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## INSECTICIDE ASSAY

# Analysis of Mixtures of Isomers of Demeton

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The isomers of demeton [O,O-diethyl O(and S)-ethyl-2-mercaptoethyl phosphorothioate] are determined in their mixtures by titration with bromine before and after alkaline hydrolysis. The reliability of the method is shown by the results of titrations of known mixtures of the isomers and related compounds. Duplicate analyses can be made in less than 40 minutes.

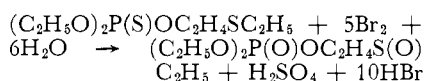
DEMETON (Systox, Chemagro Corp.), a commercial insecticide, consists of two isomeric forms, O,O-diethyl O-ethyl-2-mercaptoethyl phosphorothioate (thiono isomer), and O,O-diethyl S-ethyl-2-mercaptoethyl phosphorothioate (thiol isomer) (3, 7). Both isomers have insecticidal properties but differ in their chemical and physiological behavior, and the determination of the isomer content of experimental mixtures is of considerable importance for the evaluation of such mixtures as insecticides.

Gardner and Heath (3) separated the isomers by column chromatography and determined the phosphorus content of the eluted fractions. Henglein, Schrader, and Mühlmann (5) and Hensel and Kessler (6) have described determinations of the isomers based on infrared absorption by the P → S and P → O bonds. Hensel and Kessler gave methods based on the different rates of alkaline hydrolysis of the thiol and thiono isomers, and on determinations of the sulfate formed from the thiono sulfur when the isomer mixtures were oxidized with nitric acid.

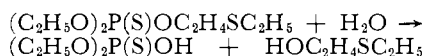
All of these methods are subject to interferences by impurities. The presence of foreign esters interferes in the hydrolysis method. Other phosphates may interfere with the infrared (4) and chromatographic methods. Nitric acid oxidation gave unreliable results for the analysis of demeton isomer mixtures (6).

The procedure reported herein determines the isomers in a mixture by titration with bromine before and after alkaline hydrolysis. The method is rapid and requires no special equipment.

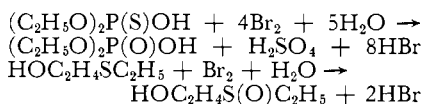
The thiono isomer reacts with 10 bromine equivalents:



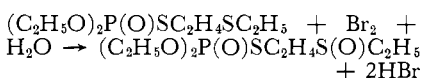
After hydrolysis,



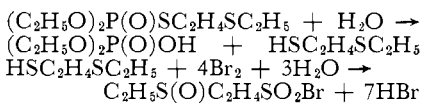
the same amount of bromine is required for the titration,



The thiol isomer requires two bromine equivalents before hydrolysis,



and eight bromine equivalents after hydrolysis,



The diethyl phosphoric acid does not react with bromine.

Siggia and Edsberg (8) reported a method for determining alkyl sulfides and thiols by titrating these compounds with 0.1N potassium bromate-potassium bromide in acetic acid solution. Their titration procedures have been used in the method to be described.

### Reagents

Bromate-bromide, 0.1000N. Dissolve 2.784 grams of high-grade potassium bromate and 15 grams of potassium bromide in water and make to 1 liter.

Sodium hydroxide, 2N, in methanol-water. Dissolve 8 grams of sodium hydroxide in 50 ml. of water and add 50 ml. of methanol.

Glacial acetic acid and hydrochloric acid. Use high-purity reagents.

### Procedure

**First Titration.** Weigh accurately a sample of 0.1 gram into a 300-ml. Erlenmeyer flask. Add 40 ml. of glacial acetic acid, 25 ml. of concentrated hydrochloric acid, and 10 ml. of water. Titrate the mixture with the bromate-bromide solution to a faint permanent yellow color, which persists about 1 minute. Make a reagent blank titration in the same manner, adding water to make the total volume about the same as that of a titrated sample.

**Second Titration.** Weigh accurately a sample of 0.1 gram into a 25-ml. glass-stoppered volumetric flask, and add 7 ml.

**Table I. Determination of Some Sulfur Compounds by Bromine Titration**

Compound, Samples	Found as % of Theoretical	Refractive Index $n_D^{25}$	Density, G./Ml. at 25° C.
2-Hydroxydiethyl sulfide			
A	99.9	1.4828	1.012
B	99.3	1.4828	1.014
C	99.9	1.4828	1.013
2-Mercaptodiethyl sulfide			
A	96.5	1.5252	1.029
B	96.5	1.5262	1.031
C	96.3	...	...
<i>O,O,O</i> -Triethylthionate			
A	97.6	1.4458	1.068
B	97.3	1.4458	1.069

of 2*N* sodium hydroxide-methanol solution. Shake in a wrist shaker for 15 minutes at room temperature (about 25° C.). At the end of the shaking period, rinse the contents of the flask into a 250-ml. beaker with 40 ml. of glacial acetic acid. Add 25 ml. of concentrated hydrochloric acid and 10 ml. of water, and titrate as above.

**Calculations.** Correct the titrations for the blank, and calculate the milliliters of bromate-bromide required for exactly 0.1 gram of the samples,

$$\frac{\text{titration, ml.}}{\text{sample weight, gram} \times 10} = \text{ml. required for 0.1000-gram sample}$$

Let *A* equal the titration of 0.1000-gram sample before hydrolysis, and *B* the titration after hydrolysis. Then, solving two simultaneous equations,

$$\begin{aligned} \text{Thiol isomer (per cent)} &= 4.306 (B - A) \\ \text{Thiono isomer (per cent)} &= 3.445 A - 0.8611 B \end{aligned}$$

**Experimental**

The validity of the oxidation equations given above was examined by titrating the isomers of demeton and their mixtures and related compounds. Siggia and Edsberg (8) obtained accurate results from the bromate-bromide titration of alkyl sulfides, and precise but low re-

sults with mercaptans. Their results were 3% low for ethanethiol and 8% low for 1-pentanethiol.

In this laboratory, the titration of three samples of 2-mercaptodiethyl sulfide, obtained by replicate syntheses (2), gave results 3.5% low (Table I). The low results may be due to the method, or to the impurity of the mercaptan.

The only constant available for estimating the purity of the mercaptan is a boiling point of 188° C. given by Demuth and Meyer (7). The mercaptans used in this work distilled at 65° C. at 8-mm. pressure, and contained 52.6% sulfur (Carius method) as compared to 52.5% theoretical. Two samples of 2-hydroxydiethyl sulfide prepared by the method of Windus and Marvel (9), and one redistilled commercial sample, gave theoretical results with the titration. The two samples of *O,O,O*-triethylthionate were technical grade compounds obtained from two commercial sources (Table I).

It is difficult to obtain the two isomers of demeton in a high state of purity, and to evaluate the small amounts of impurities nearly always present. The analyses of the demeton isomers shown in Table II are from samples prepared in this laboratory and from other laboratories.

The sulfate formed from the phosphorus-linked thiono sulfur was precipitated as barium sulfate from the titrated mixtures. These sulfate determinations,

possibly affected by the salts and reaction products in the titration mixture, lacked high precision, and varied by 1% or more. However, the approximate agreement between the calculated thiono demeton from the sulfate determination and the titration leaves little doubt that the semipolar sulfur is quantitatively oxidized to sulfate.

The determination of the thiol isomer is not affected by the presence of oxidizable impurities unless their reactivity with bromine is changed by the alkaline hydrolysis. The determination of thiono demeton is affected by these impurities.

Consideration has been given to various ways of showing the reliability of this method. None of the cited methods for determining the isomers has had any general acceptance as "standard," and the consistency of the data given in Tables I and II for mixtures of the isomers and their components is a more convincing indication of the reliability of the proposed method than a table of comparative analyses.

The end point of the titration can be determined within 2 or 3 drops for reasonably pure samples, but some difficulty may be encountered with highly colored impure samples.

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**Table II. Analysis of Demeton by Bromine Titration Method**

Compound	% Isomers by Bromine Titration			Refractive Index $n_D^{25}$	Density at 25° C.	Thiono Isomer from Sulfate <sup>a</sup>
	Thiol	Thiono	Inert			
Thiol Demeton A	92.5	2.6	4.9	1.4938	1.137	1.5
B	92.1	4.5	3.4	1.4952	1.132	...
Thiono Demeton A	2.7	96.2	1.1	1.4843	1.113	97.3
B	1.4	97.6	1.0	1.4833	1.113	96.6
Mixtures of Thiono and Thiol Demeton						
Mixture 1						
Calculated	27.00	70.85	2.15	...	...	70.85
Found	26.96	70.94	2.10	...	...	71.9
Mixture 2						
Calculated	67.93	28.16	3.90	...	...	28.16
Found	68.3	27.9	3.8	...	...	27.8

<sup>a</sup> Calculated from sulfate determination on titrated sample.