethrin, 'pyrethrins' I, 'pyrethrins' II, and mixture of 'pyrethrins' I and II (1:1), the electrolytic solution of which is left at 25±0.2°C. for a certain period of time.

		Time		
		30 min.	1 hr.	3 hrs.
α-dl-trans-Allethrin ($13.3\times10^{-4}M.$)	Wave height, cm.	4.78	4.79	4.78
	Half-wave potential, v.	−1.27	−1.27	−1.27
'Pyrethrins' I ($14.2\times10^{-4}M.$)	Wave height, cm.	5.10	5.08	5.11
	Half-wave potential, v.	−1.25	−1.25	−1.25
'Pyrethrins' II ($11.4\times10^{-4}M.$)	Wave height, cm.	3.82	3.81	3.82
	Half-wave potential, v.	−1.23	−1.23	−1.23
Mixture of 'pyrethrins' I and II (1:1) ($12.8\times10^{-4}M.$)	Wave height, cm.	4.44	4.43	4.44
	Half-wave potential, v.	−1.24	−1.24	−1.24

6. Results of determination of total pyrethrins in pyrethrum flowers and extracts, and determination values of α-dl-trans-allethrin or mixture of 'pyrethrins' I and II (1:1) added to the samples.

Pyrethrum flowers and extracts, from various sources, were analysed. The results are shown in Figs. 12~15 and Tables 12~14. The reduction waves of samples No. 1~No. 4 (Pyrethrum flowers of 1952 production), and those of samples No. 8 ~No. 10 (Pyrethrum extracts) are a two-step wave, and those of samples No. 5~No. 7 (Pyrethrum flowers of 1953 production) are a one-step wave (the second waves are so obscure that it can not be recognized as such). The wave forms and the half-wave potentials of the first waves of samples No. 1~No. 4, samples No. 8 ~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~

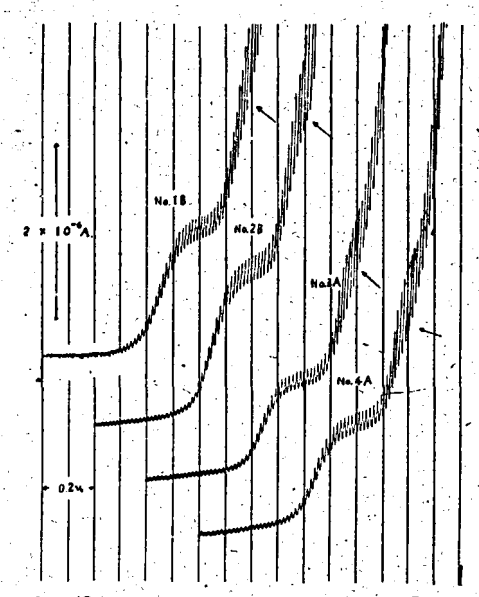

Fig. 12—Polarograms of pyrethrum flowers. Each polarogram begins at −0.80 v.

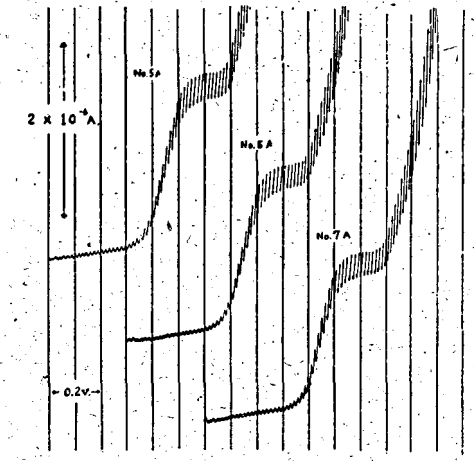

Fig. 13—Polarograms of pyrethrum flowers. Each polarogram begins at −0.80 v.

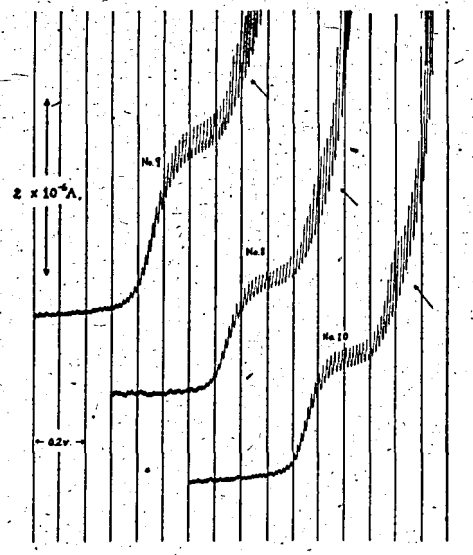

Fig. 14—Polarograms of pyrethrum extracts. Each polarograms begins at −0.80 v.

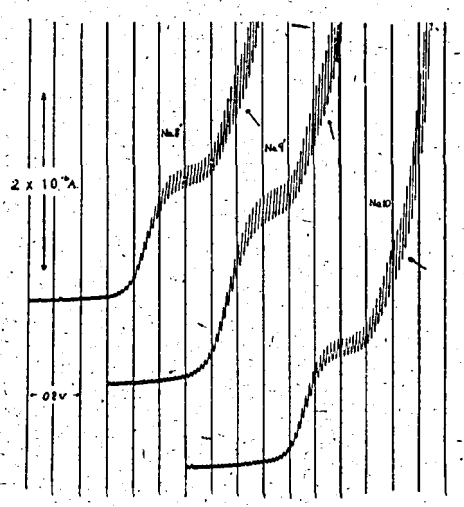

Fig. 15—Polarograms of pyrethrum extracts (The solvent is removed by means of high vacuum). Each polarogram begins at −0.80v.

Table 12

Results of polarographic determination of total pyrethrins in pyrethrum flowers produced in June~July, 1952, and analytical values of standard matters added to those pyrethrum flowers

			No. 1		No. 2		No. 3	No. 4
			A	B	A	B		
Pyrethrum flower	Sample,	g.	5.0002	4.9980	5.0003	5.0027	5.0010	4.9900
	Half-wave potential,	v.	−1.25	−1.25	−1.25	−1.25	−1.25	−1.25
	Wave height, found,	cm.	2.82	2.82	3.90	3.85	2.68	2.80
	Total pyrethrins value,	%	0.56	0.56	0.77	0.76	0.53	0.56
α-dl-trans-allethrin added to the sample	Half-wave potential,	v.	−1.26	—	−1.26	—	−1.26	−1.26
	Wave height, found,	cm.	5.15	—	5.97	—	5.05	4.44
	Wave height, calcd.,	cm.	5.05	—	5.70	—	5.02	4.40
	Error,	%	+2.0	—	+4.7	—	+0.6	+0.9
Mixture of 'pyrethrins' I and II added to the sample	Half-wave potential,	v.	−1.25	—	−1.25	—	−1.25	−1.25
	Wave height, found,	cm.	5.27	—	5.60	—	4.75	4.95
	Wave height, calcd.,	cm.	5.01	—	5.47	—	4.94	5.01
	Error,	%	+5.2	—	+2.4	—	−3.8	−1.2

Table 13

Results of polarographic determination of total pyrethrins in pyrethrum flowers produced in June~July, 1953, and analytical values of standard matters added to those pyrethrum flowers

			No. 5		No. 6		No. 7	
			A	B	A	B	A	B
Pyrethrum flower	Sample,	g.	5.0000	5.0072	4.9968	5.0014	5.0058	5.0000
	Half-wave potential,	v.	−1.25	−1.25	−1.25	−1.25	−1.25	−1.25
	Wave height, found,	cm.	5.39	5.29	5.15	4.91	4.80	4.50
	Total pyrethrins value,	%	1.03	1.05	1.02	0.97	0.95	0.89
α-dl-trans-allethrin added to the sample	Half-wave potential,	v.	−1.26	−1.26	−1.26	−1.26	−1.26	−1.26
	Wave height, found.	cm.	6.68	6.68	6.50	6.57	6.19	6.00
	Wave height, calcd.,	cm.	6.65	6.65	6.52	6.52	6.00	6.00
	Error,	%	+0.5	+0.5	−0.3	+0.8	+3.2	0
Mixture of 'pyrethrins' I and II added to the sample	Half-wave potential,	v.	—	−1.25	—	−1.25	—	−1.25
	Wave height, found,	cm.	—	6.32	—	6.06	—	5.90
	Wave height, calcd.,	cm.	—	6.30	—	6.17	—	6.00
	Error,	%	—	+0.3	—	−1.8	—	−1.7

Table 14

Results of polarographic determination of total pyrethrins in pyrethrum extracts and analytical values of standard matters added to those pyrethrum extracts

			No. 8		No. 9		No. 10		No. 8'	No. 9'	No. 10'
			A	B	A	B	A	B			
Pyrethrum extracts	Sample,	mg.	301.2	309.2	308.0	298.6	322.4	315.6	306.0	304.4	309.0
	Half-wave potential,	v.	−1.25	−1.25	−1.25	−1.25	−1.25	−1.25	−1.25	−1.25	−1.25
	Wave height, found,	cm.	3.54	3.64	4.05	4.88	3.61	3.38	3.64	5.02	3.50
	Total pyrethrins value,	%	11.7	11.7	15.9	16.2	11.1	10.6	11.8	16.4	11.2
α-dl-trans-Allethrin added to the sample	Half-wave potential,	v.	−1.26	—	−1.26	—	−1.26	—	—	—	—
	Wave height, found,	cm.	5.64	—	6.25	—	5.50	—	—	—	—
	Wave height, calcd.,	cm.	5.42	—	6.04	—	5.40	—	—	—	—
	Error,	%	+4.1	—	+3.5	—	+1.9	—	—	—	—
Mixture of 'Pyrethrins' I and II added to the sample	Half-wave potential,	v.	−1.25	—	−1.25	—	−1.25	—	—	—	—
	Wave height, found,	cm.	5.55	—	6.15	—	5.48	—	—	—	—
	Wave height, calcd.,	cm.	5.42	—	6.04	—	5.40	—	—	—	—
	Error,	%	+2.4	—	+1.8	—	+1.5	—	—	—	—

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 [1,2] 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 α-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 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 14, 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 C 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.[9] 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 have to 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 Mμ.
α-dl-trans-allethrin	224 Mμ.

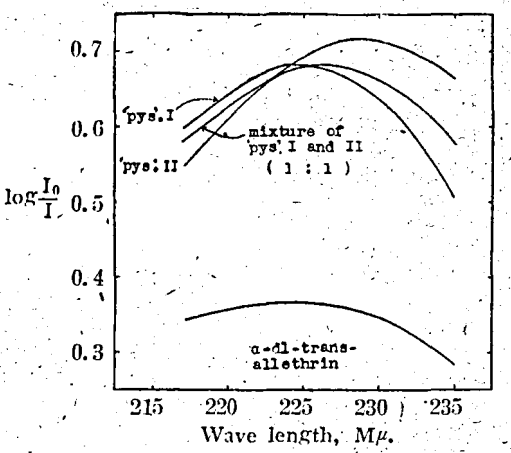

Fig. 16—Absorption curves for 'pyrethrins' I and II, and mixture of 'pyrethrins' I and II (1:1), and α-dl-trans-allethrin alcoholic solution. (2×10^{-5} M., Cell: 1 cm.)

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 α-dl-trans-allethrin and pyrethrin or cinerin have like structures, and that the wave length of absorption maximum of α-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 the mixture of 'pyrethrins' I and II (1:1) roughly agrees to that of the total pyrethrins. The authors, in this light, concluded that 226 Mμ. is the most suitable wave length for measuring the absorbency of solutions containing pyrethrins.

Table 15

Relations between concentration of 'pyrethrins' I and II and mixture of 'pyrethrins' I and II (1:1) and α-dl-trans-allethrin, and absorbency (optical density)

Concentration 10^{-5} M.	Absorbency			
	'Pyrethrins' I	'Pyrethrins' II	Mixture of 'Pyrethrins' I and II(1:1)	α-dl-trans-Allethrin
0.5	0.174	0.182	0.170	0.094
1.0	0.330	0.364	0.332	0.185
2.0	0.685	0.717	0.681	0.365
3.0	1.015	1.076	1.007	0.541
Wave-length, measured, Mμ.	224	229	226	226

Data for the plot of concentrations *vs.* absorbency were obtained by determining the absorbency at 226 Mμ. of suitable concentration of mixture of 'pyrethrins' I and II (1:1) or α-dl-trans-allethrin. The results are as shown in Table 15 and Fig. 17. The standard theoretical equations obtained from these data cross the axis of coordinates at zero point, and are as follows :

Mixture of 'pyrethrins' I and II (1:1)··

$$A_1 = 0.337\,C \cdots\cdots (10)$$

α-dl-trans-allethrin ·····

$$A_2 = 0.179\,C \cdots\cdots (11)$$

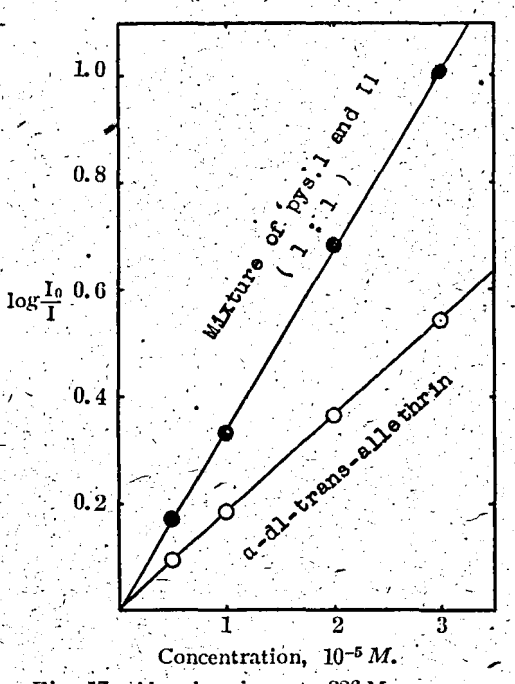

Fig. 17—Absorbencies at 226 Mμ. *vs.* concentrations of mixture of 'pyrethrins' I and II (1:1), and α-dl-trans-allethrin.

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

'pyrethrins' I···$A_3 = 0.339\,C \cdots\cdots (12)$

'pyrethrins' II···$A_4 \doteq 0.337\,C \cdots\cdots (13)$

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

Eqs. 10 and 11 are related :

$$A_1 = 1.883\,A_2 \cdots\cdots (14)$$

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 α-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 and arrive at equation of concentration *vs.* absorbency of mixture of 'pyrethrins' I and II (total pyrethrins). The appoximate 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. The spectrophotometer

was operated in strict accordance with the Direction Book, and the recommendations by Shukis et al.[9] were also conformed to.

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 Mμ.

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^{-5} M.

The solution was then added to a calibrated silica spectrophotometer cell and the absorbency was determined at 226 Mμ., with the use of 95% ethyl alcohol 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 Tables 16 and 17, and the ultraviolet absorption spectra taken on the solution of samples are shown in Fig. 18.

Table 16

Results of spectrophotometric determination of total pyrethrins in pyrethrum flowers produced in June~July, 1952

		No. 1		No. 2		No. 3	No. 4
		A	B	A	B		
Sample,	g.	5.0002	4.9980	5.0003	5.0027	5.0010	4.9900
λ_{max},	Mμ.	221	222 226	226	222 225	224	224
Absorbency at 226Mμ., found		0.295	0.305	0.399	0.397	0.357	0.345
Total Pyrethrins Value,	%	0.60	0.62	0.81	0.81	0.73	0.71

Table 17

Results of spectrophotometric determination of total pyrethrins in pyrethrum flowers produced in June~July, 1953

		No. 5		No. 6		No. 7	
		A	B	A	B	A	B
Sample,	g.	5.0000	5.0072	4.9968	5.0014	5.0058	5.0000
λ_{max},	Mμ.	221	222	223	223	225	225
Absorbency at 226Mμ., found		0.660	0.642	0.620	0.600	0.512	0.586
Total Pyrethrins Value,	%	1.35	1.31	1.27	1.22	1.04	1.20

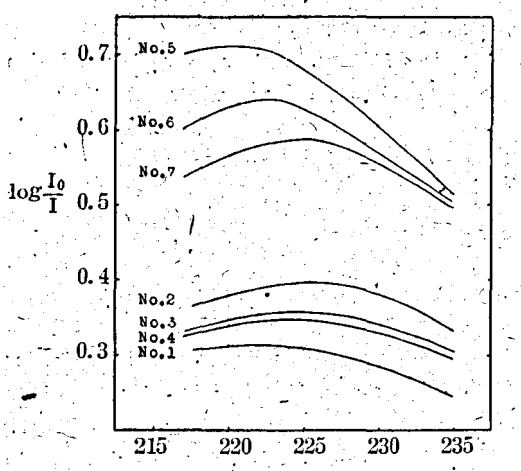

Fig. 18—Absorption curves for the samples of pyrethrum extracts.

A careful observation of these spectra reveals the following :

1) The wave lengths of maximum of ultraviolet absorption of the samples of these pyrethrum flowers are 221~226 Mμ., and are shorter than that of the mixture of 'pyrethrins' I and II (1:1).

2) Compared with those of 'pyrethrins' I and II, and the mixture of 'pyrethrins' I and II (1:1), these spectra show less sharp bending at the peak, and are less steeper, especially where wave length is shorter.

These phenomena mean that, in addition to the total pyrethrins in the flowers, other substances contaminating the samples are also absorbed. The method, therefore, is greatly influenced by contamination, and the values determined are very likely to be higher than the actual values.

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 (as shown in Table 18, the 6th line) than had been expected.

Then, in accordance with the method of Shukis et al. [9], kerosene solution, the solvents, had

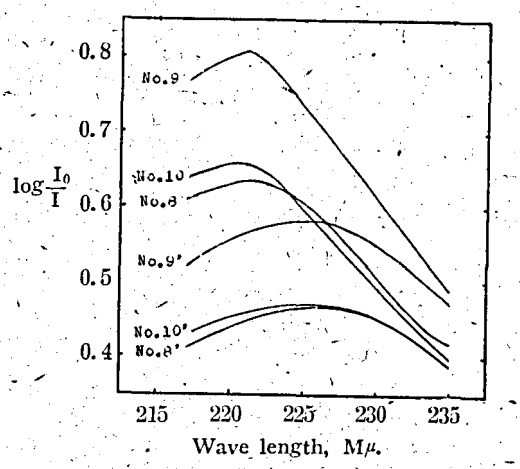

Fig. 19—Absorption curves for the samples of pyrethrum extracts.

Table 18

Results of spectrophotometric determination of total pyrethrins in pyrethrum extracts produced in Nov., 1952

		No. 8		No. 9	No. 10	No. 8'	No. 9'	No. 10'
		A	B					
Sample,	mg.	301.2	309.2	308.0	322.4	306.0	304.4	309.0
λ_{max},	Mμ.	221	221	221	221	226	225	226
Absorbency at 226 Mμ., found		0.581	0.589	0.710	0.585	0.467	0.580	0.470
Total Pyrethrins Value,	%	19.7	19.4	23.5	18.5	15.6	19.4	15.6

been removed from samples No. 8~No. 10 by the undermentioned method, 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 4.2×10^{-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 18, in the 6th line, and are noticeably lower than the corresponding values in No. 8~No. 10.

CONCLUSION

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

With these and α-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 19. 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 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 caluculate 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 20. The determination of the pyrethrins I and II in the same sample as above conducted by the

Table 19

Results of investigation on the applicability of the four determination methods to the pure matters and pyrethrum products

	Pure matter					Pyrethrum flowers and extracts		
	Simple matter		Mixture of pys. I and II					
	Pys. I	Pys. II	Pys. I	Pys. II	Total pys.	Pys. I	Pys. II	Total pys.
Polarographic method	○	○	×	×	○	×	×	○
Spectrophotometric method	○	○	×	×	○	×	×	×
Seil's method	○	×	○	×	×	○	×	×
Mercury-reduction method	×	×	×	×	×	×	×	×

1. ○ and × respectively show the possibillity and impossibility of determination.
2. Pys: pyrethrins.

Table 20

Total Pyrethrins Values : Comparative results obtained on samples of pyrethrum flowers and extracts*

Sample	No.	Produced in	Total Pyrethrins Values, %					
			Polarographic method		Spectrophotometric method		Seil's method	Mercury-reduction method
			A	B	A	B		
Pyrethrum flowers	1	June~July, 1952	0.56	0.56	0.60	0.62	0.60	0.66
	2		0.77	0.76	0.81	0.81	—	0.90
	3		0.53	—	0.73	—	0.51	0.59
	4		0.56	—	0.71	—	0.65	0.71
	5	June~July, 1953	1.03	1.05	1.35	1.31	1.08	1.19
	6		1.02	0.97	1.27	1.22	1.02	1.18
	7		0.95	0.89	1.04	1.20	1.03	1.15
Pyrethrum extracts	8	Nov., 1952	11.7	11.7	19.7	19.4	14.4	15.0
	9		15.9	16.2	23.5	—	15.9	16.8
	10		11.1	10.6	18.5	—	14.9	15.3
	8'	Nov., 1953	11.8	—	15.6	—	—	—
	9'		16.4	—	19.4	—	—	—
	10'		11.2	—	15.6	—	—	—

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

Table 21

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

Sample	No.	Produced in	Pyrethrins I and II Value, %					
			Seil's method		Mercury-reduction method		The authors' method	
			Pys. I	Pys. II	Pys. I	Pys. II	Pys. I	Pys. II
Pyrethrum flowers	1	June~July, 1952	0.30	0.30	0.37	0.29	0.30	0.26
	2		—	—	0.46	0.44	—	—
	3		0.26	0.25	0.33	0.26	0.26	0.27
	4		0.28	0.37	0.35	0.36	0.28	0.28
	5	June~July, 1953	0.53	0.55	0.68	0.51	0.53	0.51
	6		0.48	0.54	0.64	0.54	0.48	0.52
	7		0.46	0.57	0.61	0.54	0.46	0.46
Pyrethrum extracts	8	Nov., 1952	6.1	8.3	6.7	8.3	6.1	5.6
	9		7.1	8.8	8.1	8.7	7.1	9.0
	10		5.9	9.0	6.8	8.5	5.9	5.0
	8'	Nov., 1953	—	—	—	—	—	—
	9'		—	—	—	—	—	—
	10'		—	—	—	—	—	—

* These determinations were performed duriug the period Sep. 1~15, 1953.

authors', the Seil's and the mercury-reduction methods resulted in Table 21. 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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On the Toxicity of Rotenone and Pyrethrin upon the Insects Easily Cultured in the Laboratory. Yasuyuki MIYAHARA [Research Laboratory of Hokko Chemical Co. Ltd., Tokyo., former Central Laboratory of S. M. R. Co, Dairen and Laboratory of Agricultural Chemicals Investigations, National Agricultural Experiment Station, Tokyo.]
Received Aug. 27, 1953. *Botyu-Kagaku* **18**, 169, 1953 (with English résumé, 175)

27. 実験室にて飼育し易い昆虫類に対するロテノーン及びピレトリンの毒性に就て

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接触毒物の殺虫効力を正確に試験するのに適当な感受性を有し，而も飼育の容易な昆虫を探すため，ピレトリン及びロテノーンの標準殺虫剤を調製し，16種の昆虫（成虫或は幼虫）の殺虫試験を行つた所，アヅキザウ成虫及びナガシンクヒ成虫が殺虫効力試験用の供試虫として有望と思はれた。

I. 緒　論

ロテノーン及びピレトリン等の毒物を，接触的に昆虫に作用せしむる際の致死作用は，昆虫の中枢神経を麻痺せしむることに依ると云はれるが，其の殺虫機構に就ては学説区々で，まだ不明の点が多く，将来昆虫の生理解剖学的研究に俟つ処が甚だ多いようである。(6) (8) (10) (12)

是等接触毒物の毒性は，受毒昆虫の種によつて差異の存するは勿論，同一種の昆虫に対しても，飼育方法，性，齢等諸種の要因によつて影響せられることは Philips 及び Swingle(13), McIndoo(7), Davidson(3) 等の研究によつても明らかである。

Ginsburg 及び Schmitt(6) によれば，ロテノーンは蜜蜂よりも昆虫に対して毒性大なるに反し，ピレトリンは之と逆の毒性を示すといひ，Swingle(14) は *Ascia rapae* に対してロテノーンは消化中毒剤なるに反し，ピレトリンは接触毒として作用することを述べ，Trapmann 及び Nitsche(15) は鱗翅目の18種の昆虫の幼虫を用ひて此の両種毒物の殺虫力を比較して，ピレトリンはロテノーンよりも殺虫効力大なることを知つた。又 Hartzell(15) は *Tenebrio* の幼虫や *Melanoplus* の成虫の中枢神経に対して，ピレトリンはロテノーンに較べて破壊作用の強いことを報じた。森山(9)は蚕虫類及び家蠶幼虫の体表にロテノーン溶液を撒布し，時間の経過と死の症徴及び致死時間等に就いて研究した。Craufurd-Benson(1) はデリス殺虫剤の室内試