Degradation of Commercial Ethylene Bisdithiocarbamate Formulations to Ethylenethiourea under Elevated Temperature and Humidity

Warren R. Bontoyan* and Jack B. Looker

The formation of ethylenethiourea (ETU) in 18 commercial formulations was studied under controlled laboratory conditions of elevated temperature and humidity. The study revealed a large increase of ETU in certain formulations after 0 to 39 days' storage and smaller increases in others. The rate and amount of increase is apparently dependent upon storage time and chemical decomposition of other degradation products. Methods of ETU analysis and identification are described.

Recent studies have reported ethylenethiourea (ETU) to be a possible cause of thyroid cancer in albino rats (Ulland *et al.*, 1972). It is also a degradation product of ethylene bisdithiocarbamates. Because ethylene bisdithiocarbamates are extensively used on the nation's agricultural food crops, the possible ETU residue resulting from ethylene bisdithiocarbamates degrading in the field or on harvested crops is of major concern. Theoretically ETU could be present on all crops treated with ethylene bisdithiocarbamates. Aside from ETU formed under field conditions, consideration must be given to the initial ETU content in the commercial package. If ETU does prove to be harmful, a high ETU content in the package may be a possible hazard to applicators. If it becomes necessary to set new tolerances for ethylene bisdithiocarbamates on food crops, the limits may be difficult to determine by specified use patterns if those patterns are determined by the amount of ETU expected from ethylene bisdithiocarbamate degradation under field conditions. Previous studies have demonstrated a wide range of initial ETU in commercial packages of Zineb [zinc ethylenebis(dithiocarbamate)], Maneb [manganese ethylenebis(dithiocarbamate)], and zinc–manganese coordination materials (Bontoyan *et al.*, 1971). Adding to the problem is the possible further formation of ETU in commercial products stored at elevated temperature and humidity, as determined in this experiment.

METHODOLOGY

Gc Method. Although a gas chromatographic method for the determination of ehtylenethiourea (ETU) was available (Onley and Yip, 1971), a more direct analytical procedure avoiding the necessity of making a derivative was developed. This saved time and eliminated several variables such as extraction of ETU from the ethylene bisdithiocarbamates and formation and extraction of the 1-bromobutane derivative.

Thermal conductivity was chosen as the detection system so that ETU could be collected unchanged from the eluting material for confirmatory identification by infrared spectroscopy and mass spectrometry.

After trying several of the more common solvents and glc liquid phases, methanol and Carbowax 20M were chosen as the best combination with regard to stability, polarity, and solubility. The use of methanol as the extracting solvent also eliminates any possibility of introducing the dithiocarbamates in the gas chromatographs. Using the method as described below, 0.1% ETU in ethylene bisdithiocarbamates is easily detectable and quantitatively repeatable in the range of from 8 to 10 μg. Less than this amount is qualitatively detectable from 2 to 4 μg, however the low wide peaks make quantitative measurement variable.

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Reagent. The reagent used was 2-imidazolidinethione (ethylenethiourea).

Apparatus. The following equipment was used: Beckman GC-2A thermal conductivity gas chromatograph modified exhaust port tube (see under collection of ETU); and Blue M "Vapor-temp" controlled Relative Humidity Chamber, Model VP-100, Blue M Electric Co., Blue Island, Ill.

ETU Standard and Operational Parameters. A standard solution of ETU (2 mg/ml of MeOH or 2 μg of ETU/μl of solution) is prepared by dissolving 0.1 g of ETU in 50 ml of MeOH treated with anhydrous sodium sulfate. Forty microliters (40 μl or 80 μg) is injected into the gc with the following parameters: column, 3 ft × ¼ in. stainless steel with 2% Carbowax 20M on 80/100 Chromosorb W AW DMCS treated; column temperature, 220°; helium pressure, 40 psi; helium flow rate, 100 ml/min; filament current, 250 mA; chart speed, 0.5 in./min; and attenuation, 2.

At these conditions, a retention time of approximately 13 min was found, as was a peak area of approximately 1200 mm^2.

The standardization curve using peak area *vs.* μl of ETU standard solution was linear from 5 to 80 μl (10 to 160 μg of ETU). However, when using sample solutions, an injection of 80 μl was too large. Interfering substances and/or moisture from the sample caused the solvent peak to be retained as long as 6–8 min and to tail another 8–10 min, causing the ETU peak to come out on a curve. Smaller injections usually corrected this and gave a much cleaner separation of ETU.

Collection of ETU for Infrared and Mass Spectrometry. The standard exhaust port tube (4 in.) and heat block were replaced with a 6-in. length of ⅛ in. stainless steel tubing. A 3-in. piece of Teflon tubing was placed over the last 1½ in. and the entire length from the detector block to the outer end of the Teflon tubing was wound with a ½ in. flexible heating tape and heated to 230°.

The ETU was collected in a 6-in. piece of 3 mm glass tube placed 1½ in. into the Teflon tubing. No special cooling was needed. The ETU condensed within a 1-in. length of the air-cooled tube just outside of the heated portion.

For the infrared identification, the ETU was washed from the glass tube with methanol onto potassium bromide in a mortar and ground with a pestle until dry. The resulting mixture was formed into a micropellet and a full scan was made from 2.5 to 40 μ on a Perkin-Elmer 621 infrared spectrophotometer.

For mass spectrographic confirmation, the condensed ETU was washed with methanol into a 1-ml Kuderna–Danish concentrator tube and evaporated to a convenient workable volume.

EXPERIMENTAL SECTION

The experiment was set up to analyze 18 different commercial formulations. These consisted of six 80% Zineb,

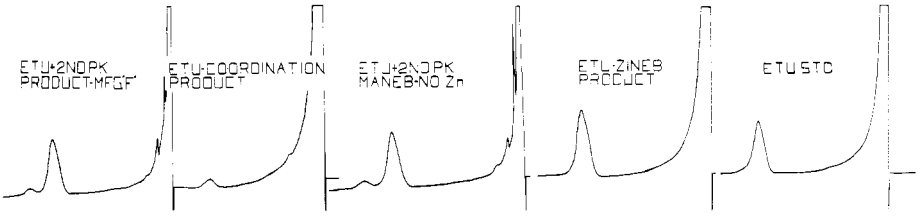


Figure 1. (left to right). Chromatograms of coordination product containing ETU peak and second peak, coordination product containing ETU, Maneb product containing ETU peak and second peak, no Zn ion present, Zineb product containing ETU, and standard ETU.

seven 80% Maneb, and five 80% zinc-manganese coordination wettable powders. Six different manufacturers' products were represented. These formulations were subjected to controlled laboratory conditions chosen to approximate climatic storage conditions in warehouses or sheds in the southeastern United States. Portions were removed periodically and used for the determination of ETU by gas–liquid chromatography as follows.

Eight 1-g portions of each of the 18 formulations were weighed into screw-cap test tubes (16 × 150 mm). These 144 tubes with caps off were placed in open racks in a glass-covered Blue M "Vapor-temp" controlled humidity chamber. The glass cover was enclosed in brown wrapping paper to keep light from the samples and the chamber was kept at a temperature of 120°F and a relative humidity of 80%. These conditions were maintained with very little variation. Samples (one tube of each of the 18 formulations) were withdrawn at intervals of 3, 7, 11, 17, 24, 31, and 39 days. This method of handling the samples was chosen to minimize variation in conditions.

Immediately upon removal, each tube was capped and allowed to stand for several minutes to cool and allow excess moisture on the outside to evaporate. Each was then weighed uncapped and no significant change was observed.

About 5 g of granular anhydrous sodium sulfate was added to each tube and 5 ml (10 ml as the ETU content increased) of methyl alcohol was added. The tubes were capped and shaken occasionally over at least a 1-hr period and then centrifuged until a clear liquid layer was obtained. An aliquot of 5 to 40 μl (depending on the ETU content) was injected into the gc with parameters as described above. ETU content was calculated using peak area measurements. Several portions of the effluent were collected for infrared and mass spectrometer identification. Recovery of ETU from spiked formulations is shown in Table I.

The condensed ETU peak effluents of initial, 7, and 24 days were trapped in glass capillaries at the end of the detector outlet and were identified as ETU by infrared spectrophotometry and mass spectrometry. Figure 1 is the gas chromatogram of ETU standard and ETU in formulations and Figure 2 is the infrared spectrum of ETU standard and the trapped ETU from a sample at 24 days.

The mass spectrometry identification was made on a Perkin-Elmer gas chromatograph mass spectrometer Model 270 and operated at 80 eV. Both standard ETU

Table I. Recovery by glc of ETU Added to Formulations

Percent ETU added to formulation	% recovered
0.1	50
0.5	60
1.0	70
5.0	80–90
10.0	80–90

Table II. Initial and Final ETU Content of Three Different Types of Commercial Formulations of Ethylene Bisdithiocarbamates

Sample no.[a]	Type of product	% ETU		Manufacturer	Remarks
		Initial	39 days		
1	Zineb, 80%	0.32	10.44	A	
2	Zineb, 80%	0.26	6.24	B	
3	Zineb, 80%	2.02	9.14	C	
4	Zineb, 80%	0.16	3.48	C	
5	Zineb, 80%	0.22	4.24	D	
6	Zineb, 80%	0.65	3.56	D	
7	Maneb, 80%	1.26	14.54	E	
8	Maneb, 80%	0.53	11.45	F	
9	Maneb, 80%	0.69	7.49	F	Contained 0.8% Zn
10	Maneb, 80%	0.43	13.60	D	
11	Maneb, 80%	0.08	0.66	C	Contained 1.0% Zn
12	Maneb, 80%	0.05	0.58	C	Contained 1.0% Zn
13	Maneb, 80%	0.05	0.69	C	Contained 1.0% Zn
14	Coordination,[b] 80%	0.04	0.22	C	
15	Coordination,[b] 80%	0.31	10.50	F	
16	Coordination,[b] 80%	0.05	0.25	C	
17	Coordination,[b] 80%	0.03	0.22	C	
18	Coordination,[b] 80%	0.02	0.13	C	

[a] Sample 3 is *ca.* 2 yr; all others 1 year or less. [b] Ethylene bisdithiocarbamate, 72%; Zn^{2+}, 2%; Mn^{2+}, 16%.

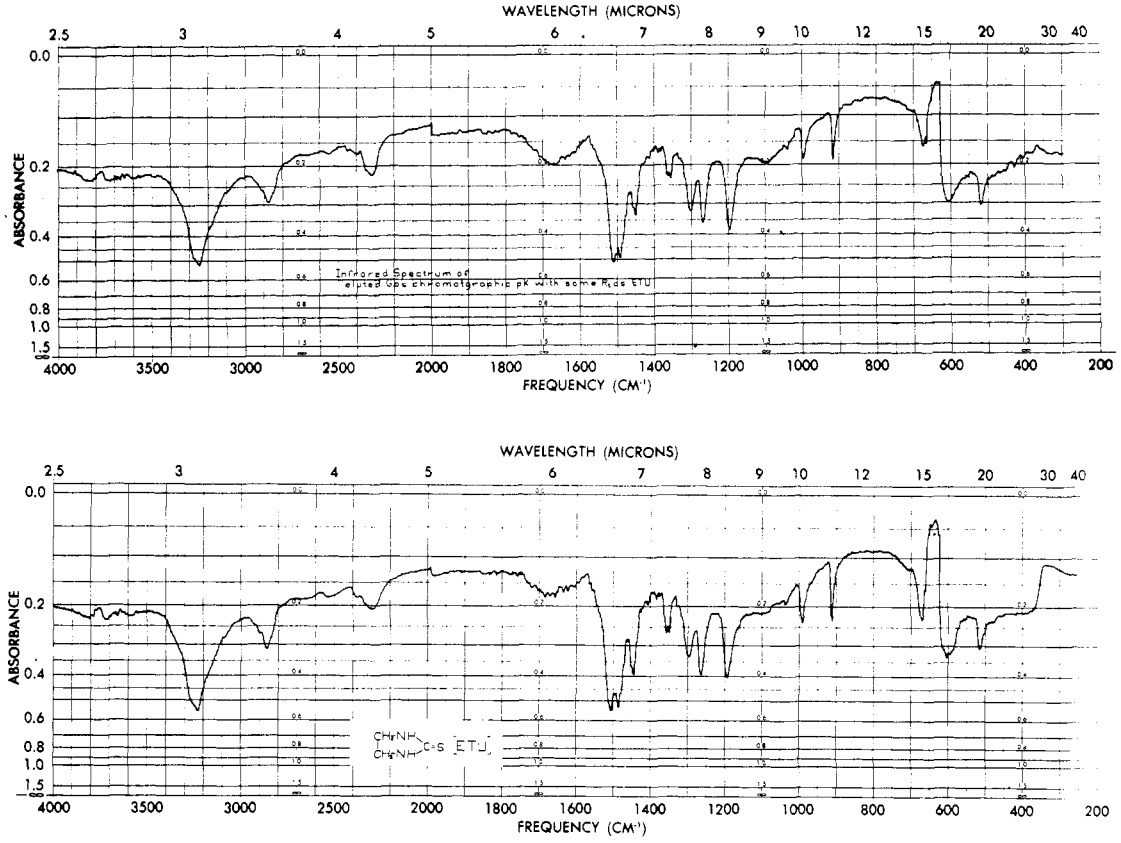


Figure 2. Infrared spectrum of ETU standard (bottom) and of ETU from sample (top).

and sample effluents had the same fragmentation pattern with M = 102.

A qualitative test for zinc was made on all samples according to Feigl (1958). All products lited in Table II contained zinc, except for products numbers 7, 8, and 10.

RESULTS

The first group of samples considered are the 80% Zineb formulations. In Table II, each of the six different commercial Zineb products is listed. Sample 3 has the highest initial ETU content and is the oldest of the Zineb samples. Its age is approximately 2 years, while the other five are 1 year or less. These Zineb samples with the exception of no. 3 have an original ETU content well below 1%, but by increasing the storage time the ETU content at the end of 39 days increased by 5.5 to 32.3 times. Figure 3 shows the average percent ETU formed in the Zineb samples trom 0 days to 39 days. It was observed that sample 3 during the 17-39 day period had an ETU formation rate comparable to the newer Zineb samples. Figure 3 indicates that the ETU formation in Zineb products appears to follow a straight line increase up to 39 days, at which time the experiment ended.

The Maneb products also present interesting data. Of the seven samples studied, four formed a much greater amount of ETU and at a greater rate than the other three (Figure 3). These four had amounts 10.9 to 31.6 times greater than the original ETU content and were all well above the 10% level after 39 days of storage. The other three samples increased from 8.3 to 13.8 times over the initial ETU content but were well below the 1% level after 39 days of storage. These latter three products were manufactured by the same company and all contained 1% zinc

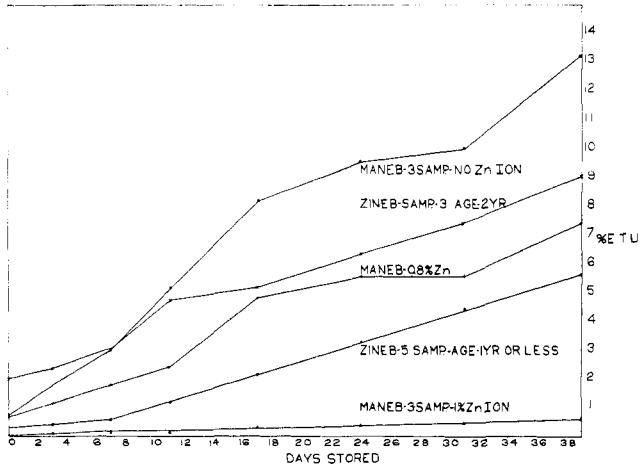


Figure 3. ETU found in formulated products from 0 to 39 days under controlled laboratory storage conditions.

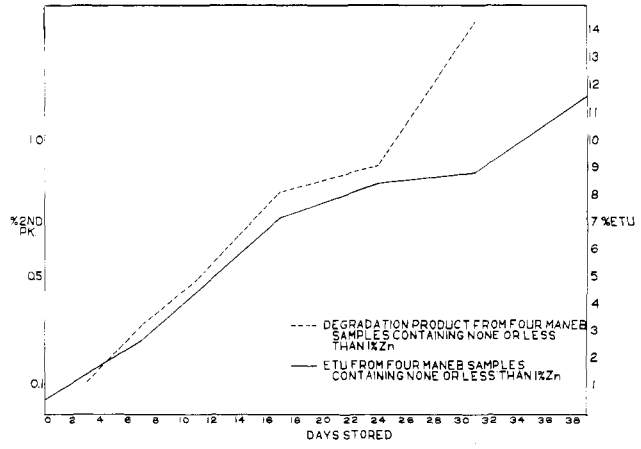


Figure 4. Rate of ETU and material represented by the second peak formed in Maneb formulations.

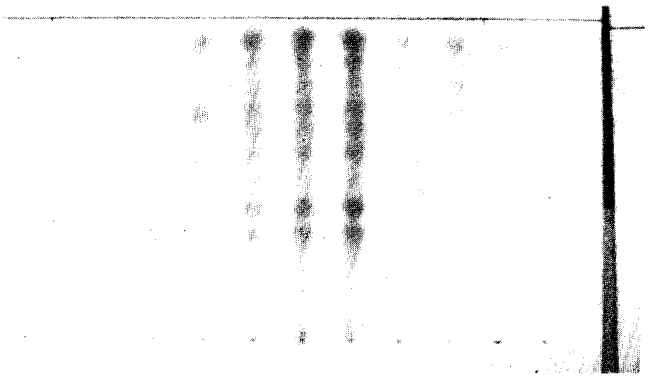
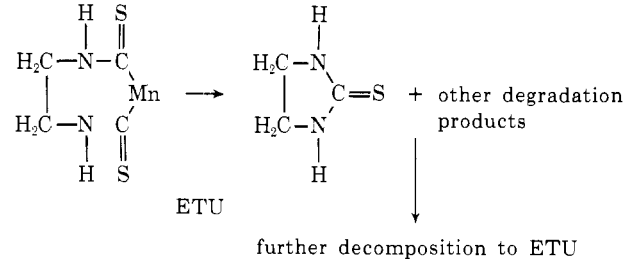
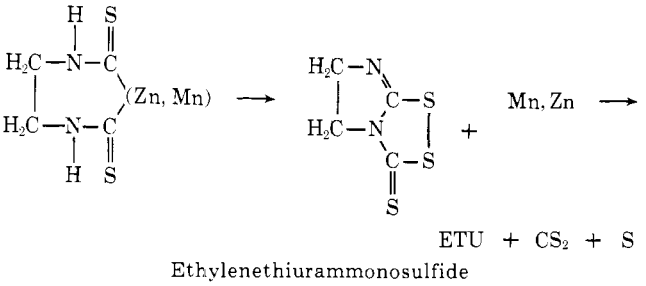


Figure 5. Thin-layer plate under ultraviolet radiation showing, from left to right, four concentrations of ETU standard (0.05, 0.1, 0.5, and 1 μg), four extracts of a Maneb sample at 3, 11, 17, and 24-day intervals, and of a Zineb sample at 3, 11, 17, and 24-day intervals.

as part of the inert ingredients. However, one of the other four Maneb samples (no. 9) contained 0.8% zinc, which apparently was not enough to keep the percent ETU formation as low as the three containing 1% (assuming the zinc ion concentration to be the factor in keeping down ETU formation). The results indicate that the rate at which ETU is formed in Maneb samples containing none or less than 1% zinc is different from the Zineb or the Maneb products containing 1% zinc. The rate of ETU formation is greatest from 0 to 17 days, proceeds at a slower rate from 17 to 31 days, and then increases at a greater rate from 31 to 39 days. When gas chromatographing these four Maneb samples (containing none or less than 1% Zn), an extra peak was observed at *ca.* 1.5 min after the ETU peak. The greatest average rate of increase in this peak's area generally corresponded to the greatest average rate of ETU formed, and its lowest average rate corresponded to the lowest average rate of ETU formed. This may be evidence that the second peak degrades to ETU. Therefore the formation of ETU may be a result of two decomposition reactions as shown by the equation:



Investigators (Fishbein and Fawkes, 1965) think ETU may be formed by the degradation reaction. (The structure for ethylenethiurammonosulfide as shown below does not correspond to that published by the investigators Fishbein and Fawkes. It corresponds with the structure as reported by Benson *et al.*, (1972).)



Ethylenethiurammonosulfide

Figure 4 shows the rate of ETU formation and the material represented by the second peak. The second peak is expressed as percent ETU. It is apparent that Maneb products containing none or less than 1% Zn degrade more rapidly and form ETU at a greater rate than Zineb or the Manebs containing 1% zinc. Further support is evidenced by Figure 5 (tlc plate under uv radiation) (Bontoyan *et al.*, 1971) showing a comparison of an 80% Maneb (no Zn) and a Zineb product spotted at 4-day intervals while being subjected to elevated temperature and humidity. The first four spotting points are of standard ETU, ranging from 0.5 to 2 μg; the next four are of the Maneb and the next four of the Zineb sample.

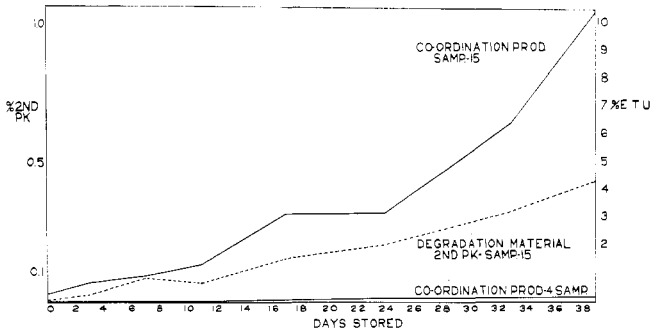


Figure 6. Rate of ETU and material represented by the second peak formed in coordination formulations.

Of the five zinc-manganese coordination products studied, four were manufactured by company C. As shown in Table II and on Figure 6, these four had an initial ETU content of less than 0.1% and less than 0.5% after 39 days. The one product manufactured by company F had a significantly higher initial ETU content than the other four and the ETU content after 39 days was 46.3 times greater than those of manufacturer C. As was the case with the Manebs containing no Zn, a second degradation peak was observed and followed the same trend as shown on Figure 6.

In general, the results show that the three types of ethylene bisdithiocarbamate products studied will, at elevated temperature and humidity, degrade to ETU. The rate and amount formed is greatest in the Maneb products containing no zinc. The Zinebs degrade at a less rapid but more steady rate than Manebs containing little or no Zn, and the coordination products (company A) degrade very slowly and form a very small amount of ETU. No explanation can be given for the greater amount of ETU found in the coordination sample of manufacturer F which follows the same trends as the Manebs with no Zn. Further studies under actual storage conditions must be made on these products before any realistic conclusion can be made as to the formation of ETU in commercial products.

ACKNOWLEDGMENT

The authors thank Ute Klingebiel and Jack Plimmer for their assistance in obtaining mass spectrographic analyses.

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Received for review March 2, 1972. Accepted December 19, 1972. Presented at the Symposium on Origin and Fate of Ethylenethiourea Fungicides, 164th Meeting of the American Chemical Society, New York, N. Y., August 1972.

Other papers presented at the 164th National Meeting of the American Chemical Society in the Symposium on the Origin and Fate of Ethylenethiourea Fungicides but not printed in this issue include: "The Determination of Ethylenethiourea in Apples," by W. H. Newsome (this article appeared in *J. Agr. Food Chem.* 20, 967 (1972)); "Determination of Ethylenethiourea in Food Crops," by John H. Onley, Robert W. Storherr, Randall R. Watts, and Nathan F. Ives; "The Fate of Ethylenethiourea in Taro Culture," by John W. Hylin; and "Determination of Ethylenethiourea Residues in or on Potatoes Using High Speed Liquid Chromatography," by Ronald F. Cook.

Synthesis of Bioactive Compounds: Juvenile Hormone Mimetics Affecting Insect Diapause

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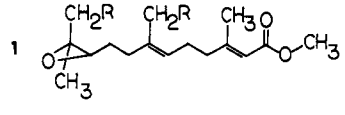
A number of juvenile hormone (JH) mimetics based on the farnesyl and geranyl skeleton have been synthesized. They have been bioassayed on the basis of their ability to prevent or terminate diapause in the adult cereal leaf beetle (*Oulema melanopus* L., Chrysomelidae). Oviposition and mortality data for treated insects are described. A theory concerning the molecular requirements of JH mimetics in diapause disruption, based on intramolecular steric and electronic factors, is discussed.

Recent years have shown a truly dramatic increase in the synthesis of juvenile hormone mimetics. This interest may be jointly ascribed to the potential these compounds have as "third generation" pesticides and to the synergistic cooperation between chemists and entomologists. Biological evaluation of these materials has been, almost exclusively, *via* metamorphosis effects on larvae or pupae. To date only limited studies on the disruption of adult insect diapause by external application of juvenile hormone (1, R = CH_3) and a few of its mimetics have been conducted (DeWilde, 1968; Slama, 1971). Artificial control of adult diapause could well offer an alternate means of bioassay of candidate hormonomimetic materials. Such substances can be utilized to supply the experimenter with a source of postdiapause insects from field-collected or laboratory-reared specimens. And, finally, disruption of diapause during unfavorable environmental conditions might afford a means of insect control.

Scheme I



The cereal leaf beetle, *Oulema melanopus* L., is having increasingly significant effects as a pest of small grains in the Northeast and North Central regions of the United States. Laboratory rearing studies (Connin *et al.*, 1968) indicated that the beetles underwent an apparent adult diapause, which required storage of 10–12 weeks to obtain mating and consistent egg production. Connin *et al.* (1967) had reported that diapausing beetles could be put into a postdiapause, sexually active condition by topical application of methyl juvenate (1, R = H). Later it was discovered (Connin and Hoopingarner, 1971) that diapause could either be prevented or terminated by treatment with this JH mimetic and that only the female need be treated.

Accordingly, we report an investigation into the feasibility of screening JH mimetics by prevention or termination of diapause in this beetle, the postdiapause state being readily discernible by observation of initiation and extent of oviposition. The compounds selected for this study were from among those shown by previous investigators to have significant juvenilizing effects on various preadult insect species. In addition, several different series of compounds were synthesized as part of an initial structure–activity study based on variations in the juvenile hormone molecule (1, R = CH_3).

ANALYTICAL AND PURIFICATION PROCEDURES

Infrared spectra were determined on a Perkin-Elmer 237B grating instrument as films between salt plates. Nuclear magnetic resonance spectra were run in CCl_4 on either a Varian A-56/60 or T60 instrument, using TMS as the internal standard (τ scale). Mass spectra were determined using the direct probe inlet of a Dupont 21-490 spectrometer at ambient temperature, unless otherwise noted, at 20 and 70 eV ionization potential as required.

Gas–liquid chromatographic analyses were performed on a Varian 1400 instrument equipped with a flame ionization detector and using helium (99.997% purity) as the carrier gas. Operating temperatures were: detector and inlet, 270°; column, in the range 160–250°. Columns used were 2 m × 2 mm i.d. glass, packed with 3% XE60 on 60–80 mesh Chromosorb W or 4% EGSP-Z on 80–100 mesh Gas Chrom Q. On-column injection was used. Carrier gas flow rates and column temperatures were adjusted to optimize conditions for each compound. Integration was by triangulation.

Analytical thin-layer chromatography utilized either E. Merck silica gel GF-254 (10–40 μ) on 75-mm plates, 0.1-mm thick, or Analtech Inc. "Uniplates" 0.25-mm silica gel GF on 5 × 20 cm, 0.25-mm thick precoated plates. Solvent systems employed were (for hexane–ether): I, 4:1; II, 3:1; III, 2:1; and IV, 1:1. Preparative tlc was carried out on 20 × 20 cm plates coated with E. Merck silica gel GF-254 (10–40 μ) 0.5-mm thick. Column chromatography utilized E. Merck silica gel, 0.05–0.2 mm, Brockmann activity II–III, with a weight ratio of 20–30:1 (support to

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