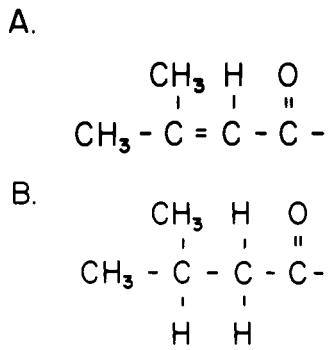

Figure 2. (A) β-Methylcrotonyl moiety. (B) Isovaleryl moiety.

The nmr spectrum of TR-1 also showed that there were two methyl groups present in addition to the two contained on the β-methylcrotonyl moiety (Figure 1B). These had chemical shifts of δ 1.96 and 0.98 (chloroform-*d*) and were not coupled to any other protons.

The methyl group at δ 1.96 experienced an upfield shift to δ 1.24 in the spectrum of TR-2, while the other methyl group remained essentially unchanged (δ 1.03) (Figure 1B).

Two 1-proton doublets in the TR-1 spectrum at δ 4.97 (J = 8 Hz) and 6.00 (J = 8 Hz) (Figure 1A) were absent in the TR-2 spectrum (Figure 1B). This presumably was caused by the loss of a double bond during reductive cleavage of TR-1.

The mass spectrum of TR-1 showed prominent losses of 15 (CH_3), 18 (H_2O), and 84 (C_5H_8O). The latter demonstrated that the same cleavage that occurred *via* chemical reduction also occurred in the mass spectrometer, with the exception that two hydrogen atoms were added to each fragment during reductive cleavage (*m/e* 86 ($C_5H_{10}O$) and *m/e* 429 ($C_{22}H_{27}N_3O_6$) compared to *m/e* 84 (C_5H_8O) and *m/e* 427 ($C_{22}H_{25}N_3O_6$)) *via* mass spectral fragmentation of TR-1.

Therefore, the small fragment from TR-1 probably existed as a β-methylcrotonyl moiety instead of an isovaleryl moiety (Figure 2, A and B). Further evidence for this was provided in the nmr spectra of TR-1 and TR-2. The chemical shifts of the two CH_3 groups lost from TR-1 during reductive cleavage were positioned at δ 1.68 as a 6-proton singlet in chloroform-*d* and at δ 1.63 and 1.74 as a 2-3 proton singlet in dimethylsulfoxide-d_6, (Figure 1, A and B), while the two methyl groups of isovaleraldehyde 2,4-DNP were positioned further upfield at δ 1.04 and 0.94 (in acetone-d_6) as a 6-proton doublet (J = 7.0 Hz). The chemical shifts of the two methyl groups of TR-1 are consistent with a β-methylcrotonyl rather than with an isovaleryl moiety. The nature of the linkage of this moiety in TR-1 is not known.

Tremorgenic compounds previously have been reported to be produced by *Aspergillus flavus* (Wilson and Wilson, 1964), *Penicillium spp* (Ciegler, 1969; Hou *et al.*, 1971; Wilson *et al.*, 1968) and *Aspergillus fumigatus* (Yamazaki *et al.*, 1971). Although all reported tremorgens appear to be indole alkaloids, TR-1 bears a closer chemical similarity to the fumitremorgins reported by Yamazaki *et al.* (1971). TR-1 and the fumitremorgins contain three nitrogen atoms (compared to one nitrogen for tremorgens from *Penicillium spp*) and an apparent 6-*O*-methoxy substitution of the indole ring, seemingly absent in the Penicillium tremorgens. However, comparisons of other physical and chemical data demonstrated that TR-1 was not identical to the fumitremorgins.

X-Ray crystallography studies are underway to determine the absolute chemical structure of TR-1.

LITERATURE CITED

Agurell, S., Holmstedt, B., Lindgren, J., *Acta Chem. Scand.* **23**, 903 (1969).
Ciegler, A., *Appl. Microbiol.* **18**, 128 (1969).
Cole, R. J., Kirksey, J. W., Moore, J. H., Blankenship, B. R., Diener, U. L., Davis, N. D., *Appl. Microbiol.* **24**, 248 (1972).
Hou, C. T., Ciegler, A., Hesseltine, C. W., *Appl. Microbiol.* **21**, 1101 (1971).
Neuss, N., "Physical Data of Indole and Dehydroindole Alkaloids," Vol. I and II, Lilly Research Laboratories, 1964.
Wilson, B. J., Wilson, C. H., *Science* **144**, 177 (1964).
Wilson, B. J., Wilson, C. H., Hayes, A. W., *Nature (London)* **220**, 77 (1968).
Yamazaki, M., Suzuki, S., Miyaki, K., *Pharm. Bull. Jap.* **19**, 1739 (1971).

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Photolysis of Parathion (*O,O*-Diethyl-*O*-(4-nitrophenyl)thiophosphate). New Products

O,O,S-Triethylthiophosphate was identified as the major product of the photolysis of parathion in aqueous THF or ethanol. Minor products were *O,O,O*-triethylthiophosphate, paraoxon, and triethylphosphate, which was formed by secondary photolysis of paraoxon.

Cook and Pugh (1957) and later Koivistoinen and Merilainen (1962) identified paraoxon (diethyl-4-nitrophenylphosphate, **2**) as one of the products from the photolysis of parathion (*O,O*-diethyl-*O*-(4-nitrophenyl)thiophosphate, **1**). Frawley (1957) and later Arterberry and Durham (1961) and Kimura (1963) have shown that 1 and 2 are inhibitors of cholinesterase. Frawley and Cook (1958) found the anticholinesterase activity of aqueous emulsions of 1 increased upon irradiation with light.

Quinby and Lemmon (1958) reported cases of parathion poisoning among farm workers, sometimes at relatively long intervals after spraying, when the parathion had reached "safe" levels. Milby *et al.* (1964) studied similar anomalous poisoning and found paraoxon on the foliage of trees which were sprayed with **1**. These authors also observed a higher incidence of poisoning among people who worked in fields repeatedly sprayed with **1**. The implication of this work is that **2** produced by the interaction of sunlight with **1** causes the poisoning of farm workers.

With these results in mind we initiated a study of the

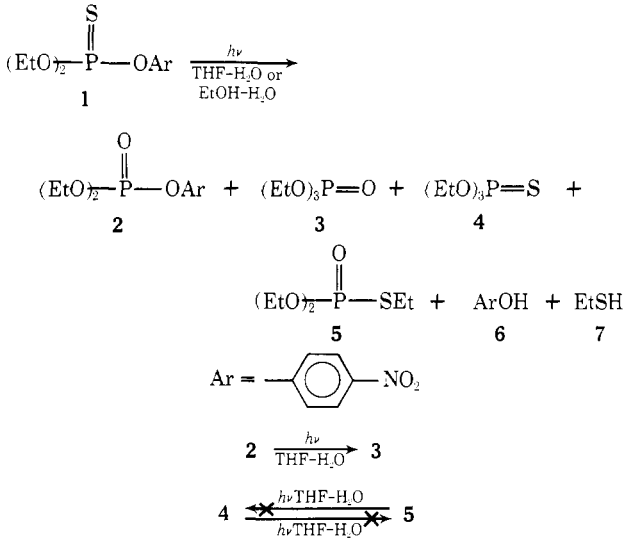

Figure 1. Photolysis of parathion and related esters.

mechanism of the photooxidation of 1 to **2.** However, during the course of our work, Pellegrini and Santi (1972) reported a synergistic mammalian toxicity between malathion and *O,O,S*-trimethylthiophosphate. In view of this work and the lack of quantitative data on the photochemistry of **1**, we wish to report the product study of the photolysis of 1 and **2.**

EXPERIMENTAL SECTION

All photolysis experiments were conducted in a Rayonet photochemical reactor using 2537, 3000, and 3500 Å lamps. Photoproducts were identified by glc retention time, mass spectra, and infrared spectrum and by comparison with an authentic sample. Mass spectra were recorded on a Perkin-Elmer Hitachi RMU-6 single focusing mass spectrometer with an ionizing voltage of 70 eV; infrared spectra were recorded on a Perkin-Elmer Model 273 spectrophotometer; and gas chromatograms were obtained on a Hewlett-Packard Model 700 chromatograph equipped with a thermal conductivity detector and either of two stainless steel columns, 6 ft 10% SE-30 silicon gum rubber and 4 ft 10% Carbowax 20M. Yields were determined with glc and the internal standard (deoxybenzoin recrystallized twice from ethanol) method. The internal standard was added after irradiation. Authentic samples of **1** and **2** were purchased from Analabs, Inc., and triethylphosphate (**3**) and *O,O,O*-triethylthiophosphate (**4**) were from Matheson Coleman and Bell. Samples of **1** (Toy and Beck, 1950), **2** (Schrader, 1947), and *O,O,S*-triethylthiophosphate (**5**) (Bracha and O'Brien, 1968) were prepared by previously reported methods. Singlet oxygen was generated by the procedure described by Foote *et al.* (1968).

Photolysis of 1. Solutions of **1** (1%) were prepared by dissolving 0.2 g of **1** in 18 ml of either 80% ethanol–20% H_2O (by weight) or 80% tetrahydrofuran–20% H_2O (by weight) and were irradiated for different amounts of time at 2537 Å (irradiation at 3000 and 3500 Å did not change the product distribution). Prior to photolysis, oxygen was removed by either bubbling prepurified N_2 into the solution or by three freeze–thaw cycles. After irradiation was completed, the solvent was removed at the aspirator and the residue chromatographed. The results are summarized in Table I. In ethanol–water, parathion completely disappeared after 45 hr of irradiation time, while in tetrahydrofuran–water, it disappeared after 13 hr. When **1** is refluxed in either solvent for 2 days in the dark, no reaction could be detected.

Table I. Product Yields

Products	Hr	1 (unreacted), %	2, %	3, %	4, %	5, %	6, %	7, %
THF–H_2O	3	31	1	5	1	12	0	detected only
	7	4	0	6	3	36	0	
	10	2	0	6	3	37	0	
EtOH–H_2O	10	66	3	2	0.5	15	1	detected only
	20	29	1	8	1	39	1	

Photolysis of 2. Paraoxon (0.15 g, 5.4×10^{-4} mol) was dissolved in 18 ml of 80% tetrahydrofuran–20% H_2O (by weight) and irradiated at 2537 Å for 6 hr. After evaporation of solvent, 16% of **2** remained and 31% of triethylphosphate was found by glc. Since we observed 4-nitrophenol (**6**) to suffer decomposition when irradiated at 2537 Å, it was not observed as was the case for parathion irradiated in THF–H_2O.

Singlet Oxygen and 1. Singlet oxygen was generated by bubbling molecular oxygen into a solution of methylene blue dissolved in ethanol containing 1% of **1.** The solution was irradiated with a 650-W quartz–iodine lamp for 10 hr. The solution was injected into the gas chromatograph. The chromatogram showed no decrease in the concentration of **1** nor the presence of **2.** The esters **4** and *O,O*-diethyl-*O*-(4-tolyl)thiophosphate, *O,O,O*-triphenylthiophosphate, and triphenylphosphine sulfide were irradiated under similar conditions and no reaction was detected.

RESULTS AND DISCUSSION

Neither singlet oxygen nor the interaction of ground state triplet oxygen with excited parathion is responsible for the formation of paraoxon. Rather, water either reacting with excited parathion or with some intermediate generated from excited parathion is the source of oxygen of the P=O bond of paraoxon.

The photolysis of **1** in either aqueous tetrahydrofuran or aqueous ethanol gave *O,O,S*-triethylthiophosphate (**5**) as the major product, lesser amounts of *O,O,O*-triethylthiophosphate (**4**), triethylphosphate (**3**), and paraoxon (**2**), and traces of ethanethiol (**7**) and 4-nitrophenol (**6**). Subsequent photolysis of **2** under identical conditions as **1** gave triethylphosphate (**3**), a result which shows that **3** arises from secondary photolysis of **2** during the photolysis of **1** (Figure 1). Since the thiophosphates **4** and **5** do not absorb light in the region 2500–8000 Å, it is not surprising that the esters **4** and **5** neither photolyze nor interconvert under the photolysis conditions.

Two molecules of **1** are required to produce **4** and **5** because the product distribution from the photolysis of **1** is similar in either aqueous tetrahydrofuran or aqueous ethanol. Clearly, the third ethyl group of esters **3, 4,** and **5** does not arise from solvent. In analogy with **1** we believe **3** is formed from two molecules of **2.**

Finally, while the amount of **2** produced by the photolysis of **1** does not seem significant enough to be responsible for the anomalous poisoning of orchard workers, the recent report (Pellegrini and Santi, 1972) of the synergistic mammalian toxicity between *O,O,S*-trimethylthiophosphate and malathion suggests that the anomalous poisoning may be caused by an analogous synergistic toxicity between **1** and **5.** While **5** has not been found in the environment, no one has looked for it either. The buildup of **5** on trees from repeated spraying of **1** and a potentiation of the toxicity of **1** by **5** could account for the higher incidence of poisoning in orchards repeatedly sprayed with **1.**

LITERATURE CITED

Arterberry, J. D., Durham, W. F., *Arch. Environ. Health* **3**, 476 (1961).
Bracha, P., O'Brien, R. D., *Biochemistry* **7**, 1555 (1968).

Cook, J. W., Pugh, N. D., *J. Ass. Offic. Agr. Chem.* **40,** 277 (1957).
Foote, C. S., Wexler, S., Audo, W., Higgins, R., *J. Amer. Chem. Soc.* **90,** 975 (1968).
Frawley, J. P., *J. Agr. Food Chem.* **5,** 346 (1957).
Frawley, J. P., Cook, J. W., *J. Agr. Food Chem.* **6,** 28 (1958).
Kimura, M., *Chem. Pharm. Bull. (Tokyo)* **11,** 44 (1963).
Koivistoinen, P., Merilainen, M., *Acta Agr. Scand.* **12,** 267 (1962).
Milby, T. H., Ottoboni, F., Mitchell, H. W., *J. Amer. Med. Ass.* **189,** 351 (1964).
Pellegrini, G., Santi, R., *J. Agr. Food Chem.* **20,** 944 (1972).
Quinby, G. E., Lemmon, A. B., *J. Amer. Med. Ass.* **166,** 740 (1958).
Schrader, G., BIOS Final Report No. 714, 1947.
Toy, A. D. F., Beck, T. M., *J. Amer. Chem. Soc.* **72,** 3191 (1950).

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Metal Coordination Compounds of Thiabendazole

Several metal coordination compounds of the fungicide thiabendazole, 2-(4′-thiazolyl)benzimidazole (TBZ), have been prepared and the solubilities in water and the pH of the solutions have been determined. Fungicidal activity of the compounds = TBZ against an isolate of *Penicillium* and of *Fusarium*.

Thiabendazole, 2-(4′-thiazolyl)benzimidazole (TBZ), has been widely used for plant disease control since its fungicidal properties were reported by Staron and Allard (1964). In an earlier investigation (Miller *et al.*, 1971), the formation of metal salts of TBZ were noted which were similar to metal salts of the parent compound benzimidazole, as reported by Skraupt (1919) and Feigl and Gleich (1928). The present compounds herein reported are believed to be coordination compounds of thiabendazole, similar to the nickel compounds of 2-(hydroxymethyl)benzimidazole and methylbenzimidazole, as reported by Artemenko and Chistyakova (1970), and the cobalt compounds of 2-benzylbenzimidazole, as reported by Artemenko *et al.* (1972). The formulas were NiL_2X_2 or NiL_3X_2 and CoL_2X_2, in which L = benzimidazole derivative and X = one of several monovalent anions.

This report describes the synthesis and some physical and biological properties of several of these coordination compounds.

EXPERIMENTAL SECTION

The 1:1:2 molar ratio (metal–TBZ–chloride) cupric compound was prepared by dissolving 1.5 g of TBZ in 40 ml of boiling ethanol containing 0.27 ml of 12 *N* HCl and adding 1.28 g of $CuCl_2 \cdot 2H_2O$ in ~25 ml of ethanol. The pale green $CuTBZCl_2$ precipitated at once. The mixture was refluxed ~2 hr on the steam bath. The compound was separated by centrifugation and washed in the centrifuge tube with ethanol.

The 1:1:2 zinc compound was prepared by dissolving 1.5 g of TBZ in 50 ml of boiling ethanol and adding 1.0 g of $ZnCl_2$. The white $ZnTBZCl_2$ quickly precipitated. The mixture was refluxed for ~2 hr and filtered hot. The insoluble crystals were purified by ethanol extraction in a ASTM Method D147 extraction apparatus (VWR Scientific =27630-000). As crystals formed in the solvent the solvent was poured off, and further crystallization occurred on cooling. More ethanol was added to the extraction apparatus and the extraction continued until the crude $ZnTBZCl_2$ had been dissolved and crystallized.

Cupric 1:2:2 compound was prepared by dissolving 2.0 g of TBZ and 0.86 g of $CuCl_2 \cdot 2H_2O$ in 100 ml of hot water containing 0.85 ml of 12 *N* HCl. The solution was warmed on the steam bath for ~2 hr. Dark green crystals formed. An additional 500 ml of boiling water was added and stirred on a hot plate to effect solution. The solution was filtered hot and the filtrate allowed to crystallize. This compound may be recrystallized from ethanol using the ASTM extraction apparatus in the same manner as the $ZnTBZCl_2$ to give green $Cu(TBZ)_2Cl_2 \cdot 2H_2O$.

The cobalt 1:2:2 compound was prepared in the same manner as the $Cu(TBZ)_2Cl_2 \cdot 2H_2O$, except that 1.2 g of $CoCl_2 \cdot 6H_2O$ was used with 2.0 g of TBZ. The solution was filtered hot and evaporated to dryness at room temperature. The dry crude cobalt TBZ chloride was extracted in an ASTM extraction apparatus with ethanol. The pink ethanol-insoluble compound remaining in the thimble was $Co(TBZ)_2Cl_2 \cdot 2H_2O$.

Zinc 1:2:4 compound was prepared by dissolving 14.6 g of TBZ in 200 ml of hot water containing 7.0 ml of 12 *N* HCl. When the solution was complete, 4.5 g of $ZnCl_2$ in 20 ml of 6 *N* HCl was added and the solution heated (~95°) on the steam bath for 1 hr. The solution was filtered hot and the filtrate evaporated to dryness on the steam bath. The dry crude compound was transferred to a thimble and extracted with hot ethanol in an ASTM extraction apparatus. The $Zn(TBZ)_2Cl_4$ was very soluble in ethanol and was quickly separated from the small amount of insoluble $ZnTBZCl_2$. On cooling the solution, crystals of $Zn(TBZ)_2Cl_4$ separated.

The 1:2:2 nickel compound was prepared by dissolving 2.0 g of TBZ and 1.2 g of $NiCl_2 \cdot 6H_2O$ in 100 ml of water containing 5.0 ml of 12 *N* HCl and warming on the steam bath for ~2 hr. The solution was filtered hot and evaporated to dryness on the steam bath. The dried material was placed in a thimble of an ASTM extraction apparatus and extracted with ethanol. Any $Ni(TBZ)_3Cl_2$ present was rapidly extracted by the hot ethanol, and the pale blue powder remaining in the thimble was $Ni(TBZ)_2Cl_2 \cdot 2H_2O$.

The 1:3:2 nickel compound was prepared by dissolving 3.0 g of TBZ and 1.2 g of $NiCl_2 \cdot 6H_2O$ in 100 ml of ethanol and refluxing 15 min on the steam bath. The solution was filtered hot and evaporated to incipient crystal formation. Recrystallization of the purple crystals of $Ni(TBZ)_3Cl_2 \cdot 3H_2O$ was from ethanol.

Cobalt TBZ gluconate (GLU) was prepared by heating 10 m*M* each of $CoCO_3$ and glucono-*d*-lactone in 100 ml of water on the steam bath until most of the carbonate had dissolved. The solution was filtered and 10 mM of TBZ was added. The heating on the steam bath was continued