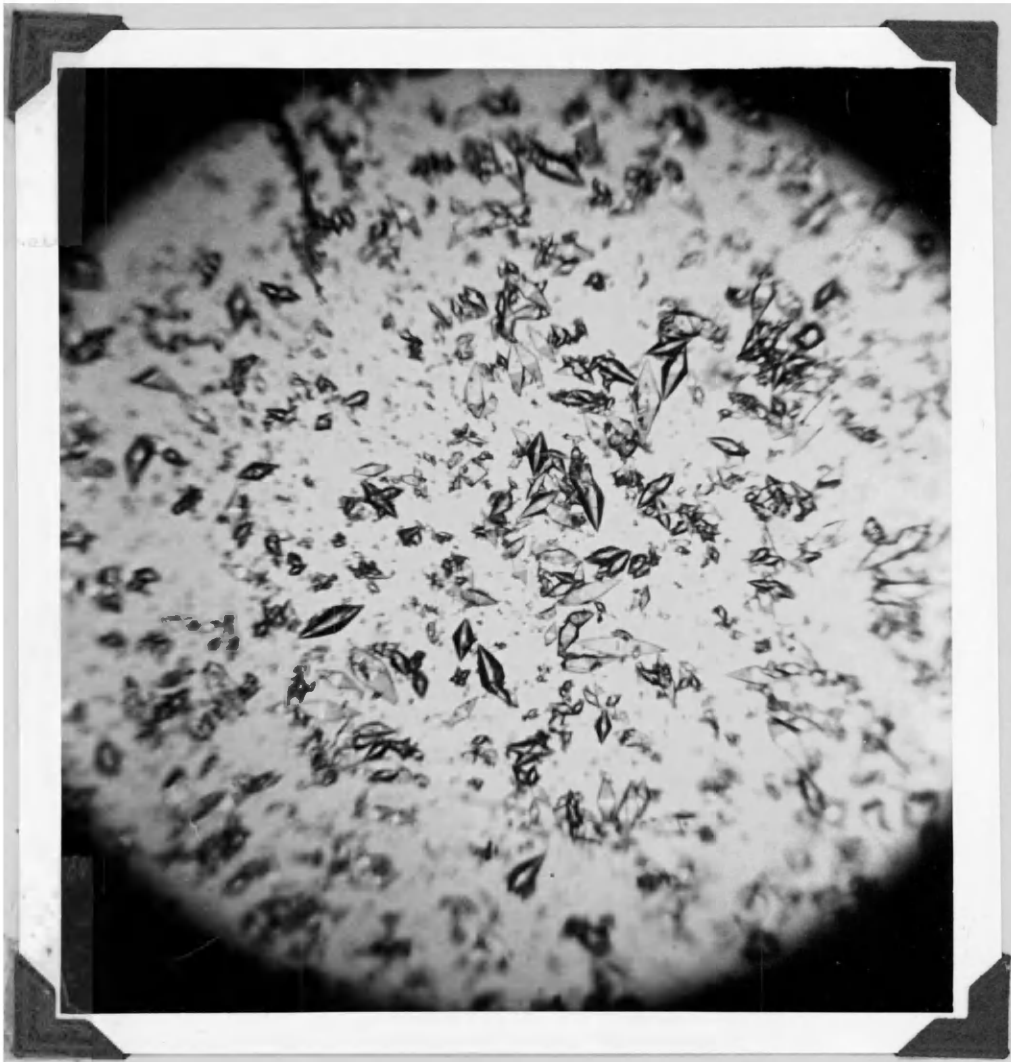


FRONTISPIECE



Pyrethrosin

(Total magnification about 250X)

**A STUDY OF PYRETHROSIN, AN INSECTICIDALLY
INERT CONSTITUENT OF PYRETHRUM FLOWERS**

By

William Alexander Stanton

**Thesis submitted to the Faculty of the Graduate School
of the University of Maryland in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy**

1941

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FOREWORD

The isolation of the chemical individual pyrethrosin from pyrethrum flowers was first reported in the years 1890-91 by several investigators (18, 28), but no great amount of work was carried out on the substance. In recent years the compound has again been isolated (3,21) by laboratories interested in the constituents of pyrethrum flower insecticides. In the laboratories of the Insecticide Division of the Bureau of Entomology and Plant Quarantine of the United States Department of Agriculture the substance was isolated (21) from petroleum ether extracts of pyrethrum flowers. The same laboratory (21) identified pyrethrosin as the main constituent of a still residue and pipe-line scale sent to them by McCormick and Company, Baltimore, Maryland, makers of pyrethrum insecticides. Because of its insecticidal inertness the Government Laboratory had no justification for continuing work on the chemical nature and properties of pyrethrosin. In consequence, this problem and a large quantity of crude and pure pyrethrosin was turned over to the Organic Chemistry Department of the University of Maryland as the basis for a series of academic researches.

HISTORICAL INTRODUCTION

While investigating the nature of the active insecticidal principles of pyrethrum flowers (*chrysanthemum cinerariaefolium*) Thoms (28), in 1891, isolated an insecticidally inert constituent for which he proposed the name "pyrethrosin". This substance proved to be identical with a compound supplied to him by a druggist friend who also had obtained it from pyrethrum extracts.

Marino-Zucco (18), an Italian, had reported a year earlier (in 1890) in a paper entitled "About A New Alkaloid Obtained Out of Chrysanthemum Extract" the isolation of a compound with some of the characteristics of pyrethrosin. Thoms had no doubt that his "pyrethrosin" was identical with the compound obtained by Marino-Zucco.

Thoms knew the general properties of pyrethrosin as we know them today. However, he found a melting point of 189° , as compared to the true melting point of $200-201^{\circ}$. On the basis of combustion data alone he assigned it a formula of $C_{34}H_{44}O_{10}$, this being exactly twice the formula now accepted.

The compound was lost sight of until 1934, when the Chinese investigators Chou and Chu (3), working on benzene extracts of pyrethrum flowers isolated a compound

melting at 200° and having $(\alpha)_D^{20} = -30$ in chloroform. These workers, again on the basis of combustion data only, suggested $C_{10}H_{13}O_3$ for the empirical formula although their analytical data better fit the present accepted formula $C_{17}H_{22}O_5$. They called this compound "Chrysanthine".

In 1937 Rose and Haller (21) reported the isolation from pyrethrum flowers of a chemical individual which seemed to be identical with that obtained by Chou and Chu and which they called "chrysanthin" having dropped the final "e" of chrysanthine in the light of the newer standards of nomenclature.

The compound as obtained by Rose and Haller melted at 201° when recrystallized from ethyl acetate and $177-178^{\circ}$ when recrystallized from ethanol. It had an optical rotation $(\alpha)_D^{25} = -30.5$ in chloroform ($c = 5.16$) and on the basis of combustion analyses and molecular weight determinations was given the formula $C_{17}H_{22}O_5$. $(\alpha)_D^{25} = -38.1$ in alcohol ($c = 0.315$) was also reported.

In 1939 Schecter and Haller (25) showed that chrysanthin and pyrethrosin were identical and recommended the adoption of the name "pyrethrosin" which had been proposed by Thoms. This suggestion

has been adopted by our laboratory.

It is interesting to note here that although pyrethrosin has been shown to be non-toxic to insects, Chou and Chu (3) report it to be toxic to rabbits within 48 hours if injected hypodermically in quantities approximating .005 per cent of body weight.

Rose and Haller having available originally only a small amount of material made only a preliminary examination of pyrethrosin. However, their findings for the most part have been substantiated by the present work. They were unable to show the presence of methoxyl groups by the Zeisel method or the presence of hydroxyl groups by the acetic anhydride-pyridine reagent. Also, they were unable to prepare an oxime or semicarbazone. They found that on hydrogenation with platinum oxide catalyst two atoms of hydrogen were adsorbed and that chromic acid oxidation in aqueous acetic acid caused the loss of two hydrogens with the formation of a neutral compound $C_{17}H_{20}O_5$. In a probable attempt to establish pyrethrosin as a flavone-like individual they fused the compound with potassium hydroxide and obtained a small quantity of substance said by them to have a strong phenol-like odor. Their product gave a red-violet color with

ferric chloride reagent.

Rose and Haller described the reaction of pyrethrosin with dilute aqueous alkali as being "perhaps the most characteristic". They found that it readily dissolved on warming with 5 per cent aqueous sodium hydroxide, and that on acidification with dilute hydrochloric acid, water soluble products were obtained. One of these was acetic acid and the other an acid only slightly soluble in ether and chloroform, and which the authors described as being difficult to crystallize. Their examination showed this to be $C_{15}H_{26}O_7$ with a melting point of 190-192°. However, if dried at 110° a molecule of water was lost and the new acid obtained melted at 210-211°. This acid was shown to possess one hydroxyl through the use of acetic anhydride-pyridine reagent but no acetylation product could be obtained. Attempts to prepare derivatives in which the carboxyl group was esterified were also unsuccessful. Acetyl determinations run on pyrethrosin were not particularly satisfactory, indicating in one case less than one acetyl per molecule and in the other slightly more than one per molecule.

In a later paper, Schecter and Haller (25)

report several additional properties of pyrethrosin. They found that on warming with 20-25 per cent aqueous hydrochloric acid, pyrethrosin gave pink-violet to violet solutions which gave amorphous yellow precipitates on dilution. In a similar manner concentrated sulfuric acid gave a yellow-brown solution. These authors also reported the failure of pyrethrosin to give a tetra-nitromethane test for unsaturation as well as its failure to decolorize a chloroform solution of bromine. Pyrethrosin did, however, reduce cold aqueous potassium permanganate solution.

Wingate (29) has run active hydrogen and carbonyl determinations on pyrethrosin using methyl magnesium iodide in di-n-amyl ether according to the modified Zerevitinoff method as developed by Kohler, Stone, and Fuson (17). Results of the work led to the conclusion that no active hydrogen atoms and/or carbonyl groups were present.

In at least one instance pyrethrosin has been confused with another substance. Kimington and Koets (20), in 1936, during the course of some work on geigera aspera, a plant that produces a vomiting sickness in sheep, isolated a compound which they called "geigerin" and which they thought to be identical with the pyrethrosin isolated by Thoms. Schechter and

Haller, in a paper previously mentioned (25), undertook the comparison of geigerin and pyrethrosin on the basis of their recorded properties and showed that geigerin and pyrethrosin are different substances.

Although geigerin has been shown to be $C_{15}H_{20}O_4 \cdot H_2O$ (20) and thus positively not identical with pyrethrosin it must be stated that the two compounds are related, if not strictly so in a chemical sense, at least in a physiological sense. Both must be included in the class of compounds that are coming to be called "bitter principles". More and more plant sources are yielding chemical compounds of varied chemical characteristics which are extremely bitter to the taste. Of the bitter principles discussed in the literature, those isolated from certain species of helenium are particularly interesting. Some of their molecular formulas have been shown to be $C_{17}H_{22}O_5$ - the same as pyrethrosin. A compound isolated (2) by workers at the University of Tennessee has been called helenium and one isolated (6) by Clark of the Bureau of Entomology and Plant Quarantine of the United States Department of Agriculture has been called tenulin. Clark (6) believes that helenium is identical with isotenulin. Isotenulin is obtained from tenulin by treatment with dilute alkali and also has the formula $C_{17}H_{22}O_5$. Inspection of Table I

shows at once that pyrethrosin is different from both tenulin and isotenulin or helenium.

TABLE I
Brief Comparison of Pyrethrosin, Tenulin,
Isotenulin and Helenium

Property	Pyrethrosin	Tenulin (6,7,8)	Isotenulin (6) Helenium (2)
melting point, °C	200-201	193-195	162 (6) 158 (2)
optical rotation	$(\alpha)_D^{25} = -33.9$ (c = 5.64 in CHCl ₃) $(\alpha)_D^{20} = -38.1$ (c = 0.32 in absolute EtOH)*	$(\alpha)_D^{25} = -21.6$ (c = 5.2 in EtOH)	-----
formation of a crystalline dibromide	no	yes	yes
reaction with dilute alkali	deep-seated with no crystalline product	yields iso- tenulin and another crystalline product	-----

* value of Rose and Haller (21)

EXPERIMENTAL PART

The melting points recorded in this section were taken by two methods: (1) in thin-walled glass capillaries in a liquid bath in the customary manner and (2) on a microscope hot stage. Figure I is a photograph (actual size) of the microscope hot stage built and used by the author. The sample is placed on a 15 mm. microscope cover glass placed in the inner depression and covered with a second glass of the same kind. A 23 mm. polarimeter cover glass fits in the large depression. The depth of the inner depression is sufficient to allow for a slight air space between the glass covering the sample and the top glass. The stage is heated electrically by means of a five ohm resistance (three feet of #24 nichrome wire) wrapped on a porcelain ring and placed in an annular depression in the bottom of the block. The heater is insulated from the block by means of asbestos paper. Connection to a Variac is made through the binding posts shown. The Variac can be calibrated to give any desired rate of heating at any desired temperature. The usual rate of heating is one degree per minute. The temperature of melting is read by means of the thermometer although provision has been



Figure 1.

made for insertion of a thermocouple through a hole bored into the block along the radius opposite the thermometer. The junction of the thermocouple would lie just below the sample in the small depression just off the axial hole that passes the light for illuminating the sample.

Both thermometers used were calibrated against standards of known melting point. The melting points are therefore corrected. The method of taking is indicated by the use of "cap." for capillary and "stage" for the microscope method in parentheses following the melting point symbol m.p. Also used are the designations "s" for softening or shrinking - in general any change in the sample preliminary to melting, "m" for melting range, "d" for darkening, and "de" for decomposition with gaseous evolution or bubbling. Thus the melting point in a capillary of a compound that melts at 192-195° with decomposition and with preliminary softening at 185° would be designated:

m.p. (cap.) s185, m192-195de

All temperatures are given in degrees Centigrade.

Preparation and Physical Properties of Pure Pyrethrosin

I. Preparation

Pure pyrethrosin was prepared from the material supplied by the United States Department of Agriculture. This material melted at 199-201°. It was recrystallized three times from ethanol and dried after grinding in an agate mortar in an Abderhalden dryer for nine hours at 100° over phosphorus pentoxide.

II. Physical appearance and taste

Physical appearance and taste

The crystals of pyrethrosin (see Frontispiece) are regular octahedra when crystallized from ethanol. If crystallized slowly the tendency is towards elongation at the expense of thickness. Pyrethrosin is very bitter to the taste.

III. Melting point

m.p. (cap.) m200-201°

m.p. (stage) s197.5, m199.5-202°

IV. Solubility

Pyrethrosin is only slightly soluble in hot and cold water but more so in cold ethanol. In the latter solvent it dissolves to the extent of approximately 7.5 g. per 100 ml. at the boiling point and 1.5 gms. at room temperature. It is readily soluble in cold

chloroform, pyridine, and dioxane. It is insoluble in ether and very slightly soluble in benzene and petroleum ether.

V. Specific rotation

1.411 g. of pyrethrosin dissolved in 25 ml. of chloroform gave an observed rotation of -3.82° when used in a two decimeter tube at 25° with a sodium vapor lamp as the light source.

$$(\alpha)_D^{25^\circ} = -33.9 \quad (c = 5.64 \text{ in chloroform})$$

VI. Combustion analyses

See Table II.

VII. Molecular weight by the Rast Camphor method*

.275 mg. of pyrethrosin in 7.225 mg. camphor ($k = 41.5$, m.p. = 177.6°) gave a depression of 5.1° .

Molecular weight = 305

Calculated for $C_{17}H_{22}O_5 = 306.35$

VIII. Saponification equivalents

.2 to .3 g. of pyrethrosin were weighed into a 125 ml. Erlenmeyer flask. 25 ml. of standard aqueous

* The author is indebted to Mr. R. I. Longley, Jr. for this analysis.

alkali were added and the mixture warmed on the steam bath for one hour. A wad of cotton was used to stopper the flask loosely. The pyrethrosin was completely in solution after four or five minutes. At the end of the heating period the determination was completed in the usual manner by titrating with standard acid. See Table III for results.

TABLE II

Combustion Analyses of Pyrethrosin

Sample mg.	H ₂ O mg.	% H	CO ₂ mg.	% C
4.256	2.743	7.21	10.384	66.60
3.547	2.206	7.37	8.192	66.79
3.681	2.356	7.22	8.927	66.72
4.330	2.759	7.13	10.551	66.49
3.649	2.393	7.33	8.890	66.48
	average	7.25		66.61
	average of 15 runs	7.28		66.54
	calculated for C ₁₇ H ₂₂ O ₅	7.24		66.65

TABLE III

Saponification Equivalents of Pyrethrosin

sample g.	ml. of .2N alkali used	saponification equivalent
.2257	7.28	155.2
.2353	7.69	153.0
.2331	7.59	153.2
.2388	7.73	154.5
	average	153.9
	calculated for C ₁₇ H ₂₂ O ₅	153.2

Potassium Hydroxide Fusion of PyrethrosinExperiment I

4 g. of pyrethrosin and 6 ml. of water were mixed with 40 g. of potassium hydroxide in a nickel crucible. The mixture was gradually warmed with a strong Bunsen flame until most of the water was driven off and then heated to 250° where it was held for ten minutes. The fused melt took on a blood red color. After cooling the solidified melt was dissolved in several hundred ml. of water and acidified to congo paper with fairly strong sulfuric acid, whereupon a brownish non-crystalline mass precipitated. The whole dark colored mixture was then extracted several times with a suitable volume of ether which removed all of the brown color and brownish precipitate and caused precipitation of a considerable amount of potassium sulfate. The ether extracts which had been separated from the water and potassium sulfate were then extracted with ten per cent sodium bicarbonate solution. The bicarbonate solution removed some of the brown color and gave a solution possessing a purple fluorescence. Evaporation of the extracted ether solution, without drying it, yielded a small amount of brown oily residue. The bicarbonate solution was then acidified with acid and extracted with ether. This again

removed all of the brown color. Evaporation of the ether yielded a brown evil-smelling tar. Attempts to crystallize both oils met with failure.

The small amount of alkali-soluble oil was distilled at atmospheric pressure without fractionation. The highest boiling point attained was 240° . The distillate was a light greenish colored oil which darkened on standing overnight to air. This oil gave no positive ferric chloride test but decolorized alkaline potassium permanganate and bromine water. It was lighter than water and dissolved readily in ether, carbon tetrachloride, ethanol and cold concentrated sulfuric acid. It was insoluble in dilute hydrochloric acid and turned a deep blue color on standing with concentrated alkali. The oil gave no Schiff's test and no hydrazone could be formed.

Experiment II

The fusion and separation as described above were repeated on 20 g. of pyrethrosin. In this experiment the ether solution remaining from the bicarbonate extraction was dried over anhydrous magnesium sulfate for 18 hours. After filtering, the ether was removed by evaporation. About 2-3 ml. of a brownish oil with a phenolic-like smell were obtained. Attempts to make a benzoate by the Schotten-Baumen reaction, an aryloxyacetic acid by the method of Shriner and Fuson (26), and to obtain a

bromination product, all met with failure.

The bicarbonate-soluble fractions from both fusion experiments were combined, acidified to congo and distilled with steam. The distillate smelled of pyroligneous acid. The residue was cooled and the insoluble tar induced to solidify. This solid was broken up and filtered off. No crystallization could be effected from any of the common solvents or mixtures of them. An attempt to make a p-phenylphenacyl ester resulted in failure as did attempts to make a methyl ester with both diazomethane and dimethyl sulfate and alkali.

Typical Experiment on the Treatment of Pyrethrosin
with Aqueous Hydrochloric Acid

1.5 g. of pyrethrosin were added to a solution consisting of 40 ml. of concentrated hydrochloric acid and 25 ml. of water. Almost all of the pyrethrosin dissolved at once. This solution was filtered through a hardened filter paper and warmed on the steam bath. In a minute or two the color was green. This rapidly grew darker, turning pink-violet and finally a deep violet. Soon a precipitate began to come out of solution and the solution appeared to be green again. After about six minutes total heating a deep orange-yellow precipitate was filtered off. The remaining filtrate was a clear violet. The precipitate was washed with distilled water until silver nitrate gave no precipitate in the washings. The precipitate which was amorphous was well dried in air. It melted to a brown oily liquid from 135-155°. Attempts to recrystallize the material failed. The dried precipitate was a dark orange-yellow powder. Dilution of the filtrate produced an additional quantity of amorphous material with the same general characteristics that was greenish-yellow in color.

5.211 mg. gave 3.281 mg. of water and 12.922 mg.

carbon dioxide

C = 67.66% H = 7.04%

4.891 mg. gave 3.023 mg. of water and 12.150 mg.

carbon dioxide

C = 67.79% H = 6.91%

Although this substance gave a brilliant Beilstein test, no positive halogen test could be obtained by dissolving in either warm aqueous or alcoholic alkali and adding silver nitrate and nitric acid. Fusion with sodium peroxide, however, in the Parr method for halogen indicated a trace to be present.

Alkaline Hydrolysis of Pyrethrosin and Dihydro-
pyrethrosin

Experiment I

(This experiment was essentially a repeat of the experiment performed by Rose and Haller (21) from which they obtained a crystalline acid).

4 g. of pyrethrosin were added to 25 ml. of water containing 2.5 g. of sodium hydroxide. On warming on the steam bath the pyrethrosin dissolved and the solution became slightly yellow in color. After about fifteen minutes the solution was acidified to Congo with dilute sulfuric acid, the solution becoming colorless. The solution at this point smelled strongly of acetic acid.

The mixture was steam distilled, until the distilling liquid was no longer acid, the distillate neutralized with sodium hydroxide, and evaporated to dryness. This residue was dissolved in 20 ml. of 50 per cent ethanol and 1.5 g. p-phenylphenacyl bromide added. The mixture was refluxed for one hour and then cooled. The product was recrystallized from aqueous ethanol and melted 110-111°. A mixed melting point with known p-phenylphenacyl acetate (m.p. 111-112°) showed no depression.

The solution remaining from the steam distillation was concentrated on the steam bath under a water pump

vacuum to a small volume. Ethanol was added and the sodium sulfate which precipitated was filtered off. The solution was then evaporated almost to dryness and taken up in absolute ethanol and cooled. The precipitated sodium sulfate was again removed. The solution was then concentrated to an almost water-white syrup which was quite soluble in water, methanol, ethanol, ethyl acetate, and like solvents, but almost insoluble in benzene, ether, petroleum ether, chloroform and the like. All attempts to crystallize this syrup failed.

Attempts to make a methyl ester of the syrup through the use of diazomethane, dimethyl sulfate and alkali, and methyl alcoholic hydrochloric acid all failed. Likewise, no p-phenylphenacyl ester could be prepared.

Treatment with boiling acetic acid and with acetic anhydride in the presence of pyridine both failed to give back pyrethrosin or any other crystalline material. Likewise, no acetylation was obtained with magnesium metal and acetyl chloride.

Experiment II

4 g. of finely powdered pyrethrosin and 1.5 g. of sodium hydroxide were added to 50 ml. of water. This mixture was left standing for twenty-four hours with

occasional shaking. Some little material did not completely dissolve. This was filtered off and after recrystallization from alcohol proved by mixed melting point to be unchanged pyrethrosin. The solution was neutralized with dilute sulfuric acid and placed in a beaker in a desiccator over phosphorus pentoxide. This was left standing with frequent stirring until dry. No crystals were obtained although recrystallization of the syrup was attempted from many solvents.

Experiment III

Samples of pyrethrosin were saponified with alkali and the solutions obtained carefully evaporated to dryness. The syrups obtained were treated:

1. With methyl iodide in an attempt to make a methyl ester.
2. With acetic anhydride and pyridine in an attempt to acetylate an hydroxyl group which should be present after hydrolysis of a lactone, if present.
3. With dinitrophenylhydrazine in order to make a carbonyl derivative of a carbonyl group obtained by hydrolysis of an enol ester or lactone.

No products were obtained from these attempts.

Experiment IV

A number of quantitative saponifications were carried out. A weighed sample (.2 to .3 g.) of pyrethrosin was added to 10 ml. of standard alkali contained in a 125 ml. Erlenmeyer flask. The mixture was then warmed on the steam bath for the desired length of time or allowed to stand at room temperature. In some cases neutral alcohol was added to increase the solubility. At the end of the saponifying treatment the solution was cooled if necessary and the residual alkali titrated with standard acid. The results are given in Table IV.

Experiment V

Some quantitative experiments designed to show re-lactonization are given in Table V. Samples were completely saponified by warming on the steam bath for one hour. The solution was then titrated to neutrality. An excess of 10 ml. of standard acid was added and the solution left standing. At the end of the desired time the residual acid was determined.

TABLE IV

Quantitative Saponification Experiments on Pyrethrosin and Dihydropyretrosin

Run	Substance	Sample g.	Conditions of the saponification	Ml. .3250N* acid used	Ml. acid needed for 2 equiv.	Ml. acid needed for 1 equiv.	% of one equiv. used	% complete saponification
1	pyrethrosin	.2770	thirty minutes on the steam bath	5.12	5.36	2.78	-	96
2	"	.2376	ten minutes on the steam bath	4.02	4.58	2.29	-	88
3	"	.2297	two minutes on the steam bath	3.10	4.45	2.23	-	70
4	"	.2244	ten minutes cold (10 ml. alcohol added to aid soln.)	2.60	4.34	2.17	-	60
			then 10 ml. more alkali added, and retitrated	2.95	4.34	2.17	-	68
5	"	.2011	dissolve in 10 ml. alcohol, add alkali back titrate as rapidly as possible; 2½ min. required	2.00	3.90	1.95	103	51
6	"	.2049	repeat of 5 but only 1 min. req'd.	1.99	3.96	1.98	100	50
7	"	.2921	repeat of 5 but several min. req'd.	2.91	5.64	2.82	104	52
			then allowed to stand while end point shifted, ¼ hr.	2.80			99.5	50
8	#dihydro-pyrethrosin	.2287	repeat of 5, 5-6 minutes required	4.40	2.20	1.62	74	37
9	"	.2492	repeat of 8	4.78	2.39	1.82	76	38
10	"	.2486	repeat of 5, but end point obtained in 1 minute**	4.78	2.39	2.34	98	49
			end point changed rapidly, finally	4.78	2.39	1.87	78	39

* all calculations given in terms of acid.

** this titration probably accurate to only .05 ml.

the dihydropyretrosin used was the mixture obtained from hydrogenation.

TABLE V

Experiments on Relactonisation of Saponified
Pyrethrosin and Dihydropyrethrosin

Run	Substance	Sample g.	Time hrs.	ml. .3250 N acid used up	Acid Req'd. for one equiv.	% of one equiv.
1	pyrethrosin	.2299	1	.44	2.22	20
2	"	.2334	25	1.11	2.26	49
3	*dihydro- pyrethrosin	.2335	1	.21	2.26	9
4	"	.2533	19	.89	2.43	37

* The dihydropyrethrosin used was the mixture obtained
from hydrogenation.

Experiment VI

One g. of dihydropyrethrosin (the mixture obtained by hydrogenation) was treated with 1.5 g. of sodium hydroxide in 50 ml. of water. On warming on the steam bath the dihydropyrethrosin soon dissolved and the solution became a light yellow in color. After one hour the solution was worked up as in Experiment I for pyrethrosin. Acetic acid was identified as its p-phenylphenacyl ester and an uncrystallisable syrup was obtained. This syrup failed to add diazomethane, failed to give an ester with p-phenylphenacyl bromide, failed to go back to dihydropyrethrosin with acetic anhydride and pyridine, and failed to give a hydrazone with 2,4-dinitrophenylhydrazine.

Addition of Diazomethane to the Syrup from Alkaline
Saponification of Pyrethrosin

The syrup obtained from 15 g. of pyrethrosin by complete hydrolysis with alkali was treated with an ethereal solution of the diazomethane obtained from 20 g. of nitrosomethylurea. The syrup was not particularly soluble in ether but on standing twenty-four hours with frequent shaking and maceration gradually changed in appearance to a white amorphous looking material. The excess diazomethane was evaporated off with the ether. Recrystallization of the residue from slightly aqueous methyl alcohol gave about 2.5 g. of crude yellowish material. After several recrystallizations from the same solvent the melting point was 195-196^o with evolution of gas. Three more recrystallizations from aqueous methanol yielded a small amount (0.1 g.) of white material crystallizing as thin fragile needles.

m.p. (cap.) 197.5, m198.5-199 de

The technique for recrystallization was to dissolve the material in the smallest possible amount of boiling methanol and to add water drop by drop until a cloudiness began to appear. The solution was then allowed to stand in a current of air so that crystallization could take

place slowly. The material was dried for four hours in vacuo over phosphorus pentoxide at 100°.

m.p. (cap.) 195, m204-204.5de

4.304 mg. gave 2.755 mg. water and 9.890 mg. carbon dioxide

C = 62.71% H = 7.16%

3.090 mg. gave 2.020 mg. of water and 7.064 mg. carbon dioxide

C = 62.53% H = 7.31%

*3.933 mg. gave .311 ml. of nitrogen at 22.6° and 764 mm.

N = 9.18%

5.465 mg. gave .430 ml. of nitrogen at 22.5° and 765 mm.

N = 9.15%

Calculated for $C_{16}H_{24}O_5N_2$

C = 62.73% H = 7.24% N = 9.14%

It was later discovered that this product could be more satisfactorily recrystallized from propanol, the yellow color being removed more quickly. Attempts to obtain a saponification equivalent on this compound gave consistently high and scattered results depending on the boiling time. The longer the boiling, the higher

* The author is indebted to Mr. L. Parks for these analyses.

the result. Values of 349, 350, and 399, 398^{**} were obtained for a theory of 306.

Acetylation of the Diazomethane Addition Product
of the Syrup from Alkaline Saponification of Pyrethrosin

About 100 mg. of material constituting the second and third crops obtained in the purification of the diazomethane addition product of the syrup from saponified pyrethrosin were dissolved in 10 ml. of acetic anhydride to which 2 ml. of dry pyridine had been added. This mixture was left standing for five days, at the end of which time it was poured into a small amount of ice and water. A crystalline material precipitated out and was filtered off. This melted with bubbling from 173-181°. Two recrystallizations from aqueous ethanol gave about 25 mg. of fine needles. This was dried in vacuo for three hours at 100° over barium oxide.

m.p. (cap.) 178, 179-80°

4.116 mg. gave 2.430 mg. water and 9.313 mg. carbon dioxide

C = 61.75% H = 6.61%

3.923 mg. gave 2.349 mg. water and 8.840 mg. carbon dioxide

C = 61.49% H = 6.70%

** The author is indebted to Mr. L. Goldman for these analyses.

*2.78 mg. gave .191 ml. of nitrogen at 28.5° and 767 mm.

$$N = 7.83\%$$

1.85 mg. gave .131 ml. of nitrogen at 38° and 770 mm.

$$N = 7.83\%$$

Calculated for $C_{20}H_{26}O_6N_2$

$$C = 61.52\% \quad H = 6.71\% \quad N = 8.04\%$$

Calculated for $C_{18}H_{24}O_5N_2$

$$C = 62.06\% \quad H = 6.95\% \quad N = 7.17\%$$

* The author is indebted to Mr. A Sadle for the nitrogen analyses.

Acid Hydrolysis of Pyrethrosin

4 g. of pyrethrosin were added to 40 ml. of 50 per cent sulfuric acid and refluxed for one and one-half hours. The solution turned purplish, then yellowish, and gradually became darker as more and more charring occurred. At the end of the heating period, 25 ml. of water were added. About 30 ml. of liquid were distilled off. This distillate was neutralized with dilute sodium hydroxide and evaporated to dryness. 15 ml. of 50 per cent alcohol were added and the solution made just acid with concentrated hydrochloric acid. .3 g. of p-phenylphenacyl bromide were added and the solution was refluxed for one hour. The product obtained on cooling was filtered off and recrystallized from ethanol. It melted 112-113° and showed no depression of its melting point when mixed with a sample of p-phenylphenacyl acetate of melting point 111-112°.

Trans-esterification of Pyrethrosin

2 g. of pyrethrosin were added to 100 ml. of absolute methanol. To this was added several drops of a dilute solution of sodium ethylate. All of the pyrethrosin did not dissolve at once. This mixture was left standing for ten days with occasional shaking. After three days nearly all of the pyrethrosin had gone into solution. After the first day a strong odor of methyl acetate was noticeable.

At the end of the standing period the solution was concentrated under a water pump vacuum to about 10 ml. Water was added and the solution cooled. A small amount (less than .05 g.) of crystalline material was obtained that melted from 171-180°. After two recrystallizations from aqueous alcohol about 20 mg. of fine white needles were obtained. This was dried at 100° for two hours in vacuo over phosphorus pentoxide.

m.p. (cap.) 3181, m184-186

2.328 mg. gave 1.536 mg. of water and 5.632 mg. of carbon dioxide

C = 66.02% H = 7.40%

3.740 mg. gave 2.480 mg. of water and 8.993 mg. of carbon dioxide

C = 65.62% H = 7.42%

3.077 mg. gave 1.993 mg. of water and 7.397 mg. of
carbon dioxide

C = 65.50 % H = 7.25%

The residual solution worked up to a colorless
syrup which resisted all attempts at crystallization.
It failed to go back to pyrethrosin when treated with
acetic anhydride and pyridine.

Typical Pyrolysis of Pyrethrosin

A small quantity of pyrethrosin was placed in a
small distilling flask having a short fractionating side
arm containing a thermometer. The receiver was arranged
so that it could be cooled in ice water. Heat was
applied to the bath by means of a metal bath.

The pyrethrosin melted at a bath temperature of
about 210°. From 210 to 250° a small amount of clear liquid
boiling at 110-115° came off. This smelled strongly of
acetic acid and solidified in ice water (freezing point,
acetic acid, 18°). A second fraction boiling at 110-140°
was obtained when the bath temperature was raised to 300°.
This too smelled of acetic acid but was dark colored. When
the bath temperature was raised to 400° a quantity of
dark viscous oil distilling from 200-350° was obtained.

The residue on cooling solidified to a dark glassy solid.

The first fraction was converted to its p-phenylphenacyl ester and proved to be p-phenylphenacyl acetate, melting at 110-112°.

On converting the second fraction to its p-phenylphenacyl ester only that of acetic acid could be obtained, although several recrystallizations were required to obtain it pure.

The third fraction was redistilled under a water-pump vacuum. It boiled at 210-260°. The refluxing distillate was a greenish blue.

In one experiment the liquid distilling below 300° was not separated but converted directly to its p-phenylphenacyl ester. This ester crystallized in plates. It was recrystallized three times from aqueous alcohol and dried for four hours in vacuo over phosphorus pentoxide at 56°. About 10 mg. were obtained pure.

m. p. (cap.) 889, m91.5-92

2.636 mg. gave 1.269 mg. of water and 7.073 mg. of carbon dioxide

C = 73.22% H = 5.39%

2.619 mg. gave 1.256 mg. of water and 7.040 mg. of carbon dioxide

C = 73.35% H = 5.37%

Calculated for $C_{23}H_{30}O_5$ ($C_{12}H_9COCH_2OOCR$)

C = 73.45%

H = 5.36%

Attempts to obtain other esters from the mother liquor failed, as did attempts to obtain this ester in larger quantity.

Pyrolytic Distillation of Hydrogenated Pyrethrosin

Several grams of syrup obtained from a complete hydrogenation of pyrethrosin using Raney Nickel Catalyst at a pressure of about 10 atmospheres and a temperature of 60° were transferred to a small distilling flask with alcohol. The alcohol was removed under a water pump vacuum and a bath temperature up to 100° . After all of the solvent was removed the vacuum was broken and the bath temperature raised to $200-220^\circ$. This caused a small amount of material of boiling point $115-120^\circ$ to come off. This distillate solidified in ice water and was unmistakably acetic acid. A vacuum of 1-2 mm. of mercury was then applied. At a bath temperature of $210-240^\circ$ smooth boiling occurred with little or no charring. The distilling liquid boiled at $170-200^\circ$ and solidified to a clear glassy mass which resisted attempts at crystallization.

Reaction of Pyrethrosin with Alcoholic Ammonia

A saturated solution of ammonia in absolute ethanol was prepared by passing gaseous ammonia through absolute ethanol that was cooled in an ice-water bath for one hour. 4 g. of pyrethrosin were added to 300 ml. of the ammoniacal solution and left standing in the icebox. Overnight the pyrethrosin completely dissolved. After two days the solution was concentrated to a small volume and diluted with an equal volume of water. On standing, a small amount of crystalline material separated out. This was filtered off and amounted to about .2 g. After one recrystallization from alcohol the melting range was 216-218° with preliminary softening at 214°. Two more recrystallizations from alcohol gave about .05 g. of fine white needles. These were dried in vacuo at 100° for three hours over phosphorus pentoxide.

m.p. (cap.) s218, m220-221

4.449 mg. gave 2.956 mg. of water and 10.585 mg. of carbon dioxide

C = 64.93% H = 7.43%

4.598 mg. gave 3.032 mg. of water and 10.912 mg. of carbon dioxide

C = 64.70% H = 7.38%

Calculated for $2C_{17}H_{22}O_5 + NH_3$

C = 64.85% H = 7.52%

This compound showed a remarkable tendency to become charged when ground in a mortar. It gave no nitrogen test on fusion with sodium nor ammonia when warmed with concentrated alkali. Dumas' nitrogen determination did, however, show nitrogen to be present. The analyst reported that a white sublimate formed in the cool exit end of the combustion tube.

*4.89 mg. gave .113 ml. of nitrogen at 26.1° and 766.6 mm.

$$N = 2.66\%$$

9.06 mg. gave .195 ml. of nitrogen at 20.1° and 762.0 mm.

$$N = 2.41\%$$

Calculated for $2C_{17}H_{22}O_5 + NH_3$

$$N = 2.23\%$$

An attempt to prepare an acetate of this compound with acetic anhydride in the presence of pyridine failed.

The residual solutions were combined and evaporated down. On cooling a whitish oil came out of solution. This was taken up in alcohol, filtered and allowed to evaporate under a slow stream of air. A thick oil was again obtained and left standing. On observation several days later crystals had appeared. This substance melted

* The author is indebted to Mr. L. T. Crews for these analyses.

at 130-145°. After two recrystallizations from alcohol the melting range was 154-156°. From the filtrates a second crop was obtained which, after one recrystallisation from aqueous propanol, melted at 154-156°. The two crops were combined and recrystallized one time from cyclohexane. About 10 mg. of fine needles were obtained. This was dried for two hours in vacuo at 78° over phosphorus pentoxide.

m.p. (cap.) 155-156

2.255 mg. gave 1.538 mg. of water and 5.334 mg. of carbon dioxide

C = 64.55% H = 7.63%

2.059 mg. gave 1.396 mg. of water and 4.824 mg. of carbon dioxide

C = 65.94% H = 7.59%

A second attempt to obtain this product in larger quantity failed. Seeding of the oil obtained from the treatment failed to produce crystals.

The residual solutions were worked up to a thick syrup which failed to give off ammonia on warming with concentrated alkali and from which no dinitrophenyl hydrazone could be prepared. An attempt to acetylate the syrup with acetic anhydride and pyridine also failed.

Addition of Diazomethane to Pyrethrosin

3 g. of pyrethrosin were dissolved in 50 ml. of pyridine. To this was added the ether solution of the diazomethane prepared from 5 g. of nitrosomethylurea. As the ethereal solution was slowly added the color of the diazomethane disappeared and a white precipitate settled out. Soon an excess of diazomethane was present and the solution took on a light yellow color. After all of the diazomethane was added the mixture was allowed to stand for five hours at room temperature. The excess diazomethane was then driven off by warming in the hood and the precipitate removed by filtration. About 2.3 g. of product melting about 195° with decomposition was obtained. This material was recrystallized from ethanol (about 500 ml. were required), after which it melted at $196-197^{\circ}$. It crystallized in long, thin, white needles. A small sample was again recrystallized from ethanol and dried for three hours in vacuo at 100° over phosphorus pentoxide.

m.p. (cap.) 193 , 196.5 de

4.136 mg. gave 2.587 mg. water and 9.431 mg. of carbon dioxide

C = 62.22% H = 7.00%

4.303 mg. gave 2.637 mg. water and 9.758 mg. of
carbon dioxide

C = 61.88% H = 6.86%

Calculated for $C_{18}H_{24}O_5N_2$

C = 62.03% H = 6.95%

This product failed to react with acetic anhydride
in the presence of dry pyridine at room temperature. The
material was recovered unchanged.

The filtrate from the insoluble product above was
concentrated, taken up in alcohol and reconcentrated to
a small volume. About .5 g. of white crystalline
material melting at 120-125° was obtained. This was
recrystallized from ethanol (about 25 ml. were required).
It then seemed to melt at 118-123° but to a semi-liquid
which became clear with bubbling at 165-169°. The
material was recrystallized four more times from ethanol,
each recrystallization raising the temperature at which
the preliminary melting occurred until finally a pure
substance was obtained. This was dried three hours in
vacuo at 100° over phosphorus pentoxide.

m.p. (cap.) 168-169°

3.811 mg. gave 2.381 mg. of water and 8.706 mg.
of carbon dioxide

C = 62.33% H = 6.99%

4.393 mg. gave 2.730 mg. of water and 10.009 mg. of carbon dioxide

C = 62.18% H = 6.95%

Calculated for $C_{18}H_{24}O_5N_2$

C = 62.06% H = 6.95%

Decomposition of More Insoluble Diazomethane

Addition Product of Pyrethrosin

One-tenth g. of the more insoluble diazomethane addition product of pyrethrosin were placed in a small test tube and slowly heated over a free flame. On melting the substance degassed to a viscous oil. After allowing to cool, this oil was taken up in ethanol and thrown out with water. The crude material obtained melted at 125° with preliminary softening at 110° . Two recrystallizations from aqueous alcohol gave about 15 mg. of pure product. This was dried for two hours in vacuo at 78° over barium oxide.

m.p. (cap.) 118, 133-135

3.219 mg. gave 2.160 mg. of water and 7.935 mg. of carbon dioxide

C = 67.27% H = 7.51%

2.256 mg. gave 1.520 mg. of water and 5.573 mg. of carbon dioxide

C = 67.41% H = 7.54%

Calculated for $C_{18}H_{24}O_5$

C = 67.46% H = 7.55%

HYDROGENATION OF PYRETHROSIN

The apparatus used throughout was one of the standard assemblies built by the American Instrument Company, Silver Spring, Maryland, for pressure hydrogenations. It consisted mainly of bombs, bomb holder with heater and a shaker assembly for the bomb holder. Temperature was controlled and observed by means of a Brown automatic potentiometric controller and a thermocouple inserted in the bomb wall. Pressure could be followed both by the main gauge on the shaker assembly, through which all hydrogen entered, and a smaller gauge which could be inserted in the line before the inlet to the bomb.

Experiment I (Preliminary)

10 g. of pyrethrosin was added to 300 ml. of absolute alcohol in a one liter bomb. Approximately 1 g. of Raney Nickel Catalyst prepared under absolute alcohol was added. The hydrogenation was run for one hour at room temperature and a pressure of 100-125 atmospheres. There was a rapid drop of several atmospheres at the beginning of the period.

The material that came out of the bomb was all

in solution. This solution, after filtering off the catalyst, was concentrated to a small volume. After cooling and standing for several hours, 4.5 g. of crystalline material melting at 190-207° was obtained. This material was recrystallized three times from ethanol and dried for three hours in vacuo at 100° over phosphorus pentoxide.

m.p. (cap.) 196, m206-211

m.p. (stage) m170-204

4.033 mg. gave 2.828 mg. of water and 9.786 mg. of carbon dioxide

C = 66.22% H = 7.85%

Calculated for $C_{17}H_{24}O_5$

C = 66.21% H = 7.85%

The residual solution was worked up to a thick syrup from which no other crystalline material could be obtained. An attempt to fractionate the crystalline material was made, using the melting point as the criterion of separation. This, however, was not successful. After the first few crystallizations the direction of change of the melting point could not be predicted. Combination of samples of like melting point would, in one instance, give a higher melting point, while in others, a combination of samples of the same melting point as before would give a lower melting point.

Experiment II

10 g. of pyrethrosin was added to 300 ml. of absolute alcohol on a 1 liter bomb. 1.5 g. of copper-chromite catalyst was added. The hydrogenation was run at 175° for five and one-half hours at about 175 atmospheres. The solution which came out of the bomb was green in color. It worked up to a thick greenish material from which no crystalline material was isolated.

Experiment III

This experiment was run in a bomb in which .0135 moles of acetone required a pressure drop of 60.5 pounds of hydrogen to give isopropyl alcohol mole for mole under the same conditions.

2.5 g. (.00816 moles) of pyrethrosin was added to 40 ml. of absolute alcohol in a 120 ml. bomb along with approximately 1 g. of Raney Nickel Catalyst. The hydrogenation was started with a pressure of 109 pounds. After two hours at room temperature the pressure had dropped to 70.5 pounds and the rate of adsorption had become almost nil.

Hydrogen used up by pyrethrosin	38.5 lbs.
Hydrogen required if pyrethrosin took up hydrogen mole for mole	37.0 lbs.

The pressure was then raised to 120 pounds and the temperature allowed to rise to 60°. At the end of six hours the rate of drop had become almost zero. After cooling to room temperature again the pressure read 45.5 pounds.

Total hydrogen used up by pyrethrosin	123.0 lbs.
Hydrogen required if one mole of pyrethrosin took up three moles of hydrogen	121.0 lbs.

On working up the product only a clear water-white syrup was obtained that resisted crystallization.

Experiment IV

This experiment was run in a bomb in which .0135 moles of acetone required under the same conditions a pressure drop of 50.0 pounds of hydrogen to give isopropyl alcohol mole for mole.

2.5 g. (.0082 moles) of pyrethrosin was dissolved in 40 ml. of dioxane in a 120 ml. bomb. Approximately 1 g. of Raney Nickel Catalyst was added. In six hours at room temperature the pressure drop was from 123.5 to 96.0 pounds.

Hydrogen used up	27.5 lbs.
Hydrogen required if being taken up mole for mole	28.0 lbs.

The temperature was then raised to 100° and the reaction allowed to proceed for six more hours. On cooling to room temperature the pressure was 56.0 pounds.

Hydrogen used up 67.5 lbs.

Hydrogen required if
pyrethrosin took up $2\frac{1}{2}$ moles
per mole 70 lbs.

Working up of this material gave only a syrup and no crystalline material.

Experiment V

Experiment IV was repeated using alcohol as a solvent. In a similar manner, approximately 2.5 moles of hydrogen were taken up. However, on working up the solution, almost a gram of crude dihydro material was obtained.

Experiment VI

This hydrogenation was run in a bomb calibrated with acetone under the same conditions. .0135 moles of acetone required a pressure drop of 50.0 pounds to give isopropyl alcohol mole for mole.

5 g. (.016 moles) was dissolved in 40 ml. of dioxane in a 120 ml. bomb. About 2 g. of Raney Nickel Catalyst

prepared under dioxane was added. During five hours at room temperature a pressure drop from 120 to 60 pounds was observed.

Hydrogen used up	60 lbs.
Hydrogen required by .016 moles of pyrethresin taking up hydrogen mole for mole	58 lbs.

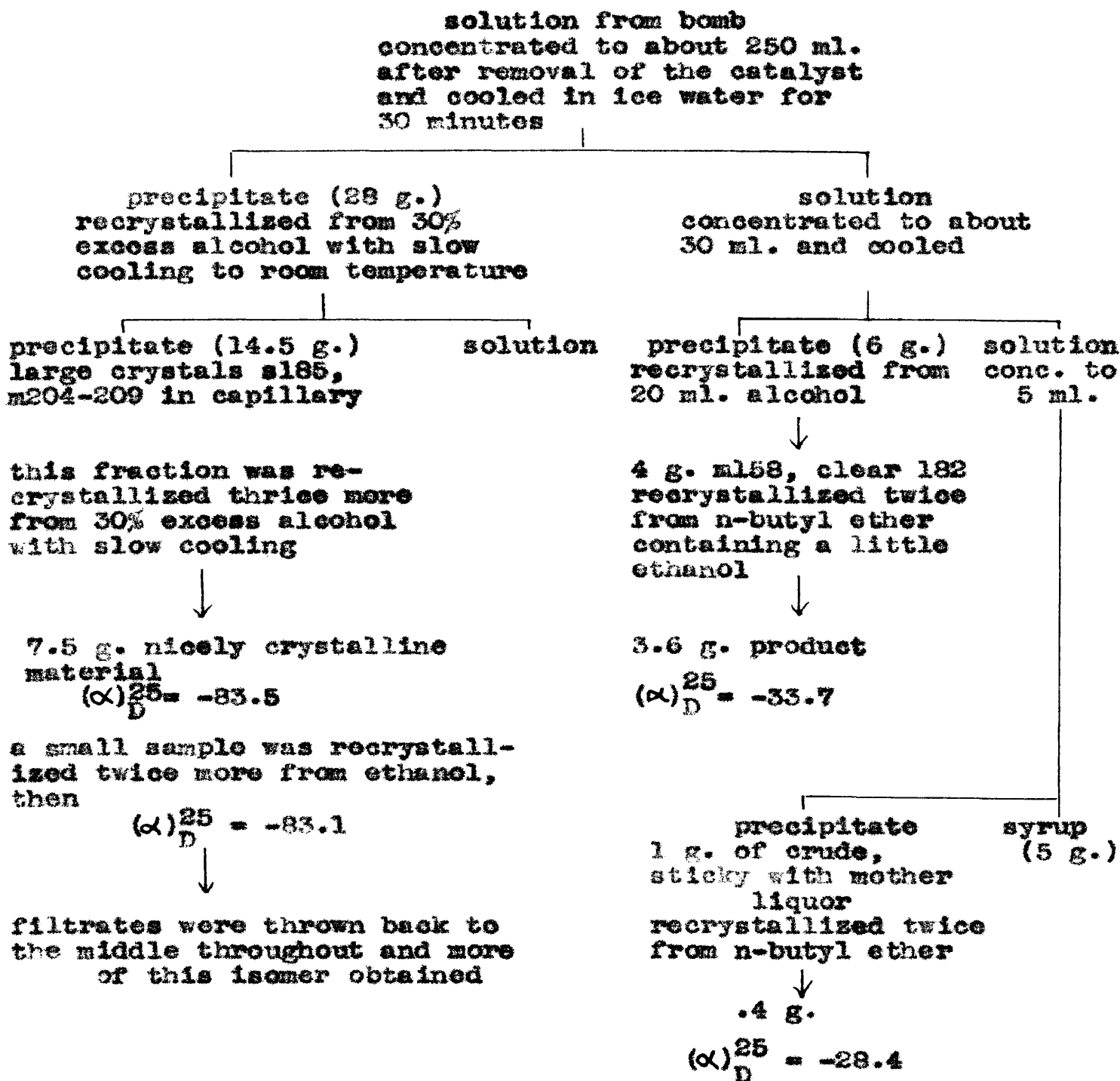
After the catalyst was filtered off, the solution was taken up in a large quantity of alcohol and then concentrated to about 30 ml. This was allowed to cool slowly. About 2 g. of large rod-like crystals were removed by filtration. This was called fraction I. The residual solution was then cooled in an ice bath and about 1 g. of crystals called fraction II was obtained. The mother liquor was then concentrated to a small volume and on cooling yielded about 1 g. of fraction III. The following observed rotations were obtained using as nearly as practicable the same concentration of material in chloroform (about .35 g. per 25 ml.). Also given are the approximate capillary melting points.

Fraction	Observed rotation	Melting point
I	2.00°	s195, m200-205
II	1.25°	s160, semi-liquid 188, clear 202
III	0.75°	s150, semi-liquid 160, clear 182

As it appeared that rotation was a surer method of following a separation than melting point as tried in Experiment IV, this experiment was repeated using 40 g. of pyrethrosin. The simplified fractionation scheme is given in Figure 2. It was also found that observation of the crystalline form was of value in following the fractionation, particularly in determining the purity of the more insoluble form. All rotations are for solutions in chloroform of about the same concentration (about .35 g. in 25 ml.).

FIGURE 2

Simplified Chart of the Fractionation Carried
out on Product of Hydrogenation Experiment V



By repeated removal of the more insoluble form from the precipitates, a fraction was obtained that soon reached constant rotation. This fraction, if allowed to cool slowly, forms long hexagonal rods. Crystals 12 mm. long and 1.5 mm. in thickness are readily obtained. Several grams of this material were dried in vacuo for two hours at 100°.

m.p. (cap.) 175, 195-200 to semi-liquid, clear at 202
 m.p. (stage) 165, considerable liquid forming at 180,
 clear at 198

4.303 mg. gave 3.021 mg. of water and 10.467 mg. of
 carbon dioxide

C = 66.38% H = 7.87%

4.199 mg. gave 2.973 mg. of water and 10.195 mg. of
 carbon dioxide

C = 66.26% H = 7.92%

Calculated for $C_{17}H_{24}O_5$

C = 66.21% H = 7.85%

.3310 g. in 25 ml. of chloroform rotated the plane
 of polarized light -2.21° when used in a two decimeter
 tube at 25° with a sodium vapor lamp as light source.

$(\alpha)_D^{25} = -83.5$ ($c = 1.32$ in chloroform)

.2335 g. used up 7.63 ml. of .20 N alkali in a
 saponification experiment. Saponification equivalent
 = 153.1.

.2533 g. used up 8.18 ml. of .20 N alkali in a saponification experiment. Saponification equivalent = 155.0.

Calculated for $C_{17}H_{24}O_5$

Saponification equivalent = 154.2.

Combination of the filtrates from the more insoluble form and continuous removal of that form from them failed to give, from the mother liquors, a precipitate of minimum rotation in the opposite direction. The lowest rotation observed was $(\alpha)_D^{25} = -22.7$, and this on a fraction obtained in the middle of the fractionations to the extent of about .5 g. The direction of change of rotation on recrystallization of a sample could not be predicted. Ethanol, n-dibutyl ether, and dioxane were the only solvents from which satisfactorily crystalline material could be obtained readily. In all of these the one form was more insoluble than the other. In all, some thirty-five recrystallizations were made. For purposes of comparison, several grams of an intermediate fraction were prepared and dried.

m.p. (cap.) s155, m160, clear 172

m.p. (stage) s148, m162-167, clear 185

3.439 mg. gave 2.422 mg. of water and 8.362 mg. of carbon dioxide

C = 66.35%

H = 7.88%

2.832 mg. gave 2.005 mg. of water and 6.842 mg.
of carbon dioxide

C = 65.93% H = 7.92%

Calculated for $C_{17}H_{24}O_5$

C = 66.21% H = 7.85%

.3425 g. in 25 ml. of chloroform rotated the plane
of polarized light -1.02° when used in a two
decimeter tube at 25° with a sodium vapor lamp for
light source.

$(\alpha)_D^{25} = -37.2$ ($c = 1.37$ in chloroform)

Phthalic Monoperacid Oxidation of Pyrethrosin
and Dihydropyrethrosin

A sample (3-.4 g.) of the substance to be oxidized was weighed out into a 25 ml. volumetric flask. To this was added 10 ml. of a phthalic monoperacid solution in dioxane (about .05 N.) prepared according to the directions of Böhme (1). The flask was shaken to dissolve the solid and then allowed to stand the desired length of time at room temperature. The solution was then made up to volume with pure dioxane and a 5 ml. aliquot taken. This was added to 30 ml. of a 20 per cent potassium iodide solution in a 125 ml. Erlenmeyer flask and allowed to stand for ten minutes with occasional shaking. The liberated iodine was then titrated with standard sodium thiosulfate. Other 5 ml. aliquots were titrated at later times. Since the peracid solution deteriorates on standing the strength of the solution used was determined from a control experiment carried through the same procedure. The difference between the amounts of thiosulfate used for titration of the control and the sample is a measure of the peracid used up by the substance being studied. Cinnamyl alcohol was used as a known. The dihydropyrethrosin used in this experiment was the more insoluble isomer (α)_D^{25°} = -83.6 (n_D = 1.32 in chloroform). Table VI gives the results.

TABLE VI
Phthalic Monoperacid Oxidation of Pyrethrosin
and Dihydropyrethrosin

Run sample g.	Cinnamyl alcohol		Pyrethrosin			Dihydro- pyrethrosin*		
	II	III	I	II	III	I	II	III
	.2920	.2148	.3844	.4093	.3826	.4016	.3111	.2944
Time in hrs.	moles of peracid used per mole sample							
1.5	-	.92	-	-	.10	-	-	.21
6.0	-	.97	-	-	.55	-	-	.37
20.5	1.01	-	-	.74	-	-	.74	-
21.5	-	-	.72	-	-	.73	-	-
28.75	-	.98	-	-	.60	-	-	.50
45.0	-	-	.75	-	-	.75	-	-
67.5	-	-	.81	-	-	.44	-	-
73.5	.65	-	-	.63	-	-	.44	-
74.5	-	.31	-	-	-	-	-	-
90.0	-	-	.63	-	-	.20	-	-

* More insoluble isomer (α)_D²⁵ = -83.5 (c = 1.32 in
chloroform)

Product from the Phthalic Monoperacid Oxidation of
Pyrethrosin

The residual solutions (containing about 0.3 g. pyrethrosin) from the phthalic monoperacid oxidation studies on pyrethrosin were combined and left standing until all of the peracid was decomposed. The solution was then concentrated to dryness under a current of air at room temperature. The crystals so obtained consisted mostly of phthalic acid. This material was extracted several times with boiling chloroform, the phthalic acid being very slightly soluble. The chloroform extracts were combined and evaporated to dryness. The syrup obtained crystallized readily from ethanol. After two recrystallizations from ethanol its melting point was constant. Its behavior on melting was very much like pyrethrosin itself. The product (50 mg.) was dried in vacuo for two hours at 78° over barium oxide.

m.p. (cap.) 212, m215-217 with final clearing at 233

3.116 mg. gave 1.892 mg. of water and 7.207 mg. of carbon dioxide

C = 63.12% H = 6.80%

3.267 mg. gave 2.048 mg. of water and 7.568 mg. of carbon dioxide

C = 63.23% H = 7.01%

Calculated for $C_{17}H_{22}O_6$

C = 63.34% H = 6.88%

Product from Phthalic Monoperacid Oxidation of
Dihydropyrethrosin

This product was quite difficult to purify and could not be obtained from the residual solutions from the oxidation studies.

0.5 g. of dihydropyrethrosin (α) $D_{25}^{25} = -83.5$ ($c = 1.32$ in chloroform) and 18 ml. of the peracid solution were mixed and left standing until all of the peracid was decomposed (several days). The solution was then worked up according to the same procedure as used above for the similar solution obtained from pyrethrosin. The crystalline product obtained required six recrystallizations from aqueous propanol before its melting point became constant. About 20 mg. of fine white needles were obtained. These were dried in vacuo for one and one-half hours at 100° over barium oxide.

m.p. (cap.) s221, m228-230d

2.866 mg. gave 2.019 mg. of water and 6.829 mg. of carbon dioxide

C = 65.27% H = 7.86%

3.211 mg. gave 2.197 mg. of water and 7.686 mg. of carbon dioxide

C = 65.32% H = 7.65%

From the mother liquor was obtained a small amount of unreacted dihydropyrethrosin.

The Determination of Active Hydrogen Atoms and
Carbonyl Groups in Pyrethrosin

The active hydrogen atoms and carbonyl groups present in pyrethrosin were determined by using the modified Zerevitinoff apparatus of Kohler, Stone, and Fuson (17). In addition to the Grignard reagent in di-n-amyl ether and the pyrethrosin, pyridine to the amount of 5 ml. was added to increase the solubility. A blank determination using pyridine was run to obtain the correction necessary because of the pyridine and to obtain the strength of the Grignard solution being used. The data are given in Table VII.

TABLE VII

Determination of Active Hydrogen Atoms and Carbonyl
Groups in Pyrethrosin

Run	I	II	III	IV
Time of heating for reaction with active hydrogen atoms in minutes	10	20	20	20
millimoles pyrethrosin	.318	.676	.638	.569
millimoles active hydrogen atoms	.206	.612	.496	.478
millimoles active hydrogen atoms per millimole pyrethrosin	.649	.905	.775	.840
millimoles Grignard reagent consumed by carbonyls	.643	1.14	1.07	-
millimoles of carbonyl groups per millimole pyrethrosin	2.02	1.68	1.68	-
total millimoles Grignard reagent used per millimole pyrethrosin	2.67	2.59	2.46	-

Negative Experiments carried out in Attempts to
Make Carbonyl and Hydroxyl Derivatives of
Pyrethrosin and Dihydropyretrosin

The following methods have failed to yield hydroxyl derivatives with both pyrethrosin and dihydropyretrosin:

1. Phenyl isocyanate in absolute chloroform.
2. Metallic sodium.
3. Diazomethane.
4. Acetic anhydride and pyridine.

In addition, the following methods have failed with pyrethrosin only:

1. Acetic anhydride and anhydrous sodium acetate.
2. Magnesium and acetyl chloride.
3. The reaction product of pyrethrosin and methyl magnesium iodide plus acetic anhydride.

The following methods have failed to yield carbonyl derivatives of both pyrethrosin and dihydropyretrosin:

1. 2,4-dinitrophenylhydrazine and hydrochloric acid in ethyl cellosolve.
2. Hydroxylamine hydrochloride and sodium acetate.
3. Hydroxylamine hydrochloride, trace of pyridine in ethanol solution in a sealed tube at 100°.
4. Semicarbazide hydrochloride and pyridine in ethanol.
- * 5. Hydrazine hydrate and alkali.

Many of the above experiments have been repeated several times with slight variations in conditions.

* Only tried for pyrethrosin.

Product from an Attempt to make an Oxime of
Dihydropyrethrosin

.5 g. of dihydropyrethrosin (the pure isomer) and .5 g. of hydroxylamine hydrochloride were added to 10 ml. of ethanol containing about 1 ml. of pyridine in a pyrex tube of about 15 mm. external diameter and 30 cm. long which had been sealed at one end. After cooling in an ice-water bath this tube was sealed off at a length of about 20 cm. and then heated for three hours at 100°. At the end of the heating period the tube was again cooled and then carefully opened. After removal, the solution was evaporated to dryness, the residue taken up in alcohol and evaporated again until crystals began to appear. After cooling, the crystals were filtered off. These melted at 213-218° with preliminary softening at 198°. After two recrystallizations from benzene about fifteen mg. of flaky white plates were obtained. The sample was dried in vacuo for two hours at 100° over barium oxide.

m.p. (cap.) s216, m219-221

3.554 mg. gave 2.534 mg. of water and 8.594 mg. of carbon dioxide

C = 65.99% H = 7.97%

2.717 mg. gave 1.932 mg. of water and 6.609 mg. of carbon dioxide

C = 66.38% H = 7.96%

Calculated for $C_{17}H_{24}O_5$

C = 66.21% H = 7.85%

6.934 mg. in 1.503 ml. of chloroform gave an observed rotation of $+.02^\circ$ when read in a one decimeter semi-micro polarimeter tube at 26° using a sodium vapor lamp as light source.

$$(\alpha)_D^{26} = +.4 \text{ (c = .47 in chloroform)}$$

The same product was also obtained from an attempt to make a semicarbazone of dihydropyretrosin using semicarbazide hydrochloride and pyridine in ethanol. A mixed melting point of the two products showed no depression.

CONCLUSIONS

Pyrethrosin is a white, optically active, crystalline compound with a decidedly bitter taste. In solution it is neutral to litmus and does not take up alkali. The crystals have an octahedral shape, but on casual observation appear to be diamond-shaped (see Frontispiece). Pure pyrethrosin melts in a capillary at $200-201^{\circ}$ when the rate of heating is fairly rapid (ten to fifteen degrees a minute), until the neighborhood of the melting point is reached. If heated slowly, the material slowly changes to a glassy-like mass which refused to become a liquid even at 300° . If observed under the microscope softening occurs at 197.5° and the melting range is $199.5-202^{\circ}$. This is typical of the differences in melting points taken by the capillary and hot stage methods. Preliminary changes which occur are much more easily seen and the end of melting is much more accurately determined by the microscope method. The criterion of complete fusion in the microscope method is the disappearance of the property of double refraction in polarized light.

Pyrethrosin above its melting point rapidly assumes a yellowish appearance and decomposes with

bubbling. Melted pyrethrosin will not solidify to a crystalline material.

Rose and Haller (21) reported a form of pyrethrosin melting at 178° if ethanol was used as the recrystallization solvent. The normal form melting at $200-201^{\circ}$ was obtained by them when ethyl acetate was used. In the present work only the high melting form has been obtained from a variety of solvents, ethanol, absolute ethanol, pyridine, acetic anhydride, chloroform, ethyl acetate, and dioxane.

This leads to the speculation that the compound worked on by Rose and Haller was in some way different from pyrethrosin. The sample worked on by them was obtained only in small quantity from pyrethrum extracts of their own, whereas the sample now being studied was obtained from a commercial source. Purification of the two samples was brought about, however, in the same general way. At least one worker in the same laboratory (private communication from M. S. Schechter) has since failed to obtain the low melting form. Examination in our laboratory of Rose and Haller's low melting sample (supplied by the Government laboratory) revealed a melting range of $183-198^{\circ}$. Recrystallization of the sample from ethanol removed a slightly yellow color and showed the material to be identical with the ordinary

high melting form. This of necessity leads to the conclusion that there is only one form of pyrethrosin.

Pyrethrosin contains only the elements carbon, hydrogen, and oxygen. A comparison of combustion, equivalent weight and molecular weight data found for pyrethrosin with those calculated for various theoretical possibilities is given in Table VIII. Examination shows at once that the formula is $C_{17}H_{22}O_5$.

Although any of the formulas $C_{17}H_{20-24}O_5$ are possible from the molecular weight and saponification data, only $C_{17}H_{22}O_5$ agrees for the combustion data. In a similar way, $C_{34}H_{44}O_{10}$ gives good agreement for combustion and saponification data, but does not agree with the molecular weight data. The formula $C_{17}H_{22}O_5$ is further confirmed by combustion and saponification data for dihydropyrethrosin which are also included in Table VIII, and by combustion data for other derivatives.

It is difficult to run fast molecular weight determinations on pyrethrosin, as the compound probably associates and/or polymerizes to give high values. The longer the period of heating, the higher the results. The value given, 305, was the lowest or limiting value obtained.

Rose and Haller also arrived at a molecular formula of $C_{17}H_{22}O_5$ by a similar series of determinations.

TABLE VIII
 Comparison of Combustion, Saponification Equivalent,
 and Molecular Weight Data for Pyrethrosin and Di-
 hydro-pyrethrosin with Those of Various
 Probable Formulae

Substance	Molecular weight	% C	% H	Saponification equivalent
$C_{17}H_{20}O_5$	304.33	67.09	6.62	152.2
$C_{17}H_{22}O_5$	306.35	66.65	7.24	153.2
Pyrethrosin	305	66.54	7.28	153.9
$C_{17}H_{24}O_5$	308.36	66.21	7.85	154.2
Dihydro- pyrethrosin	-	66.31	7.81	154.1
$C_{17}H_{26}O_5$	310.38	65.78	8.44	155.2
$C_{34}H_{44}O_{10}$	612.70	66.65	7.24	153.2

Early Attempts to Show Pyrethrosin to be a Flavone-
like Substance Containing a Pyrone Nucleus

Although not a colored substance, there were early indications based on the preliminary work of Rose and Haller (21) that pyrethrosin might be related to the flavones, i.e., might contain a pyrone nucleus. Thus, an alkali fusion using the general directions that had been used by Karrer (16) for splitting certain anthocyanidins was carried out in an attempt to split the molecule into known phenolic and acidic substances. No typical clean-cut reaction was obtained and no products were identified. In all probability a great deal of the decomposition which occurred was due to the heating alone and not to the combination of heat and alkali.

When pyrethrosin is warmed with aqueous hydrochloric acid stronger than 15 per cent, a variety of colored solutions can be produced depending upon the length of time involved. On standing or on diluting with water these solutions all yield yellowish amorphous precipitates. Attempts to purify these materials by recrystallization were fruitless. However, analysis of a carefully washed and dried sample showed only traces of halogen to be present when it was expected that the

compound might be a salt of the oxonium type. Carbon and hydrogen determinations best fit the formula $C_{17}H_{22}O_5$, that of pyrethrosin.

Attempts to make an oxonium salt by the procedure used by Karrer (16) for making pyryllium salts of the flavones failed. When flavones are warmed in 8 per cent hydrogen chloride solution in dry methanol and the resulting solution diluted with ether, a colored precipitate of an amorphous oxonium salt is obtained. Although these materials can not be recrystallized, analysis of a carefully prepared and dried sample gave values indicated by the theory. Pyrethrosin was recovered from such a treatment unchanged.

Pyrethrosin also fails to give positive reactions with lead acetate, tartaric acid and ferric chloride reagents. These are standard (12) color and precipitation tests for pyrones.

Some of the tests for anthocyanidins and anthocyanins were also tried but these gave a mixture of conflicting indications.

The conclusion to be drawn, of course, is that pyrethrosin is not a flavone-like material. In all probability the amorphous materials obtained from the hydrochloric acid treatment are polymers or isomers of pyrethrosin.

Pyrethrosin, An Ester of Acetic Acid

Pyrethrosin readily reacts with dilute alkali to show a saponification equivalent of 154, half of its molecular weight. Hydrolysis is approximately 72 per cent complete after two minutes warming with alkali and 88 per cent after ten minutes. Twenty minutes heating shows the reaction to be 95 per cent complete while thirty to sixty minutes heating gives complete saponification.

One of the products of alkaline hydrolysis is readily identified as acetic acid. This immediately suggests the presence of an acetyl group. In the light of the experience of Rose and Haller (21), who found acetyl values above and below, but still in the neighborhood of one per molecule by different methods, and in the light of the lability of the molecule to acidic and alkaline reagents, no acetyl determinations were run. The lability of the pyrethrosin molecule to alkali is indicated by the attempts to isolate a second product or products from the alkaline hydrolysis. Although Rose and Haller (21) report the isolation of an acid $C_{15}H_{24}O_6$ that was difficult to crystallize, so far in the present work no crystalline product has been obtained from working up the residues from saponification. In

all cases, and many attempts have been made, only a thick syrupy material has resulted. Many attempts have been made to acetylate this material back to pyrethrosin or a pyrethrosin-like substance but with no success, perhaps the failure being as much due to the fact that the starting material is probably a complex mixture rather than to the lack of a hydroxyl or enol capable of being acetylated.

Many attempts have also been made to isolate a product from hydrolysis through the hydroxyl and/or carboxyl that might be liberated by the second equivalent of alkali used up. It was one of these attempts that yielded a product which helps to establish an acetyl as being present in pyrethrosin. The syrup obtained from alkaline hydrolysis was treated with diazomethane in the hope of obtaining a methyl ester. This treatment led to a crystalline product which was looked upon for a time as the desired methyl ester of an acid obtained by hydrolysis. Although on melting this product degassed to a thick glassy-like mass, as would be expected of a pyrazoline obtained by addition of diazomethane to a double bond, no test for nitrogen was obtained from a sodium fusion of the material, although the test was repeated carefully several times. However, ignition with copper oxide in a closed system did indicate

nitrogen to be present and carbon, hydrogen and nitrogen determinations soon showed the formula to be $C_{16}H_{22}O_4N_2$.

The theoretical addition of diazomethane to pyrethrosin would produce a compound of formula $C_{18}H_{24}O_5N_2$. The difference between this formula and $C_{16}H_{22}O_4N_2$ is C_2H_2O . C_2H_2O is the difference between an acetylated and a free hydroxyl group. The reactions of pyrethrosin to produce $C_{16}H_{22}O_4N_2$ would then be:

pyrethrosin plus water minus acetic acid plus diazomethane gives the diazomethane addition product of an alcohol obtained from pyrethrosin by hydrolytic removal of an acetyl group.



The low yields obtained in this treatment help to confirm the suggestion that the original syrup was a mixture and not a pure substance. That this must be so is readily shown by the fact that no second equivalent of alkali has been used up in the formation of this product.

Acetylation of this product gives a crystalline material melting at 179-180°. Analysis, however, does not show whether it is a monoacetate or diacetate. Carbon and hydrogen values agree very well for a diacetate but nitrogen values are low for a monoacetate, and much too high for a diacetate. Saponification equivalents on these materials are worthless. The parent substance $C_{16}H_{22}O_4N_2$ gave inconsistently high results depending

upon the length of time boiled. The acetate obtained, however, if a monoacetate, is not identical with either of the diazomethane addition products of pyrethrosin itself which have the same empirical formula.

Having thus obtained some evidence for an acetyl group in pyrethrosin other evidence was sought for and found.

Two attempts to hydrolyze pyrethrosin to acetic acid with five per cent sulfuric acid failed. When heated with sulfuric acid of this concentration, the crystals of pyrethrosin disappeared with the formation of an insoluble oil. This oil and surrounding solution gradually took on a yellowish color as heating was continued. If some of the solution was diluted with alcohol a yellowish amorphous precipitate was obtained. This precipitate was similar to the product obtained by treating pyrethrosin with hydrochloric acid, and could not be recrystallized. Steam distillation of the mixture to remove acetic acid was carried out, the distillate neutralized and evaporated to dryness and the residue thus obtained treated with p-phenylphenacyl bromide in alcohol in an attempt to obtain the p-phenylphenacyl ester of acetic acid. On working up the residue from the steam distillation a yellowish glassy-like resin

was obtained. This resin resisted all attempts at crystallization, first forming an oily tar that then reresinified.

An attempt to hydrolyze pyrethrosin with hot 50 per cent sulfuric acid, however, did yield acetic acid. These conditions produced considerable charring and doubt may be expressed as to whether or not the acetic acid resulted by simple hydrolysis. In view of the fact that in many cases the equilibrium in the reaction:



lies well to the right in the presence of acid, the failure of pyrethrosin to hydrolyze readily in dilute acid in the absence of special efforts to displace the equilibrium is not unexplainable.

When pyrethrosin is added to absolute methyl alcohol to which a trace of sodium ethylate has been added, most of the sample dissolves on standing overnight and cannot be recovered as pyrethrosin. The odor of methyl acetate is unmistakable. One experiment performed in an attempt to drive off the methyl acetate, saponify it and identify the acetic acid produced, failed. The major product of such a treatment is a syrup which yields a very small amount of crystalline material which on the basis of carbon and hydrogen determinations is no expected compound.

also, no acceptable empirical formula can be calculated from the data. Attempts to convert this syrup back to pyrethrosin all failed. The trans-esterification, however, which came about, is evidence for an ester linkage in the molecule.

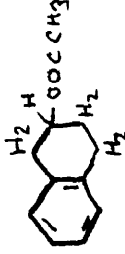
Several pyrolytic experiments were carried out in an attempt to obtain simple degradation products whose identification would offer an insight into the fundamental carbon skeleton of pyrethrosin. When pyrethrosin is heated just above its melting point it degases to a resinous tar and a small amount of liquid distills off. The major part of this liquid is acetic acid. However, in all probability the distillate contains a small amount of pyrethrosin carried over by sublimation and other degradation products. In one experiment, instead of isolating the p-phenylphenacyl ester of acetic acid, the p-phenylphenacyl ester of another acid was obtained in small quantity. Combustion data alone indicate this ester to be derived from an acid $C_9H_{10}O_4$, but no support has been obtained for this as attempts to obtain the material again have failed.

If the residue from the distillate is raised to about 300° a thick viscous material distills off. On cooling, this solidifies to a clear glass that resists all attempts at crystallization. Nothing is known as to the nature of this material except that it appears to be highly unsaturated

since it takes up bromine rapidly with considerable darkening.

In one experiment the set-up was so arranged that the distillation could be carried out under nitrogen and so that the issuing gases could be passed through barium hydroxide solution. No deposit of barium hydroxide was obtained thus indicating no loss of carbon dioxide on heating.

The loss of acetic acid on heat treatment is a characteristic of acetates (15). Esters of the type:



readily lose acetic acid with the formation of a dihydro-naphthalene by simple distillation. Also known are cases where acetic acid is lost with a change of ring size.

There is no evidence, however, on which to base a speculation as to the way in which the ester pyrethrosin loses acetic acid, as no work has been carried out on the fraction distilling after the acetic acid has come off.

The three facts then, (1) loss of acetic acid on heating above the melting point; (2) the trans-esterification; and (3) the isolation of a product differing from the parent by only an acetyl group are evidence that pyrethrosin is an ester of acetic acid.

When dihydropyrethrosin (the mixture resulting from

hydrogenation) is treated with aqueous sodium hydroxide in the same way as outlined for pyrethrosin, similar results are obtained. Acetic acid, identified as its p-phenylphenacyl ester, and a syrupy material are the products. This syrup like that from pyrethrosin also has resisted all attempts to crystallize it. An attempt to add diazomethane to this syrup failed as did an attempt with acetic anhydride and pyridine to convert it back to dihydropyrethrosin. Also, when a syrup obtained when pyrethrosin took up six atoms of hydrogen per mole was subjected to pyrodistillation, acetic acid was still obtained. These latter facts would indicate that the ester linkage of pyrethrosin persisted unchanged through hydrogenation, and would help eliminate the possibility that acetic acid could be obtained from pyrethrosin as outlined above by some complex mechanism involving the unsaturation of the system.

The general conclusion must be, therefore, that pyrethrosin contains a simple ester linkage, and that the acid fragment is acetic acid. Whether this ester linkage is one of a primary, secondary, tertiary or enolic hydroxyl cannot be stated.

Pyrethrosin a Lactone

That pyrethrosin is not a di-ester of acetic acid, and that the second equivalent of alkali used up might be consumed by hydrolyzing such a second ester linkage is established by the existence of $C_{16}H_{22}O_4N_2$, the diazomethane addition product of the syrup obtained from saponification. The solution left after the saponification of pyrethrosin was acidified and then steam distilled until the distillate was no longer acid. This removed all of the acetic acid and any other volatile acids. Working up of the residue then yielded $C_{16}H_{22}O_4N_2$, a compound differing from the diazomethane addition product of pyrethrosin by only one acetyl group. This, then, is not only evidence that pyrethrosin is a monoacetate but that there are no other ester linkages involving any other volatile acid.

$C_{16}H_{22}O_4N_2$ also offers some evidence for the nature of the linkage using the second equivalent of alkali. The formation of $C_{16}H_{22}O_4N_2$ from pyrethrosin requires the use of only one equivalent of alkali, whereas two are readily used up. Obviously then, the linkage that uses up the second equivalent of alkali is readily re-synthesized from its component parts. In obtaining $C_{16}H_{22}O_4N_2$, the necessary condition for such a resynthesis,

the presence of acid was fulfilled.

Whether the existence of $C_{16}H_{22}O_4N_2$ is due to the resynthesis of a lactone or to the resynthesis of an ester from non-volatile alcoholic and acidic fragments cannot be definitely stated. As mentioned before, many attempts to isolate acidic and alcoholic components from saponified solutions have been made, but no free acids or alcohols or their derivatives have been obtained. Thus, no direct evidence is as yet available.

Quantitative saponification studies tend to throw some light on the subject. Samples of pyrethrosin which had been saponified completely were titrated to neutrality, and after addition of an excess of standard acid left standing at room temperature. The excess acid added was about four or five times that which would be required in using up one equivalent and the solution was about one-tenth normal with respect to this acid. In one hour, one-fifth of one equivalent had been used up and after twenty-five hours almost one-half of one equivalent had been consumed. A similar experiment run on dihydropyrethrosin gave the same results. After nineteen hours almost four-tenths of one equivalent had been used. This latter fact is evidence that in pyrethrosin itself the acid was not being used up by any mechanism involving the active double

bond which can be shown to be present.

These results lead to a conclusion that a lactone is being reformed. Such a conclusion is natural since, although hydroxy and keto acids are known which do not exist in any but the lactone state, others lactonize slowly. Under the conditions it is hardly possible that any alcohol and acid fragments would recombine to the extent found.

Special attempts to open the lactone ring were made. Solutions of saponified pyrethrosin were worked up without acidification. The supposed salt thus resulting was then treated in various ways in efforts to not only tie up the acid part of the ring but also the liberated hydroxyl. Even these attempts failed to give crystalline esters.

The failure to obtain derivatives from the many treatments mentioned so far again calls up the possibility that after saponification a mixture of substances exists from which it is not possible to isolate a crystalline derivative. If a lactone alone were present in the molecule, the set-up necessary for producing a mixture on saponification might be present, the simplest mixture being that of the lactone and free acid. If the lactone were of an enol and a carboxyl, then the free acid might exist in keto-enol forms. Pyramided on top of this might be the possibilities that there is further keto-enol

tautomerism due to the presence of an enol acetate and that re-lactonization might occur involving another hydroxyl in the molecule rather than the one saponified.

The possibility of keto-enol tautomerism in the alcoholic fragment from saponification led to attempts to prepare carbonyl derivatives of the syrups obtained. No carbonyl derivatives, however, have yet been prepared.

One of the standard methods for opening lactone rings is treatment with alcoholic ammonia at low temperature. Pyrethrosin was subjected to such a treatment and two products were obtained, one in about five per cent overall yield, and the other in less than one per cent yield. Analysis of the second product gave no clue as to its formula, and an attempt to prepare it a second time failed.

Analysis of the first compound showed it to contain carbon, hydrogen, nitrogen and oxygen but not to be any of the sought after nitrogen derivatives of pyrethrosin. In fact, the nitrogen percentage was only about one-half of that expected. This led to the conclusion that the product was impure.

In reviewing the literature on ammonolysis of lactones at a later time, a peculiar parallelism was found. Ruzicka (22) and Hansen (13), working independently, had both obtained from a sesquiterpene compound known as isocalantolactone on treatment with alcoholic ammonia a

crystalline product which consisted of two molecules of lactone plus one molecule of ammonia. Calculation for the same occurrence with pyrethrosin gave carbon and hydrogen values agreeing excellently with the observed. Agreement of the observed nitrogen values were not as good, 2.41 per cent, and 2.66 per cent being observed for a theoretical of 2.21. An isomer of iscalantolactone, known as alantolactone and differing from it only with respect to the position of two double bonds which are present in both molecules, behave normally when treated with alcoholic ammonia and yielded the desired amide.

Ruzicka (22) offered an explanation for the formation of the compound consisting of two moles of lactone plus a molecule of ammonia following some work on a large quantity of the substance. He believed that a molecule of ammonia added across one of the double bonds in iscalantolactone and that the amine thus formed then added to a second molecule. This explanation is very plausible and could apply to pyrethrosin which can be shown to have at least one very active ethylenic linkage.

The general conclusion to be reached, although based to be sure on more or less indirect evidence and analogy, is that a lactone ring is present in the pyrethrosin molecule and accounts for the use of the second equivalent of alkali required in saponification.

Some Further Saponification Studies

Although pyrethrosin cannot be titrated directly with alkali, if an excess of standard aqueous alkali is added to an alcoholic solution and the excess alkali immediately back-titrated with standard acid, at least one equivalent of alkali is always used up. Three experiments gave 1.00, 1.03, and 1.04 equivalents used. The longer time the back-titration required, the higher the value. The solution from the experiment giving the value 1.04 was allowed to stand for forty-five minutes during which time the end-point shifted slightly, back to .995 equivalents used. As yet no quantitative experiment to determine whether the one equivalent liberates only acetic acid or whether the second reaction is also involved has been devised. Some special procedure, possibly involving the separation of the liberated sodium acetate quantitatively by a solubility method will be necessary.

Similar experiments carried out on dihydropyrethrosin add to the available data. If an excess of standard alkali is added to an alcoholic solution of dihydropyrethrosin and quickly back-titrated with standard acid, .98 of one equivalent is used up but the end-point quickly changes to an equilibrium value of .78 equivalents used up. Two

other experiments gave equilibrium values of .74 and .76 of one equivalent.

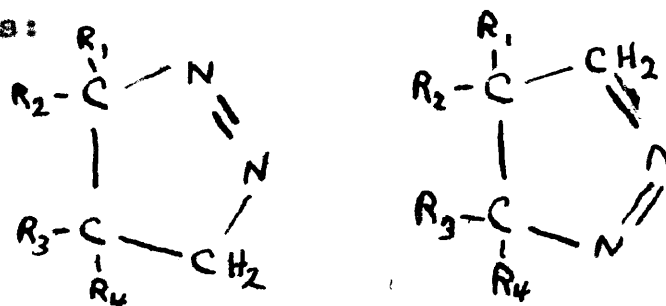
No attempt is made to interpret the above results on the basis that not enough data are available. Attempts to prepare hydroxyl and carbonyl derivatives by working up the solutions from such partial saponification experiments have also failed.

The Unsaturation of the Molecule

Pyrethrosin readily reduces cold alkaline permanganate solution but does not decolorize bromine water. It will take up bromine, however, from a concentrated solution in chloroform but with apparent decomposition and no pure product. It also fails to give a positive test with tetranitromethane, a reagent often useful for the detection of double bonds (10).

When it was observed that a pyrazoline was formed from the syrup obtained by alkaline saponification of pyrethrosin by treatment with diazomethane, it was suspected that the same double bond might be present in pyrethrosin. On addition of diazomethane to pyrethrosin itself two pyrazolines of formula $C_{18}H_{24}O_5N_2$ were formed, one quite insoluble in alcohol and melting at 196.5° and formed in about six times the quantity of the second which was fairly soluble in alcohol. The latter was considerably

more difficult to purify and melted at 168-169°. The more insoluble addition product readily lost nitrogen at its melting point to give the expected compound. The other, however, although it degased readily at its melting point, gave no pure crystalline product although the experiment was repeated several times. The nature of the decomposed material was entirely different from that obtained from the more insoluble form. Had the two products given the same cyclopropane derivative it could have been concluded that the diazomethane had added to the molecule in both possible ways:



The failure to give the same decomposition product indicates then that the two addition products are either stereoisomers or addition products of different double bonds. If the latter were the case the isolation of a product that had added two molecules of diazomethane would be expected. None, however, was found. Also, it would be expected that at least one diazomethane addition product would be obtained from dihydropyretrosin. None, however, could be made, the material being recovered unchanged.

The more insoluble addition product $C_{18}H_{24}O_5N_2$ was saponified with alkali in the hope of obtaining the diazomethane addition product of the syrup obtained from saponification of pyrethrosin $C_{16}H_{22}O_4N_2$. Considerable decomposition occurred and no product could be isolated.

The fact that pyrethrosin takes up one mole of hydrogen readily at room temperature and low pressure in the presence of a catalyst is another indication of the presence of unsaturation in the system. The material obtained by hydrogenation of pyrethrosin in alcohol in the presence of Raney Nickel Catalyst at room temperature and a pressure of six to eight atmospheres showed the wide melting range of $180-207^\circ$, although analysing for $C_{17}H_{24}O_5$. Attempts to fractionate this material using melting point as a guide were fruitless, the direction of change of the melting point of a sample on recrystallization being unpredictable. It was observed, however, that if the material was roughly separated into fractions there was a difference in rotation and crystalline form. By fractionation with these properties as a guide considerable progress was made. Repeated removal of the more insoluble form finally yielded a sample of constant rotation that crystallized in long hexagonal rods. Its specific rotation at 25° using a sodium vapor lamp as light source was -83.1 ($c = 1.32$ in chloroform). Its

melting point, however, was still over a range, from 195-202° with preliminary softening at 175°. No sample of maximum opposite rotation could be separated. The lowest specific rotation obtained was -22.7 ($c = 1.4$ in chloroform). This sample had a melting point of 158-170° with preliminary softening at 148° but was obtained in the center of the fractionation triangle rather than at the side. After a certain point it seemed impossible to remove the remaining amounts of the more insoluble form by crystallization. Only ethanol, di-n-butyl ether, and dioxane gave nicely crystalline material. In all of these solvents the one form was the more insoluble. A similar difficulty was observed in purifying the product obtained if a hydrogenation was stopped just short of one mole of hydrogen taken up. After the removal of the first dihydropyrethrosin in large crystals by allowing the solution to cool slowly, it was impossible to remove the unreacted pyrethrosin from the remainder, the presence of the pyrethrosin being unmistakable under the microscope. It is interesting to note that a mixed melting point of pyrethrosin and dihydropyrethrosin shows no depression or apparent change in the melting range of the dihydropyrethrosin. All of the melting points reported above were taken in capillaries. Under the microscope the ranges were even wider. In many cases at the end of the

melting point range solution rather than melting appeared to be the phenomenon going on.

All of the crystalline products isolated from hydrogenation experiments have analyzed for dihydropyrethrosin. It would thus seem that hydrogenation of the double bond was generating isomeric forms. This would agree with the results obtained when diazomethane was added to pyrethrosin. The similarity not only exists due to the fact that two products are formed but that the solubility of the two are in both cases widely different. That a double bond was hydrogenated and not some other functional group reduced is indicated by the fact that dihydropyrethrosin does not add diazomethane. Thus the double bond which adds diazomethane must also be the one that is hydrogenated.

On complete hydrogenation at 60° and approximately six to eight atmospheres with Raney Nickel Catalyst, pyrethrosin took up three moles of hydrogen per mole. The only product was a syrup. In this hydrogenation there was only a steady dropping off in the pressure during the adsorption of the second two moles of hydrogen, and no break which would indicate a successive addition of these two moles. In two experiments, in which approximately two and one-half moles of hydrogen were used up, one run in dioxane gave only a syrup while one run in alcohol gave about 35 per cent of dihydropyrethrosin.

This would indicate a concurrent addition of the second two moles in alcohol but a successive addition in dioxane. There was no observed break in the rate at which the hydrogen was adsorbed in the latter experiment. It should be mentioned here that all of the nickel catalysts used were four to eight months old. None would catch fire spontaneously if allowed to dry in air. This, of course, brings to mind that catalytic hydrogenation work is as much of an art as a science and that any conclusions drawn from quantitative experiments of the kind reported here must of necessity be speculative to some degree.

Pyrethrosin hydrogenated at 175° and 175 atmospheres in the presence of copper chromite catalyst, also produced a syrup. A summary of the hydrogenation experiments is given in Table IX. In all cases it will be noted that the reaction in dioxane was much slower, possibly due to a less active catalyst.

TABLE IX
Summary of Hydrogenation Experiments on Pyrethrosin

Expt.	g. used	ml.	Solvent	Cat.	Time hrs.	Temp.	P _i lbs.	P _f lbs.	H _r lbs.	H _u lbs.	M	Product
I	10	300	abs. ethanol	about 1 g.Ni(R)	1.0	room	100-125 atm.	-	-	-	-	some cryst. material anal. for dihydro obtained
II	10	300	"	1.5 g. Cu-Cr-O	5.5	175°	175 atm.	-	-	-	-	product a syrup only
III	2.5	40	"	about 1 g. Ni(R)	2.0	room	109.0	70.5	37.0	38.5	1	
					6.0	60°	120.0	45.5	37.0	74.5	2	
					Total				37.0	123.0	3	product a syrup only
IV	2.5	40	dioxane	"	6.0	room	123.5	96.0	28.0	27.5	1	
					6.0	100°	96.0	56.0	28.0	40.0	1.5	
					Total				28.0	67.5	2.5	product a syrup only
V	2.5	40	abs. ethanol	"		(similar to IV)				2.5	about 1 g. crystalline dihydro obtained	
VI	5.0	40	dioxane	about 2 g. Ni(R)	5.0	room	120.0	60.0	50.0	60.0	1	see experi- mental part

Symbols:

P_i = initial pressure
P_f = final pressure
H_r = hydrogen required per mole of hydrogen per mole of pyrethrosin
H_u = hydrogen taken up
M = moles hydrogen taken up per mole pyrethrosin
cat. = catalyst

Having established the presence of at least one double bond a quantitative study was indicated. On treating pyrethrosin with phthalic monoperoacid according to the scheme used by Böhme (1) in studying the presence of double bonds in all manner of simple compounds, only .74 to .81 double bonds were indicated per mole of pyrethrosin. Oddly enough, .74 to .75 double bonds were indicated to be present per mole of dihydropyrethrosin. The product obtained from the peracid oxidation of pyrethrosin analyzed for the expected oxide $C_{17}H_{22}O_6$. However, on melting it behaved much like dihydropyrethrosin, showing a wide range from 215-233°. As only a small quantity of the material was obtained no attempt was made at fractionation. However, the possibility that it is a mixture of stereoisomers remains. The product obtained from the peracid oxidation of dihydropyrethrosin was much harder to obtain in nicely crystalline form but melted sharply at 228-230°. It did not analyze for an expected oxide. The nearest empirical formula on the basis of combustion data alone would be $C_{16}H_{22}O_5$ (C = 65.25%, H = 7.52%) - dihydropyrethrosin that had lost a methylene group. No attempt is made to explain the formation of such a substance as further work should be carried out to prove definitely that the compound is $C_{16}H_{22}O_5$. It should be noted here that a small amount of unchanged

dihydropyrethrosin was recovered from the mother liquors.

Interpretation of these results is difficult. It is hard to understand why a double bond active enough to add diazomethane and hydrogen as readily as it does should fail to react quantitatively with phthalic monoperoacid. Incidentally, the failure of dihydropyrethrosin to give an epoxide is further indication that the bond which adds diazomethane is also the bond that is hydrogenated. Cinnamyl alcohol run as a known in these oxidation studies went 92 per cent to completion in one and one-half hours, while pyrethrosin was reacting only to the extent of about 10 per cent. At the end of twenty and one-half hours cinnamyl alcohol had gone 101 per cent to completion while pyrethrosin had only gone 74 per cent, its maximum for the run. It is interesting to note that after a certain maximum is reached, the amount of peracid in the solution seems to increase at the expense of the previous oxidation, e.g., after seventy-three and one-half hours the sample of cinnamyl alcohol that had shown 1.01 equivalents of peracid used up at twenty and one-half hours showed only .65 equivalents as being used. The cause of this was not investigated save in one run (number II) to keep nitrogen rather than air over the solutions. No difference was observed in the results. The results of these experiments have been given in Table VI.

More difficult to explain than the failure of the

reaction to go to completion is the fact that dihydro-pyrethrosin behaves in the same manner as does pyrethrosin, except in the important matter of the products obtained. Were the active double bond in pyrethrosin a part of a conjugated system, as in an alpha-beta unsaturated ketone, perphthalic acid might not be expected to react (9) as the peracids seldom attack this type of system. Alkaline hydrogen peroxide, however, does form epoxides with such a system (9). An attempt to oxidize pyrethrosin with this latter reagent gave only a syrupy material, in all probability the result of the action of the alkali alone.

The formation then, of a dihydropyrethrosin, an epoxide, and diazomethane addition products is excellent evidence for the presence of one highly active double bond in the molecule. How the other two moles of hydrogen are used up during hydrogenation and whether or not other double bonds are present, are questions that remain to be answered.

The Skeleton of Pyrethrosin

The isolation of a possible ammonia addition product of pyrethrosin offered the first direct connection with other naturally occurring compounds. There are sesquiterpenes related to the alant substances already mentioned that contain up to four oxygen atoms and which show similarities to pyrethrosin. As a speculation, pyrethrosin might be an acetyl derivative of a sesquiterpene derivative $C_{15}H_{20}O_4$.

Having failed to establish the presence of an aromatic system in pyrethrosin by alkali fusion, it seemed likely, on the basis of theoretical considerations, that such a system might arise from dehydrogenation experiments and that the fundamental carbon skeleton of pyrethrosin might be deduced in this way. Work now being carried on in this laboratory by Mr. H. D. Anspen is bearing out this conclusion. So far, by palladium-charcoal dehydrogenation of pyrethrosin, an azulene has been isolated and an aromatic fraction obtained. The azulene is probably $C_{13}H_{14}$. Work in progress on the aromatic fraction indicates it to be a substituted naphthalene or a mixture of substituted naphthalenes.

If the azulene is $C_{13}H_{14}$, only four carbons of the original molecule are left to be accounted for. This

would certainly eliminate the possibility that the second equivalent of alkali used up in the saponification is by an ester linkage in preference to a lactone, as dehydrogenation would split the molecule at the ester linkage.

The isolation of an azulene also connects pyrethrosin with a group of essential oils consisting of sesquiterpene alcohols and ketones. At least one of these, beta-vetivone (24) has been shown to have the azulenic skeleton present in the original molecule. However, azulenes are also formed from other classes of terpene compounds (14), which have been shown not to contain the azulenic skeleton.

It should be mentioned here that although pyrethrosin arises from pyrethrum flowers and might be expected to be structurally related to other substances isolated from this same source, its characteristics are entirely different and no connection has as yet been found.

A Consideration of the Molecular Formula

An idea as to the nature of the pyrethrosin molecule can be obtained, at least in a limiting sense, from a mathematical consideration of its molecular formula. Whenever an hydroxyl group present in a molecule is replaced by a hydrogen atom the number of hydrogen atoms present in the molecule remains the same, and only a loss of oxygen appears in the molecular and generalized formulas. When a simple ether linkage is replaced by hydrogen, not only oxygen is lost but also as many $-CH_2-$ groups as there are carbons lost. The latter, of course, has no effect on the generalized formula. Whenever a cyclic ether oxygen, or a carbonyl oxygen is replaced, two hydrogen atoms must be added to the molecule in order to satisfy the bonds liberated by the removal of the oxygen. In the case of esters, methylene groups may be lost when the group is replaced and no hydrogens are required for the connecting oxygen, as this becomes a simple ether linkage after the carbonyl oxygen is replaced.

By replacing the oxygen of a molecule with hydrogens, the formula of the theoretical hydrocarbon to which it is related is obtained. The completely saturated aliphatic hydrocarbon to which all molecules of seventeen carbon atoms are related is $C_{17}H_{36}$, corresponding to C_nH_{2n+2} .

For every double bond added to the molecule, two hydrogens may be subtracted. Likewise, for every acyclic ring. For every triple bond added, however, four hydrogens could be subtracted, and so on.

If all of the oxygens of pyrethrosin were carbonyls its basic formula would become $C_{17}H_{32}$, each oxygen having been replaced by two hydrogens. This corresponds to C_nH_{2n-2} , and would permit the existence in pyrethrosin of two double bonds, or two acyclic rings, or one double bond and one acyclic ring, and so on. At present, one of the oxygen atoms of pyrethrosin appears to be bound in a cyclic ether linkage (in the lactone group) requiring two atoms of hydrogen if replaced, two appear to be carbonyl oxygens, also requiring two hydrogens apiece on replacement, and a fourth is the connecting oxygen of an ester requiring no hydrogens when replaced. Thus, stripping the molecule of these four oxygens gives $C_{17}H_{26}^0$, corresponding to $C_nH_{2n-6}^0$. If the fifth oxygen were a carbonyl or cyclic ether oxygen, two more hydrogens would be required and the formula would become $C_{17}H_{28}$, corresponding to C_nH_{2n-6} . This would allow the equivalent of four double bonds in the molecule. If the fifth oxygen were a hydroxyl or ether oxygen, no additional hydrogens would be required and the formula would become $C_{17}H_{26}$.

corresponding to C_2N_{2n-8} . This would allow for the equivalent of five double bonds. The dehydrogenation work now in progress appears likely to show the presence of two acyclic rings. The present work has indicated at least one double bond. This would leave then, one or two double bonds to be accounted for, depending upon the nature of the fifth oxygen.

The Nature of the Fifth Oxygen Atom

Although it seems unlikely that a naturally occurring compound containing five oxygen atoms should have no simple oxygenated functional group, the experimental evidence thus far obtained for the most part indicates this to be the case. Pyrethrosin has resisted all attempts to make carbonyl or hydroxyl derivatives even though many schemes have been tried. However, active hydrogen determinations by the Zerewitinoff method show .65 to .91 active hydrogens per mole. Carbonyl determinations run at the same time show 1.68 to 2.02 groups per mole. In view of the fact that there are two carbonyl groups present as parts of the ether and lactone systems the carbonyl results are worthless. Wingate's failure (29) to get results is explained by the insolubility of pyrethrosin in the reagent used. In the present work pyridine was

used to bring about solution.

Accounting for the active hydrogen, however, is another matter. This could only result from an ordinary hydroxyl group, an enol, or the type of active hydrogen found in acetylenes. Unless the hydroxyl were very much sterically hindered, as might be indicated by the fractional values obtained, the experimental work indicates no group of this kind as being present. The fractional value obtained might also indicate that pyrethrosin is a mixture of fairly stable keto-enol forms but derivatives in this case should still be possible. No specific attempts have been made to remove water from the molecule by dehydration, for any of the usual methods for bringing about dehydration, like heating with formic acid or sulfuric acid would be doomed to failure because of the instability of the molecule in such reagents. Likewise, no work has been done in an effort to locate a possible active methylene group, although groups of such a type would be expected to react with metallic sodium.

It was hoped that hydrogenation of a double bond of pyrethrosin would modify the character of the fifth oxygen sufficiently so that it could be detected. This was not the case, however, because dihydropyrethrosin also resists all attempts to make carbonyl and hydroxyl

derivatives. It should be mentioned here that hydrogenation does, however, increase the stability of the molecule towards alkali, at least to some extent. Although no product other than acetic acid can be isolated on saponification, dihydropyrethrosin does not color up and decompose like pyrethrosin in alkaline media. Also, on melting it will resolidify to a crystalline material, that on the first remelting, melts only several degrees lower than the true melting point.

Attempts to determine or show the presence of methoxyl, a group often found in naturally occurring substances, in pyrethrosin by the modified Viebock-Schwappach method of Clark (5) led to results indicating a small amount of methoxyl. Percentages ranging from about five-tenths to one and five-tenths were obtained but depended entirely upon the length of heating. Clark (4), mentioned above as the modifier of the Viebock-Schwappach methoxyl determination, reported obtaining small amounts of methoxyl with some gossypol derivatives when none was present. In consequence, it must be concluded that methoxyl groups are lacking. The same argument would hold for the absence of ethoxyl groups, these being detectable along with methoxyl.

The presence of an ether oxygen as part of a ring must not be overlooked as a possibility. However,

if an epoxide, the treatment with methyl alcoholic hydrogen chloride might have been expected to have opened the ring (11) and given a crystalline chlorohydrin. Crystalline chlorohydrins have been obtained from compounds of the terpene series (27), by addition of hypochlorous acid. In view of the ease with which diazomethane adds to pyrethrosin a similar reaction would be expected. No attempts, however, were made to obtain such a product.

If the fifth oxygen is a part of a cyclic ether system larger than an epoxide, no indications have been found except its indifference.

The Stereochemistry of the Molecule

Although pyrethrosin is an optically active compound, little is as yet known as to its stereochemistry. When a mole of hydrogen is added catalytically with the saturation of a double bond a mixture of optical isomers is produced. No indications as to the mechanism of their formation, however, is as yet at hand. In addition, in some recently completed work the treatment of the pure isomer obtained by hydrogenation with pyridine and hydroxylamine hydrochloride in ethanol solution in a sealed tube in an attempt to make an oxime, gave an optically inactive

compound that still analyzes for dihydropyrethrosin, but which has a melting point that is much sharper and which is almost twenty degrees higher than the starting material. An attempt to make a semicarbazone in a similar manner by allowing the material to stand in ethanol solution in the presence of pyridine and semicarbazide hydrochloride gave the same product. No attempt is made to account for this transformation on the basis of insufficient evidence except to say that it is well known that pyridine (and similar weak bases) cause racemization and epimerization of optically active centers.

The Present Status of the Problem

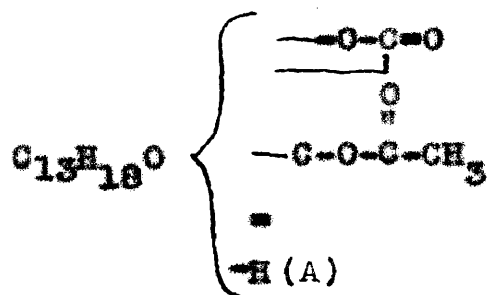
It may be stated with certainty that pyrethrosin is an ester of acetic acid containing at least one very active double bond, and that until proved otherwise, a lactone grouping must also be considered as being present. Nothing can be said with certainty regarding the nature of the fifth oxygen or the possibility of one or more other unsaturations in the molecule. A source of active hydrogen, however, is present.

Dehydrogenation work now being carried out will in all probability establish the central carbon system of the molecule and connect pyrethrosin with one or more

classes of known terpene compounds.

The failure to obtain pure products from hydrolytic work has slowed down the investigation. This failure indicates the arrangement of the molecule to be complex and quite unstable. The only pure crystalline derivatives whose relations to pyrethrosin are known without doubt have arisen from additions to the double bond. In addition, all of the other crystalline materials have been obtained in very small yields. Oxidative work is now indicated as the source of degradation products from which to carry on the investigation.

The formula for pyrethrosin may now be written:



SUMMARY

1. Pyrethrosin, the white, optically active, crystalline compound isolated from pyrethrum flowers, has a molecular formula of $C_{17}H_{22}O_5$.
2. It is an ester of acetic acid, but the type of hydroxyl esterified with the acetic acid has not been characterized.
3. There are strong indications that a lactone group is present.
4. At least one very active double bond is present, from which crystalline diazomethane addition products, dihydrogenated products, and an epoxide have been formed.
5. The presence of somewhat less than one active hydrogen per mole has been determined but its origin is as yet unexplainable.
6. The nature of the fifth oxygen atom and the fundamental carbon skeleton have not yet been determined.
7. Other as yet fully uninterpreted data regarding pyrethrosin and its derivatives have been presented.

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