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CONSTITUENTS OF PYRETHRUM FLOWERS. XVI. HETEROGENEOUS NATURE OF PYRETHROLONE

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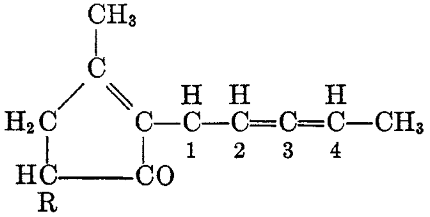
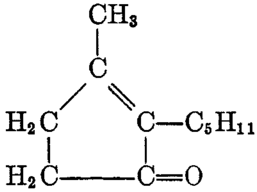
In a previous article (1) the known facts concerning the nature of the pentadienyl side chain of pyrethrolone, the substituted cyclopentenolone component of the pyrethrins, were considered in conjunction with absorption data furnished by pyrethrone (desoxypyrethrolone). It was concluded that pyrethrolone and pyrethrone should be represented by formula I, containing the cumulated system as originally proposed by Staudinger and Ruzicka (2).



Pyrethrolone (R = OH)
Pyrethrone (R = H)

I

The principal evidence for the cumulated system, and against the conjugated system suggested by Ruzicka and Pfeiffer (3), may be briefly presented as follows: Pyrethrolone and pyrethrone apparently failed to undergo the Diels-Alder reaction. On hydrogenation, pyrethrone was converted into tetrahydropyrethrone, which proved to be identical with dihydrojasomone, of known structure II (4).

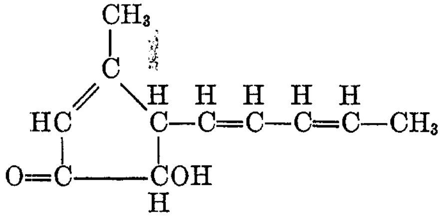


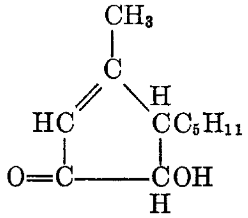
II

This compound results also on hydrogenation of pyrethrolone, its ethers, its acetyl derivative, and the pyrethrins themselves, and its formation shows the presence in all these derivatives of a double bond within the ring system.

Both pyrethrolone and pyrethrone furnished acetaldehyde on ozonization, this fact locating one double bond in position 3,4 in the side chain. The choice between the cumulated and the conjugated system seemed then to depend upon the establishment of the position of the remaining double bond. Evidence in favor of position 2,3, and hence for the cumulated system, was furnished by ultraviolet absorption data obtained on pyrethrone as compared with those observed on a reference compound with a five-membered side chain containing such a system. The reference compound, 1-cyclohexyl-2,3-pentadiene, showed no absorption in the wave-length region concerned. With the assumption of a double bond in the *alpha*,*beta*-position in the cyclopentenone nucleus of pyrethrone, as in formula I, the data obtained excluded the presence of a conjugated system in the side chain in conjugation with the nuclear double bond and the keto group. With position 1,2 therefore excluded, 2,3 was the only possible location for the remaining double bond.

Gillam and West (5) have recently reopened the question, not only of the nature of its side chain, but of the structure of pyrethrolone in other respects. They conclude, on the basis of absorption results, that the conjugated system is present in the pyrethrolone side chain, but that a trienone system with the *alpha*,*beta*-unsaturated ketonic feature of the molecule is not involved. That two separated chromophores are present apparently follows from the nature of the absorption spectrum of pyrethrolone as compared with that of tetrahydropyrethrolone, in which only the side-chain chromophore has been eliminated by hydrogenation. By subtracting the values furnished by the tetrahydro compound from those observed for pyrethrolone, the hypothetical values for the side-chain chromophore could be deduced. From these results Gillam and West conclude that pyrethrolone should be represented by formula III and tetrahydropyrethrolone by a corresponding formula IV with a saturated side chain.


III


IV

In an earlier publication Gillam and West (6) also reported results on the absorption spectra of tetrahydropyrethrolone and dihydrojasmone (tetrahydropyrethrone). The curves plotted for the two compounds were so close together that the conclusion was justified that they contained the same essential features. It is unforuntate that a spectrographic examination of pyrethrone (desoxypyrethrolone) was not made and the data compared with those furnished by tetrahydropyrethrone. Our observations on pyrethrone included only the wave-length at maximum absorption and the intensity which was regarded as adequate for the exclusion of a trienone chromophoric system. The values (λ max. 235 mμ; log. $\epsilon = 4.2$) are not in agreement with those observed by Gillam and West for pyrethrolone.

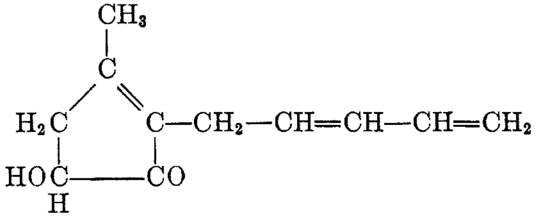
If the pyrethrolone structure does in fact contain two separated chromophores, one in the side chain and the other consisting of the *alpha*,*beta*-unsaturated ketonic component, formula I is untenable. But the alternative formula III is also unacceptable because it is inconsistent with the following chemical facts: While it would account for the formation of acetaldehyde, it would not provide for that of malonic acid on oxidation. Since pyrethrolone is optically active, its reduction product, pyrethrone, should also be optically active, because it would still contain an asymmetric center in the nucleus at the *beta* carbon in formula III. But pyrethrone is actually optically inactive and cannot be resolved. Tetrahydropyrethrone obtained *via* the chloro compound from tetrahydropyrethrolone, which should be optically active according to formula III, is also inactive. Some of these discrepancies are explained by the assumption that reductions involving the elimination of the oxygen atom are accompanied by an isomerization whereby the nucleus as in formula III rearranges to that in formula II.

The structures of tetrahydropyrethrone and jasmone are so well established from the chemical standpoint as to be indisputable, since they are based on the nature of their oxidation products, which include levulinic acid, and especially on their syntheses (7), which definitely locate the position of the side chain relative to the ketone group in the sense of formula II.

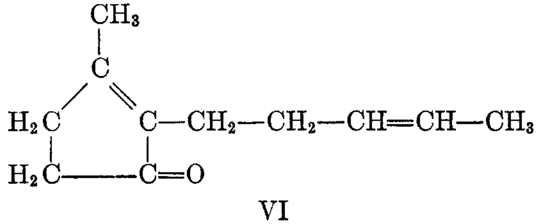
If, then, formula I for pyrethrolone is to be rejected on the grounds of absorption results, the chemical facts provide a much stronger reason against the acceptance of formula III. But if the chemical facts are to be reconciled with the absorption data, a new conception for the distribution of the double bonds in pyrethrolone and pyrethrone is required.

The results reported by Gillam and West have stimulated us to extend our own investigations on this problem.

In a recent private communication, R. B. Woodward, of Harvard University, has suggested that pyrethrolone be assigned formula V and that pyrethrone corresponds to formula VI.



V



VI

These formulas would satisfy the absorption results, but they account for the formation of acetaldehyde from pyrethrolone only in a very strained manner,—*i.e.*, with the intermediary formation of malonaldehydic acid, and formula VI is contradicted by analytical results.

At this point it may be well to mention some observations concerning pyrethrolone which have been considered unimportant. Its distillation point is not constant, and the distillate always exhibits striations which indicate lack of homogeneity. The yield of acetaldehyde on ozonization does not exceed 30% of the theory, and a considerable quantity of formaldehyde is always formed. The values obtained on analysis of pyrethrolone and its derivatives were usually slightly high for hydrogen and low for carbon, as calculated for compounds with a double unsaturated side chain. The same observations have been made in the case of pyrethrone.

We have, then, an indication that pyrethrolone may be a mixture, possibly containing a compound with a side chain similar to that in VI, an assumption that the results to be described tend to confirm.

Although the boiling range of pyrethrolone is small, upon fractional distillation of the crude product a very marked, progressive increase in the refractive index of the successive fractions was observed (8). The tetrahydropyrethrolone prepared by hydrogenation of each of the fractions, however, showed only very slight differences in refractive index.

Pyrethrone prepared from unfractionated pyrethrolone could also be separated into fractions with similar differences in refractive index, and semicarbazones prepared from the separate fractions showed different melting points. Pyrethrolone methyl ether and acetylpyrethrolone could also be fractionated with similar results.

Positive evidence that pyrethrolone is a mixture was furnished by the results obtained upon determination of the carbon-linked methyl groups in fractions of pyrethrolone and of all the derivatives mentioned. The method employed is, with slight modifications, the one described by Pregl (9). The fractions obtained from pyrethrolone gave terminal methyl values decreasing with increase in boiling range and refractive index. The same was true for the fractions of its derivatives. Although complete separation was in no case attained, the fraction with the highest refractive index and boiling range would be presumed to contain the largest proportion of the conjugated component of structure V, while the fraction with the lowest boiling range and refractive index would contain the largest proportion of the component or components differing from V in having a side chain with the grouping $C{=}CH{-}CH_3$, the compound of structure I not being excluded.

Pyrethrone as prepared in our earlier work, and on which our own spectrographic data were obtained, probably consisted largely of the compound with this grouping.

This demonstration of the heterogeneous nature of pyrethrolone and its derivatives reconciles the apparent contradictions that have arisen between the chemical and the physical facts.

Since analyses of samples of pyrethrolone semicarbazone from mixtures of pyrethrin semicarbazones show 1.2 to 1.3 moles of terminal methyl groups, the question arose whether one of the components is characteristic of pyrethrin I and the other of pyrethrin II. This apparently is not the case, for the analysis of pyrethrolone semicarbazone prepared from pure pyrethrin II semicarbazone (m.p. 164°) also indicated the presence of terminal methyl groups corresponding to 1.3 moles, and pyrethrolone prepared from it furnished on distillation fractions with the same characteristic differences as did pyrethrolone from mixed pyrethrin semicarbazones. It appears now that, unless in the preparation of the original pyrethrin semicarbazones a change affecting the double bonds has occurred, the pyrethrins are more complex mixtures than was previously supposed, possibly comprising at least four structurally different compounds with the possible existence of geometric variations as well.

EXPERIMENTAL

Fractionation of pyrethrolone. Pyrethrin semicarbazone was prepared in the usual manner from a purified pyrethrum concentrate (10) and recrystallized from acetone. This product, which has been shown to be a mixture of the semicarbazones of both pyrethrins but predominantly of pyrethrin I, was saponified and the pyrethrolone semicarbazone recrystallized from methanol. The product that first separated consisted of compact spherical groups of crystals. A second fraction formed flat but separated prisms. Both showed the same melting point, 210–212° (uncorr.), and the same composition.

Anal. Calc'd for $C_{12}H_{17}N_3O_2$: C, 61.26; H, 7.28.
Found,[1] Fraction I: C, 61.04, 60.85, 61.51; H, 7.43, 7.52, 7.57.
Fraction II: C, 61.30, 60.67; H, 7.67, 7.48.
Terminal methyl: (I) 1.20, 1.18; (II) 1.14, 1.17.

Except for an apparent difference in solubility which may later prove of importance, both these fractions will be regarded as the same compound. In the experiments to be described no fractional crystallization of pyrethrolone semicarbazone was made, the total product being employed. It was hydrolyzed in the usual manner, with aqueous potassium bisulfate. Sixteen grams of the crude pyrethrolone was distilled in an ordinary Claisen flask at an oil-bath temperature of 165–170°, and four fractions were collected. The refractive index of each fraction was determined, and each was analyzed for terminal methyl. The results are indicated in Table I.

Each of the four fractions was then separately hydrogenated, using palladium-calcium carbonate catalyst in ethanol solution; all fractions absorbed about the calculated volume of hydrogen in a few minutes. The resulting tetrahydro derivatives were distilled, and the refractive index was determined for each distillate. With a bath temperature of 170° and at 1 mm. pressure, all four hydrogenated fractions distilled almost completely between 135° and 136°. The four distillates showed only slight differences in refractive index, n_D^{26} for fraction I being 1.4907, and for fraction IV, 1.4900. It must be concluded, therefore, that the differences in physical properties among the fractions as shown in Table I are due to the distribution of the components present in pyrethrolone, which depends mainly on the nature or degree of unsaturation in the side chain.

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[1] These values, as well as those recorded in previous articles, are, with respect to hydrogen, high beyond the limit of error. A mixture of 75% of the compound $C_{12}H_{17}N_3O_2$ and 25% of one of similar formula but with two more hydrogen atoms would have a calculated value for hydrogen of 7.49% and for carbon 61.11%, while 75% of the $C_{12}H_{17}N_3O_2$ compound with 25% of the one of formula $C_{11}H_{17}N_3O_2$ would have a value for hydrogen of 7.38% and for carbon 60.74%.

Pyrethrolone was also prepared from pure pyrethrin II semicarbazone (m.p. 164°), fractionated, and the refractive index determined on three fractions obtained through a temperature range of 138–140° at 1 mm. pressure. The values n_D^{27} found for the succeeding fractions were 1.5278, 1.5343, and 1.5405.

Pyrethrolone methyl ether and acetylpyrethrolone were prepared from unfractionated samples of pyrethrolone, the derivatives fractionated, and refractive index and terminal methyl determinations made on the fractions. The results, which are given in Table II, show relations similar to those observed with pyrethrolone.

TABLE I

REFRACTIVE INDEX AND TERMINAL METHYL VALUES OF FRACTIONS OF PYRETHROLONE

FRACTION	WEIGHT, g.	B.P./0.8 MM. °C	n_D^{27}	TERMINAL METHYL[a]
1-A[b]	0.75	140	1.5221	1.64, 1.65
1	2.70	137–139	1.5297	1.46, 1.48
2	3.95	139–141	1.5329	1.35, 1.36
3	3.55	141–144	1.5367	1.22, 1.27
4	2.15	144–145	1.5416	1.15, 1.11

[a] Moles of acetic acid per mole of pyrethrolone (m.w. 178).

[b] Obtained by redistillation of fraction 1.

TABLE II

REFRACTIVE INDEX AND TERMINAL METHYL VALUES OF FRACTIONS OF METHYL ETHER, AND ACETYL DERIVATIVES OF PYRETHROLONE

FRACTION	WEIGHT, G.	DIST. RANGE, °C[a]	n_D^{27}	TERMINAL METHYL[b]
		Pyrethrolone methyl ether		
1		104–105	1.5009	1.35, 1.37
2		105–106	1.5049	
3		106–107	1.5112	1.04, 1.03
		Acetyl pyrethrolone[c]		
1	3.85	125–128	1.5043	2.53, 2.53
2	3.85	128–133	1.5120	2.24, 2.16
3	1.65	133–137	1.5188	2.00, 2.00

[a] At 1 mm. pressure for pyrethrolone methyl ether, and at 4 mm. for acetylpyrethrolone.

[b] Moles of acetic acid per mole of compound.

[c] Distilled with 18-cm. indented column, bath temperature 165–170°.

Pyrethrone. Another preparation of pyrethrolone was reduced with aluminum amalgam as previously described. After filtration of the ethereal solution, the solvent was removed and the crude residue was dissolved in 7 parts of ethanol and 1 of pyridine. A solution of 1 part of semicarbazide hydrochloride in 1.25 parts of water was added and the solution allowed to stand overnight. The crude semicarbazone was filtered and washed with methanol and then with water (m.p. 210–215°). The yield was about 65% of the weight of the pyrethrolone taken. The crude material was hydrolyzed with oxalic acid according to the original procedure (11), and 6 g. of crude pyrethrone was obtained from 11 g. of the semicarbazone. This material was distilled from a flask provided with an 18-cm. indented column, with a bath temperature maintained at 110–112° and at 1 mm. pressure.

Fraction 1 (2.7 g.) was collected between 80° and 84° and showed the refractive index n_D^{28} 1.5059 and terminal methyl values of 1.56 and 1.60. It furnished a semicarbazone melting at 211–213° (uncorr.) with decomposition.

Anal. Calc'd for $C_{12}H_{17}N_3O$: C, 65.7; H, 7.80.
$C_{12}H_{19}N_3O$: C, 65.1; H, 8.60.
Found: C, 64.78, 64.74; H, 8.77, 8.59.
Terminal methyl, 1.65, 1.69.

One and two-tenths grams of fraction 1, hydrogenated with palladium-calcium carbonate catalyst in ethanol solution, absorbed about 210 ml. of hydrogen (corr.) in 10 minutes. Pyrethrone (m.w. 162) requires 330 ml. and dihydropyrethrone (m.w. 164) 163 ml. of hydrogen per 1.2 g. for saturation of the side chain. The product of the reaction was isolated and distilled at 77–78° (2 mm. pressure). The semicarbazone melted at 176° as reported for tetrahydropyrethrone.

It may be assumed that fraction 1 consists mostly of dihydropyrethrone of structure VI, possibly formed by 1,4-addition of hydrogen to pyrethrone of structure V by the aluminum amalgam reduction. It is possible, however, that the side chain as in VI or its next lower homolog may have been originally present in one of the pyrethrolone components.

Fraction 2, which was collected in the amount of 2.7 g. between 84° and 90°, showed the refractive index n_D^{28} 1.5251 and terminal methyl values of 0.99 and 0.97. It furnished a semicarbazone melting at 214–216° with decomposition.

Anal. Found: C, 65.57, 65.50; H, 8.17, 8.14.
Terminal methyl, 1.03, 1.04.

These data indicate that in fraction 2 pyrethrone with the conjugated side chain predominates.

SUMMARY

It is shown that pyrethrolone is not a homogeneous compound, as previously assumed, but a mixture of components differing with respect to the nature of the side chain. These components can be partially separated by distillation and show marked differences in refractive index. By determination of the carbon-linked methyl groups in successive fractions, it is shown that one component possesses the conjugated system of double bonds, while the other contains a side chain terminating with the group $C{=}CH{-}CH_3$. Several derivatives were shown to be mixtures corresponding to the two systems of unsaturation.

With the demonstration of the heterogeneous nature of pyrethrolone, the apparent discrepancies between absorption results and chemical facts are explained, and the revisions of the formulas proposed by Gillam and West become unnecessary. The mixture of closely related compounds to which the name "pyrethrolone" has been assigned consists predominantly of the compound of structure V.

Beltsville, Md.

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