Propylene oxalate exists in at least two mutually interconvertible forms: a monomer and a polymer. Monomeric propylene oxalate polymerizes much less rapidly than ethylene oxalate.

Trimethylene oxalate (m. p. 86°) prepared from ethyl oxalate and trimethylene glycol is a linear condensation polymer. It shows no tendency to depolymerize spontaneously. At high temperature it undergoes thermal decomposition, and one of the products of this reaction is the dimeric 14-membered heterocycle, m. p. 187°. This is stable and shows no tendency to polymerize further.

Hexamethylene oxalate and decamethylene oxalate prepared by the action of the glycols on ethyl oxalate are linear condensation polymers.

Ethyl(*p*-hydroxyethyl)-oxalate and ethylene-bis-*m*-bromobenzoate are described.

WILMINGTON, DELAWARE

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[CONTRIBUTION FROM THE RESEARCH LABORATORY OF MCLAUGHLIN GORMLEY KING COMPANY]

# STUDIES ON PYRETHRUM FLOWERS. IV. THE RELATIVE TOXICITY OF PYRETHRINS I AND II

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Staudinger and Harder[1] have shown that the two pyrethrins do not occur in *Pyrethrum* flowers in equal proportions. This conclusion has been confirmed by Tattersfield and Hobson.[2] The amount of pyrethrin II, in the flowers examined by these writers, was from 38 to 172% of the pyrethrin I content. If the two pyrethrins are equally toxic to insects, the proportion in which they are present is of little importance, and a determination of the total pyrethrin content would indicate the toxic value of a given sample. If, however, there is a considerable difference in the toxicity of the two pyrethrins, it would be necessary to determine the proportion of each present in a sample in order to establish its insecticidal value.

Staudinger and Ruzicka[3] state that pyrethrin I is somewhat more toxic than pyrethrin II. Gnadinger and Corl[4] also found that pyrethrin I is slightly more toxic. Tattersfield, Hobson and Gimingham[5] found that pyrethrin I is about ten times more toxic than pyrethrin II, and they attempted to prove that the toxicity of *Pyrethrum* flowers depends almost entirely on the pyrethrin I content.

In view of the contradictory conclusions reached by different investi-

[1] Staudinger and Harder, *Ann. acad. sci. Fennicae*, **29A**, 1–14 (1927).

[2] Tattersfield and Hobson, *J. Agr. Sci.*, **19**, 433–437 (1929).

[3] Staudinger and Ruzicka, *Helv. Chim. Acta*, **7**, 449 (1924).

[4] Gnadinger and Corl, THIS JOURNAL, **51**, 3054 (1929).

[5] Tattersfield, Hobson and Gimingham, *J. Agr. Sci.*, **19**, 266–296 (1929).

gators, it was considered advisable to isolate the pyrethrins and determine their toxicity by the most accurate biological method available. At the same time the toxicity of *Pyrethrum* flowers assayed by the method of Gnadinger and Corl[4] was determined, thus deciding whether or not this method is a true measure of the toxicity to insects.

Through the courtesy of Mr. E. B. Phillips, Chief Chemist, the biological experiments were conducted at the laboratory of the Sinclair Refining Company, East Chicago, Indiana. This company was invited to take part in the work because of its experience in testing *Pyrethrum* insecticides biologically, and because its facilities for this work are unexcelled. The experiments were carried out under the supervision of Mr. N. J. Gothard, Assistant Chief Chemist, and Mr. A. G. Grady, Entomologist. We express our appreciation to the Sinclair Refining Company, Mr. Phillips and his assistants; without their coöperation the work could not have been completed.

## Experimental

**Isolation of Pyrethrins I and II.**—The isolation of pyrethrin I from *Pyrethrum* flowers by the method of Staudinger and Ruzicka[6] is not particularly difficult. It should be noted, however, that neither Staudinger and Ruzicka nor Tattersfield, Hobson and Gimingham isolated pyrethrin II direct from the flowers. Instead, they saponified a mixture of the semicarbazones of pyrethrin I and II, obtaining eventually, after appropriate treatment, the alcohol pyrethrolon and the two acids, chrysanthemum monocarboxylic acid and chrysanthemum dicarboxylic acid methyl ester. Part of the pyrethrolon and the monocarboxylic acid were esterified to form pyrethrin I and another portion of the pyrethrolon was combined with the dicarboxylic acid methyl ester to yield pyrethrin II. Staudinger and Ruzicka[7] have pointed out that these partially synthesized pyrethrins do not yield semicarbazones of sharp melting point, and suggest that a change has possibly taken place in the side chain of the pyrethrolon or that stereoisomers are present. Tattersfield, Hobson and Gimingham[5] also mention that their synthetic pyrethrin II possibly contained an isomer which lowered the toxicity. Gnadinger and Corl[4] were able to isolate the semicarbazone of pyrethrin II directly from the flowers in fairly pure condition by repeated crystallization of the mixed semicarbazones of pyrethrin I and II.

For the present work, 215 kg. of Japanese *Pyrethrum* flowers was treated in substantially the manner described by Staudinger and Ruzicka.[6] About 500 g. of white, crystalline, mixed semicarbazones of pyrethrin I and II, melting at 60–90° was obtained. This material was repeatedly crystallized from 90% alcohol, 60% alcohol and a mixture of one part benzene and three parts of petroleum ether. In this way were obtained 40 g. of pyreth-

[6] Ref. 3, p. 184.

[7] Ref. 3, pp. 451–453.

rin I semicarbazone melting at 115–117° and 30 g. of pyrethrin II semicarbazone, melting point 55–61°. Further crystallization of the latter finally yielded a product melting at 54–58° whose melting point was not lowered by further crystallization. In addition, a large quantity of mixed semicarbazones of pyrethrin I and II was obtained.

**Purity of the Semicarbazones.**—About 1 g. of the pyrethrin I semicarbazone, melting at 115–117°, was saponified by boiling with a solution of 1 g. of sodium hydroxide in 100 cc. of 90% methanol. The alkaline solution was then acidified with sulfuric acid and distilled with steam. The chrysanthemum monocarboxylic acid in the distillate was determined by titration and from this the percentage of pyrethrin I semicarbazone in the original material was calculated. The chrysanthemum dicarboxylic acid in the residue from the steam distillation was determined by extracting with ether and titrating the residue obtained by evaporating the ether solution. Blanks were run on the reagents. In the same manner, the percentage of pyrethrin I semicarbazone in the pyrethrin II semicarbazone melting at 54–58° was determined.

Analysis of pyrethrin I semicarbazone

Volatile acids calculated as pyrethrin I semicarbazone.....96.94%
Non-volatile acids calculated as pyrethrin II semicarbazone.....8.10%

Analysis of pyrethrin II semicarbazone

Volatile acids calculated as pyrethrin I semicarbazone.....16.94%

The pyrethrin I semicarbazone was 97% pure, while the pyrethrin II semicarbazone contained 17% of semicarbazone of pyrethrin I. The total acidity found in the analysis of pyrethrin I semicarbazone was 5% higher than the theoretical amount. This is probably due to the formation of acid compounds by the action of the sodium hydroxide on pyrethrolon semicarbazone. Staudinger and Ruzicka[8] found that pyrethrolon yielded dehydropyrethrolon, a compound having weak acid properties, on prolonged digestion with alcoholic sodium hydroxide.

**Purity of the Isolated Pyrethrins.**—The semicarbazones were converted into the pyrethrins by digestion with oxalic acid solution.[9] The crude pyrethrins so obtained were extracted with petroleum ether and the petroleum ether solution was washed once with 10% potassium carbonate solution, three times with 1% sodium hydroxide, three times with water, three times with 3% potassium permanganate, three times with water, once with 1% sodium hydroxide and three times with water. It was then filtered and distilled *in vacuo* to constant weight at a maximum temperature of 40°.

The monocarboxylic acid in the pyrethrin I was determined as previously

[8] Ref. 3, p. 220.
[9] Ref. 3, p. 194.

described, by saponification and distillation with steam, and was calculated to pyrethrin I. The percentage of pyrethrin I was also determined from the copper reducing power.[4]

Analysis of pyrethrin I

Pyrethrin I calculated from volatile acid.....79.7%
Pyrethrin I calculated from copper reducing power.....79.5%

*Anal.* Calcd. for $C_{21}H_{30}O_3$: C, 76.31; H, 9.16. Found: C, 76.51, 76.20; H, 9.06, 8.89.

Similarly the amounts of monocarboxylic acid and dicarboxylic acid in the pyrethrin II were determined and calculated to pyrethrin I and II, respectively.

Analysis of pyrethrin II

Pyrethrin I calculated from volatile acid.....23.3%
Pyrethrin II calculated from non-volatile acid.....84.3%

*Anal.* Calcd. for $C_{22}H_{30}O_5$: C, 70.54; H, 8.08. Found: C, 72.62, 72.00; H, 8.17, 8.09.

The pyrethrin I was 80% pure and contained little pyrethrin II. The ultimate analysis, however, showed practically the theoretical percentage composition for pyrethrin I. The pyrethrin II contained about 20% pyrethrin I and 80% pyrethrin II. As in the analysis of the pyrethrin I semicarbazone, the total acidity was about 7% higher than the theoretical, probably because of the presence of acid decomposition products of pyrethrolon. Ultimate analysis confirmed that the material was a mixture of about 20% pyrethrin I and 80% pyrethrin II.

**Preparation of Solutions for Biological Experiments.**—Solutions of pyrethrin I and pyrethrin II were prepared by dissolving the accurately weighed pyrethrins in a highly refined mineral oil. These solutions were made within twelve hours from the time the pyrethrins were isolated and in the interval the latter had been kept in vacuum flasks. These precautions were taken to insure that the pyrethrins were not oxidized by exposure to the air. The mineral oil used was water white, nearly odorless and tasteless; specific gravity 0.785 at 15.6°; distilling range, 180–240°. The pyrethrin solutions were perfectly clear and were colorless. Solutions were prepared containing 150, 125, 100, 75, 50, 25 and 10 mg. of the isolated pyrethrins per 100 cc. The actual amounts of pyrethrins in these solutions were calculated from the analyses given above, which showed that the pyrethrin I was 80% pure and the pyrethrin II consisted of 77% pyrethrin II and 23% of pyrethrin I.

Extracts were prepared from four samples of *Pyrethrum* flowers, which had been assayed by the method of Gnadinger and Corl, using the same oil employed for making the pyrethrin solutions. The ground flowers were macerated for seventeen days with frequent shaking. The extracts were

then filtered and kept in well filled bottles in a dark closet. The composition of these extracts is fully described in Table I.

TABLE I

EXTRACTS OF ASSAYED FLOWERS (*Pyrethrum cinerariaefolium*)

| No. | Description | Pyrethrins 6/3/29 | Pyrethrins 1/28/30 | Pyrethrins Av. | Composition of oil extracts Flowers, g. | Oil, cc. | Pyrethrins, mg. per 100 cc. |
|---|---|---|---|---|---|---|---|
| 6 | Dalmatian open, 1926 crop | 0.60 | 0.60 | 0.59 | 63.5 | 497.5 | 75 |
| | | .56 | | | | | |
| 7 | Dalmatian half open, 1925 crop | .38 | .33 | .36 | 69.5 | 497.3 | 50 |
| | | .38 | | | | | |
| 13 | Dalmatian open, 1926 crop | .43 | .39 | .42 | 59.5 | 497.6 | 50 |
| | | .43 | | | | | |
| 82 | Japanese, 1929 crop | | .97 | .96 | 104.2 | 996.0 | 100 |
| | | | .97 | | | | |
| | | | .93 | | | | |

From the extract of sample 6, dilutions were prepared containing 60 and 40 mg. of pyrethrins per 100 cc.; from sample 82, solutions containing 80, 60, 40, 20 and 10 mg. per 100 cc. were made. All of the solutions were kept in the dark when not in use.

**Biological Experiments.**—Many attempts have been made to evaluate powdered *Pyrethrum* by actual tests on insects. Flies, roaches, bedbugs, bees, aphids and other insects have been employed for this purpose. The principal causes of error in these tests were lack of uniformity in the vitality of the insects used and inability to apply the same dosage of *Pyrethrum* powder to each insect. When it is considered that *Pyrethrum* flowers contain from 100 to 150 times the lethal dose of pyrethrins for these insects, it is not surprsiing that the results were meaningless. The value of these methods is best illustrated by the fact that closed Dalmatian flowers were generally considered superior to open and Japanese flowers even as late as 1929. It is now known that open flowers are more toxic than closed and that Japanese flowers contain about twice the amount of pyrethrins found in Dalmatian flowers.[10]

Within the last three years, however, methods for evaluating the *Pyrethrum*-oil sprays have been developed which yield good results. Probably the most accurate biological method for evaluating these sprays is the method of Peet and Grady,[11] which, briefly, is as follows.

About 100 flies, five days old, are placed in a chamber 1.83 by 1.83 by 1.83 meters in size, whose walls have been rendered non-absorbent with sodium silicate. The temperature of the chamber is kept at 25.6°. Twelve cc. of *Pyrethrum*-oil spray is introduced through four one-half inch holes near the ceiling, using a special type of atomizer under a constant pressure of 0.88 kg. per sq. cm. At the end of ten minutes the flies clinging

[10] Gnadinger and Corl, THIS JOURNAL, **52**, 680–688 (1930).

[11] Peet and Grady, *J. Econ. Entomol.*, **21**, 612 (1928).

to the ceilings and walls are counted and those which have dropped are carefully placed in wire gauze cages containing bread and milk and cheesecloth saturated with water, and are allowed to stand overnight at 25.6° and about 45% humidity. At the end of twenty-four hours, the number of disabled flies that have died or recovered is recorded. Unless water is supplied to the disabled flies, nearly all will die regardless of the toxicity of the spray. As soon as the flies have been removed from the test chamber, the walls are carefully wiped and the chamber is ventilated for twenty minutes by means of a blower and fan which draw air through a number of sliding doors in the chamber; it is then ready for another test.

The flies used in the test are carefully bred for the purpose in an insectary, held at constant temperature and humidity, in substantially the manner described by Grady.[12] By this method flies are available the year round. These flies are more resistant than wild flies, and since the age of each culture is definitely known, the vitality of the flies is more uniform.

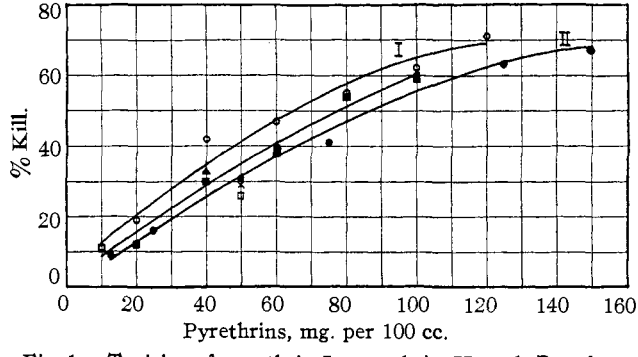


Fig. 1.—Toxicity of pyrethrin I, pyrethrin II and *Pyrethrum* flowers to flies. ○, Pyrethrin I; ●, Pyrethrin II; ■, Flowers. No. 82; ▲, Flowers. No. 6; □, Flowers. No. 13; ×, Flowers. No. 7.

Thus the method of Peet and Grady controls, as far as possible, the vitality of the insects, temperature, concentration of spray in the test chamber, pressure at which the spray is applied, time the insects are subjected to the action of the spray and humidity.

All of the biological tests presented in Table II were made by Mr. Grady and were observed by one of us. The temperature throughout the experiments was 25.6° and the humidity 43–50%. The same atomizer was used in all experiments. The results of every experiment are recorded with the exception of five which were obviously incorrect. The controls were not sprayed but were kept in the same kind of cage and under the same conditions as the disabled flies. The values for pyrethrin I and pyrethrin

[12] Grady, *J. Econ. Entomol.*, **21**, 598 (1928).

II and *Pyrethrum* flowers, after correcting for the kill due to the oil alone, are plotted in Fig. 1; the curve of the pyrethrin I solution is based on 100% pure pyrethrin I. The material from which the pyrethrin II curve was prepared consisted of 23% pyrethrin I and 77% pyrethrin II.

TABLE II

TOXICITY OF PYRETHRINS I AND II AND PYRETHRUM FLOWERS TO FLIES (*M. domestica*)

| No. of tests | Description of soln. | Pyrethrin content mg. per 100 cc. I | II | Total | Av. no. flies | Disabled in 10 min., av., %[a] | Condition of disabled flies in 24 hrs. Recovered, av., %[a] | Dead Max., %[a] | Dead Min., %[a] | Dead Av., %[a] |
|---|---|---|---|---|---|---|---|---|---|---|
| 7 | Oil only | 0 | 0 | 0 | 97 | 19 | 15 | 6 | 1 | 4 |
| 7 | Pyrethrin I | 120 | | 120 | 97 | 95 | 20 | 83 | 68 | 75 |
| 4 | Pyrethrin II | 34 | 116 | 150 | 97 | 99 | 28 | 76 | 66 | 71 |
| 4 | Pyrethrin I | 100 | | 100 | 96 | 95 | 29 | 71 | 57 | 66 |
| 4 | Pyrethrin II | 29 | 96 | 125 | 99 | 98 | 31 | 73 | 60 | 67 |
| 6 | Flowers No. 82 | 39 | 61 | 100 | 99 | 96 | 33 | 69 | 53 | 63 |
| 6 | Pyrethrin I | 80 | | 80 | 97 | 96 | 33 | 66 | 50 | 63 |
| 5 | Pyrethrin II | 23 | 77 | 100 | 94 | 97 | 33 | 71 | 57 | 64 |
| 5 | Flowers No. 82 | 31 | 49 | 80 | 95 | 96 | 38 | 67 | 52 | 58 |
| 3 | Pyrethrin I | 60 | | 60 | 98 | 92 | 41 | 52 | 50 | 51 |
| 3 | Pyrethrin II | 17 | 58 | 75 | 96 | 96 | 51 | 48 | 40 | 45 |
| 6 | Flowers No. 82 | 23 | 37 | 60 | 96 | 94 | 52 | 52 | 33 | 42 |
| 4 | Flowers No. 6 | | | 60 | 95 | 92 | 48 | 46 | 40 | 44 |
| 5 | Pyrethrin I | 40 | | 40 | 94 | 90 | 44 | 52 | 40 | 46 |
| 4 | Pyrethrin II | 11 | 39 | 50 | 103 | 93 | 58 | 42 | 29 | 35 |
| 5 | Flowers No. 82 | 16 | 24 | 40 | 93 | 91 | 57 | 44 | 26 | 34 |
| 4 | Flowers No. 6 | | | 40 | 105 | 82 | 45 | 42 | 30 | 37 |
| 4 | Flowers No. 7 | | | 50 | 96 | 81 | 47 | 40 | 30 | 34 |
| 4 | Flowers No. 13 | | | 50 | 95 | 78 | 48 | 38 | 26 | 30 |
| 3 | Pyrethrin I | 20 | | 20 | 98 | 69 | 46 | 29 | 18 | 23 |
| 3 | Pyrethrin II | 6 | 19 | 25 | 96 | 83 | 63 | 20 | 19 | 20 |
| 3 | Flowers No. 82 | 8 | 12 | 20 | 91 | 76 | 60 | 21 | 13 | 16 |
| 3 | Pyrethrin I | 10 | | 10 | 91 | 50 | 35 | 20 | 8 | 15 |
| 3 | Pyrethrin II | 3 | 10 | 13 | 101 | 64 | 51 | 17 | 8 | 13 |
| 3 | Flowers No. 82 | 4 | 6 | 10 | 93 | 65 | 50 | 22 | 10 | 15 |
| 7 | Controls (not sprayed) | 0 | 0 | 0 | 92 | | | 0 | 0 | 0 |

[a] Per cent. of number of flies used.

Inspection of Table II indicates at once that the difference in toxicity between pyrethrin I and pyrethrin II cannot be great. Calculations from the kill (Fig. 1) at concentrations of 120, 100, 80, 60 and 40 mg. per 100 cc. show that pyrethrin II is at least 77% as toxic as pyrethrin I. The kill obtained with the extract of flowers No. 82 is further proof that pyrethrin II is nearly as toxic as pyrethrin I. Sample No. 82 was analyzed by three different laboratories, both by Tattersfield's method and the method of Gnadinger and Corl. These analyses are given in Table III.

The ratio of pyrethrin I to pyrethrin II in Sample No. 82 is 1:1.5 but the

TABLE III

ANALYSIS OF *Pyrethrum* FLOWERS NO. 82

| Laboratory | Tattersfield method, pyrethrins I, % | | II, % | | Total, % | | Gnadinger and Corl method, total pyrethrins, % | |
|---|---|---|---|---|---|---|---|---|
| A | 0.39 | 0.38 | 0.60 | 0.60 | 0.99 | 0.98 | 0.92 | |
| B | | | | | | | .95 | 0.95 |
| C | | | | | | | .97 | .97 |
| | | | | | | | .97 | .93 |

kill is only slightly less than with pure pyrethrin I and is somewhat greater than with pyrethrin II. The toxicity of Sample No. 6 is also between that of pyrethrin I and pyrethrin II while the toxicity of Nos. 7 and 13 is about the same as that of pyrethrin II. Therefore the toxicities of oil extracts of *Pyrethrum*, whose total pyrethrin content has been determined by the copper reduction method, fall between the toxicity of pyrethrin I solution and the toxicity of pyrethrin II solution of the same pyrethrin content, made by dissolving the pyrethrins in the same oil. Furthermore, it will be seen from Tables I and II that when extracts of *Pyrethrum* flowers, of widely different pyrethrin content, are diluted to the same pyrethrin content, as determined by the copper reduction method, the toxicities are substantially the same.

Table II affords an idea of the accuracy of the biological method. It should be noted that the percentage of flies disabled in ten minutes is not an index of the toxicity of the spray.

## Summary

1. Pyrethrins I and II of known purity have been isolated directly from *Pyrethrum* flowers.

2. The toxicity of pyrethrins I and II to house flies (*Musca domestica*) has been determined by the method of Peet and Grady.

3. The toxicity of oil solutions of pyrethrins I and II has been compared with the toxicity of oil extracts of *Pyrethrum* flowers assayed by the copper reduction method.

4. The comparative toxicity of extracts of *Pyrethrum* flowers, of widely different pyrethrin content by the copper reduction method, has been determined.

5. Pyrethrin II is approximately 80% as toxic to flies as pyrethrin I.

6. The determination of the total pyrethrin content of *Pyrethrum* flowers by the copper reduction method of Gnadinger and Corl is an accurate index of their toxicity.

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