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Studies on Pyrethrum Flowers. V. The Presence of Pyrethrolon and Methyl Pyrethrolon in the Flowers

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In 1929 Tattersfield, Hobson and Gimingham[1] published two chemical methods for evaluating pyrethrum flowers which were modifications of methods proposed by Staudinger and Harder.[2] One of these methods depended on the saponification of the pyrethrins, which are esters, and titration of the resulting acids. The other consisted of isolating the pyrethrins as semicarbazones, with subsequent estimation of the pyrethrins from the nitrogen content of the semicarbazones, determined by the Kjeldahl method. The results obtained by the two methods were in good agreement.[1] In the same year Gnadinger and Corl[3] described a method based on the copper-reducing action of the ketone group present in both pyrethrins. Martin and Tattersfield[4] have shown that this method agrees excellently with their own. Hartzell and Wilcoxon[5] also have found good agreement between Tattersfield's acid method and the copper reduction method. Martin and Tattersfield[4] have used the principle of the writers' method to develop a fourth method which also gives results that agree with the Gnadinger and Corl method.

Thus it has been found that the four chemical methods, depending on three different reactions, yield concordant results. Martin and Tattersfield,[4] Hartzell and Wilcoxon,[5] and the writers[6] have found that the pyrethrin content, determined chemically, is correlated with the toxicity determined by biological tests on different insects.

Ripert[7] has condemned all of the methods for evaluating pyrethrum, both chemical and physiological. He claims to have isolated the methyl ether of pyrethrolon from pyrethrum flowers and states that this compound is present to the extent of 0.1 to 0.4% and would interfere with the copper reduction method, being estimated as pyrethrins without having any toxicity to insects.

In order to make clear the significance of this statement, it should be recalled that the pyrethrins are esters of the ketone alcohol pyrethrolon with chrysanthemum monocarboxylic acid or chrysanthemum dicarboxylic acid methyl ester, and the copper reducing action of the pyrethrins is due to the presence of the ketone group in the alcoholic component (pyrethrolon) of the esters.

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(1) Tattersfield, Hobson and Gimingham, *J. Agr. Sci.*, **19**, 266–296 (1929).
(2) Staudinger and Harder, *Ann. Acad. Sci. Fennicae*, **A29**, 1–14 (1927).
(3) Gnadinger and Corl, THIS JOURNAL, **51**, 3054 (1929).
(4) Martin and Tattersfield, *J. Agr. Sci.*, **21**, 120 (1931).
(5) Hartzell and Wilcoxon, *Contribution from Boyce Thompson Institute*, **4**, 107–117 (1932).
(6) Gnadinger and Corl, THIS JOURNAL, **52**, 3300 (1930).
(7) Ripert, *Ann. fals.*, **24**, 325–341 (1931).

Ripert does not give the details of the method he employed for isolating the methyl ether of pyrethrolon from the flowers and the proof of its identity which he presents is not conclusive. His statement that the compound would interfere in the copper reduction method is not supported by any evidence.

When the copper reduction method was developed, the writers realized that pyrethrolon might be present in the flowers; the possible presence of the methyl ether of pyrethrolon was not considered. At that time pyrethrolon was prepared and its properties were investigated.

Neither pyrethrolon nor its methyl ether was found in the flowers by Staudinger and Ruzicka;[8] the thoroughness of their work makes it improbable that quantities of the methyl ether as great as Ripert reports would be overlooked. Nevertheless, it was considered advisable to investigate Ripert's claims. The results of this investigation are herewith presented.

Experimental Part

Staudinger and Ruzicka[9] called attention to the fact that the pyrethrins in petroleum ether solution are not appreciably attacked by potassium permanganate solution. They also found that pyrethrolon[10] in aqueous suspension, and pyrethrolon methyl ether[11] are immediately oxidized by potassium permanganate solution. Staudinger and Ruzicka did not investigate these reactions quantitatively nor under exactly the same conditions. The writers[12] had found that the pyrethrins in petroleum ether are scarcely attacked by potassium permanganate, while pyrethrolon under these conditions was completely oxidized and removed from the petroleum ether solution.[13] Hence this procedure might afford a means for separating the pyrethrins from pyrethrolon and possibly from pyrethrolon methyl ether.

Effect of Potassium Permanganate Solution on Pyrethrins I and II.—The semicarbazones of pyrethrins I and II were separated from 15 kg. of Japanese *Pyrethrum cinerariaefolium* by a slight modification of Staudinger and Ruzicka's[8] method. The mixed semicarbazones melted at 75 to 90° and were converted into mixed pyrethrins I and II with oxalic acid solution.[8] The crude pyrethrins were purified by the writers' method[14] and were immediately dissolved in sufficient petroleum ether to make the pyrethrin content about 3.5 g. in 1000 cc. This stock solution was kept in the dark. Analysis by Tattersfield's acid method showed that the mixed pyrethrins consisted of 51.3% pyrethrin I and 48.7% pyrethrin II.

The extent to which the pyrethrins in petroleum ether are oxidized by permanganate was determined in the following manner.

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(8) Staudinger and Ruzicka, *Helv. Chim. Acta*, **7**, 177–259, 377–458 (1924).

(9) Ref. 8, p. 189.

(10) Ref. 8, p. 228.

(11) Ref. 8, p. 222.

(12) Ref. 3, p. 3056.

(13) Ref. 3, p. 3060.

(14) Ref. 6, p. 3303.

An aliquot of the stock petroleum ether solution of pyrethrins was measured into a separatory funnel and diluted with sufficient petroleum ether to make the volume 100 cc.; 25 cc. of 2% potassium permanganate solution was added and the funnel was vigorously shaken for one minute. Immediately 20 cc. of 5% sodium bisulfite solution and 5 cc. of 15% sulfuric acid were added and the funnel was gently shaken until the excess of permanganate was destroyed. In some cases 50 cc. of 2% permanganate solution was used; in every case an excess of permanganate solution was present.

The petroleum ether and aqueous layers were separated; the former was washed with 25 cc. of water and the aqueous layer was extracted with an equal volume of petroleum ether. This second petroleum ether extract was washed with the 25 cc. of water used to wash the original petroleum ether solution. The combined petroleum ether extracts were filtered into a 400-cc. beaker. A second aliquot of the stock solution of pyrethrins, equal to the first, was measured into another 400-cc. beaker and was diluted with petroleum ether to the same volume as the solution which had been treated with permanganate. The two petroleum ether solutions were then evaporated on a steam-bath and the pyrethrin content of the residues was determined by the writers' method. The results of these experiments are given in Table I.

TABLE I

EFFECT OF WASHING PYRETHRINS IN PETROLEUM ETHER WITH 2% PERMANGANATE SOLUTION

Aliquot taken, cc.	Pyrethrins found Not treated, mg.	Pyrethrins found Treated with $KMnO_4$, mg.	Pyrethrins lost by $KMnO_4$ treatment mg.	Pyrethrins lost by $KMnO_4$ treatment %
50	185.4	174.1	11.3	6.1
40	141.9	137.2	4.7	3.3
35[a]	135.6	128.6	7.0	5.2
30	106.3	100.7	5.6	5.3
25[a]	85.9	84.0	1.9	2.2
20	70.2	66.8	3.4	4.8
10	34.6	33.6	1.0	2.9
			Av.	4.3

[a] 50 cc. of $KMnO_4$ solution used.

The aliquots taken were selected to correspond to the pyrethrin content which would be present in pyrethrum flowers when using 15 g. of flowers for assay. The loss of pyrethrins by the permanganate treatment varied from 2.2 to 6.1%, with an average of 4.3%.

Effect of Permanganate Solution on Pyrethrolon.—Part of the mixed semicarbazones of pyrethrins I and II was saponified at 0° as recommended by Staudinger and Ruzicka.[15] The resulting pyrethrolon semicarbazone was converted into pyrethrolon by prolonged shaking with benzene and sodium bisulfate solution.[16] This pyrethrolon (0.898 g.) was completely dissolved in petroleum ether, in which it was but slightly soluble. The petroleum ether solution was washed with 3% permanganate solution, which was instantly reduced. The washings with permanganate were continued until an excess of permanganate remained. The petroleum ether solution was then filtered and distilled in vacuum at 45° to constant weight; the residue weighed 0.012 g., that is, 98.7% of the pyrethrolon was oxidized and removed from the petroleum ether solution, by the permanganate treatment.

Effect of Permanganate Solution on Pyrethrolon Methyl Ether.—A second portion of the mixed semicarbazones of pyrethrins I and II was saponified at 0° and the pyre-

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(15) Ref. 8, p. 196.

(16) Ref. 8, p. 216.

throlon semicarbazone so obtained (m. p. 200–203°) was used to synthesize pyrethrolon methyl ether, employing the procedure of Staudinger and Ruzicka.[17] Thirteen grams of pyrethrolon semicarbazone yielded 4.7 g. of pyrethrolon methyl ether. A small portion of the pyrethrolon methyl ether was treated with semicarbazide hydrochloride; the semicarbazone of pyrethrolon methyl ether so obtained melted at 187°. This melting point is slightly higher than recorded by Staudinger and Ruzicka (183°), probably because of the presence of a small amount of pyrethrolon.

The remainder of the pyrethrolon methyl ether was dissolved in petroleum ether, the concentration being 0.643 g. per 100 cc. Aliquots of this solution were treated with permanganate solution exactly as described above in the case of the pure pyrethrins, and the copper reducing power of the permanganate treated solution was compared with that of a solution of the same pyrethrolon methyl ether content which had not been treated with permanganate. Since no tables have been calculated for the copper reducing power of pyrethrolon methyl ether, it was necessary to express its reducing power, in Table II, in terms of the amount of dextrose having equivalent copper reducing power.

TABLE II

EFFECT OF TREATING PYRETHROLON METHYL ETHER IN PETROLEUM ETHER WITH 2% PERMANGANATE SOLUTION

Pyrethrolon methyl ether, mg.	Permanganate soln. used, cc.	Dextrose equivalent in reducing power to pyrethrolon methyl ether		Pyrethrolon methyl ether lost by $KMnO_4$ treatment, %
		Not treated with $KMnO_4$, mg.	Treated with $KMnO_4$, mg.	
128.6	25	13.84	6.25	54
128.6	50	13.84	0.00	100
64.3	25	6.41	0.00	100

In the first experiment (Table II) the permanganate solution was completely reduced by about half the pyrethrolon methyl ether taken. When the amount of permanganate was doubled, or the quantity of pyrethrolon methyl ether was halved, the copper reducing power of the pyrethrolon methyl ether was completely destroyed. The amounts of pyrethrolon methyl ether taken were several times greater than would be encountered in assaying pyrethrum flowers containing the amount of pyrethrolon methyl ether Ripert claims to have found. It should also be noted that the copper reducing power of pyrethrolon methyl ether is somewhat less than that of the pyrethrins. This was also true of pyrethrolon.[18]

Effect of Permanganate on Petroleum Ether Extract of Pyrethrum Flowers.—From the foregoing experiments it is quite clear that the pyrethrins can be separated, with slight loss, from pyrethrolon or pyrethrolon methyl ether by treating them, in petroleum ether, with 2% potassium permanganate solution.

According to Ripert,[19] methyl pyrethrolon is the form in which the reserve of pyrethrolon is stored by the plant, pending esterification with the chrysanthemum acids by metabolic processes. If this is the case one would expect to find larger quantities of methyl pyrethrolon in freshly harvested flowers than in flowers several months old. In Table III are given the analyses of samples of pyrethrum flowers assayed as soon as possible after harvesting. Two series of 15-g. samples of the ground flowers were extracted with petroleum ether in Soxhlet extractors. One series of petroleum ether extracts was assayed by the writers' method. Another series of petroleum ether extracts was first treated with 2% permanganate solution exactly in the manner described above, before assaying.

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(17) Ref. 8, p. 222.

(18) Ref. 3, p. 3060.

(19) Ref. 7, p. 340.

TABLE III

EFFECT OF PERMANGANATE TREATMENT ON PYRETHRIN CONTENT OF FRESHLY HARVESTED PYRETHRUM FLOWERS

Where grown	Harvested	Assayed	Pyrethrin content of flowers Not treated with $KMnO_4$, %	Pyrethrin content of flowers Treated[a] with $KMnO_4$, %	Loss of pyrethrins by $KMnO_4$ treatment %	Loss of pyrethrins by $KMnO_4$ treatment % of pyrethrin content
Colorado	7/14/32	7/21/32	1.04	1.00	0.04	3.8
Wakayama, Japan	6/15/32	8/9/32	1.00	0.94	.06	6.0
Spalato, Dalmatia	5/27/32	7/19/32	0.84	.79	.05	5.9
				Av.	.05	5.2

[a] 50 cc. of 2% $KMnO_4$ used.

The average loss in pyrethrin content of the flowers by the permanganate treatment is 0.05% equivalent to 5.2% of the total pyrethrins present. This is only 0.9% greater than the loss with pure pyrethrins (4.3%, see Table I). This indicates that pyrethrolon and pyrethrolon methyl ether are not present in flowers shortly after harvesting.

Ten additional samples of pyrethrum flowers from different sources were assayed ten months after they were harvested. These samples were assayed by the copper reduction method both with and without the permanganate treatment. The results are compared in Table IV.

TABLE IV

EFFECT OF PERMANGANATE TREATMENT ON PYRETHRIN CONTENT OF PYRETHRUM FLOWERS TEN MONTHS OLD

Where grown	Grade	Pyrethrin content of flowers Not treated with $KMnO_4$, %	Pyrethrin content of flowers Treated with $KMnO_4$, %	Loss of pyrethrins due to $KMnO_4$ treatment %	Loss of pyrethrins due to $KMnO_4$ treatment % of pyrethrin content
Rose, Dalmatia	Half closed	0.70	0.59	0.11	15.7
Trogir, Dalmatia	Mixed	.76	.65	.11	14.4
Trogir, Dalmatia	Closed	.71	.59	.12	16.9
Hokkaido, Japan	......	.76	.68	.08	10.5
Hokkaido, Japan	......	.79	.69	.10	12.6
Hokkaido, Japan	......	.71	.59	.12	16.9
Hokkaido, Japan	......	.85	.72	.13	15.3
Hokkaido, Japan	......	.86	.72	.14	16.3
Hokkaido, Japan	......	1.01	.85	.16	15.8
Cyprus	Closed	0.75	.63	.12	16.0
			Av.	.12	15.0

The loss in pyrethrin content averaged 0.12% of the weight of the flowers, equivalent to 15.0% of the pyrethrins present. The losses are fairly constant for flowers from different countries and of different pyrethrin contents. Correcting for the loss due to the effect of permanganate on pure pyrethrins (4.3%), the average loss in these ten-month old flowers, due to other oxidizable material, is 10.7% of the pyrethrins present. In the freshly harvested flowers the corrected loss was 0.9%.

The writers[20] have shown that the pyrethrin content of pyrethrum flowers becomes gradually reduced as the flowers age, the loss amounting to about 30% in one year. This loss of pyrethrins is due to oxidation or to a molecular rearrangement and is accelerated by light.[5,21] The altered pyrethrins are almost insoluble in petroleum ether, the

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(20) Gnadinger and Corl, *Ind. Eng. Chem.*, **24**, 901 (1932).

(21) Tattersfield, *J. Agr. Sci.*, 396-417 (1932).

solubility being 3.6 mg. per 100 cc. at 20°. Their copper reducing power is slightly higher than that of the pyrethrins. Whether they are oxidized in petroleum ether solution by permanganate was not determined because of their low solubility in that solvent.

In ether, however, a considerable oxidation of altered pyrethrins occurs when the permanganate treatment is applied. An ether solution containing 88 mg. of pyrethrins altered by exposure to air at room temperature was washed with 25 cc. of saturated permanganate solution. The ether solution was evaporated and the residue was weighed; 62 mg. of altered pyrethrins was lost by oxidation. A second ether solution containing 176 mg. of altered pyrethrins was treated with permanganate in the same manner as the pure pyrethrins, as previously described. The loss by the permanganate treatment was 45 mg. The altered pyrethrins are apparently more readily attacked than pyrethrins I and II and it is quite probable that the altered pyrethrins in the samples reported in Table IV were oxidized by the permanganate treatment. This would account, in part, for the fact that old flowers show a greater loss in pyrethrin content when treated with permanganate than new flowers similarly treated.

Summary

1. A method is described for separating pyrethrolon and its methyl ether from pyrethrins I and II.

2. The pyrethrins in petroleum ether are not readily oxidized by dilute permanganate solution, the loss averaging about four per cent.

3. Pyrethrolon and pyrethrolon methyl ether, in petroleum ether, are instantly oxidized by dilute permanganate solution and removed from the petroleum ether.

4. Pyrethrins altered by exposure to air are, in ether solution, appreciably oxidized by permanganate solution.

5. Freshly harvested pyrethrum flowers assayed by the permanganate method show about the same loss of pyrethrins as solutions of pure pyrethrins of the same strength.

6. Pyrethrum flowers ten months old show a somewhat greater loss by the permanganate method, probably because of the oxidation of altered pyrethrins.

7. No evidence of any appreciable amount of pyrethrolon or pyrethrolon methyl ether was found in flowers from America, Dalmatia or Japan.

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