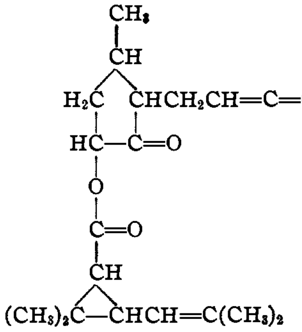
[CONTRIBUTION FROM DIVISION OF INSECTICIDE INVESTIGATIONS, BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE, UNITED STATES DEPARTMENT OF AGRICULTURE]

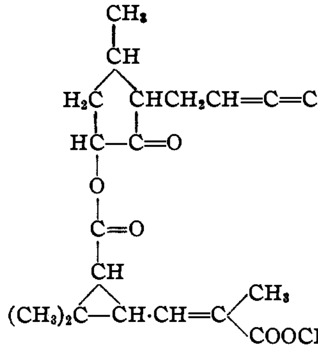
Constituents of Pyrethrum Flowers.[1] II. Isolation of Pyrethrin II

BY F. B. LAFORGE AND H. L. HALLER

The researches of Staudinger and Ruzicka[2] on the insecticidal principles of pyrethrum flowers are too well known to require detailed comment. In brief, the two active constituents, pyrethrin I and pyrethrin II, were shown to be esters of the cyclic ketonic alcohol pyrethrolone with chrysanthemum monocarboxylic acid and chrysanthemum dicarboxylic acid methyl ester, respectively, as represented by the formulas



Pyrethrin I



Pyrethrin II

Pyrethrin I was isolated from a partly purified extract of the flowers as the crystalline semicarbazone, which was hydrolyzed to the free ester, a liquid that could be distilled in a high vacuum. It was also prepared by esterification of pyrethrolone with chrysanthemum monocarboxylic acid.

The semicarbazone of pyrethrum II was not obtained pure, and the free ester was prepared only by esterification of pyrethrolone with chrysanthemum dicarboxylic acid methyl ester. The alcoholic and acidic components used in these partial syntheses of the two pyrethrins were obtained from mixtures of the semicarbazones of both.

Although the formulas of the pyrethrins proposed by Staudinger and Ruzicka have remained unquestioned, the authors themselves refer to the unsatisfactory properties of the semicarbazones, especially of the resynthesized pyrethrins, and point to the possibility of a change in the arrangement of the double bonds in the side chain of the alcoholic component and to possible steric changes during the reaction processes.[3]

To reduce the risk of changes that may occur in connection with the employment of semicarbazones, the pyrethrins should be prepared directly and under as mild conditions as possible.

Pyrethrum flowers are now purchased on the basis of their total pyrethrin content which is usually up to 1% or slightly more. Various analytical methods have been developed for their evaluation, some of which indicate only the total pyrethrin content, others the separate amounts of the two pyrethrins.

Because a wide difference of opinion exists as to the insecticidal value of each of the two compounds, it is of practical as well as of scientific importance to establish their relative toxicities. This can be best accomplished by isolating both pyrethrin I and pyrethrin II in pure condition and testing each one separately on insects.[4]

Extracts of the flowers made with organic solvents contain, in addition to the pyrethrins, comparatively large quantities of fats, waxes, fatty acids and other known and unknown substances which are regarded as inert from an insecticidal standpoint. The separation of the pyrethrins from these substances, which must constitute the first step in their isolation, has offered such unusual difficulties as to discourage attempts for its accomplishment.

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(1) The first paper in this series by Haller and Acree, "Determination of Pyrethrin II," appeared in *Ind. Eng. Chem., Anal. Ed.*, **7**, 343 (1935).

(2) H. Staudinger and L. Ruzicka, *Helv. Chim. Acta*, **7**, 177 (1924).

(3) Staudinger and Ruzicka, *ibid.*, **7**, 448 (1924).

(4) With this object in view Gnadinger and Corl, THIS JOURNAL, **51**, 3054 (1929), have repeated the isolation of the mixed semicarbazones by the method of Staudinger and Ruzicka, and have separated that of pyrethrin II to the extent of obtaining a product melting at 56–59°. The analytical figures given by them for the free ester obtained from their semicarbazone indicate, however, that it was still a mixture of about 70% pyrethrin II and 30% pyrethrin I.

Staudinger and Ruzicka and all subsequent investigators have employed petroleum ether extractives as starting material. Dissolving this material in methyl alcohol containing a little water and refrigerating, effects a partial separation of fats and waxes. The material remaining in solution is transferred to petroleum ether, and the fatty acids are removed by extraction with aqueous alkali carbonate, an operation that is exceedingly unsatisfactory owing to the formation of emulsions. By this method preparations of 50 to 60% total pyrethrin content are obtained. These are employed for the isolation of the pyrethrins as semicarbazones.

The initial difficulties in the isolation of the pyrethrins, involving the removal of the fats, waxes and acids from the original extractive, are eliminated in the following process, which is described in detail in the experimental part of this article.

The petroleum ether extractive used contained about 30% of total pyrethrins, pyrethrin I and pyrethrin II being present in about equal amounts. Addition of a small quantity of water to an acetic acid solution of this material precipitated the fats and waxes in a semi-solid form, which, after hardening by cooling, were readily removed by filtration.

By addition of water to the filtrate and extraction with petroleum ether, a product was obtained with a total pyrethrin content of 60 to 65% but still containing fatty acids and other impurities.

For the extraction of the acids, the only suitable solvent that did not involve the formation of emulsions was aniline. By agitation of the pyrethrin concentrate in this solvent with aqueous potassium carbonate the fatty acids were removed. The product separated from the aniline by acid was a concentrate containing 65 to 70% of total pyrethrins.

When this mixture was dissolved in a mixture of acetic acid and petroleum ether and sufficient water was added to cause separation of the two liquids, the pyrethrin II tended to concentrate in the acid layer, and the pyrethrin I, together with most of the impurities, concentrated in the petroleum ether layer. By isolation of the material in the acid layer and several repetitions of the process, a concentrate was obtained containing 80 to 83% pyrethrin II and 3 to 8% of pyrethrin I.

For the final purification the concentrates were fractionated in a molecular still at a comparatively low temperature, yielding pyrethrin II of practically 100% purity.

Although it is possible to obtain fairly large quantities of the concentrate containing about 80% pyrethrin II, the losses in distillation are considerable. Pyrethrin II so obtained is a colorless, very viscous liquid. Instead of being dextrorotatory, as recorded by Staudinger and Ruzicka for the synthetic compound, it showed a slight levo rotation.

It is to be regarded as probable that the pyrethrin II which is isolated by the process described in this article is different from the pyrethrin II obtained by partial synthesis. It is also quite possible that one or more other compounds having the same empirical formula and general nature as pyrethrin II are present in pyrethrum extracts.

Work is now in progress on this phase of the problem and on the direct isolation of pyrethrin I.

Experimental

Preliminary Purification of Oleoresin.—The starting material, a commercial concentrated petroleum ether extract. consisted of a thick brown liquid with a total pyrethrin content of about 30%, in which the pyrethrins were present in about equal amounts. After removal of the remaining solvent by warming under reduced pressure (an essential step), 450 g. of the material was divided into nine 50-g. portions.

To each portion 75 cc. of glacial acetic acid was added, and the mixture was stirred until practically all had dissolved. The same quantity of acetic acid to which 15 cc. of water had been added was mixed with the solution, causing the separation of a semi-solid mass of material. The containers were then cooled in an ice-bath for about half an hour, after which the contents were filtered separately on cold Büchner funnels. The solid material consisted of fats and waxes with comparatively little occluded pyrethrins. The green filtrates were combined and after addition of decolorizing carbon were again filtered. The acetic acid solution was then divided into two or three portions and strongly diluted with water, and the separated oil was extracted with petroleum ether. A small quantity of insoluble dark sirup was discarded. The petroleum ether solution was washed with water, a little sodium chloride being added to break slight emulsions. After the solution had been dried with sodium sulfate, most of the solvent was recovered by distillation on the steam-bath; the remainder was removed under reduced pressure. The same method was used in isolating all pyrethrum concentrates referred to below from their acetic acid solutions.

The quantity of concentrate obtained from 450 g. of the original material was 140 g. It contained, besides other impurities, fatty acids, which were removed by dissolving the material in 400 cc. of freshly distilled aniline and agitating the solution for about ten hours at −10° with a solution of 140 g. of potassium carbonate in 600 cc. of water. The aniline solution readily separated from the slimy soap solution, which was drawn off and on acidification yielded the extracted fatty acids. The aniline solution was washed three times with saturated bicarbonate solution and then, with constant stirring, was slowly run into 400 cc. of concentrated hydrochloric acid and 400 g. of cracked ice. These procedures were usually carried out in two operations with half the quantities stated. The separated oil was taken up in a suitable quantity of petroleum ether. A small quantity of insoluble material was discarded. The residue obtained from the washed and dried petroleum ether solution consisted of 90 g. of yellow oil containing 38.9% of pyrethrin II and 29.2% of pyrethrin I. Only slight variations from these values were observed in similar runs.

The analytical results for pyrethrin II were obtained by the methoxyl method of Haller and Acree,[5] those for pyrethrin I by the method of Seil.[6] In some instances pyrethrin II was also determined by the method of Seil as a check on the methoxyl method. It was observed that concentrates with a content of pyrethrin II of the order of 80% as determined by the methoxyl method showed a value about 5 or 6% higher by the Seil method. Practically 100% pyrethrin II usually showed an apparent pyrethrin I content of 2 to 3% by the Seil method. This is not surprising, because abnormally large samples had to be employed to obtain a significant volume in the titration, and the values observed evidently were very close to the limits of error of the method.

Separation of Pyrethrin II from Pyrethrin I by Differential Solubility.—The 90 g. of partly purified material obtained by the process just described was dissolved in 200 cc. of glacial acetic acid and 400 cc. of petroleum ether in a separatory funnel, and 20 cc. of water was added in small portions with vigorous shaking.

The addition of water caused the solution to separate into two layers, a petroleum ether fraction (P) and an acetic acid fraction (A). The latter was washed with six 40-cc. portions of petroleum ether, yielding an acid fraction (A-1).

The combined petroleum ether washings were added to the petroleum ether fraction (P), and the resulting solution was washed with six 40-cc. portions of acetic acid containing 10 to 12% of water. These acid washings were combined (A-2).

The acid-washed petroleum ether solution (P-1) yielded about 35 g. of a product containing 43% pyrethrin I and 14.4% pyrethrin II. This was set aside for the isolation of pyrethrin I.

The acid solution A-2 was extracted once with 240 cc. of petroleum ether and then with two 60-cc. portions of the same solvent. The combined petroleum ether washings were designated P-2, and the washed acid solution was designated A-3.

The two acetic acid solutions A-1 and A-3 were combined, and the dissolved material was isolated by the addition of water and extraction with petroleum ether. The quantity of concentrate so obtained was 30 g. It contained 72.2% of pyrethrin II and 14.2% of pyrethrin I. The material will be referred to as of stage I.

This product was dissolved in 100 cc. of acetic acid and 200 cc. of petroleum ether, and 14 cc. of water was added in small portions, with vigorous shaking after each addition. The separated acid solution was washed with four 20-cc. portions of petroleum ether. The washings and the separated petroleum ether layer were added to solution P-2.

The dissolved material in the acid solution (A-4) was isolated by dilution with water and extraction with petroleum ether and 20 g. of material containing 76.5% of pyrethrin II and 7.4% of pyrethrin I was obtained (stage II).

At stage III, which is reached by repeating the solution in acetic acid–petroleum ether, addition of water and subsequent washing of the acid layer with petroleum ether, using corresponding quantities of solvents, the yield was about 14 g. of material containing 82.7% of pyrethrin II and 6% of pyrethrin I. It is necessary to repeat the process whenever at stage III the pyrethrin II content falls below 80%, but it is not possible by further repetition to raise it much above 83% when the proportions originally present were of the order given.

All petroleum ether extracts except P-1 were combined with P-2. The quantity of material obtained from these combined extracts was 25 g. When treated in a second series of operations according to the procedure described, it yielded 8 g. of concentrate containing at stage III about the same percentages of the two pyrethrins as the corresponding final product from the first series.

As the separation and concentration of pyrethrin II proceeds, the concentrates become progressively more viscous and lighter in color and more difficultly soluble in petroleum ether. A concentrate of more than 70% of pyrethrin II separates in part even from a moderately dilute petroleum ether solution on cooling, but this is of no advantage in purification, as the separated product carries the impurities with it.

The process described could probably be replaced by a continuous one involving the same principle, if the object were to prepare large quantities of concentrates of high pyrethrin II content.

Preparation of Pure Pyrethrin II from the Concentrate by Distillation in a Molecular Still.—From 8 to 10 g. of solvent-free concentrate containing about 80% of pyrethrin II and 3 to 8% of pyrethrin I was distilled in a Hickman-type molecular still of suitable size.[7] The still was heated in a small oil-bath kept at the constant temperature of boiling chlorobenzene (131°), the pressure as recorded by the mercury gage being about 10^{-6} mm. The distillation usually required eight to ten hours.

In the earlier trial runs a number of fractions were collected and analyzed to determine at which points the distillation should be interrupted. In later distillations three fractions were usually collected. The first fractions contained most of the pyrethrin I and the more volatile impurities. The second fractions generally consisted of 80 to 90% of pyrethrin II and were combined for redistillation. The third fractions were practically 100%

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(5) H. L. Haller and Fred Acree, Jr., *Ind. Eng. Chem., Anal. Ed.*, **7**, 343 (1935).

(6) H. A. Seil, *Soap*, [5] **10**, 89 (1934).

(7) K. Hickman, *J. Franklin Inst.*, **213**, 119 (1932).

pyrethrin II. There was always more than 50% loss due to polymerization in the still, and the still residue consisted of a glassy mass almost insoluble in all solvents. The yield of pure pyrethrin II was in no case more than 20% of the quantity of material distilled, but an additional quantity was obtained on redistillation of the second fractions.

The analytical results and physical constants for several samples are given below.

Preparation A.—*Anal.* Calcd. for $C_{22}H_{30}O_5$: C, 70.54; H, 8.08; $1CH_3O$, 8.28. Found: C, 70.63, 70.84; H, 7.69, 7.69; CH_3O, 8.10. Calcd. to pyrethrin II (mol. wt. 374), 97.72; $n^{30}D$ 1.5247; $n^{20}D$ 1.5285. *Rotation:* 0.135 g. in 2.28 cc. ether at 20° rotated $-0.34°$; l = 0.96 dm.; $[\alpha]^{20}D$ -6.0. 0.235 g. in 2.28 cc. benzene at 20° rotated $-0.42°$; l = 0.96 dm.; $[\alpha]^{20}D$ -4.2.

Preparation B.—*Anal.* Found: CH_3O, 8.24, 8.33. calcd. to pyrethrin II (mol. wt. 374), 99.66, 100.38; $n^{29}D$ 1.5258.

Preparation C.—*Anal.* Found: CH_3O, 8.07, 8.04; $n^{20}D$ 1.5259.

Preparation D.—*Anal.* Found: CH_3O, 8.00; $n^{20}D$ 1.5247.

It will be noted that none of the samples shows a pyrethrin II content of appreciably more than 100% as determined by the methoxyl method, indicating that none of them contained methyl pyrethrolone, alkyl esters of the chrysanthemum acids or other methoxyl containing impurities. These may have been present in the concentrates before distillation, but, being of much lower molecular weight, they would be removed in the more volatile fractions.

Summary

A procedure for the preparation of pyrethrin II is described.

Petroleum ether extractives of pyrethrum flowers are separated from fats and waxes by the employment of acetic acid.

The acids are removed from the concentrate by extraction in aniline solution with potassium carbonate.

Partial separation of pyrethrin II from pyrethrin I is accomplished by taking advantage of their different solubilities in a system consisting of diluted acetic acid and petroleum ether. Pyrethrin II tends to concentrate in the acid solution, pyrethrin I in the petroleum ether.

Concentrates so obtained containing about 80% of pyrethrin II and about 6% of pyrethrin I are distilled in a molecular still and yield pure pyrethrin II.

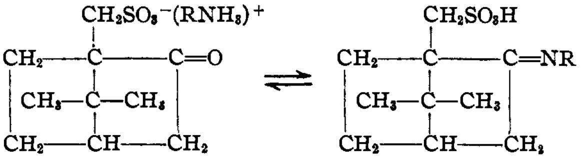
WASHINGTON, D. C. RECEIVED JULY 26, 1935

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS]

Anomalous Mutarotation of Salts of Reychler's Acid. III. Reduction of Ketimines of *d*-Camphor-10-sulfonic Acid. Formation of Sultams

BY R. S. SCHREIBER AND R. L. SHRINER

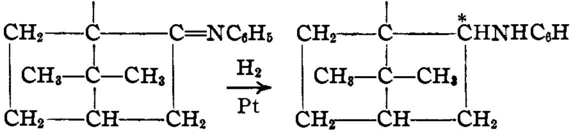
The mutarotation of salts of primary amines and Reychler's acid was ascribed[1] to the establishment of an equilibrium between the *d*-salt (I) and the *l*-ketimine (II).



I II

In order to establish the ketimine structure beyond question, the product of this reaction was subjected to catalytic reduction, since it is known that ketimines may be hydrogenated to secondary amines.[2]

The ketimine (III) obtained by the dehydration of the aniline salt of Reychler's acid readily absorbed one mole of hydrogen in the presence of platinum black and the hydrogenated product, 2-(N-phenylamino)-10-camphane-sulfonic acid (IV) was found to consist of the two diastereoisomers which would be expected because of the creation of a new asymmetric carbon atom at position 2. These isomers were separated by fractional crystallization. The α-form melted at 300–302° and had a specific rotation[3] of $-88.5°$ whereas the β-form melted at 350–351° and was dextrorotatory, $+8.5°$.



III IV

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[1] Schreiber and Shriner, THIS JOURNAL, **57**, 1306, 1445 (1935).

[2] Mignonac, *Compt. rend.*, **171**, 114 (1920); Rupe and Vogler, *Helv. Chim. Acta*, **8**, 832 (1925); Rupe and Metzger, *ibid.*, **8**, 838 (1925); **13**, 457 (1930).

[3] All specific rotations reported in this paper were determined at 25° with sodium D light.