ion. The combined washings were re-extracted by two 25-ml. portions of ether. The latter ether extract (50 ml.) was washed with 25 ml. of distilled water, then with 10 ml. of distilled water. The combined ether extract was filtered through a small cotton pad (in a buret funnel) into a tared beaker. The cotton was washed carefully with 10-ml. portions of ether after most of the ether had been evaporated from the extract in the beaker. The nordihydroguaiaretic acid tetraacetate was then dried at 105° C. for a minimum of 2 hours, and held in a vacuum desiccator prior to microanalysis.

The molecular weight of nordihydroguaiaretic acid, $C_{18}H_{22}O_4$, is 302.36. The molecular weight of nordihydroguaiaretic acid tetraacetate, $C_{26}H_{30}O_8$, is 470.50. In theory, 1.000 gram of pure nordihydroguaiaretic acid yields 1.5561 grams of nordihydroguaiaretic acid tetraacetate.

ACKNOWLEDGMENT

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Infrared Spectrophotometric Determination of Allethrin

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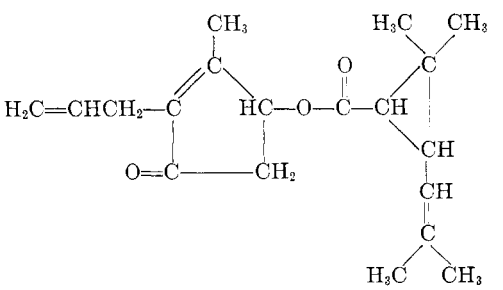
An infrared spectrophotometric method has been developed for the determination of allethrin. The intense 5.81-micron band was selected for this study, and it was ascertained that only one of the four impurities known to be present in the insecticide interfered at the analytical wave length. Chrysanthemummonocarboxylic acid anhydride was shown to be present as a minor constituent in the samples investigated. The 5.56-micron band was utilized for determining the anhydride. Allethrolone has been determined by means of its 2.86-micron absorption maximum, and standard and differential techniques have been compared. *cis-* and *trans-*allethrins exhibit different absorptivities at 5.81 microns, and the relative amounts of the isomers were determined by their absorbances at 8.70 and 8.85 microns.

ALLETHRIN is a commercially available insecticide similar to the pyrethrins in action.



There are two methods at present employed in industry for the analysis of this substance, and both have certain disadvantages. The hydrogenolysis procedure of Schechter (*18*) fails to take into account one of the interfering impurities present in commercial allethrin (chrysanthemummonocarboxylic anhydride) and, in some instances, gives erratic results when the purity of the insecticide is less than 90%. The ethylenediamine method (*12*) requires daily standardization of solutions prior to their use, the exact location of the end point is difficult to detect when off-color samples of allethrin are analyzed, and the reagents employed are rather expensive. Cueto and Dale (*3*) have published a colorimetric method for determining allethrin, and Oiwa, Shinohara, Takeshita, and Ohno (*16*) investigated the polarographic analysis of *α-dl-trans*-allethrin. Harris (*11*) has developed a chromatographic method for the insecticide, and a spectrophotometric procedure has recently been reported (*14*).

While this article was being reviewed, a paper (*15*) discussed the determination of allethrin by weighing the chromatographed 2,4-dinitrophenylhydrazone. Green and Schechter (*8*) have developed a similar method.

Elliott (*4*) has recently published entomological data on the trans content of allethrin, and his average figure of 75% agrees well with that of the author. Elliott attempted to explain the reason for the radical difference between his results and those of Gersdorf and Mitlin (*7*) by stating that it might be due to "different reactive conditions and varying nonstoichiometric ratios of the reagents in the preparations of the esters." Schechter stated that the materials supplied to Gersdorf for his studies were prepared by the procedure outlined in his original paper (*17*). Therefore, some other reason must be sought to explain the divergency.

Crombie (*4*) recently found, by infrared spectrometry, that methyl chrysanthemumate contains 68% of the trans isomer. A short while later (*10*) Harper and Sleep observed that chrysanthemumonitrile contains 73% of this isomer. By means of a modified AOAC method (*1*), the quantity of *trans*-chrysanthemummonocarboxylic acid present in the racemic acid was found to be nearly identical with that reported in this paper (*19*).

An infrared spectrophotometric method has been developed for determining allethrin, utilizing the intense 5.81-micron band (Figure 1). It was first necessary to examine the infrared spectra of the impurities occurring in commercial allethrin. Allethrolone (Figure 2), chrysanthemummonocarboxylic acid (Figure 3), its acid chloride (Figure 4), and its anhydride (Figure 5) were prepared, and it was ascertained quantitatively, by determining the absorption of purified allethrin containing known amounts of added impurities, that only allethrolone interfered at 5.81 microns in the concentrations encountered in the technical product.

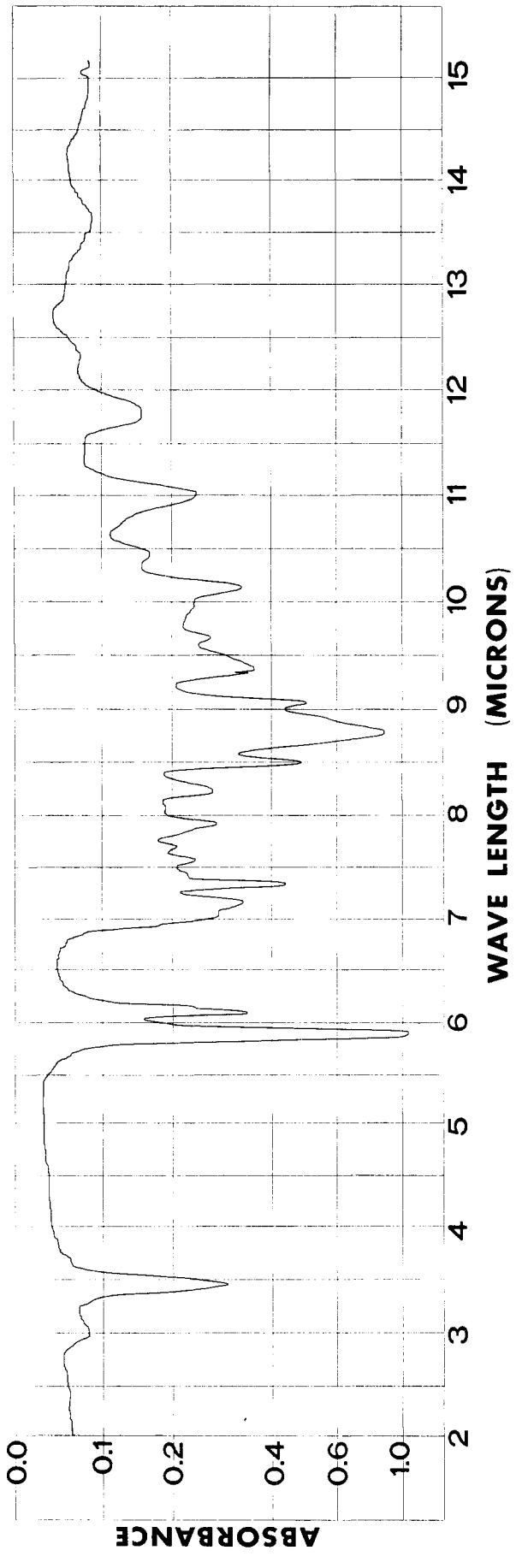


Figure 1. Infrared spectrum of allethrin (between salt plates)

The anhydride affects the absorbance of allethrin at this wave length in amounts exceeding 5%, and the largest quantity found in the insecticide samples studied was 3%.

Hogsett, Kacy, and Johnson (*12*) state that the presence of chrysanthemummonocarboxylic acid anhydride in most samples was established by chemical analysis and confirmed by infrared data, but present no actual experimental proof of its existence. The author has separated the anhydride from allethrin by partition chromatography, using nitromethane on silicic acid and *n*-hexane as the mobile solvent (*6*). Its identity was proved by comparison of its infrared spectrum with that of an authentic sample, in addition to chemical analysis.

The determination of chrysanthemummonocarboxylic acid anhydride in allethrin by the morpholine procedure (*12*) suffers from several disadvantages—the quantity of "acid chloride" must first be determined, the methanolic acid solution requires daily standardization, and, finally, a few allethrin samples have yielded negative anhydride values in the hands of the author. The latter anomaly is due to an interference with the color change at the end point, necessitating the addition of more than the theoretical quantity of reagent to bring the sample solution to match that of the blank. Substitution of a potentiometric titration would probably eliminate this effect.

The anhydride content of technical allethrin can be conveniently and rapidly determined at 5.56 microns (Figure 6). A standard curve, conforming to the Beer-Bouguer law, was drawn by employing samples of distilled allethrin to which had been added known amounts of purified chrysanthemummonocarboxylic acid anhydride. The concentration range examined was 0.1 to 6%. Attempts to determine the substance by the differential technique were unsuccessful, owing to poor instrument response even with wide slit widths and high amplification. Results obtained by the morpholine and infrared methods were in good agreement.

Allethrolone has been assayed by its ultraviolet absorption maximum at 227 mμ (*5*), but this method cannot be used in the presence of other alpha, beta-unsaturated carbonyl compounds. The keto alcohol can be determined by means of its 2.86-micron absorption band (Figure 7). Although satisfactory results have been obtained in the usual manner, by employing the differential technique the uncertainty accompanying the reading of absorbance values off the slope of a steep line is eliminated (Figure 8). Some preliminary work was carried out using the differential procedure in conjunction with an expanded wave-length scale (Figure 9), but the time consumed in calculating results outweighed the small increase in precision and accuracy. In actual practice transmittance paper is used and the area between 0% and sample amount of allethrolone is cut out and weighed analytically. All three techniques are accurate to $\pm 0.02\%$ in the concentration range 0.1 to 2.0%. The straight-line standard curves were plotted by measuring the absorbances of distilled allethrin containing varying amounts of purified allethrolone.

Although the author believes that the differential procedure is superior to the standard one, precautions must be taken to ensure that the allethrin used in the reference beam is completely free from allethrolone. Even when stored at 0° C. under nitrogen, purified allethrin develops very small amounts of allethrolone over a period of several months.

Alpha-*dl-trans*-allethrin (*18*) was first used to derive a standard curve at 5.81 microns. The analyses of several commercial allethrin samples based on this straight-line curve yielded results between 2.6 and 3.0% lower than the assays by the hydrogenolysis and ethylenediamine methods. Distilled allethrin (97.0% by both chemical procedures) was next employed as the standard. Good agreement was then observed among the three methods.

It was believed that the lack of concordance among the assays based on α-*dl-trans*-allethrin was due to a difference in absorb-

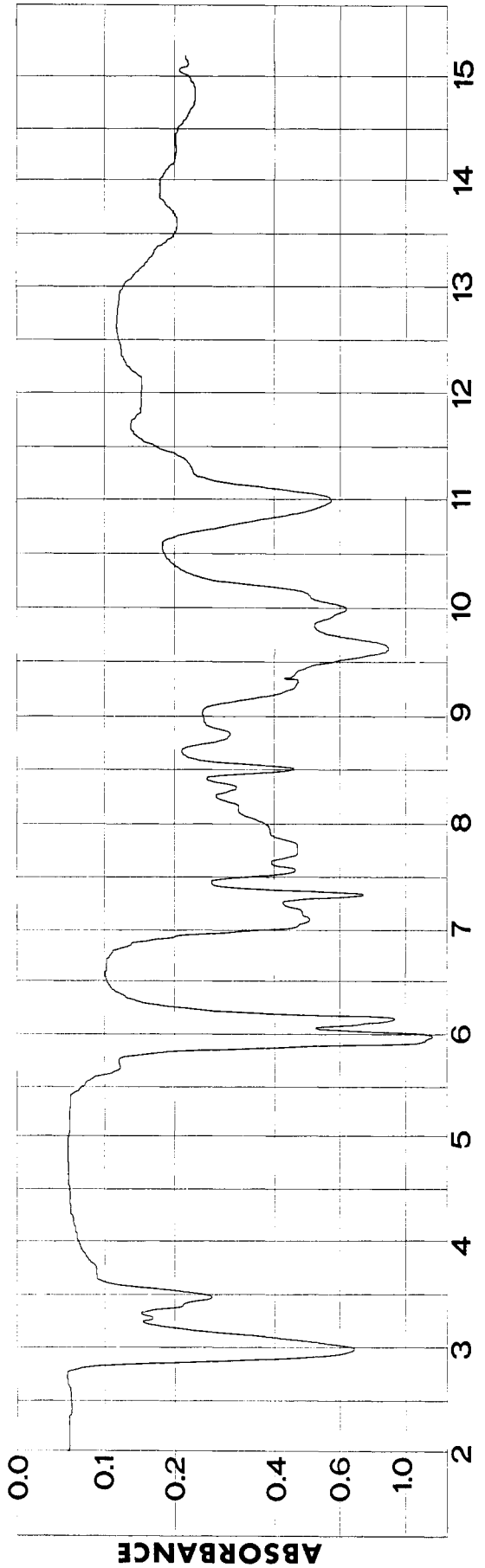


Figure 2. Infrared spectrum of allethrolone (between salt plates)

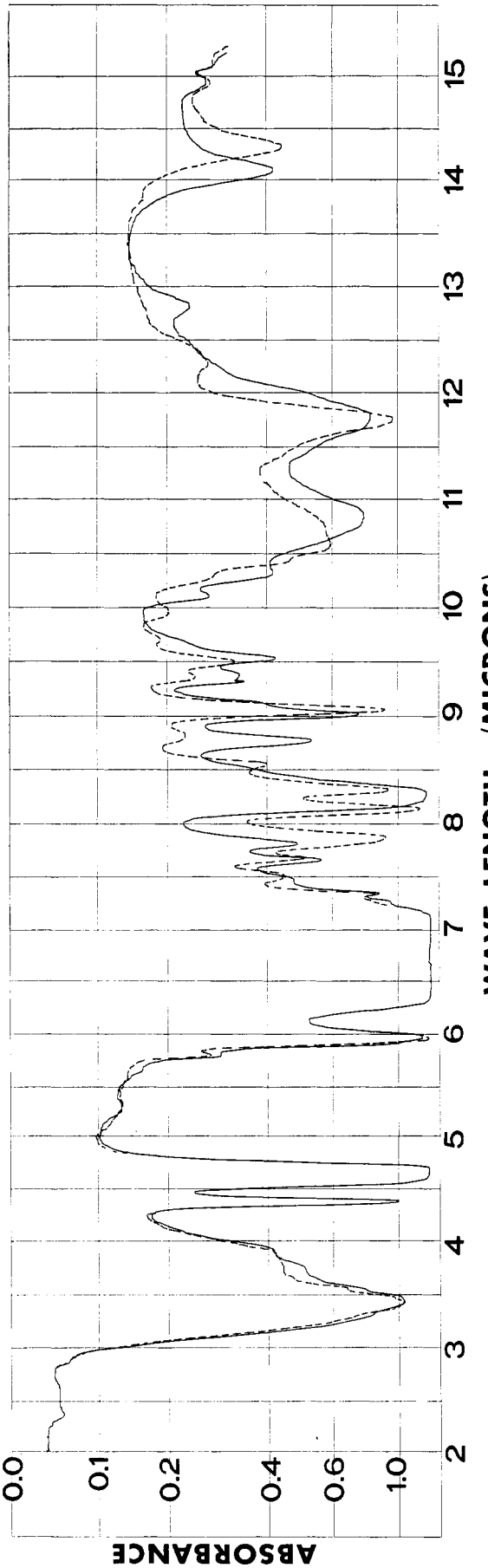


Figure 3. Infrared spectra of chrysanthemummonocarboxylic acids
—— cis, - - - trans, 2% in carbon disulfide, 0.5-mm. cell

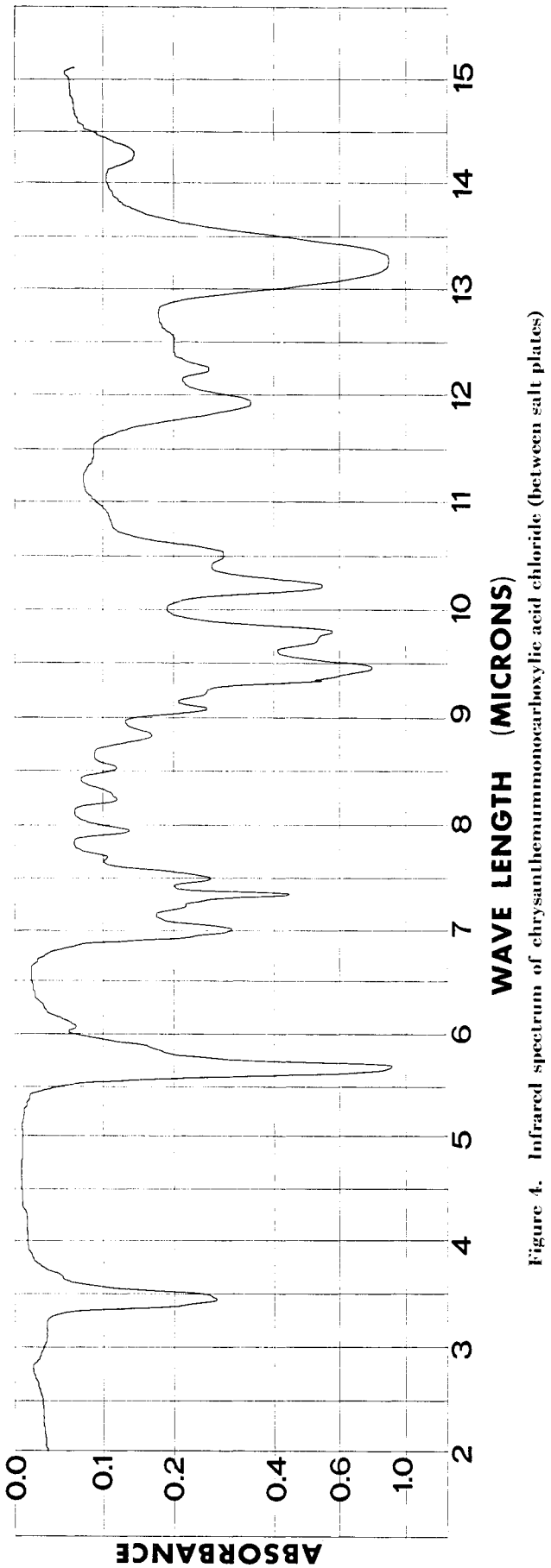


Figure 4. Infrared spectrum of chrysanthemummonocarboxylic acid chloride (between salt plates)

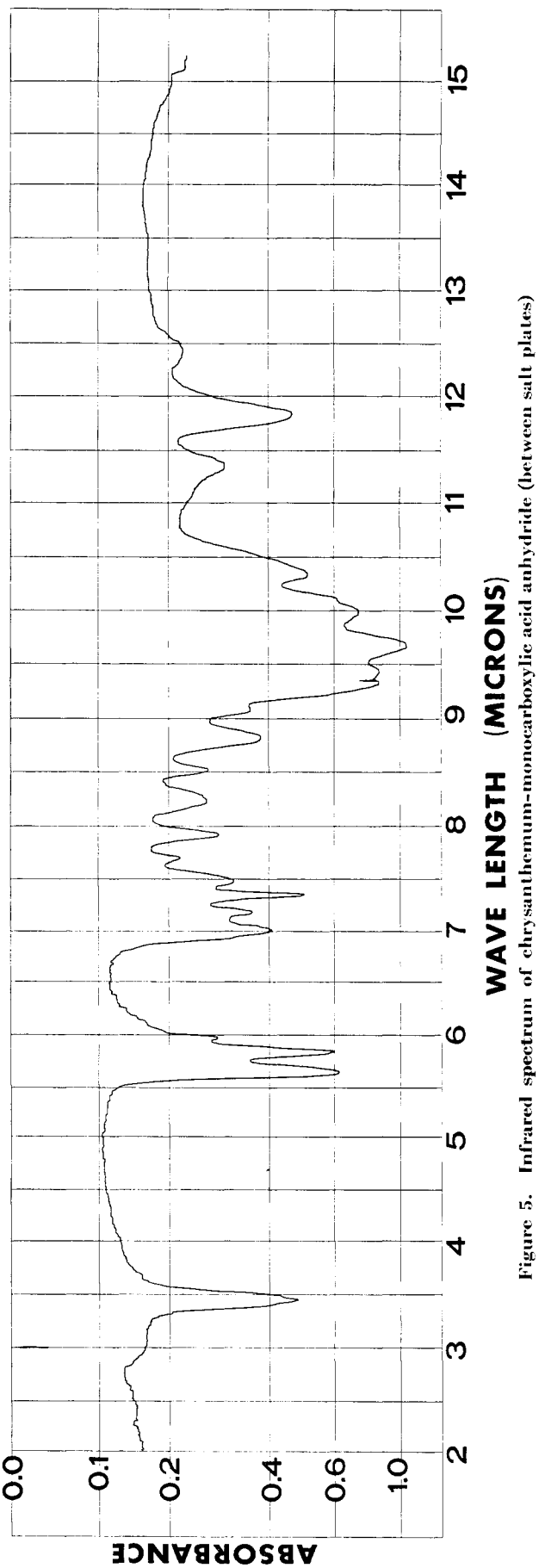


Figure 5. Infrared spectrum of chrysanthemum-monocarboxylic acid anhydride (between salt plates)

ances of the cis and trans isomers. The two geometrical isomers of allethrin were prepared and their infrared spectra compared (Figure 10). The 8.7-micron (trans) and 8.85-micron (cis) bands were selected as the basis for an analytical procedure and a straight line was obtained when log ratio of absorbances was plotted against per cent *trans*-allethrin content.

The commercial allethrin samples studied displayed a fairly constant cis-trans ratio (20 to 80). Owing to the fact that the infrared assay shows *cis*-allethrin to be 14% lower than the trans isomer, a correction factor of 0.14% must be applied for each per cent variation from 80.0% trans, the amount present in the distilled allethrin standard.

Apparently little change in cis-trans proportion occurs in the preparation of allethrin according to the synthesis of Schechter,

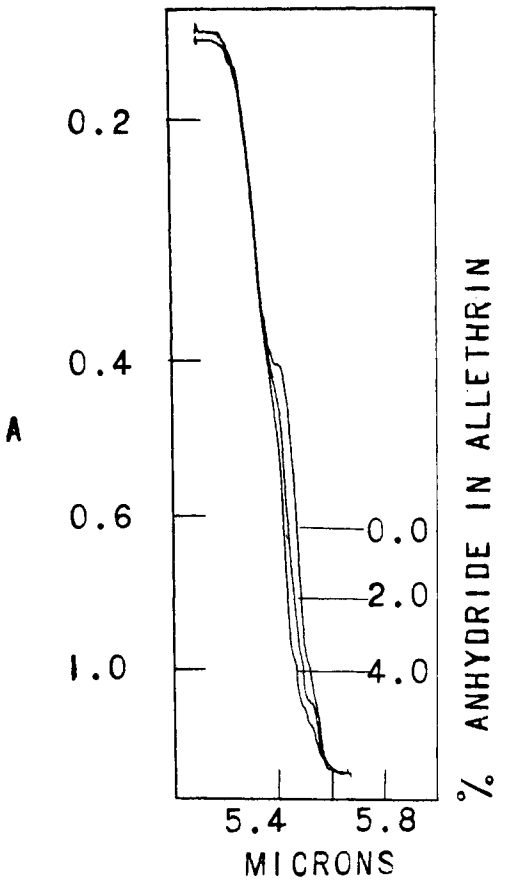


Figure 6. Anhydride content of technical allethrin

Green, and LaForge (*17*). The first intermediate exhibiting geometrical isomerism is ethyl chrysanthemumate, and the acid was isolated by saponifying the ester with 0.5*N* alcoholic potassium hydroxide. Pure *cis*- and *trans*-chrysanthemummonocarboxylic acids were prepared and their spectra recorded (Figure 3). The absorption bands at 14.04 (cis) and 14.26 (trans) were selected, and a standard curve was drawn. The acid derived from the ester assayed 77% trans.

DISCUSSION OF RESULTS

More than 50 commercial allethrin samples were analyzed by the infrared method described in this paper and checked by the hydrogenolysis and ethylenediamine procedures. Twelve representative analyses are outlined in Table I.

The results of both the ethylenediamine and infrared analytical methods are in excellent agreement in all instances except for sample E, where there is a difference of 0.8%. With the exception of samples G and H, the hydrogenolysis assays compare favorably with those of the other two methods. However, the author has not found the hydrogenolysis procedure completely reliable when applied to some commercial allethrin samples less than 90% pure. The source of the insecticide appears to be important, for erratic behavior has been observed in some samples (G and H, Table I).

Once the standard curves have been drawn, determination of allethrin requires less time by infrared spectrophotometry than by the chemical methods. Gersdorf and Mitlin (*7*) report that *dl-trans*-allethrin is approximately 50% more active than *dl-cis*-allethrin.

Preparation of ethyl chrysanthemumate by methods other than that described by Schechter, Green, and LaForge could conceivably yield an insecticide with a different cis-trans ratio. None of the chemical methods for determining the purity of allethrin is able to detect an important alteration of the molecule of this type, which directly affects its entomological activity.

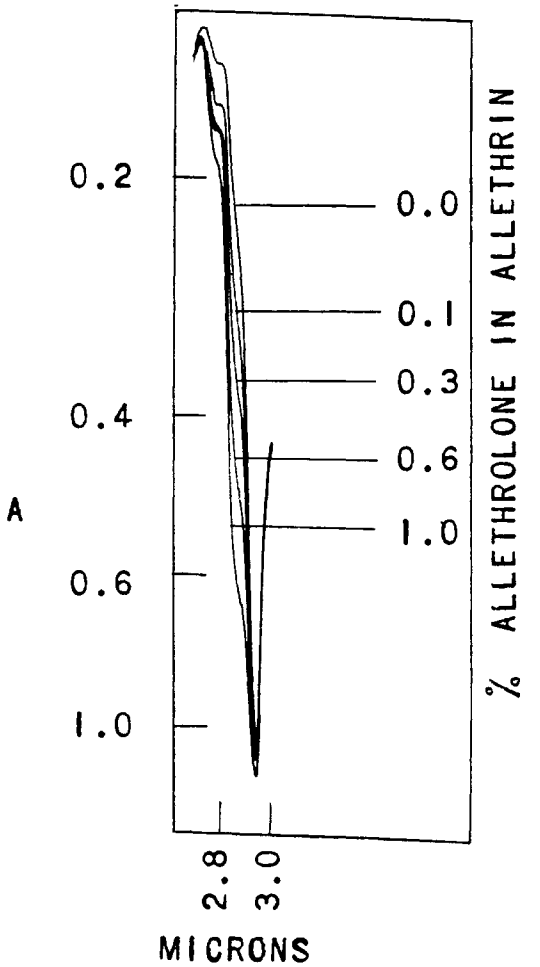


Figure 7. Determination of allethrolone in allethrin by standard technique

The data of Gersdorf and Mitlin (*7*) indicate that allethrin consists of 69% cis and 31% trans isomers, in direct contradiction to the present paper's findings. In the light of the numerous analy-

ses conducted in the author's laboratory, the entomological findings should be viewed with suspicion.

EXPERIMENTAL

A Perkin-Elmer No. 21 double-beam recording spectrophotometer was employed with the controls set for quantitative operation as recommended by the manufacturer. Standard curves were plotted as absorbance (*A*) *vs.* concentration. Where solutions were used, at every analytical wave length the absorbance due to the solvent alone was subtracted from that of the sample plus solvent.

Allethrolone (*dl*-2-allyl-4-hydroxy-3-methyl-2-cyclopenten-1-one). The keto alcohol was prepared by the method of Schechter (*17*) and purified through its semicarbazone (*5*). The material assayed 99.5% by the ultraviolet absorption method (*5*). Three different techniques were employed in determining small amounts of allethrolone in allethrin.

A. Standard Procedure. A 0.2-mm. sodium chloride sealed cell is filled and the spectrum recorded from 2 to 3 microns. The absorbance at the 2.86-micron maximum is noted and the percentage of allethrolone is read off the standard curve. Between 0.1 and 2.5% allethrolone can be determined by this procedure. Larger amounts can be analyzed by decreasing the cell thickness.

B. Differential Method. A 0.5-mm. cell containing purified allethrolone is placed in the reference beam and the material under test (0.5-mm. cell) is put in the path of the sample beam. The spectrum is scanned between 2 and 3 microns. The absorbance obtained by placing purified allethrolone in both cells is subtracted from the total absorbance found at 2.86 microns and this corrected value is used in reading the quantity of allethrolone present from the standard curve. Between 0.05 and 2.5% can be determined employing this technique.

C. Differential Method with Expanded Scale. Two 0.5-mm. sealed cells were used as in Method B. The instrument gears were changed to increase the wave-length scale to 160 cm. per micron and the spectrum was recorded from 2 to 2.9 microns.

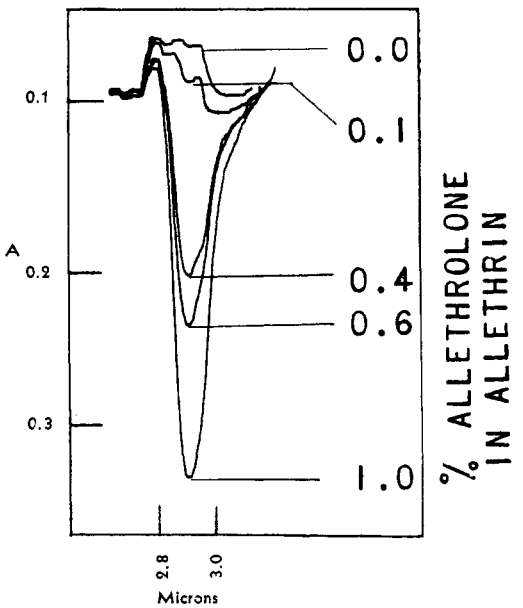


Figure 8. Determination of allethrolone in allethrin by differential technique

Table I. Infrared and Chemical Determination of Allethrin

Sample	Allethrolone	Anhydride	Trans	Purity I.R.	Purity H_2	Purity E.D.
A	0.10	0.79	80.0	93.9	93.6	93.8
B	0.20	1.9	80.5	92.3	92.1	92.0
C	0.15	2.1	80.2	92.8	93.1	93.0
D	0.11	0.80	80.9	93.6	93.9	93.6
E	0.10	0.00[a]	80.1	93.9	94.6	94.7
F	0.21	1.5	80.5	93.0	94.0	93.1
G	0.07	0.42	81.0	90.4	87.0	90.5
H	0.13	0.65	79.5	90.6	87.5	90.5
I	0.22	3.1	80.4	94.5	94.6	94.2
J	0.10	0.10	80.4	94.7	94.3	94.5
K	1.00	0.40	80.0	95.3	95.5	95.2
L	0.25	2.5	80.2	94.8	94.9	94.5

[a] Morpholine method yielded (−)1.0%.

A blank was run with purified allethrolone in both cells and the area between the blank and sample curves was cut out and weighed on an analytical balance. The allethrolone content was then read from a standard curve which was plotted in weight of paper *vs.* percentage of allethrolone. Between 0.03 and 4% can be determined by this technique.

Chrysanthemummonocarboxylic Acid Anhydride. The anhydride was prepared for the first time by reaction of equimolar quantities of the acid chloride and sodium chrysanthemumate in a flask under nitrogen. When the liberation of heat ceased, the vessel and contents were heated on a steam bath for 1 hour. The mixture was then extracted with ether and the ether was dried and distilled off. On distilling at 188° to 189° C. and 10 mm., a pale yellow, odorless liquid was obtained in 55% yield assaying 98.5 to 99.0% anhydride by the morpholine procedure (*12*). The anhydride contained 2% chrysanthemummonocarboxylic acid as determined by potentiometric titration to pH 9.5 in 50%

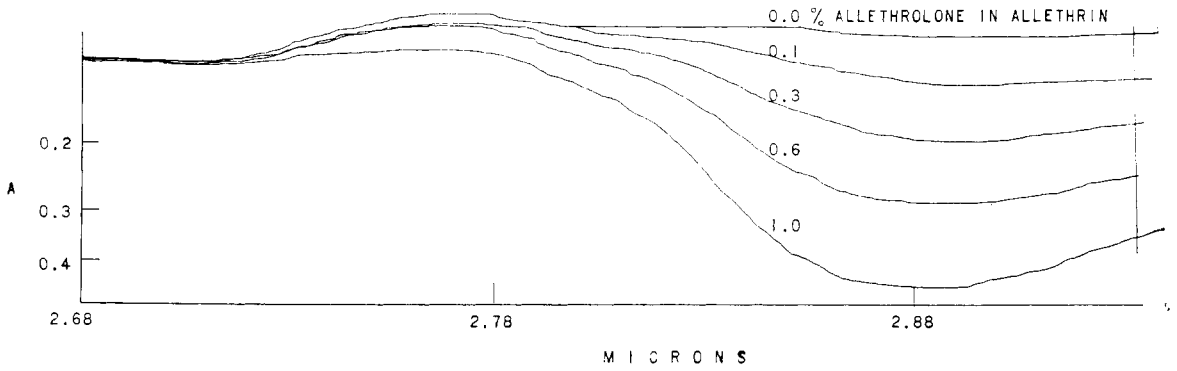


Figure 9. Determination of allethrolone by differential technique with expanded wave-length scale

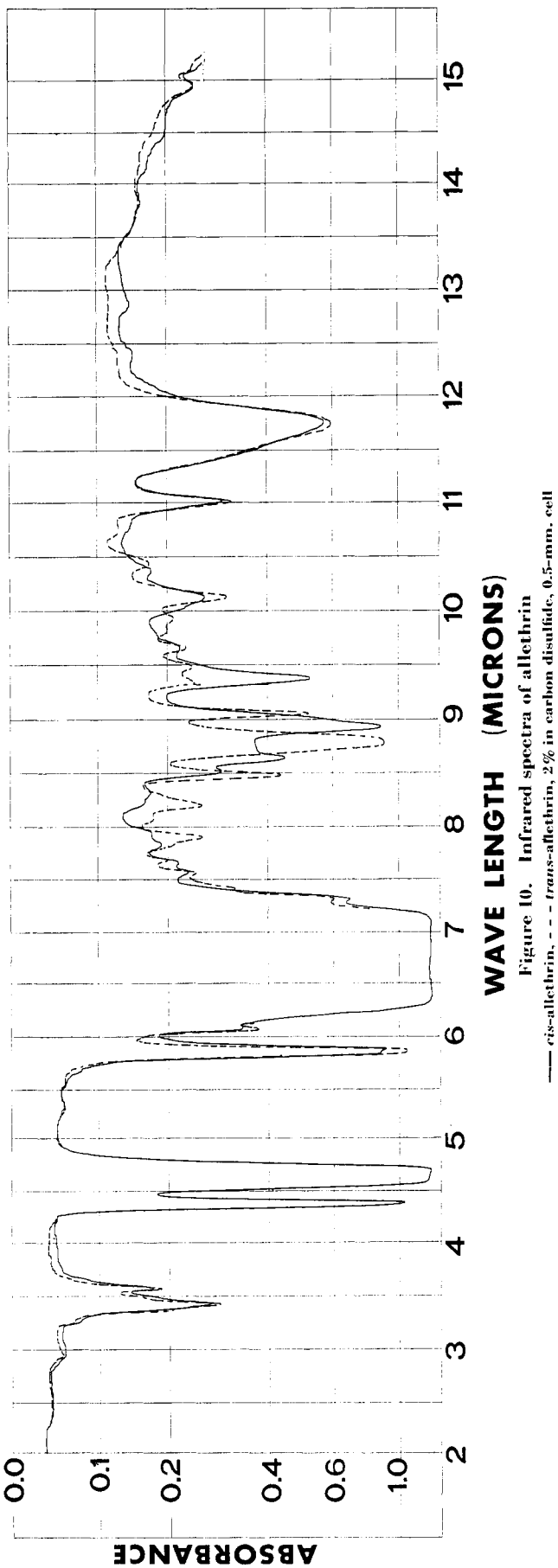


Figure 10. Infrared spectra of allethrin
—— *cis*-allethrin, - - - *trans*-allethrin, 2% in carbon disulfide, 0.5-mm. cell

ethyl alcohol–water solution. The anhydride was also prepared by refluxing chrysanthemummonocarboxylic acid with a large excess of acetic anhydride, but a lower yield of material assaying 88% resulted.

For the determination of anhydride in allethrin a 0.1-mm. cell was employed and the spectral region between 4 and 5.8 microns recorded. The analytical wave length selected was 5.56 microns; anhydride in the range of 0.1 to 6% can be determined.

Allethrin. The insecticide was prepared as directed in the literature (*17*) and approximately 500 grams were fractionated at 1 mm. A middle cut was taken and, when assayed by both hydrogenolysis and ethylenediamine methods, was found to contain 97.0% allethrin. Beer-Bouguer's law was followed in the range 0.18 to 0.28 gram per 100 ml. of carbon tetrachloride at 5.81 microns. More than 50 points were taken over a period of 2 months for the preparation of the standard curve.

For the analysis, 0.2 to 0.25 gram of allethrin was dissolved in 100 ml. of carbon tetrachloride, and transferred to a 0.5-mm. sealed absorption cell, and the region between 5.2 and 6.0 was recorded. The percentage of allethrin was then read from the standard curve. A correction must be made for the allethrolone present in the sample, by subtracting the allethrolone content multiplied by a factor of 1.1 from the total allethrin found. When anhydride is present in more than 5% amounts, an additional correction must be made. However, no commercial samples examined contained amounts necessitating a correction. Finally, the percentage of *trans*-allethrin present must also be determined and a correction made for it.

***cis*- and *trans*-Allethrin.** The two geometrical isomers were prepared according to the method of Schechter (*17*). Prepared mixtures of the isomers were dissolved in carbon disulfide (2 grams per 100 ml.) and the spectra run in a range 8.4 to 9 microns in a 0.2-mm. cell. Log ratios of absorbances at 8.7 microns (trans) and 8.85 microns (cis) were plotted against percentage trans isomer to yield a straight-line standard curve. Straight-line relationships between log A and concentration indicate to the reader that, although the solution does not follow Beer's law, the shape of the standard curve is hyperbolic in nature. A correction factor of 0.14% must be applied for each percentage variation from the trans content of the standard allethrin (80.0%).

***cis*- and *trans*-Chrysanthemummonocarboxylic Acids** (*9*, *18*). One gram of acid is dissolved in 100 ml. of carbon disulfide and transferred to a 0.5-mm. cell. The spectral region recorded lies between 13.6 and 14.6 microns. The cis form exhibits a maximum at 14.04 microns and the trans form at 14.26 microns.

Chrysanthemummonocarboxylic acid ethyl ester used in the synthesis of the acids studied was prepared by the addition of ethyl diazoacetate to 2,5-dimethyl-2,4-hexadiene (*2*, *17*).

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