Some Barthrin Isomers and Their Toxicity to Houseflies in Space Sprays

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The 6-chloropiperonyl esters of *d-trans-*, *dl-trans-*, *dl-cis-*, and *dl-cis-trans-* (barthrin) chrysanthemumic acid were synthesized. These esters and the *l-trans* ester (by inference) were, respectively, 62, 38, 29, 32, and 14% as toxic to houseflies (*Musca domestica* L.) as allethrin at the 50% mortality level. At the 95% mortality level the ratios were one third greater. Year-old sprays of barthrin were just as toxic as sprays from a freshly prepared sample. The knockdown values of barthrin and the three isomers, although excellent at highly toxic concentrations, were much inferior to that value of allethrin at comparable concentrations.

THE *dl*-ALLETHROLONE ESTER of *d-trans* - chrysanthemumic acid has been shown to be about twice as toxic to houseflies (*Musca domestica* L.) as the corresponding ester of the *dl-cis-trans* acid—that is, allethrin (*7*, *9*, *10*). *dl-trans*-Allethrin has been shown to be about twice as toxic as *dl-cis*-allethrin (*12*). With the synthesis (*1*) of barthrin (6-chloropiperonyl ester of *dl-cis-trans*-chrysanthemumic acid) and the estimation of its relatively high toxicity by the same method (*14*), there arose the question of the effects of similar structural differences in the isomers in barthrin upon their toxicity. This paper reports the results of tests designed to answer this question.

Materials

6-Chloropiperonyl alcohol was prepared from piperonyl alcohol (*16*) by the procedure of Barthel and Alexander (*1*): Boiling point 150–2° C., 4 mm. of mercury, n_D^{25} 1.5380.

d-trans-Chrysanthemumic acid was prepared from a 20% pyrethrum concentrate. The concentrate was purified by the nitromethane method of Barthel, Haller, and LaForge (*2*), and hydrolyzed according to the procedure of Campbell and Mitchell (*3*): boiling point 126–8° C., 4 mm. of mercury, n_D^{20} 1.4801.

dl-cis- and *dl-trans*-chrysanthemumic acids were separated from the commercial *dl-cis-trans* acid by fractional crystallization from pentane: melting point 50.5–2.6° C. for the cis acid and 113.8–114.5° C. for the trans acid. The constants of all intermediates were in good agreement with reported values.

The *d-trans*, *dl-cis*, and *dl-trans* esters were prepared by two methods. The first consisted in condensing 6-chloropiperonyl alcohol with the acid chloride according to the general method of LaForge and Barthel (*15*), and the second involved ester exchange between the ethyl ester of the acid and 6-chloropiperonyl alcohol according to the procedure of Barthel and Alexander (*1*). These esters exhibited identical ultraviolet and infrared absorption spectra and distilled between 180° and 182° C. at 0.6 mm.; n_D^{25} 1.5393.

Two samples of barthrin were used: one, B, prepared recently in the laboratories of the Benzol Products Co. and the other, A, prepared a year ago in the laboratories of the Entomology Research Division, U. S. Department of Agriculture.

The purity of the esters, determined by saponification, was as follows: *d-trans*, 98.9%; *dl-trans*, 99.5%; *dl-cis*, 99.5; barthrin sample A, 95.2%; and sample B, 96.0%.

The infrared and ultraviolet absorption spectra, obtained with Perkin-Elmer No. 21 double-beam and Beckman DK-2 ratio recording spectrophotometers, are given in Figures 1 and 2.

As a standard of comparison in the toxicity tests, a sample of allethrin analyzing 93.4% by the hydrogenolysis method was used.

Testing Procedure

The materials were dissolved in refined kerosine (Deobase) and diluted with the

Table I. Knockdown and Mortality of Houseflies Caused by Kerosine Sprays Containing Allethrin, Pyrethrins, and Certain Stereoisomers in Barthrin

(Six replicates, approximately 104 flies each)

Material	Concentration, Mg./Dl.	Knockdown in 25 Minutes, %	Mortality in 1 Day, %
Barthrin isomers			
d-trans	444.4	99.8	98.4
	296.3	99.5	95.4
	197.5	92.5	77.2
	131.7	52.1	39.4
	87.79	31.8	9.1
dl-trans	444.4	99.5	90.6
	296.3	96.4	68.4
	197.5	68.0	33.1
	131.7	30.8	5.2
	87.79	22.8	3.0
dl-cis	666.7	99.8	93.6
	444.4	99.1	79.6
	296.3	96.1	50.1
	197.5	71.0	11.3
	131.7	29.2	3.5
Barthrin			
Sample B	666.7	99.7	97.5
	444.4	98.9	89.9
	296.3	67.0	51.5
	197.5	64.9	17.8
	131.7	40.3	2.6
Sample A	666.7	100	96.6
	444.4	96.4	79.9
	296.3	82.2	48.9
	197.5	56.8	18.1
	131.7	37.6	2.8
	87.79	13.2	1.6
Allethrin	214.1	100	88.5
	142.8	100	77.9
	95.17	100	56.3
	63.45	100	26.0
	42.30	99.2	9.9

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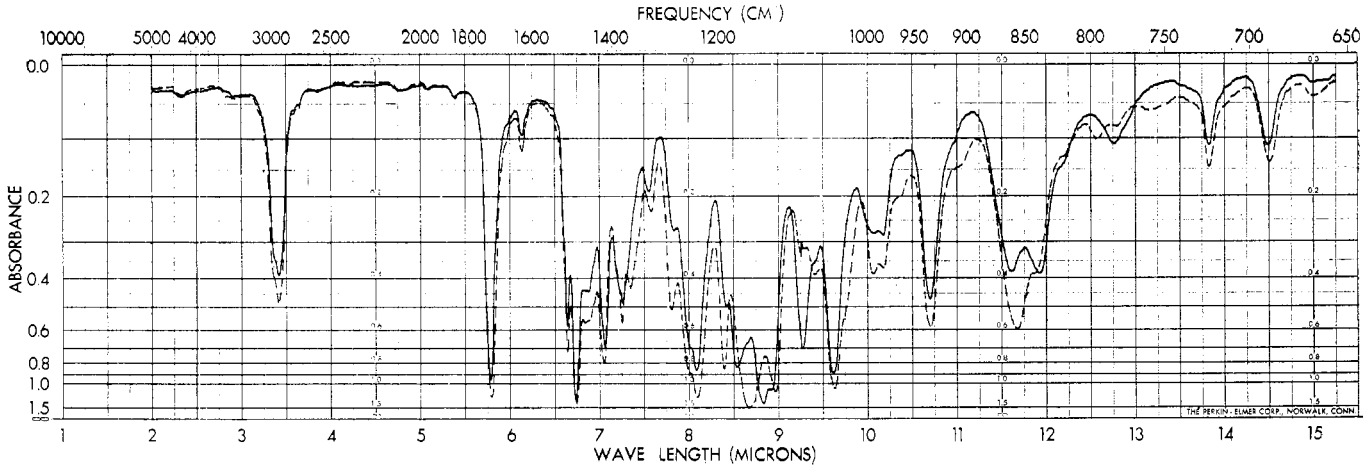

Figure 1. Infrared absorption spectra of 6-chloropiperonyl chrysanthemumate isomers taken with film between salt plates

——— *dl-cis* isomer
- - - - *dl-trans* isomer

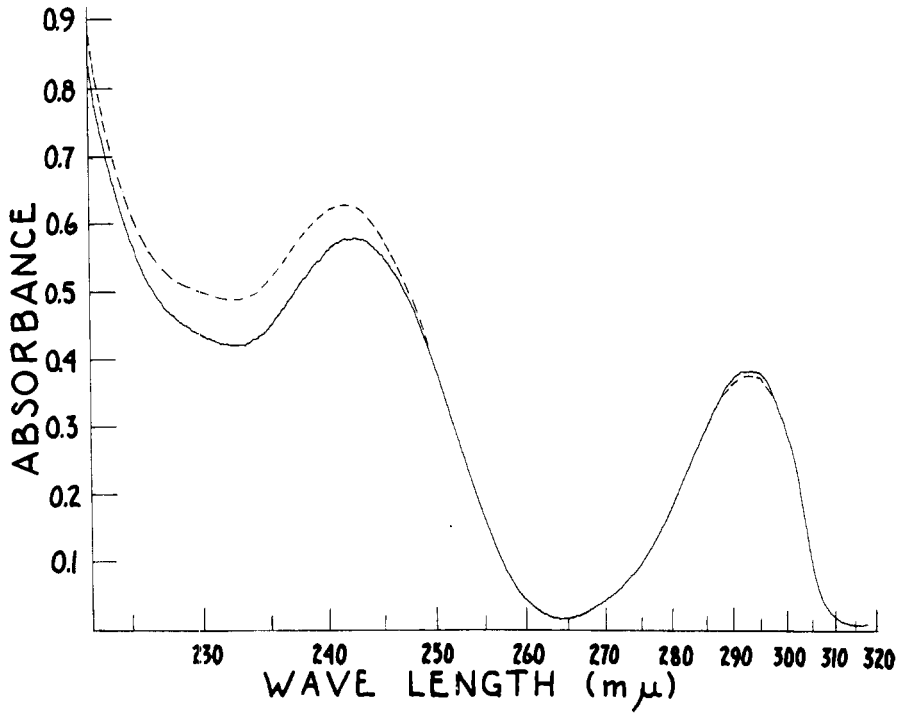

Figure 2. Ultraviolet absorption spectra of 6-chloropiperonyl chrysanthemumate isomers, 0.02 gram per liter of ethyl alcohol

——— *dl-cis* isomer
- - - - *dl-trans* isomer

Table II. Toxicity Relative to Allethrin of Barthrin and Three of Its Isomers

Material	LC_{50}, Mg./Dl.	Relative Toxicity, %	Range in Ratio between 5 and 95 Mortality Levels, %
Barthrin isomers			
d-trans	148.4 ± 4.6	61.86 ± 2.80	45.4 — 84.3
dl-trans	242.4 ± 7.7	37.87 ± 1.72	27.8 — 51.6
dl-cis	312.2 ± 9.5	29.40 ± 1.31	21.6 — 40.1
Barthrin			
Sample B	285.1 ± 8.7	32.20 ± 1.44	23.6 — 43.9
Sample A	298.1 ± 9.3	30.80 ± 1.39	22.6 — 42.0
Allethrin	91.80 ± 3.0	100	100

same solvent to furnish sprays for each at five or six concentrations selected according to preliminary toxicity tests.

Knockdown and mortality were determined by the Campbell turntable method (*4*) on houseflies of the National Association of Insecticide and Disinfectant Manufacturers' 1948 strain. All sprays were tested simultaneously and only once on each of six populations of flies. Approximately 104 flies were used in each test. The average age of the flies in each population was 2 to 3 days. The mortality and knockdown data are summarized in Table I.

Evaluation of Relative Toxicity

To evaluate relative toxicity and determine the precision of the estimates, the mortality data were subjected to probit analysis as described by Finney (*5*). Provisional individual regression lines were fitted. Just as in a previous study with piperonyl esters (*14*), it appeared that the data for these piperonyl esters were represented by lines of steeper slope than that for allethrin and that those for the isomers could be adequately represented by parallel lines. The statistical procedures based on these hypotheses were followed to obtain weighted regression equations, and subsequent analysis of chi-squares gave no evidence that the data could not be so represented. However, heterogeneity factors were required in the calculation of variances; they were 5.888 for each isomer and 4.094 for allethrin.

The final equations showing the regression of mortality, expressed in probits, on concentration in milligrams per deciliter, expressed as logarithms, are given below. The standard errors of the regression coefficients are 0.163 for the isomers and 0.236 for allethrin.

Barthrin isomers	
d-trans	$Y = 5.2326X - 6.3634$
dl-trans	$Y = 5.2326X - 7.4771$
dl-cis	$Y = 5.2326X - 8.0525$
Barthrin	
Sample B	$Y = 5.2326X - 7.8459$
Sample A	$Y = 5.2326X - 7.9475$
Allethrin	$Y = 3.6643X - 2.1925$

From these equations LC_{50}'s (median lethal concentrations) were determined and evaluations of relative toxicity were then obtained as the inverse ratio of the

pertinent pair of LC_{50}'s. The estimations together with their standard errors are given in Table II.

Because the regression lines for the isomers are of greater slope than that for allethrin, ratios of comparison with this standard will vary between low and high mortality levels. Such ratios were estimated from LC_5's and LC_{95}'s determined directly from the equations and are also given in Table II to indicate the approximate range possible.

Discussion

As indicated in Table I and demonstrated in Table II, the industrially prepared sample of barthrin was toxicologically the equal of the original preparation. The slightly higher ratios obtained, although to be expected because of the slightly higher degree of purity, were not significantly so. These ratios agree very well with that found for the original preparation a year ago when it was determined by the same method to be 31.5% as toxic as allethrin (*14*).

As was found in the case of allethrin (*12*), the trans fraction of barthrin was more toxic than the cis fraction. However, the disparity was not as great, the ratio of toxicity being 2.18 with the allethrin isomers and 1.29 with the barthrin isomers. With the latter the closeness of the relative toxicities together with the precision of their estimation does not permit a meaningful estimation of the proportion of the two fractions by this bioassay. That proportion is undoubtedly the same as in allethrin (*6*, *12*) when prepared according to the synthesis of Schechter, Green, and LaForge (*17*).

Synerholm (*18*) stated that there is practically no difference in effectiveness, when tested against houseflies in the large group Peet-Grady chamber, between the piperonyl ester prepared from the *dl-cis-trans* acid and that from the *d-trans* acid. The present results with the 6-chloro derivatives of these esters, however, show the one from the *d-trans* acid to be about twice as toxic as the other, 61.86 divided by 31.50. The ratio (1.96) is about the same as was found by this method for the two esters with the same configurational relationship in the acid component when the alcoholic component was *dl*-allethrolone, 2.01 (*7*), and when the latter component was *dl*-furethrolone, 1.73 (*8*). Two similar ratios may be inferred from the relative toxicities of certain stereoisomers of allethrin as determined by this method (*13*). The relative toxicities were calculated for the cis and trans pairs of isomers racemic in the acid component and of *d* form in the alcoholic component. This was done on the basis of equivalents according to the similar-action equation given previously (*10*). It may be stated in general form as follows:

$$p_1R_1 + (1 - p_1)R_2 = R_m$$

in which p_1 is the proportion of the first compound and R_1, R_2, and R_m are the ratios of toxicity for the first and second compounds and the mixture. A like calculation was made for the two pairs of isomers having the alcoholic component of the *l* form. Then the proper trans and cis pairs were used in a similar equation, but with the *trans-cis* proportion of 80 to 20 as found in the synthetic acid by Freeman (*6*) with the spectrophotometric method. Thus, ratios of toxicity were obtained for *d*-allethrolone *dl-cis-trans* chrysanthemumic acid ester (1.594) and for the corresponding *l*-allethrolone ester (0.279). Division of the relative toxicities of the *d-d trans* (3.367) and the *l-d trans* (0.579) isomers, by these estimations, respectively, gave 2.11 for the first comparison and 2.08 for the second. Had the 70 to 30 *trans-cis* proportion as found by bioassay against houseflies (*12*) been used, the ratios would not have differed much; they would have been, respectively, 2.22 and 2.15.

The *d-trans* ester was about 1.63 as toxic as the *trans*-racemate (242.4 divided by 148.4); therefore, it may be calculated from an equation similar to those above, that the *l-trans*-ester is about 0.37 as toxic as the *trans*-racemate, or 0.14 as toxic as allethrin.

It may be estimated that the maximum relative toxicity possible for either of the cis optically active isomers would be 0.588 as toxic as allethrin (twice the ratio for the racemate, if one active isomer were nontoxic), and even this extreme condition is not likely. Therefore, it may be concluded that, as in the allethrin esters, the *d-trans* acid also furnishes the most toxic of the barthrin isomers.

The knockdown values of barthrin and the three isomers, although excellent at highly toxic concentrations, are much inferior to that of allethrin at comparable concentrations. In a range of concentrations selected for mortality, percentage knockdown for the isomers begins to drop soon with decrease in concentration, almost as soon as does mortality, whereas for allethrin it does not begin to drop below 100% before the lowest concentration in this range is reached. In other words, the disparity between the intensity of the stimulus required to cause 50% mortality and that required to cause 50% knockdown is much less with the barthrin isomers than with allethrin. If dosage-knockdown curves are determined for the isomers—and individual graphic lines will suffice for this purpose—DC_{50}'s (median knockdown concentrations) of 113, 144, 156, 170, and 151 mg. per deciliter may be estimated for the isomers in the order of appearance in the tables. The respective ratios of LC_{50} to DC_{50} will be 1.3, 1.7, 2.0, 1.7, and 2.0, with a mean of 1.7. The ratio estimated for allethrin was about 8 (*11*).

Preliminary tests indicate that barthrin and these isomers are synergized by piperonyl butoxide about as much as allethrin and its isomers are synergized by that synergist. The intensity of synergism in 1 to 10 mixtures will probably be about threefold—that is, the toxicity of the mixtures will be three times that expected.

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