DETERMINATION OF THE DEGRADATION PRODUCTS OF ETHYLENEBIS-(DITHIOCARBAMATES) BY THIN-LAYER CHROMATOGRAPHY AND SOME INVESTIGATIONS OF THEIR DECOMPOSITION *IN VITRO*

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Ethylenebis(dithiocarbamates) exert their fungicidal effect by their "active degradation products". Of these products, Cox *et al.*[1] detected ethylenediamine and carbon disulphide, BARRATT AND HORSFALL[2] hydrogen sulphide, CLARKE *et al.*[3] ethylenethiourea, KLÖPPING AND VAN DER KERK[4] ethylenethiuram disulphide, and LUDWIG AND THORN[5] ethylenethiuram monosulphide. It has been proved that the latter degradation product was the most active and the fungicidal effect was actually due to its presence.

The analytical properties of dithiocarbamates including ethylenebis(dithiocarbamates) have already been investigated by several research workers. A detailed survey of their work is given in the monograph of THORN AND LUDWIG[6]. Their experiments, however, do not concern the determination of the degradation products: this problem has been dealt with by FISHBEIN AND FAWKES[7]. These scientists separated the degradation products on silica gel layers, using formamide or paraffin as stationary phase, and chloroform or butanol–methanol–water as the mobile phase. The chromatograms of the degradation products were evaluated only qualitatively.

In the present work a thin-layer chromatographic method has been developed, offering an easier and better separation of the degradation products. After development the separated degradation products appear on a light background as well-defined, semi-quantitatively evaluable dark spots.

This method has been used for the examination of the degradation products of some Zineb- and Maneb-containing pesticides. Before investigating the degradation process on the plant, *i.e.* in a biological environment, as a first step the decomposition *in vitro* has been studied by excluding light. These investigations, besides representing the most simple models, at the same time throw some light on the process of decomposition under storage conditions.

EXPERIMENTAL

Separation of degradation products of Zineb and Maneb

Preparation of chromatoplates. A Merck "Kieselgel G" thin layer (20 × 20 cm) after drying was activated for 10 min at 105° and then placed in a chamber of 20 % relative humidity.

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Solvent system. Chloroform–butanol–methanol–water (100:5:1:0.5). After shaking, this mixture is poured into the chromatographic trough.

Preparation of samples. The degradation products to be investigated are extracted from the differently stored samples (see later) with a mixture of chloroform–methanol (1:1). Generally, a solution of 5 ml is prepared from a test material containing 0.5 g agent, and 10 μl of this solution is placed on the starting point of the silica gel layer. The undecomposed pesticide itself is insoluble.

Detection of the developed chromatograms. After evaporation of the solvent the layer is placed in a chamber saturated with iodine vapours. The degradation products that take up iodine first appear light coloured on the mildly coloured background, and after reaction with iodine due to adsorption of iodine vapours turn more and more brown, to appear after about 15–20 min as intensely brown spots. Sulphur does not absorb iodine and its spots colour more slowly. Yet even in this case the time prescribed for keeping in the iodine vapour chamber is sufficient.

Since iodine vapours evaporate from the layer, the visualized chromatograms must be covered with a glass plate until the spots have been compared with reference spots of known amounts and semi-quantitive evaluation has been performed.

In vitro investigation of the degradation of Zineb and Maneb

The following products have been examined:

Zineb 80 (COPCI, Paris), Zineb content 80 %

Thiezene (Montecatini), Zineb content 80 %

Siaprit (SIAPA, Rome), Zineb content 45 % + 5 % ethylenethiuram monosulphide

Maneb (Lyro), Maneb content 80 %

Maneb (Sandoz), Maneb content 80 %

Of these pesticides amounts containing 0.5 g reagent were weighed, *i.e.* 0.625 g, or of Siaprit which contains 45 % Zineb, 1.111 g. The degradation of these materials has been investigated in the dark at the following temperatures and relative humidities:

(1) 10°, relative humidity 0 %
(2) 10°, relative humidity 80 %
(3) 30°, relative humidity 0 %
(4) 30°, relative humidity 25 %
(5) 30°, relative humidity 80 %.

(It is notable that in the 2nd and 4th experiments the absolute humidity was practically the same, *i.e.* 0.0075 kg/m^3 and 0.00076 kg/m^3, respectively).

The products investigated containing an insoluble reagent were first of all separated from the soluble degradation products formed during storage by extraction with chloroform–ethanol (1:1). Thus the experiments were begun with pesticides free of degradation products. An exception was Siaprit, in which besides the insoluble Zineb there is a soluble reagent component, *i.e.* ethylenethiuram monosulphide. This pesticide has thus been examined in a state corresponding to that of storage, this fact having been taken into consideration when results were evaluated. The weighed samples were spread on a filter paper and placed by means of suitable glass supports in the conditioned chamber. The investigation of the degradation products has been performed after storage for 1, 2, 3, 4, 5, 10, 20, 30, 40 and 50 days. In-

sertion of the samples has been started with the 50 days' sample, the other being inserted at appropriate intervals, so that at the end of the experiment all samples to be investigated were available at the same time.

The degradation products were extracted and reduced to the volume already prescribed and spotted on to the chromatoplates.

RESULTS AND DISCUSSION

Qualitative evaluation of the chromatograms

Fig. 1 shows a chromatogram of the reference substances as well as of the extracted degradation products of stored Zineb 80 and Lyro-Maneb.

R_F values and sensitivities are given in Table I.

TABLE I

R_F VALUES AND SENSITIVITIES OF REFERENCE SUBSTANCES AND DEGRADATION PRODUCTS OF ZINEB 80 AND LYRO-MANEB

	R_F	*Sensitivity* (μg)
EDA (ethylenediamine)	0.00	—
ETU (ethylenethiourea)	0.29	0.03
ETD (ethylenethiuram disulphide)	0.47	0.05
ETM (ethylenethiuram monosulphide)	0.75	0.15
S (sulphur)	0.94	0.50

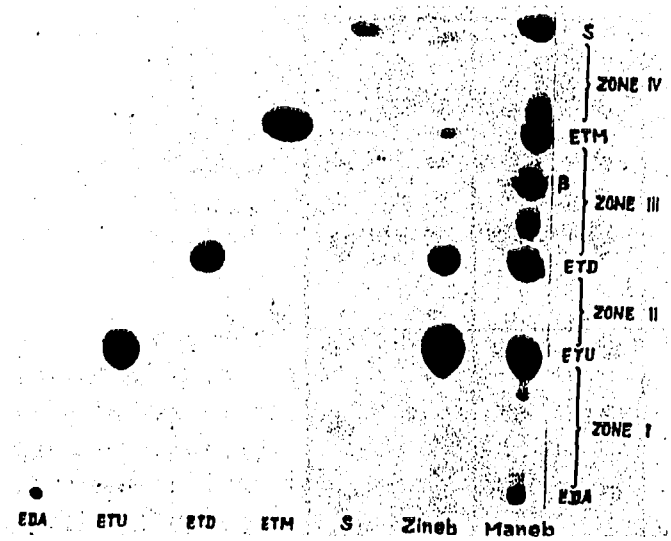

Fig. 1. Chromatogram of ethylenediamine (EDA), ethylenethiourea (ETU), ethylenethiuram disulphide (ETD), ethylenethiuram monosulphide (ETM), sulphur (S) and of the degradation

It is notable that the spectrum of the Maneb degradation products is much richer than that of Zineb. This is in conformity with—though not completely explained by—the known phenomenon also confirmed by VÉGH[8] that Maneb decomposes more readily than Zineb. These investigations showed that increased decomposition also manifests itself in a greater variety of degradation products.

Results of the degradation tests

The results of investigations concerning the degradation products of pesticides stored in the dark at various temperatures and humidities for different periods of time are shown in Fig. 2 for Zineb, and in Fig. 3 for Maneb.

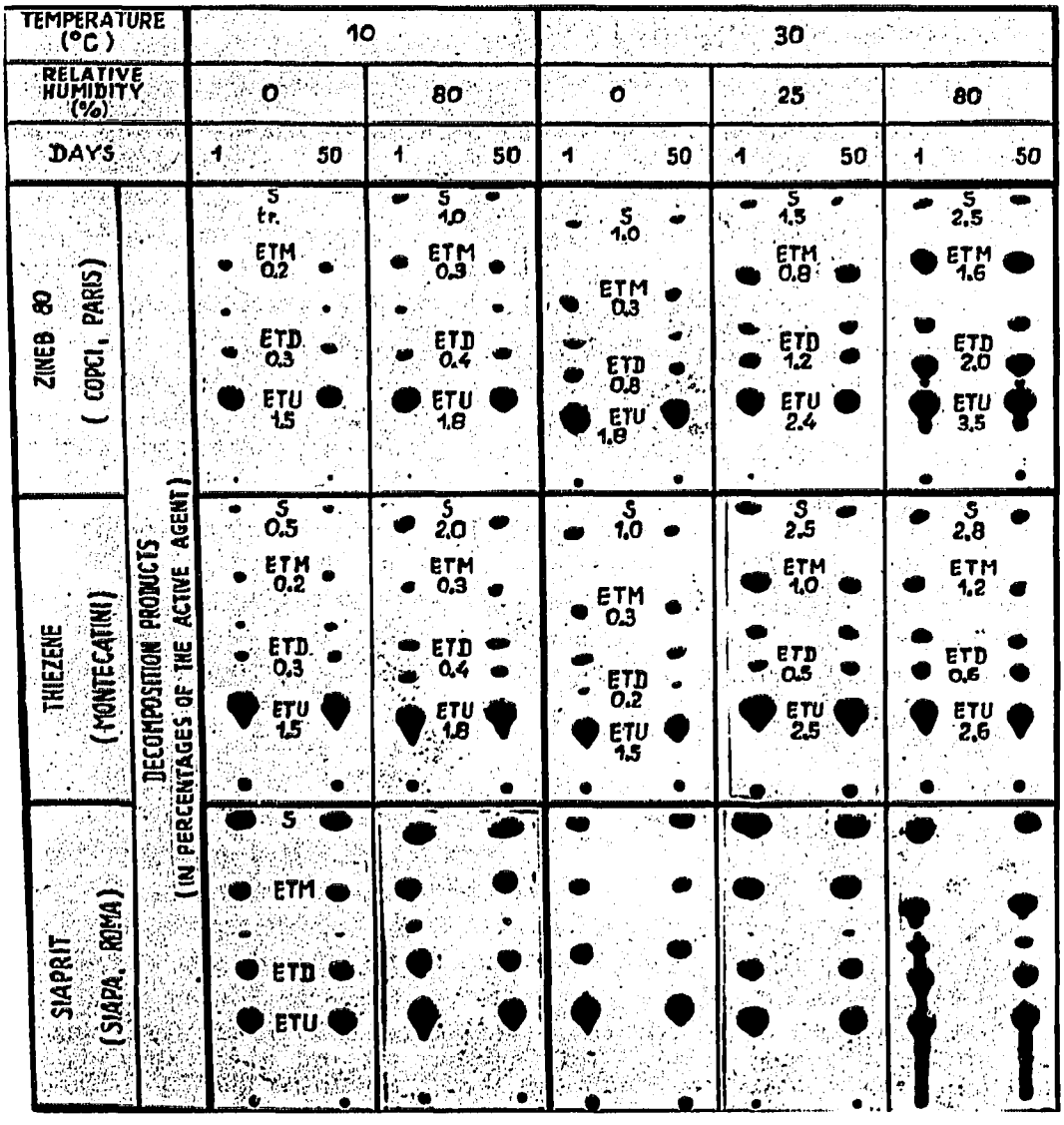

Fig. 2. Chromatographic picture of the degradation products of Zineb-containing pesticides and amount of degradation products in per cent of the initial amount of agent under different storage conditions.

It is notable that for all the pesticides and under each set of conditions degradation had already proceeded to a certain stage on the first day which did not change for 50 days. Thus in the dark and under all conditions, a condition of equilibrium is

reached within hours, and certainly within one day. (This circumstance is very favourable in respect of pesticide storing.) Since in the case of every single condition the chromatograms within the series were identical to the very end, the chromatograms of the degradation products in the figures are shown for those prepared on the first and fiftieth day of storage.

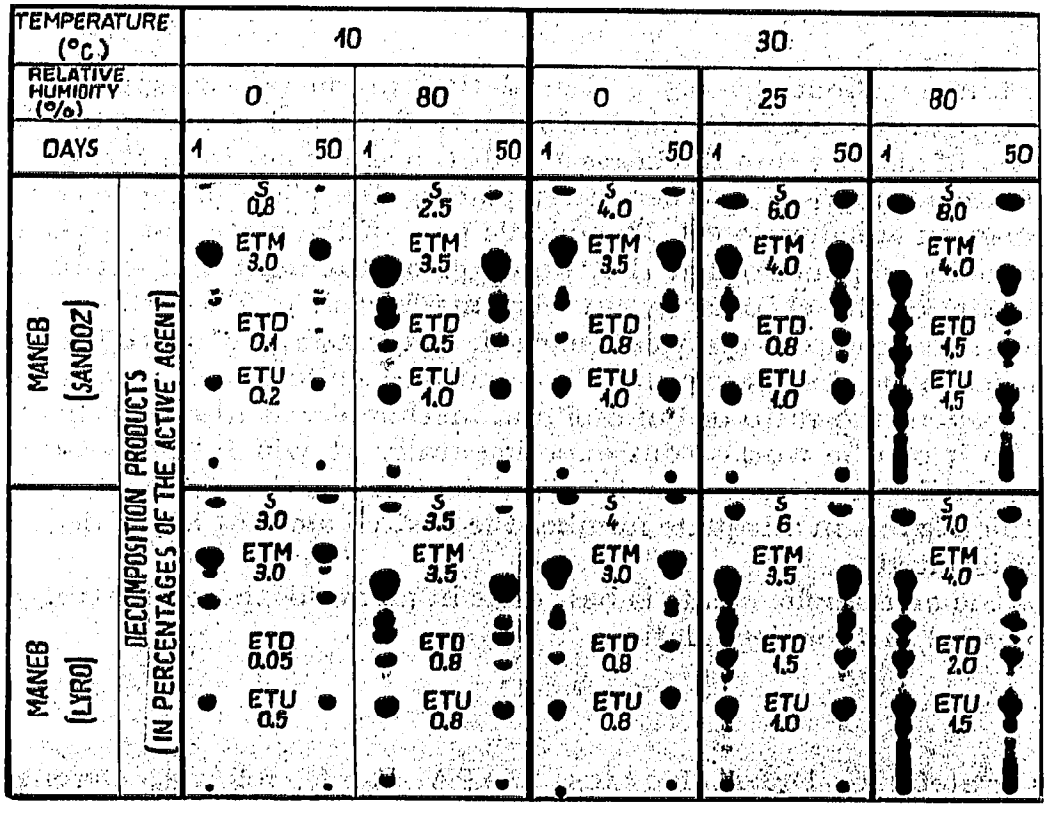

Fig. 3. Chromatographic picture of the degradation products of Maneb-containing pesticides and amount of degradation products in per cent of the initial amount of agent under different storage conditions.

The results concerning Siaprit must be handled separately. Owing to the presence of the added ETM previous extraction was not performed to clean the pesticide before storage. Accordingly the chromatograms show degradation products in greater amounts than those of the two Zineb products which underwent previous extraction. For this reason the degradation products were not evaluated quantitatively. The chromatograms shown in the figure serve only to demonstrate the degradation process. The presence of the greater ETM amounts can be observed.

Comparing Figs. 2 and 3 the phenomenon of the more pronounced degradation spectrum of Maneb shown in Fig. 1 is again obvious. With regard to both pesticides (in the case of Zineb only under the last two conditions) it is remarkable that in zone I with increasing temperature and humidity the series of spots get more intense and that in zone III the beta-front appears, in which an intensive and even under milder conditions a differentiable spot is visible. In the Maneb chromatogram another spot appears above this spot. At a higher temperature and humidity both spots join into a single large spot.

APPENDIX

Micropreparative separation of degradation products

In order to investigate the degradation products detected by the prescribed method from the viewpoint of chemistry, efficiency and toxicology, preparative separation of these degradation products has to be performed.

This can be done on thick-layer chromatoplates and by elution of the separated components.

A solution containing about 0.2–0.5 g degradation products was spread by means of a suitable apparatus or by the method already described[9] on a manually prepared silica gel layer of about 2 mm thickness at a distance of 2 cm from the lower edge, in the form of a continuous line. Then, after placing the plate in the solvent mixture, the chromatogram was developed. The middle part of the plate was covered before placing in the iodine vapour so that the two edges on the right and left-hand sides of the covered part of the chromatogram were made visible. Then the zones corresponding to the single components were marked. After scraping off the zones the material was placed in a chromatographic column and eluted with methanol.

If in a solvent system the ratio of butanol and methanol was altered a little, R_F values changed. So it can be worked out experimentally at which composition of the solvent system the degradation product to be detected would be separated the most clearly. Using thick layers in adequate number the desired amount of the degradation product can be isolated.

Evaporation of the methanolic eluate is best done in an atmosphere of nitrogen or carbon dioxide to avoid further degradation.

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SUMMARY

A method has been developed for the investigation of degradation products of ethylenebis(dithiocarbamate) type pesticides by thin-layer chromatography. The chromatograms were developed in a system of chloroform–butanol–methanol–water and were detected in iodine vapour.

By this method the degradation products of five pesticides containing Zineb and Maneb were investigated in the dark at temperatures between 10° and 30°, at varying humidity during a period of one to fifty days.

It has been shown that the degradation of Maneb yielded more degradation products than that of Zineb.

Under all conditions degradation had set in and reached equilibrium already on the first day. Under different conditions this equilibrium was of a different character.

A method has been described for the micropreparative separation of degradation products.

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