

JOURNAL OF THE WASHINGTON ACADEMY OF SCIENCES

VOLUME 42

October 1952

No. 10

BIOCHEMISTRY.—*A bioassay of some stereoisomeric constituents of allethrin.*

W. A. GERSDORFF and NORMAN MITLIN, U. S. Bureau of Entomology and Plant Quarantine.

In the study of the toxicity of pyrethroids in relation to chemical structure it has been of interest to determine the effect, if any, of differences in the arrangement of the atoms in the molecule. The relative toxicity of position isomers has been touched upon only with a comparison of two esters differing in point of attachment of the acyl group to the cyclopentenolone nucleus (LaForge et al., 1948), two pairs of esters differing in position of the double bond in the butenyl side chain (LaForge et al., 1948; Gersdorff, 1949a), two monochloro derivatives of allethrin (Gersdorff and Mitlin, 1951), and three pairs of esters of different cyclopropanecarboxylic acids (LaForge et al., 1952).

Most of the available isomers have differed only in spatial configuration. In tests with the pyrethrins and cinerins no differences in relative toxicity were demonstrated between the esters formed from the same *d-trans* acid with the *d-cis* and *dl-cis* forms of the same pentenolone (Gersdorff, 1947). Appreciable differences in toxicity were found, however, when there were differences in optical activity in the acid component, whether the cyclopentenolone possessed the 2-butenyl side chain (Gersdorff, 1949a) or the allyl side chain (Gersdorff, 1949a, b; Elliott et al., 1950; Fales et al., 1951; LaForge et al., 1952). No differences in toxicity were found between the esters of the *cis* and *trans* forms of the acid component, whether the cyclopentenolone possessed the 2-butenyl or the allyl side chain (Gersdorff, 1949a). However, only small amounts of the acids were available for the preparation of the esters (Schechter et al., 1949), so that there was some question of their purity (Gersdorff and Mitlin, 1951);

therefore, small differences in their toxicity, otherwise measurable, could have been missed.

A similar interest has continued in the studies with the stereoisomeric constituents of allethrin (*dl*-2-allyl-4-hydroxy-3-methyl-2-cyclopenten-1-one—that is, *dl*-allethrolone—acylated with a mixture of *cis* and *trans dl*-chrysanthemum monocarboxylic acids). Information on their relative toxicities and contents in allethrin would be of considerable importance. Allethrin may be considered a mixture of four racemic pairs of isomers, two being esters of the *cis* form of the acid and two of the *trans* form.

In 1950 chemists in the Bureau of Entomology and Plant Quarantine prepared the *cis* and *trans* fractions in nearly pure condition and in quantities adequate for extensive tests. A study was therefore undertaken to determine under these improved conditions the relative toxicities of the two fractions and of allethrin itself and to estimate by means of these values the amounts of the two in allethrin. By cooling distilled allethrin the chemists also obtained a crystalline compound which they identified as one of the racemic *trans* pairs and designated the α -*dl-trans* isomer of allethrin. A sample (A) of this compound was included in the study.

In 1951 one of the manufacturers of allethrin submitted to the Bureau a crystalline compound (sample C) obtained by holding commercial allethrin at 4° C. and found to be identical chemically with the Bureau's sample of the α -*dl-trans* isomer (Schechter et al., 1951). Another series of tests was made to compare the two products insecticidally, this time a newly prepared sample (B) from the distilled allethrin being used.

The oil obtained from the filtrate from the α -*dl-trans* isomer, designated the β -*dl-trans* isomer, was included in this series.

Materials.—Thus four new materials were available for comparison of toxicity—the mixture of the two *dl-cis* isomers, the mixture of the two *dl-trans* isomers, the α -*dl-trans* isomer, and the β -*dl-trans* isomer. The term “isomer” refers in each case to a pair of optical isomers. The first two mixtures were about 95 percent pure. The sample of the α -*dl-trans* isomer was pure. The β -*dl-trans* isomer contained about 5 percent of dissolved α -*dl-trans* isomer. The α -*dl-trans* isomer was represented by three separately prepared samples (A, B, and C). Two mixtures of the *dl-cis* and *dl-trans* fractions in the proportions 3:7 and 1:1 were also prepared. A distilled sample of allethrin, analyzing 95 percent by the hydrogenolysis method, and a sample of pyrethrins, 52 percent of which consisted of pyrethrin I and cinerin I (A.O.A.C. method), were used as standards of comparison.

Sprays of these materials were prepared by dissolving them in refined kerosene at concentrations selected according to preliminary tests.

Evaluation of relative toxicity.—The method of assay was based on the comparison of the relative toxicities of the components of a mixture with the relative toxicity of the mixture. These values were obtained from replicated tests at four concentrations made with the Campbell turntable. The test insect was the laboratory-reared adult house fly (*Musca domestica* L.). Approximately 100 flies, averaging 2 to 3 days in age, were used in each test. Knockdown and mortality are summarized in Table 1. The results are arranged in two series, each giving the means obtained with a different group of seven populations of flies. Tests with all materials in each group were made simultaneously.

Methods of probit analysis described by Finney (1947) were used to fit the regression lines to the mortality data and to estimate the LC 50 and its standard error for each of the materials. The estimations are given in Table 2. Relative toxicity is calculated as the inverse ratio of LC 50's. The equations for the lines showing the regression of mortality, expressed in probits, on concentration

in milligrams per deciliter, expressed as logarithms, are as follows:

Series 1:

<i>dl-cis</i> isomers	$Y = 2.902X - 0.7654$
<i>dl-trans</i> isomers	$Y = 2.902X - 0.2040$
Mixtures of <i>dl-cis</i> and <i>dl-trans</i> isomers:	
3:7	$Y = 2.902X - 0.3823$
1:1	$Y = 2.902X - 0.4390$
Allethrin	$Y = 2.902X - 0.5684$
α - <i>dl-trans</i> isomer, sample A	$Y = 3.722X - 4.0297$
Pyrethrins	$Y = 2.384X - 0.7338$

Series 2:

α - <i>dl-trans</i> isomer.	
Sample B	$Y = 3.241X - 2.8675$
Sample C	$Y = 3.241X - 2.8706$
β - <i>dl-trans</i> isomer	$Y = 2.690X + 0.2439$
Allethrin	$Y = 2.499X + 0.0583$
Pyrethrins	$Y = 2.479X - 0.8047$

In series 1 the slopes of the individually fitted lines with their standard errors were, respectively, 2.814 ± 0.097 , 2.952 ± 0.095 , 2.678 ± 0.093 , 3.054 ± 0.099 , 3.050 ± 0.101 , 3.722 ± 0.118 , and 2.384 ± 0.088 probits per unit log concentration. The results with the *cis* and *trans* fractions and their mixtures could be fitted with parallel lines, so this was done as their equations above show, the generalized regression coefficient being 2.902 ± 0.043 . The slopes of the lines for the α -*trans* isomer and pyrethrins were significantly different, however, so their individual equations are given.

In series 2 the individual slopes with their standard errors were, respectively, 3.386 ± 0.112 , 3.109 ± 0.107 , 2.690 ± 0.093 , 2.499 ± 0.093 , and 2.479 ± 0.094 . The data for the two samples of the α -*trans* compound could be fitted by parallel lines with a generalized regression coefficient of 3.241 ± 0.077 . The slopes for the three other materials are significantly different from those for the α -*trans* samples but not among themselves. However, the slope for allethrin is usually slightly greater than that for pyrethrins when compared by this method. Therefore, it was thought best to use the individually fitted lines for these three materials. That these lines will show more nearly true relationships may be seen if allethrin, the α -*trans* isomer, and pyrethrins are compared by means of LC 50's in the two series. The difference in relative susceptibility of different populations of flies has resulted in greater variation in the measurements with

pyrethrins. Therefore, differences in relative toxicity values involving pyrethrins are due essentially to the measurements made with that insecticide.

Assay.—To estimate the amounts of components in a mixture by means of relative toxicity, it is necessary to determine equivalents and to assume or establish that the joint action of the components is of the similar type as defined by Bliss (1939). It does not seem likely that synergistic or antagonistic action will occur in mixtures of components so nearly alike structurally. In the three mixtures of the *cis* and *trans* fractions there can be no pronounced action of either of these types. As shown by the LC 50's in Table 2, by the equations, or indeed by the mortalities in Table 1, the toxicities of these mixtures fall between those of the individual fractions, in themselves not greatly different in toxicity. If one of the fractions is chosen as a standard of comparison and the insecticide equivalents in terms of this standard calculated for the LC 50's of the two prepared mixtures, it will be found that they do not differ greatly from the standard. This was done in Table 2 with the *cis* fraction as the standard.

To find whether there was a significant departure from similarity, the relative toxicity on the equivalent basis was determined. This is also given in Table 2, together with its logarithm, since tests of significance are properly made on the log concentration scale. When these log ratios are compared with the minimum log ratio required to demonstrate synergism or antagonism, it is seen that there is no difference from similarity in the action of these components when mixed.

If the joint action is similar one component may be substituted for the other in a constant ratio without altering the toxicity of the mixture. The percentage composition of the two fractions in the sample of allethrin used may therefore be calculated on the basis of their relative toxicities. Thus, the sum of the equivalents of the components would equal the equivalent of the mixture, stated as a general equation as follows:

$$p_c R_c + (1 - p_c) R_t = R_m$$

in which p_c is the proportion of the *cis* frac-

TABLE 1.—KNOCKDOWN AND MORTALITY OF HOUSE FLIES CAUSED BY CONSTITUENTS OF ALLETHRIN AND THEIR MIXTURES IN KEROSENE SPRAYS
All tests replicated 7 times

Material	Concentration	Knock-down in 25 minutes	Mortality in 1 day
	Mg per ml	Percent	Percent
SERIES 1:			
<i>dl-cis</i> isomers	2.0	100	83.8
	1.0	100	50.0
	0.5	99.7	14.8
	.25	99.7	8.4
<i>dl-trans</i> isomers	2.0	100	93.0
	1.0	100	72.5
	0.5	100	41.0
	.25	100	11.2
Mixtures of <i>dl-cis</i> and <i>dl-trans</i> isomers:			
3:7	2.0	100	90.9
	1.0	100	61.0
	0.5	100	32.9
	.25	98.7	12.7
1:1	2.0	100	89.4
	1.0	100	69.5
	0.5	100	24.9
	.25	97.1	8.8
Allethrin	2.0	100	86.2
	1.0	100	62.7
	0.5	99.9	25.4
	.25	98.9	4.8
α - <i>dl-trans</i> isomer, sample A	8.0	100	97.2
	4.0	100	75.1
	2.0	99.9	26.8
	1.0	99.0	8.2
Pyrethrins	8.0	100	88.4
	4.0	100	68.3
	2.0	100	39.3
	1.0	100	17.2
SERIES 2:			
α - <i>dl-trans</i> isomer:			
Sample B	8.0	99.0	95.4
	4.0	100	74.8
	2.0	97.7	25.2
	1.0	95.0	10.9
Sample C	8.0	100	94.4
	4.0	98.9	72.9
	2.0	99.1	24.5
	1.0	90.1	13.7
β - <i>dl-trans</i> isomer	2.0	100	90.6
	1.0	100	74.1
	0.5	99.7	48.0
	.25	98.4	12.8
Allethrin	2.0	100	80.3
	1.0	100	50.9
	0.5	99.4	22.9
	.25	91.6	8.5
Pyrethrins	8.0	100	94.9
	4.0	100	69.3
	2.0	100	45.2
	1.0	100	21.7

tion and R_c , R_t , and R_m are the respective toxicity ratios of the *cis* and *trans* fractions and the mixture. The ratios may be relative to any standard, but if based on allethrin the values given in Table 2 may be introduced as follows:

$$p_c 0.85 + (1 - p_c) 1.33 = 1.00$$

from which $p_c = 0.69$ and $1 - p_c = 0.31$. Thus, according to this bioassay the *cis* isomers comprised about 69 percent and the *trans* isomers about 31 percent of the sample of allethrin used in this study.

The use of relative toxicities in such an assay may be tested by substituting the appropriate values for the two prepared mixtures in the above equation, as follows:

For the 3:7 mixture, $p_c 0.85 + (1 - p_c) 1.33 = 1.16$, from which $p_c = 0.35$.

For the 1:1 mixture, $p_c 0.85 + (1 - p_c) 1.33 = 1.11$, from which $p_c = 0.46$.

The differences from the actual proportions of the *cis* fraction used in the mixtures, 0.30 and 0.50, are within experimental error.

In series 2 the assay of the α -*dl-trans* and β -*dl-trans* isomers may proceed in the same fashion, since the two together form the total *trans* fraction. However, in this case

similar action is assumed since no prepared mixtures were tested. Since the β -*dl-trans* fraction tested still contained about 5 percent of the α -*dl-trans* isomer, a correction for this should first be made. The substituted equation for the correction is

$$0.05 \times 0.35 + 0.95 \times R_\beta = 1.62$$

from which R_β (the ratio of toxicity of the pure β -*dl-trans* isomer) is 1.69. Now the proportion of the two isomers may be obtained from the substituted equation, p_α representing the proportion of α isomer

$$p_\alpha 0.35 + (1 - p_\alpha) 1.69 = 1.33$$

from which $p_\alpha = 0.27$ and $1 - p_\alpha = 0.73$. Thus, 27 percent of the *dl-trans* fraction consisted of the α isomer and 73 percent the β isomer. Since the *trans* fraction was only 31 percent of allethrin, the two isomers comprised 8 and 23 percent of allethrin, respectively.

Discussion.—In the present study with materials of 95 percent purity, the *trans* fraction was shown to be 1.56 as toxic as the *cis* fraction. This ratio, obtained in the comparison of the separate materials, is substantiated by the relative toxicities deter-

TABLE 2.—RELATIVE TOXICITY OF SOME CONSTITUENTS OF ALLETHRIN AND THEIR DERIVED CONTENT IN ALLETHRIN

Material	LC 50		Toxicity Relative to—		Log of Ratio of Equivalents	Content in Allethrin
	Original Basis	<i>dl-cis</i> Isomers Equivalent	Allethrin, Original Basis	<i>dl-cis</i> Isomers Equivalent Basis		
	mg per dl	mg per dl				percent
Series 1:						
<i>dl-cis</i> isomers	97.0 \pm 2.3	97.0	0.85	1.0	—	69 \pm 5
<i>dl-trans</i> isomers	62.1 \pm 1.4	—	1.33	—	—	31 \pm 5
Mixtures of <i>dl-cis</i> and <i>dl-trans</i> isomers:						
3:7	71.5 \pm 1.7	99.5	1.16 (1.19) ¹	0.975	-0.011	—
1:1	74.8 \pm 1.6	95.7	1.11 (1.09)	1.014	0.006	—
Allethrin	82.9 \pm 1.8	—	1.0	—	—	—
α - <i>dl-trans</i> isomer, sample A	266.5 \pm 5.1	—	0.31	—	—	7 \pm 1 ²
Pyrethrins	254.0 \pm 6.6	—	0.33	—	—	—
Series 2:						
α - <i>dl-trans</i> isomer:						
Sample B	267.8 \pm 5.6	—	0.35	—	—	7 \pm 1 ²
Sample C	268.4 \pm 6.0	—	0.35	—	—	7 \pm 1 ²
β - <i>dl-trans</i> isomer	58.6 \pm 1.4	—	1.62 ²	—	—	24 \pm 1 ²
Allethrin	94.9 \pm 2.5	—	1.0	—	—	—
Pyrethrins	219.5 \pm 5.8	—	0.43	—	—	—
Minimum required to demonstrate synergism or antagonism					± 0.030	—

¹ Figures in parentheses calculated for similar action.

² When corrected for the presence of 5 percent of α -*dl-trans* isomer in the β -*dl-trans* fraction, the relative toxicity of 100 percent β -*trans* isomer becomes 1.69 and the content figures 8 and 23 percent.

mined for the two prepared mixtures. This substantiation is very readily shown by substituting the appropriate values in the general equation above and using for them the toxicities relative to the *cis* fraction. These ratios are not given in Table 2, but are easily obtained from the LC 50 values. Thus, the ratio for the 3:7 mixture is 1.36 and for the 1:1 mixture 1.30. The equations then become as follows:

For the 3:7 mixture, $0.3 \times 1.00 + 0.7R_t$
 $= 1.36$, and $R_t = 1.51$

For the 1:1 mixture, $0.5 \times 1.00 + 0.5R_t$
 $= 1.30$, and $R_t = 1.60$

These two indirect estimations of the relative toxicity of the *trans* fraction agree well with the direct estimation.

In series 2 the samples of the α -*dl-trans* isomer prepared at different laboratories are shown to be toxicologically identical. When these results are compared with the results obtained with an earlier preparation against different populations of flies in series 1, the agreement is still good. The significantly higher regression coefficient for this compound shows that mortality caused by it increased more rapidly with concentration than did that caused by pyrethrins. Therefore, although the two toxicants had about the same range of effective concentrations, equal mortalities were not obtained throughout the course of toxic action. However, at the 50 percent mortality level the two materials were about equally toxic. This relationship, the relatively simple chemical nature of the α -*dl-trans* isomer with the accompanying assurance of high purity and uniformity, the greater ease of handling, and the probably greater stability suggest that this compound may serve as a welcome substitute for pyrethrins as a standard for fly sprays.

The mixed ester formed by the acylation of racemic allethrolone with *l-trans* chrysanthemum monocarboxylic acid has been found to have little toxicity to house flies in comparison with allethrin (4 percent, LaForge et al., 1952). Even this may be due to a slight impurity, so that for practical purposes the allethrin equivalent of the mixed ester may be considered zero. Therefore, unless there is a mutually masking, antagonistic joint action, which is unlikely,

the two optical isomers in the ester—that is, the *l-trans* acid with *d*-allethrolone and with *l*-allethrolone—must have allethrin equivalents of zero. Now the α -*dl-trans* isomer is one of two optical pairs—*d-trans* acid with *d*-allethrolone plus *l-trans* acid with *l*-allethrolone, or *d-trans* acid with *l*-allethrolone plus *l-trans* acid with *d*-allethrolone—and the β -*dl-trans* isomer is the other pair (Schechter et al., 1951). These two pairs have been found in this study to have allethrin equivalents of 0.35 and 1.69. But one isomer in each pair, as deduced above, is nontoxic. Therefore, the remaining two isomers—*d-trans* acid with *d*-allethrolone and with *l*-allethrolone—have allethrin equivalents twice those for the mixtures, or 0.70 and 3.38. The more toxic isomer, if separated, would be the most toxic pyrethroid known, about 10 times as toxic as natural pyrethrins. If these two isomers were mixed to give the esters that would be formed by the acylation of racemic allethrolone with the *d-trans* acid, in equal proportion, this mixture would have an allethrin equivalent of 2.04, for

$$0.5 \times 0.70 + 0.5 \times 3.38 = 2.04.$$

This value has been demonstrated in actual tests with such a mixture. In four comparisons by the same method as used in the present study (Gersdorff, 1949b, and unpublished data), representing a total of 32 replications at four concentrations for each material, the estimations of the allethrin equivalent of a prepared ester of *d-trans* acid with *dl*-allethrolone were 1.98, 2.15, 2.01, and 2.04.

The ratio of toxicity of allethrin to pyrethrins in the first series, 3.06, was close to the mean of evaluations by this method, but in the second series this ratio, 2.31, was the lowest ever obtained with five or more replications at several concentrations for each insecticide.

It is shown in Table 1 that all the separated constituents of allethrin caused high knockdown of flies at the concentrations used, knockdown value in general paralleling toxic value.

Summary.—A bioassay of stereoisomeric constituents of allethrin was made by means of an evaluation of their relative toxicity

and that of their mixtures. The materials were applied as contact insecticides in refined kerosene on the Campbell turntable. The house fly (*Musca domestica* L.) was used as the test insect.

The *dl-trans* fraction of allethrin was 1.56 as toxic as the *dl-cis* fraction. The toxic action of the two fractions when applied in mixtures was identified as similar action.

The *trans* fraction was 1.33 and the *cis* fraction 0.85 as toxic as the sample of allethrin used. On this basis the *cis* isomers comprised about 69 percent and the *trans* isomers about 31 percent of allethrin.

A crystalline compound separated from the *trans* fraction was only 0.35 as toxic as allethrin and constituted 8 percent of that insecticide. The remainder of the *trans* fraction was 1.69 as toxic as allethrin and constituted 23 percent of that insecticide.

It is deduced that half of each portion of the *trans* fraction is relatively nontoxic and that, of the remaining two isomers, *d-trans* acid with *d*-allethrolone and *d-trans* acid with *l*-allethrolone, one is 0.70 and the other 3.38 as toxic as allethrin.

All the separated constituents possessed high knockdown value.

LITERATURE CITED

- BLISS, C. I. *The toxicity of poisons applied jointly*. Ann. Appl. Biol. **26**(3): 585-615. 1939.
- ELLIOTT, M., NEEDHAM, P. H., and POTTER, C. *The insecticidal activity of substances related to the pyrethrins*. I. *The toxicities of two synthetic pyrethrin-like esters relative to that of the natural pyrethrins and the significance of the results in the bioassay of closely related compounds*. Ann. Appl. Biol. **37**(3): 490-507. 1950.
- FALES, J. H., NELSON, R. H., FULTON, R. A., and BODENSTEIN, O. F. *Insecticidal effectiveness of aerosols and sprays containing esters of synthetic cyclopentenolones with natural chrysanthemum monocarboxylic acid*. Journ. Econ. Ent. **44**(2): 250-253. 1951.
- FINNEY, D. J. *Probit analysis*: 256 pp. Cambridge, 1947.
- GERSDORFF, W. A. *Toxicity to house flies of the pyrethrins and cinerins, and derivatives, in relation to chemical structure*. Journ. Econ. Ent. **40**(6): 878-882. 1947.
- . *Toxicity to house flies of synthetic compounds of the pyrethrin type in relation to chemical structure*. Journ. Econ. Ent. **42**(3): 532-536. 1949.
- . *Toxicity to house flies of new synthetic pyrethroid*. Soap and Sanit. Chem. **25**(11): 129, 131, 139. 1949.
- and MITLIN, NORMAN. *Relative toxicity of allethrin analogs to house flies*. Journ. Econ. Ent. **44**(1): 70-73. 1951.
- LAForge, F. B., GERSDORFF, W. A., GREEN, NATHAN, and SCHECHTER, M. S. *Allethrin-type esters of cyclopropanecarboxylic acids and their relative toxicities to house flies*. Journ. Organ. Chem. **17**(3): 381-389. 1952.
- LAForge, F. B., GREEN, NATHAN, and GERSDORFF, W. A. *Constituents of pyrethrum flowers. XXII. Synthesis and relative toxicity of two isomers of cinerin I*. Journ. Amer. Chem. Soc. **70**: 3707-11. 1948.
- SCHECHTER, MILTON S., GREEN, NATHAN, and LAForge, F. B. *Constituents of pyrethrum flowers. XXIII. Cinerolone and the synthesis of related cyclopentenolones*. Journ. Amer. Chem. Soc. **71**: 3165-73. 1949.
- SCHECHTER, MILTON S., LAForge, F. B., ZIMMERLI, A., and THOMAS, J. M. *Crystalline allethrin isomer*. Journ. Amer. Chem. Soc. **73**: 3541-2. 1951.

PALEONTOLOGY.—*New species of Lecanocrinus*. HARRELL L. STRIMPLE, Bartlesville, Okla. (Communicated by Alfred R. Loeblich, Jr.)

Much of the material used in the present study has been made available through the generosity of Drs. G. A. Cooper and A. R. Loeblich, of the U. S. National Museum, and Richard Alexander, at present a student at the University of Oklahoma. One rare specimen from the Haragan formation was collected by Mrs. Beverley Graffham on the occasion of the first field trip by herself and her husband, Allen Graffham, to the old Hunton town site under the guidance of Richard Alexander. Numerous specimens from the Henryhouse formation have been

collected by the author and his wife, Mrs. Melba Strimple.

The three most distinctive forms of *Lecanocrinus* found in the Henryhouse formation are described as new species: *L. brevis*, *L. erectus*, and *L. invaginatus*. One form from the Brownsport (Lobelville) formation is described as *L. lindenensis*, n. sp. The Haragan form is described as *Lecanocrinus huntonensis*, n. sp.

Abbreviations are given in the first systematic description below and are used thereafter without explanation.



Gersdorff, W A and Mitlin, N O R M A N. 1952. "A bioassay of some stereoisomeric constituents of allethrin." *Journal of the Washington Academy of Sciences* 42, 313–318.

View This Item Online: <https://www.biodiversitylibrary.org/item/122703>

Permalink: <https://www.biodiversitylibrary.org/partpdf/101843>

Holding Institution

Smithsonian Libraries and Archives

Sponsored by

Biodiversity Heritage Library

Copyright & Reuse

Copyright Status: Permission to digitize granted by the rights holder

Rights: <https://www.biodiversitylibrary.org/permissions/>

This document was created from content at the **Biodiversity Heritage Library**, the world's largest open access digital library for biodiversity literature and archives. Visit BHL at <https://www.biodiversitylibrary.org>.