[Chem. Pharm. Bull. 19(11)2373—2379(1971)]

UDC 547.24.04 : 547.25.04 : 546.74.04

Reductive Cleavage of Sulfur Containing Organophosphorus Compounds with Raney Nickel[1]

Kinzo Nagasawa, Toshiharu Yamada, and Akira Ogamo

School of Pharmaceutical Sciences, Kitasato University[2]

(Received June 2, 1971)

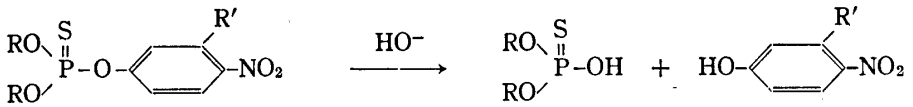
The reductive cleavage of sulfur containing organophosphorus compounds and its application to the analysis of organophosphorus pesticides were examined.

Cidial, malathion, and dimethoate having a common structure $(CH_3O)_2P(S)SR$ were treated by Raney nickel in ethanol. The non-phosphorus products characteristic to each of the original pesticides were formed, and they were identified with ethyl phenylacetate, diethyl succinate, and N-methylacetamide, respectively. Px which is a main phosphorus containing product common to these pesticides was identified with the phosphorus compound obtained by the same treatment of dimethyl dithiophosphate. The structure of Px and the reaction mechanism were discussed.

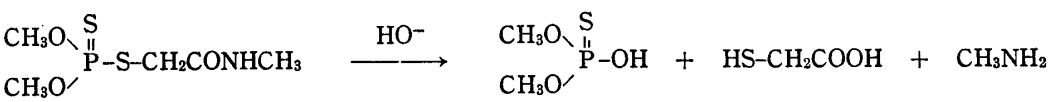
It was also proved that this reaction afforded a new method to characterize this type of organophosphorus pesticides.

Introduction

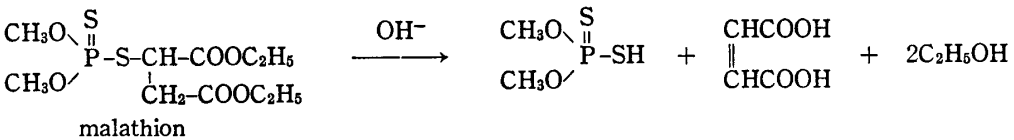
A number of the methods for analysis of organophosphorus pesticides have been described.[3,4] The determination of hydrolytic products formed from an organophosphorus pesticide is an useful analytical procedure in this field.[3-6] For example, the discrimination among methylparathion, ethylparathion, and sumithion, all of which are an aryl ester of dialkyl phosphorothionate, is easily attained by the analysis of their alkaline hydrolysis



methylparathion (R, R=CH_3, R′=H)
ethylparathion (R, R=C_2H_5, R′=H)
sumithion (R, R=CH_3, R′=CH_3)



dimethoate



malathion

Chart 1

1) This work was presented at the 91th Annual Meeting of Pharmaceutical Society of Japan, Fukuoka, April 1971.
2) Location: *Shirokane 5-9-1, Minato-ku, Tokyo.*
3) G. Zweig, "Anal. Methods for Pesticides, Plant Growth Regulators, and Food Additives," Vol. II, Acad. Press, New York and London, 1964.
4) W. Horwitz (ed.), "Official Methods of Analisis of the AOAC," 11th ed., 1970, p. 112, 114.
5) P.A. Giang and M.S. Schechter, *J. Agr. Food Chem.*, **11**, 63 (1963).
6) J. Askew, J.H. Ruzicka, and B.B. Wheals, *J. Chromatog.*, **41**, 180 (1969).

products (Chart 1).[7,8] Dimethoate, a phosphorodithioate type pesticide, is relatively stable in dilute acid, but it hydrolyses easily in alkali to dimethyl thiophosphate, methylamine, and thioglycolic acid, and the two latter compounds of them are those to be analysed.[5,9] On the other hand, malathion is cleaved by hot alkali to dimethyl dithiophosphate and maleic acid, as shown in Chart 1.[4,8]

The present authors have intended to examine a new and additional analytical procedure of sulfur containing organophosphorus pesticides. This paper deals with the reductive cleavage of $(CH_3O)_2P(S)SR$ type compounds with Raney Nickel and with the examination on analytical use of this reaction.

Experimental

Reagent and Solvent——Raney Nickel (W-2) was prepared by the conventional method[10] and stored in absolute ethanol at 0—5°. Ethanol and acetonitrile were dehydrated and redistilled by conventional methods. Other solvents and reagents, which were all special reagent grade, were used without further purification.

Sulfur Containing Organophosphorus Compounds——Cidial, malathion and dimethoate[11] were obtained from Nissan Chemical Ind. Co. Ltd. (Nihon-bashi, Chuō-ku, Tokyo). Cidial and malathion were purified by WAKOGEL C-200 column chromatography using acetonitrile as an eluant. Dimethyl dithiophosphate and dimethyl phosphite were obtained from Nippon Chemical Ind. Co. Ltd. (Kameido-machi, Kōtō-ku, Tokyo).

Thin-Layer Chromatography (TLC)——The layer (0.25 mm thick) coated with Kieselgel G (Merck) was activated by heating at 105° for 30 min prior to its use. For a preparative TLC, the layer with 0.5 mm thick was used. The following solvents were used for the chromatography: (1) acetonitrile–water (88: 12), (2) acetone, (3) benzene–ethyl acetate (1: 19), (4) methanol–acetic acid (19: 1). The samples in ethanol were spotted on the starting line 1.5 cm from the edge of the plate. The plate was developed ascendingly until the length of run was 10 cm.

The developed plates were examined by the following methods.

Bromine or Iodine Vapor Treatment: The plate is exposed to bromine or iodine vapor in a closed tank 1—2 min. Most of organic compounds give an yellow to brown spot against a creamy white background.

4-(*p*-Nitrobenzyl)pyridine·Tetraethylenepentamine(NBP–TEP Reagent):[12] The plate is sprayed with 2% 4-(*p*-nitrobenzyl)pyridine in acetone and heated at 130° for 15 min, followed by spraying with 10% tetraethylenepentamine in acetone. Fully esterified organophosphorus compounds give a blue to violet spot against a white background.

Hanes & Isherwood Reagent (H & I Reagent):[13] With this reagent, most phosphorus compounds are detectable as a greenish to blue spot.

Hydroxylamine·Ferric Chrolide ($H_2NOH \cdot Fe^{3+}$ Reagent):[14] Carboxylic acid esters are detected as an orange to brown spot against a thin yellow background.

Dibromoquinone Chlorimide (DQC Reagent):[15] Sulfur containing organophosphorus compounds give a reddish spot against a white background.

Gas Chromatography (GLC)——Gas chromatography was performed on a Hitachi Perkin-Elmer F 6-D gas chromatograph equipped with a hydrogen flame ionization detector. The stainless steel tube (1 m,

7) T. Suzuki, *Kagaku Keisatsu Ken-kyu-jo Hōkoku*, **17** (2), 173 (1964) [*C. A.*, **62**, 2199b (1965)].
8) T. Ukita, M. Irie, and S. Ishihara, *Eisei Kagaku*, **8**, 1 (1960).
9) D.A. George, D.H. Moore, W.P. Brian, and J.A. Garman, *J. Agr. Food Chem.*, **9**, 264 (1961).
10) R. Mozingo, "Organic Syntheses," Coll. Vol. III, ed. by E.C. Horning, John Wiley and sons, Inc., New York, N.Y., 1955, p. 181.
11) Cidial: O,O-Dimethyl-S-(phenyl,carboethoxymethyl)phosphorodithioate, $(CH_3O)_2P(S)SCH(C_6H_5)COOC_2H_5$.
Malathion: O,O-Dimethyl-S-(1,2-diethoxycarbonylethyl)phosphorodithioate, $(CH_3O)_2P(S)SCHCOO\text{-}C_2H_5$ $CH_2COOC_2H_5$.
Dimethoate: O,O-Dimethyl-S-(N-methylcarbamoylmethyl)phosphorodithioate, $(CH_3O)_2P(S)SCH_2CO\text{-}NHCH_3$.
12) M.E. Getz and H.G. Wheeler, *J. Assoc. Offic. Anal. Chem.*, **51**, 1101 (1968).
13) C.S. Hanes and F.A. Isherwood, *Nature*, **164**, 1107 (1949); R.S. Bandurski and B. Axelrod, *J. Biol. Chem.*, **193**, 405 (1951).
14) E. Stahl (ed.) "Thin Layer Chromatography," Spring-Verlag, 1969, p. 880.
15) J. Stenersen, *J. Chromatog.*, **38**, 538 (1968).

3 mmϕ) packed with either 10% (w/w) SE-30 on 80—100 mesh Chromosorb W or 15% (w/w) diethyleneglycol succinate on 80—100 mesh Celite 545K was used. The quantitative analysis of non-phosphorus products was carried out by an internal standard method where *n*-octanol for both ethyl phenylacetate and diethyl succinate, and 1,3-butanediol for N-methylacetamide were used as an internal standard substance. Details of the conditions used for qualitative and quantitative analyses were described in Table III.

Spectral Measurement——UV spectra were measured on an ethanolic solution of samples with a Hitachi Recording Spectrophotometer EPS-3. IR spectra were measured on a thin-film of samples with a JASCO Model IR-S.

Determination of Inorganic Sulfate and Phosphate——Inorganic sulfate was determined by Dodgson's turbidimetric method[16] and inorganic phosphate by Allen's colorimetric method.[17]

Procedure for the Reaction between Sulfur Containing Organophosphorus Compounds and Raney Nickel——To 50 mg of one of the sulfur containing organophosphorus compounds, anhydrous EtOH (10 ml) and an appropriate amount of Raney Nickel W-2 were added.[18] The mixture was heated at $83 \pm 2°$ under constant stirring for 1 hr. The reaction mixture was cooled and centrifuged (5,000 rpm) for 10 min. The Raney Nickel precipitated was washed with EtOH (20 ml × 5) and the supernatant combined with the washings was concentrated at 30° to a small volume under reduced pressure. The residue obtained was accurately diluted with EtOH to 5 ml and the resultant solution was filtrated.

Isolation of Px from the Reaction Mixture of Cidial and Raney Nickel——To 1.5 g of cidial, anhydrous EtOH (300 ml) and Raney Nickel W-2 (30 ml) were added. The mixture obtained above was reacted and worked up in a similar fashion as in the preceding procedure. After concentration of the ethanolic supernatant (300 ml) and washings (200 ml × 5), the residue was dissolved in EtOH (25 ml) and added with water (25 ml). The mixture was extracted with petr. ether (25 ml × 3) to remove ethyl phenylacetate, and the water layer separated was concentrated to about 3 ml over P_2O_5 in a desiccator under ordinary pressure. The concentrate thus prepared was submitted to the preparative TLC using acetonitrile–water (88: 12) as an eluant and the developed plate was monitored by the color reaction with 4-(*p*-nitrobenzyl)pyridine·tetraethylenepentamine.[19] The silica gel on the plate corresponding to Px band (*Rf* 0.59) was scraped off and extracted with a small volume of ether. After filtration, the ether extract was evaporated to give an oily liquid by air-spraying at room temperature.

Analysis of the Distribution of Phosphorus and Sulfur among the Fractions obtained by Raney Nickel Treatment of Cidial——The Raney Nickel recovered from the reaction mixture was transfered altogether into a beaker and added dropwise 2 ml of aqua regia. With evolving heat and hydrogen sulfide, the Raney Nickel dissolved into a solution. An additional 38 ml of aqua regia was added and the beaker covered with a watch glass was heated to boil on a heating plate for 1 hr. After digestion was completed, the content of the beaker was carefully evaporated on a boiling water bath to remove most of volatile acids. The wet residue obtained was redissolved in a minimum volume of water and applied on the top of a Dowex 50 H^+ column (25 × 2.5 cm), followed by elution with 800 ml of water. The eluant which contains no metallic cations was evaporated to dryness at 40° under reduced pressure. The syrupy material resulted was accurately diluted to 25 ml with water and submitted for the analyses of inorganic phosphate and sulfate.

Result and Discussion

Cidial, malathion, and dimethoate, each of which is a dimethyl phosphorodithioate type pesticide, were reacted with Raney Nickel in ethanol and the reaction products formed were examined by TLC. As shown in Fig. 1, each one phosphorus-negative spot (*Rf* 0.80, 0.73, and 0.46) originated from cidial, malathion, and dimethoate, respectively, and one phosphorus-positive spot (*Rf* 0.59) common to these pesticides were detected on the chromatograms. Besides these spots, a phosphorus-positive (only NBP–TEP reaction) minor spot which seemed to be characteristic to each of these pesticides was detected.

The non-phosphorus product (*Rf* 0.80) of cidial was positive to the hydroxylamine–Fe^{3+} reagent, and identified with authentic ethyl phenylacetate by TLC and GLC (Fig. 2, and Tables I and II.)

Furthermore, the ultraviolet (UV) spectrum of this product isolated by a preparative TLC was coincided with that of authentic ethyl phenylacetate ($\lambda_{max}^{ethanol}$ 254, 260, 266, 250

16) K.S. Dodgson, *Biochem. J.*, **84**, 106 (1962).
17) R.J.L. Allen, *Biochem. J.*, **34**, 858 (1940).
18) To each 50 mg of cidial, malathion, and dimethoate, 1, 3, and 5 ml (wet volume) of Raney Nickel W-2 were necessary for completion of their transformation within 1 hr, respectively.
19) M.E. Getz and R.R. Watts, *J. Assoc. Offic. Anal. Chem.*, **47**, 1094 (1964).

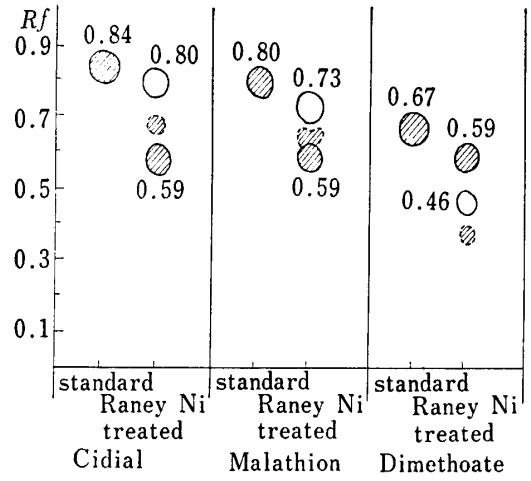
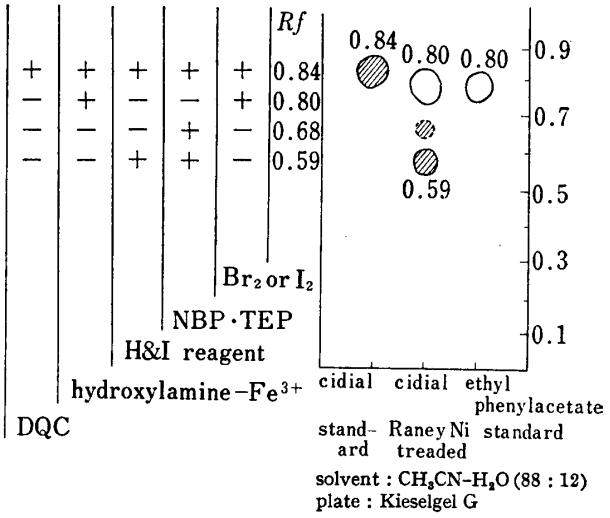


Fig. 1. Thin-Layer Chromatograms of Sulfur Containing Organophosphorus Pesticides and Their Decomposition Products by Raney Nickel Treatment

○ P negative spot ◍ P positive spot (main)
◍ P positive spot (minor)
solvent: CH_3CN–H_2O (88:12)
plate: Kieselgel G



solvent : CH_3CN–H_2O (88 : 12)
plate : Kieselgel G

Fig. 2. Thin-Layer Chromatogram of Decomposition Products of Cidial obtained by Raney Nickel Treatment

Table I. *Rf* Data of Non-Phosphorus Compounds formed by Raney Nickel Treatment of Cidial, Malathion, and Dimethoate

Sample	Solvent		
	CH_3CN–H_2O (88:12)	Benzene–ethyl acetate (19:1)	CH_3OH
Non-phosphorus compound from cidial	0.80	0.50	0.74
Ethyl phenylacetate	0.80	0.50	0.74
Non-phosphorus compound from malathion	0.73	0.27	0.78
Diethyl succinate	0.73	0.27	0.78
Non-phosphorus compound from dimethoate	0.46	0.30	0.57
N-Methylacetamide	0.46	0.30	0.57

(shoulder) nm). In a similar fashion as in the case of cidial, the non-phosphorus products (*Rf* 0.73 and 0.46) from malathion and dimethoate were identified by TLC and GLC with diethyl succinate and N-methylacetamide, respectively (Tables I and II). The above result showed that one of the reductive cleavages by Raney Nickel occurred in the S-R linkage of $(CH_3O)_2P(S)SR$, as expected.

From an analytical interest, the recoveries of these non-phosphorus products were determined by GLC. As can be seen in Table III, the recoveries of them were not so quantitative to use this reaction as an analytical tool, however the recoveries obtained were similar with those expected on the reductive desulfurization of the ordinary sulfur-containing compounds with Raney nickel.[20] This limited recovery is probably due to a strong adsorption on Raney Nickel, because the same treatment of authentic ethyl phenylacetate, diethyl succinate, and N-methylacetamide with Raney Nickel lowered their recoveries without occurrence of any other reaction product.

20) G.R. Pettit, "Organic Reaction," Vol. 12, ed. by R. Adams *et al.*, John Wiley and Sons, Inc., New York, London, 1962, p. 356.

Table II. Gas Chromatographic Data of Non-Phosphorus Compounds formed by Raney Nickel Treatment of Cidial, Malathion, and Dimethoate

Sample	Retention time (min)	Condition
Cidial	1.53	column: 10% SE-30/Chromosorb W (80—100 mesh), 3 mmϕ × 1 m; column temp.: 190° for cidial, 150° for ethyl phenylacetate; carrier gas: N_2 60 ml/min
Non-phosphorus compound from cidial	2.35	
Ethyl phenylacetate	2.35	
Malathion	10.20	column: 10% SE-30/Chromosorb W (80—100 mesh), 3 mmϕ × 1 m; column temp.: 190° for malathion, 95° for diethyl succinate; carrier gas: N_2 60 ml/min for malathion, N_2 40 ml/min for diethyl succinate
Non-phosphorus compound from malathion	14.40	
Diethyl succinate	14.40	
Dimethoate	—[a]	column: 15% diethyleneglycol succinate/Celite 545SK (80—100 mesh), 3 mmϕ × 1 m; column temp.: 120° for N-methylacetamide; carrier gas: N_2 40 ml/min
Non-phosphorus compound from dimethoate	9.0	
N-methylacetamide	9.0	

a) Dimethoate was not chromatographed at the column temperature of 90—190° under the conditions described.

Table III. Recovery of Non-Phosphorus Compounds formed by Raney Nickel Treatment of Cidial, Malathion, and Dimethoate

Sample		Recovery (%)
Ethyl phenylacetate	from cidial	74.53
	from authentic ethyl phenylacetate	74.62
Diethyl succinate	from malathion	70.00
	from authentic diethyl succinate	73.01
N-Methylacetamide	from dimethoate	92.19
	from authentic N-methyl acetamide	70.86

The phosphorus positive spot of *Rf* 0.59 (Px) common to every cidial, malathion, and dimethoate was supposed to be the same product originated from the common partial structure of these three pesticides. As shown in Table IV, the identity of Pxs obtained from them was proved by TLC using different solvent systems. It was also found that the chromatographical behavior and infrared (IR) spectrum of Px coincided with those of a main phosphorus product formed by Raney Nickel treatment of dimethyl dithiophosphate ($(CH_3O)_2$-

Table IV. *Rf* Data of the Phosphorus Compound (Px) formed by Raney Nickel Treatment of Cidial, Malathion, Dimethoate, and Dimethyl Dithiophosphate

Sample	Solvent			
	CH_3CN–H_2O (88:12)	Acetone	Benzene–ethyl acetate (1:19)	CH_3OH–AcOH (19:1)
Px-cidial	0.59	0.58	0.18	0.72
Px-malathion	0.59	0.58	0.18	0.72
Px-dimethoate	0.59	0.58	0.19	0.72
Px-dimethyl dithiophosphate	0.59	0.58	0.18	0.72
Dimethyl dithiophosphate	0.55	0.57	0.09	0.80

P(S)SH), which corresponds to the partial structure common to these pesticides. A small amount of Px was isolated by solvent extraction and preparative TLC, and it was a neutral and relatively volatile liquid. Digestion of Px with aqua regia followed by the analysis of inorganic sulfate revealed that Px had no sulfur. The IR spectrum of Px displayed major absorption peaks at 1,260 cm^{-1} (P=O), 1,188 cm^{-1} (P-OCH_3), 2,800—2,940 cm^{-1} (-OCH_3), but lacked a sharp peak at 2,400 cm^{-1} characteristic to P-H linkage.[21] Although Px and dimethyl phosphite resembled each other in major absorption peaks of their IR spectra, the lack of a peak at 2,400 cm^{-1} (P-H) in the spectrum of Px and the difference in their chromatographical behaviors discriminated the former from the latter.

Another phosphorus-positive minor spot (Fig. 1) which seems to be characteristic to each of these pesticides was not examined because of its extreme poor yield.

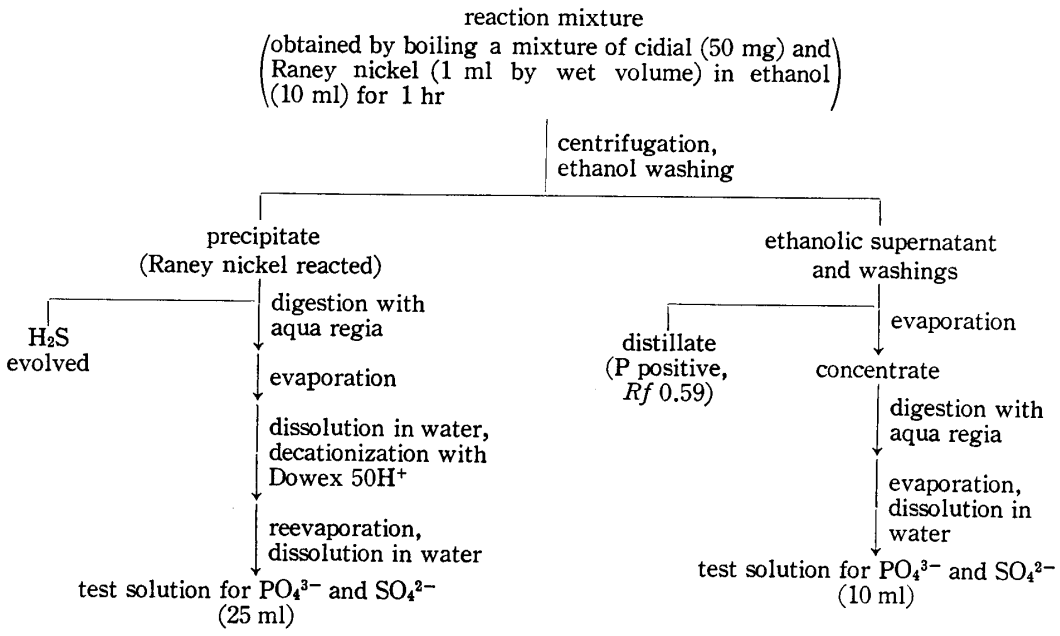


Chart 2

Table V. Distribution of Phosphorus and Sulfur among the Fractions obtained by Raney Nickel Treatment of Cidial

Fraction	Recovery	
	P (%)	S (%)
Ethanolic supernatant	14.43	0
Raney Nickel precipitated	70.14	64.86

In the course of conducting these experiments, our attention was called to the poor recovery of Px, 5—10% at most. In order to make clear this, a distribution of phosphorus and sulfur among the fractions obtained by Raney nickel treatment of cidial was examined according to the scheme presented in Chart 2. As shown in Table V, it was found that most of the phosphorus containing compounds were tightly bound to the Raney nickel precipitated and no sulfur was found in the ethanolic supernatant which contained Px and another minor phosphorus compound. During the operation shown in Chart 2, a small amount of Px was

21) R.A. McIvor, G.A. Grant, and C.E. Hubley, *Can. J. Chem.*, **34**, 1617 (1956).

detected in the ethanolic distillate by TLC, and a temporary evolution of hydrogen sulfide was observed at the beginning of wet digestion of the Raney nickel with aqua regia.

Conclusion

Cidial (I: R=$-CH(C_6H_5)COOC_2H_5$), malathion (I: R=$-\underset{\displaystyle CH_2COOC_2H_5}{\overset{\displaystyle |}{C}HCOOC_2H_5}$), and dimethoate (I: R=$-CH_2CONHCH_3$) having a common structure $(CH_3O)_2P(S)SR$ were treated by Raney Nickel in ethanol (Chart 3). Each of them was reductively cleaved to a non-phosphorus product (II: ethyl phenylacetate, diethyl succinate, and N-methylacetamide) and a main phosphorus product, Px (III).

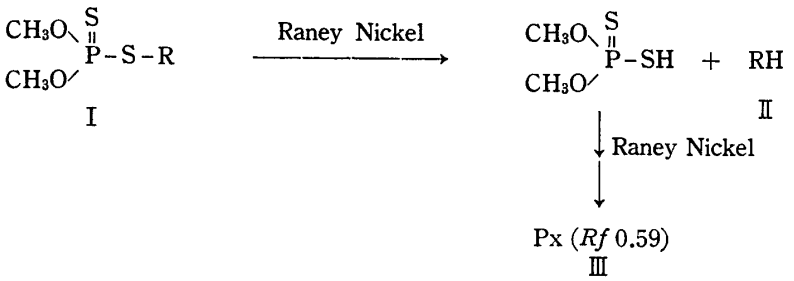


Chart 3

This reduction afforded a new method to characterize these types of pesticides, however, the recovery of these non-phosphorus products was not so good to apply this reaction on the quantitative determination of them. Px, a common product to these pesticides, was identified with the phosphorus product obtained by Raney nickel treatment of dimethyl dithiophosphate $(CH_3O)_2P(S)SH$ by TLC and IR spectrometry. From a consideration of the result obtained, it seemed probable that after a prior cleavage of the S-R linkage in $(CH_3O)_2P(S)SR$(I), the intermediary product, dimethyl dithiophosphate, was further desulfurized by Raney nickel to give a sulfur free phosphorus compound Px, which is an unknown compound having both CH_3OP- and P=O groups in its structure.