terial. Since compounds 3 and 7 were also isolated from the [*ring*-^{14}C]fonofos standard solutions, the question of their metabolic formation cannot be answered at this time. Compound 4, which is directly formed from compound 3, is probably not formed from fonofos (compound 1).

ACKNOWLEDGMENT

Special appreciation is expressed to the Bayerische Landesanstalt für Bodenkultur und Pflanzenschutz and the Institute for Ecological Chemistry, Gesellschaft für Strahlen und Umwelt forschung, both at Munich, Germany, whose hospitality made this research possible. Special thanks are given to P. R. Wallnöfer and E. Pritzel for their invaluable advice and assistance.

LITERATURE CITED

Budzikiewicz, M., Fenselaur, C., Djerassi, C., *Tetrahedron* **22**, 1391 (1966).

Djerassi, C., Mutzenbecher, G. V., Fajkos, J., Williams, D. M., Budzikiewicz, H., *J. Am. Chem. Soc.* 88, 529 (1966).

Flashinski, S. J., Lichtenstein, E. P., *Can. J. Microbiol.* **20(3)**, 399 (1974).

McLafferty, F. W., *Appl. Spectrosc.* 11, 148 (1957).

Peterson, J. I., Wagner, F., Siegel, S., Nixon, W., *Anal. Biochem.* **31**, 189 (1969).

Spiteller, G., Spiteller-Riedman, M., Houriet, R., *Monatsh. Chem.* **97**, 121 (1966).

Wegener, W. S., Schell, J., Romano, R. A., *J. Bacteriol.* **94(6)**, 1951 (1967).

Received for review November 4, 1976. Accepted February 28, 1977. This research was conducted during the senior author's study leave from the University of Wisconsin while working at the Institute for Ecological Chemistry and the Bavarian Institute for Plant Protection, both at Munich, Germany. Research supported in part by the University of Wisconsin Graduate School. Contribution by Project 1387 from the Wisconsin Agricultural Experiment Station as a collaborator under North Central Regional Cooperative Research Project 96, entitled, "Environmental Implications of Pesticide Usage".

Degradation of Tetrachlorvinphos and Its Major Metabolite 2,4,5-Trichlorophenacyl Chloride in Aqueous Media

M. Humayoun Akhtar

The degradation of the insecticide tetrachlorvinphos [2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate] and its major metabolite, 2,4,5-trichlorophenacyl chloride, was investigated in buffered aqueous media at various pHs. Rate constants and half-life studies revealed that these compounds were stable in acidic and neutral solution but unstable under alkaline conditions. The major mode of decomposition of tetrachlorvinphos in both acidic and basic media is dephosphorylation which results in the formation of 2,4,5-trichlorophenacyl chloride. In alkaline media the chloride undergoes hydrolysis, followed by aldol condensation resulting in the formation of 1-(2,4,5-trichlorobenzoyl)-2-(2,4,5-trichlorophenyl) glycerol.

Organophosphorus and carbamate insecticides are widely used in animal production. These compounds are known to degrade chemically and/or biochemically in a relatively short period after their application (Sheets, 1967). Tetrachlorvinphos [2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate], an organophosphate insecticide, is an effective foliar insecticide for a wide range of crops and is particularly active against adult and larval forms of lepidopterous pests. In animal production it is used in and around agricultural premises to control external parasites—flies, mites, ticks, etc. (Beynon et al., 1973).

The metabolism of tetrachlorvinphos in dogs and rats (Akintonwa and Hutson, 1967), dairy cows (Gutenmann et al., 1971), and the breakdown in plants and soils (Beynon and Wright, 1969) have been investigated. While hydrolysis is considered to be one of the main detoxification mechanisms for organophosphates (Muhlmann and Schrader, 1957), no information is available on the fate of tetrachlorvinphos in aqueous media. The present work was undertaken to obtain information on degradation of tetrachlorvinphos and its major metabolite 2,4,5-trichlorophenacyl chloride, in aqueous media at various pHs.

Animal Research Institute, Research Branch, Agriculture Canada, Ottawa, Ontario, K1A 0C6.

EXPERIMENTAL SECTION

Chemicals. All solvents were of pesticide grade and used as received. Analytically pure tetrachlorvinphos was prepared by the procedure described by Whetstone et al. (1966). 2,4,5-Trichlorophenacyl chloride was prepared from tetrachlorvinphos by acid hydrolysis and was purified by column chromatography on neutral alumina (BDH Chemicals) and finally recrystallized from hexane. 2,4,5-Trichlorophenacyl alcohol was prepared by hydrolysis of 2,4,5-trichlorophenacyl acetate (Cebrian, 1948). 2,4,5-Trichloroacetophenone was obtained by the reduction of 2,4,5-trichlorophenacyl iodide.

Stock solutions of tetrachlorvinphos (1.5 mg/mL) and 2,4,5-trichlorophenacyl chloride (0.5 mg/mL) were prepared in acetone and stored at -20 °C. Buffer solutions ranging from pH 6–9 were prepared by titrating 0.1 M disodium hydrogen phosphate with 0.1 M sodium hydroxide and 0.1 M phosphoric acid to the desired pH. All solutions were brought to room temperature (~25 °C) before mixing.

Rate of Hydrolysis. The hydrolysis reactions were conducted at 27 ± 2 °C in the dark. One milliliter of stock tetrachlorvinphos or metabolite solution was pipetted into a 500-mL volumetric flask covered with aluminum foil.

Table I. Rate Constants and Half-Lives for the Degradation of Tetrachlorvinphos and 2,4,5-Trichlorophenacyl Chloride at Various pHs

pH	Tetrachlorvinphos		2,4,5-Trichlorophenacyl chloride	
	k, h^{-1} [a]	$t_{0.5}$, h	k, h^{-1} [a]	$t_{0.5}$, h
5.75	$1.93 \pm 0.21 \times 10^{-4}$	3590	$1.32 \pm 0.17 \times 10^{-4}$	5250
6.5	$2.88 \pm 0.17 \times 10^{-4}$	2406	$1.65 \pm 0.22 \times 10^{-4}$	4200
7.4	$6.03 \pm 0.2 \times 10^{-4}$	1149	$2.88 \pm 0.13 \times 10^{-4}$	2406
8	$2.21 \pm 0.15 \times 10^{-3}$	313.6	$4.01 \pm 0.12 \times 10^{-3}$	172.8
9	$1.15 \pm 0.08 \times 10^{-2}$	60.3	$2.27 \pm 0.14 \times 10^{-2}$	30.5

[a] Mean values of at least five observations.

The solution was reduced to near dryness with a gentle stream of N_2 gas. The residue was dissolved in 450 mL of distilled water by stirring for 1 h. One milliliter of the approximate buffer solution was added and the remaining volume was made up with distilled water. The contents were mixed by vigorous shaking for 1 min. An aliquot (50 mL) was withdrawn immediately and placed in a 100-mL glass-stoppered volumetric flask. Hexane (50 mL) was added, the flask stoppered, and the mixture shaken vigorously for 1 min. After the layers had separated, 40 mL of the hexane extract was withdrawn into a round-bottom flask and evaporated to dryness under reduced pressure. The residue was taken up in 2 mL of carbon disulfide, and an aliquot (3 μL) was injected into the gas chromatograph. The peak height recorded from this injection, taken as 100% of the compound, was compared with that recorded from an injection of 3 μL of carbon disulfide solutions obtained from samples withdrawn at different intervals.

The disappearance of tetrachlorvinphos and 2,4,5-trichlorophenacyl chloride in aqueous media was followed by noting the decrease in peak heights of the compounds in the extracts at different time intervals. Gas chromatographic analysis of the extracts resulted in well-defined peaks for tetrachlorvinphos and 2,4,5-trichlorophenacyl chloride along with other small peaks.

Gas Chromatography (GC). The carbon disulfide extracts were analyzed for tetrachlorvinphos and its metabolites on a Packard-Becker 420 gas chromatograph equipped with a flame ionization detector (FID). Tetrachlorvinphos was determined on column 1, whereas 2,4,5-trichlorophenacyl chloride and metabolite(s) were measured on column 2. Column 1 was a 1.37 m × 4 mm (i.d.) glass tube, packed with 5% (w/w) OV-210 on 80–100 mesh Gas Chrom Q. The column, detector, and injector temperatures were 225, 295, 260 °C, respectively. The nitrogen carrier gas, hydrogen, and air flow rates were 60, 25, and 200 mL/min, respectively. Column 2 was a glass tube 1.83 m × 4 mm (i.d.) packed with 10% (w/w) DC-200 on 80–100 mesh Gas Chrom Q. Column temperature was 200 °C, while the other operating conditions were the same as those described for column 1. Under these conditions the retention times of tetrachlorvinphos (column 1) and 2,4,5-trichlorophenacyl chloride (column 2) were 2.7 and 9.2 min, respectively.

Thin-Layer Chromatography (TLC). Tetrachlorvinphos and its metabolite(s) were also analyzed on precoated (250 μm) aluminum oxide. TLC plates (Fisher Scientific Co.) were developed in benzene:hexane (1:1, v/v). The plates were then sprayed with 2-phenoxyethanol–silver nitrate reagent, air-dried for 5 min and viewed under UV light (254 nm). The compounds exhibited dark spots due to the chlorinated portion of the molecule. The R_f values for 2,4,5-trichlorophenacyl alcohol, 2,4,5-trichlorophenacyl chloride, 2,4,5-trichloroacetophenone, and compound X were 0.73, 0.69, 0.54, and 0.35, respectively.

Other Analyses. The melting point was determined on a Fisher-Jones apparatus. The IR and NMR spectra were recorded on a Beckman IR 12 spectrophotometer and a Varian A-60 spectrometer, respectively. The mass spectra were obtained on a Finnigan Model 3100 mass spectrometer interfaced with a Model 6100 computer data acquisition system. Exact mass measurements were recorded on an Associated Electrical Industries MS-9 double-beam focusing high-resolution spectrometer.

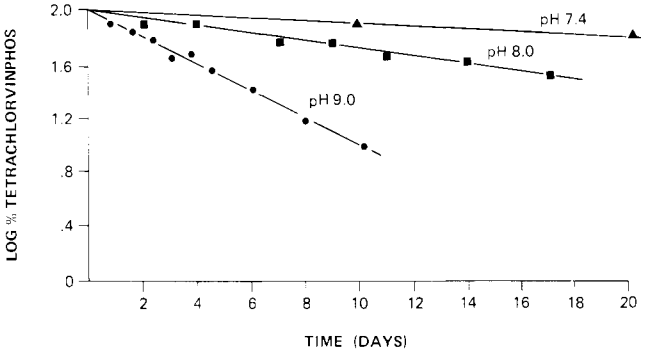

Figure 1. Effect of pH on the decomposition of tetrachlorvinphos at 27 ± 2 °C.

RESULTS AND DISCUSSION

Rate of Hydrolysis. A plot of log (percent residue of the starting compounds, i.e., I and II) vs. time gave a straight line with a slope of $-k/2.303$, e.g., for tetrachlorvinphos (Figure 1). Consequently, the multiplication of the slope by −2.303 resulted in the pseudo-first-order rate constant, k. The half-life time values for degradation at various pHs were obtained by substituting the respective values in the following equation (Ander and Sonnessa, 1965):

$$t_{0.5} = 0.693/k$$

The rates of degradation of tetrachlorvinphos and 2,4,5-trichlorophenacyl chloride at different pHs are listed in Table I. Tetrachlorvinphos and 2,4,5-trichlorophenacyl chloride are both degraded considerably more slowly in acidic than in alkaline media. The degradation of tetrachlorvinphos proceeds slightly faster than that of 2,4,5-trichlorophenacyl chloride in an acidic or neutral media, whereas the reverse is observed under alkaline conditions.

Analysis of Degradation Products. The degradation scheme of tetrachlorvinphos in aqueous media is shown in Figure 2: I, tetrachlorvinphos; II, 2,4,5-trichlorophenacyl chloride; III, 2,4,5-trichloroacetophenone; IV, trichlorophenacyl alcohol. Carbon disulfide extracts obtained from both tetrachlorvinphos and 2,4,5-trichlorophenacyl chloride decompostion experiments were analyzed for product(s) on column 1 and 2 under the GC conditions described above. In order to obtain the maximum concentrations of product(s) only the extracts of the final sampling (17 and 10 h for pH 8 and 9, respectively) were analyzed in each case. The identity of the peaks on the chromatogram

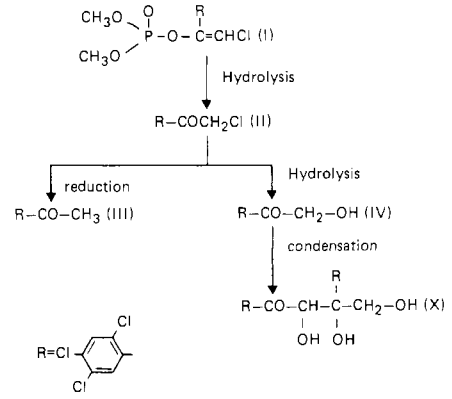

Figure 2. Possible route of degradation of tetrachlorvinphos and 2,4,5-trichlorophenacyl chloride in aqueous media.

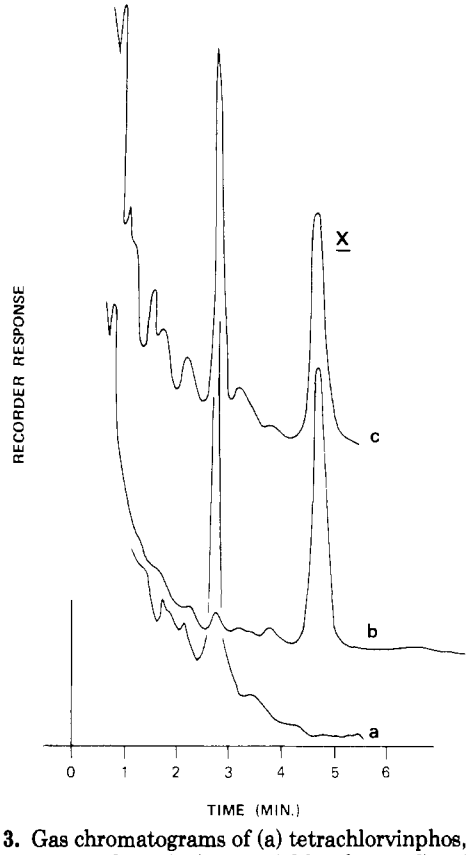

Figure 3. Gas chromatograms of (a) tetrachlorvinphos, (b) aldol condensation product (1-(2,4,5-trichlorobenzoyl)-2-(2,4,5-trichlorophenyl) glycerol), (c) an extract of degradation at pH 9. GC conditions: glass column, 1.37 m × 4 mm (i.d.), packed with 5% OV-210 on Gas Chrom Q; injector port temperature, 295 °C, column temperature, 225 °C; detector temperature, 300 °C; carrier gas (nitrogen), air hydrogen flow rates, 60, 300 and 25 mL/min, respectively; chart speed, 1.25 cm/min.

were established by comparing their retention times with those of the reference standards and by cochromatography.

The extracts obtained from tetrachlorvinphos degraded at pH 8 and 9 exhibited a major peak (column 1) with a retention time of 4.5 min (Figure 3) due to a new compound, which will be referred to as compound X. The rate of formation of compound X increased with increasing pH.

Gas chromatographic analysis on column 2 of the extracts of tetrachlorvinphos decompostion revealed that at up to pH 7.4, II was the major decompostion product (90–92%) with small quantities of III (<5%) and IV (<1%). The concentration of products I, II, and X in the final extracts at pH 8 were 34, 18, and 28%, respectively. Furthermore, the corresponding values at pH 9 were 9.8, 1, and 76%. A small peak due to III was also detected in the gas chromatograms of these extracts. The formation of III from I and II is unclear.

Assuming that the conversion of I to IV constitutes two consecutive first-order reactions, the concentration of I, II, and IV at a given time t can be calculated from the following expressions (Moelwyn-Hughes, 1964):

$$\mathrm{I} \rightarrow \mathrm{II} \rightarrow \mathrm{IV} \rightarrow \mathrm{X}$$

$$n_0 = n_1 + n_2 + n_3$$

$$n_1 = n_0 e^{-k_1 t}$$

$$n_2 = n_0 \frac{k_1}{k_2 - k_1} (e^{-k_1 t} - e^{-k_2 t})$$

where n_0 is the initial concentration of I (100%), n_1, n_2, and n_3 are the concentrations of I, II, and IV, respectively at time t; k_1 and k_2 are the rate constants for the disappearance of I and II, respectively. Substitution of the appropriate values in the above expressions yields the following composition of the mixture at pH 8: I (40.6%), II (26%), and IV (33.4%); pH 9: I (6.3%), II (6.04%), and IV (87.6%).

A comparison between the observed and the calculated values clearly indicates a major contribution of the two consecutive first-order reactions in the formation of the above noted products. The similarities between the calculated values of IV and the observed values for X further substantiate the view that the condensation of IV to form X is very fast. However, it should be pointed out that these data also indicate the involvement of some other minor degradation reactions which were not indentified in the present study.

2,4,5-Trichlorophenacyl chloride decomposed very slowly in acidic or neutral media. However, the rate of decompostion was greatly enhanced with increasing pH (Table I). The extracts obtained at pH 8 and 9 contained 2,4,5-trichlorophenacyl alcohol in trace amounts. In addition, analysis on column 1 exhibited a peak with a retention time of 4.5 min, identical with that obtained for compound X. Analysis of the extracts on column 2 revealed the presence of III (~1–2%) and IV (~1%).

In view of the foregoing it is apparent that both tetrachlorvinphos and 2,4,5-trichlorophenacyl chloride in basic media are converted into an unknown compound X with a retention of 4.5 min on column 1. This compound was also prepared by overnight treatment of a methanolic solution of 2,4,5-trichlorophenacyl chloride with 10% aqueous K_2CO_3. The organic material was extracted with ether and dried. Removal of the solvent in vacuo yielded light-yellow oil. The resultant oil was saturated with hexane to form a yellowish white solid. This solid had an identical retention time on column 1 as reported above for compound X. The melting point of the compound was 146–148 °C (uncorrected).

The identity of compound X was established by spectroscopic methods. The mass spectrum (probe) of the material had a molecular ion peak at 476 (M^+). The m/e 476 represents twice the molecular weight of 2,4,5-trichlorophenacyl alcohol ($C_8H_5Cl_3{}^{35}O_2$ = 238) which is the hydrolysis product of the phenacyl chloride. In addition, the spectrum (Figure 4) also exhibited the isotope pattern for a molecule containing six chlorine atoms, 478 (M + 2/M = 1.94), 480 (M + 4/M = 1.50), 482 (M + 6/M^+ = 0.6), 484 (M + 8/M^+ = 0.13) (Silverstein and Bassler, 1968). The base peaks at m/e 207 and 179 are attributed to $C_6H_2Cl_3CO^+$ and $C_6H_2Cl_3^+$, respectively. The molecular weight (mass spectra) for the compound X was 476.1705 (calculated for $C_{16}H_{10}Cl_6O_4$, 476.1716). The IR spectrum

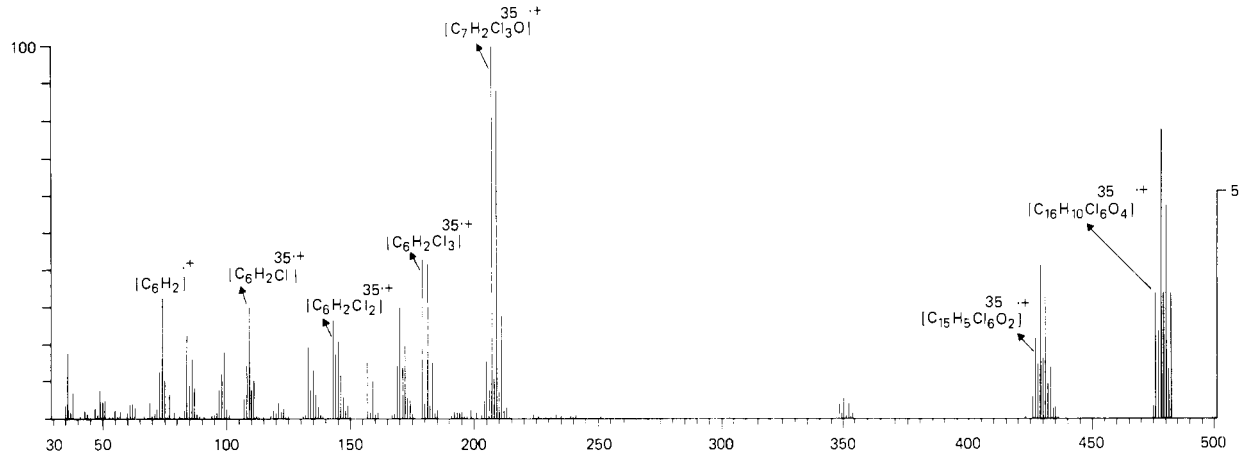

Figure 4. Mass spectrum of 1-(2,4,5-trichlorobenzoyl)-2-(2,4,5-trichlorophenyl) glycerol.

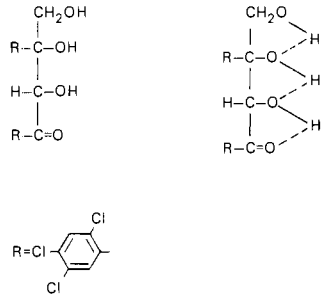

Figure 5. Suggested structure for compound X.

($CHCl_3$) had peaks at 3090, 3075 (m), 3000 (w), 1700 (s), 1585 (s), 1450 (m) cm^{-1}. The peaks at 3090 and 3075 cm^{-1} are attributed to strongly intramolecularly hydrogen bonded hydroxy stretching vibrations (Silverstein and Bassler, 1968). The resonance at other wavelengths is assigned as 3000 cm^{-1} (-C-H), 1700 cm^{-1} (aromatic -C=O), 1585 and 1450 cm^{-1} (aromatic -C=C-). The NMR spectrum in $CDCl_3$ exhibited resonance at δ 4.03 (AB quartet, 2 H, $-CH_2-$, J_{AB} = 13.5 Hz), 4.43 (s, 1 H, tertiary -C-H), and 7.3–7.7 (bm, 7 H, 4 aromatics and 3 OH's), weakly exchangeable protons, supporting the strong intramolecular hydrogen bonding viewpoint.

The foregoing spectroscopic data suggest the structure shown in Figure 5 for compound X.

The formation of 1-(2,4,5-trichlorobenzoyl)-2-(2,4,5-trichlorophenyl) glycerol is viewed as the base-catalyzed aldol condensation of 2,4,5-trichlorophenacyl alcohol.

The data indicate that both tetrachlorvinphos and its major metabolite are persistent in acidic and neutral media. However, in basic media the rate of hydrolysis of the insecticide is greatly enhanced. The major mode of decompostion of tetrachlorvinphos in both acidic and basic aqueous environments is dephosphorylation leading to the formation of 2,4,5-trichlorophenacyl chloride. In basic media, the latter undergoes hydrolysis, followed by fast aldol condensation resulting in the formation of 1-(2,4,5-trichlorobenzoyl)-2-(2,4,5-trichlorophenyl) glycerol.

The data show that while both the insecticide and its major metabolite are degraded, they would have long residual life under acidic and neutral conditions. It is interesting to note that Beynon and Wright (1969) reported that 95% of applied phenacyl chloride (II) was decomposed within 60 h in a medium loam soil (pH 8, water content 19%). They also observed that in the absence of rain and winds, I (Gardona) was not persistent on foliage in the green house. It should be realized, however, that the conditions employed in the above study were probably more rigorous than those which might be encountered in the field. A study on the mode of decomposition of these compounds in in vivo and in vitro systems is in progess.

ACKNOWLEDGMENT

The author gratefully acknowledges the help of S. I. M. Skinner, R. Gibb, and A. Hogg for spectrometric analysis.

LITERATURE CITED

Akintonwa, D. A. A., Hutson, A. A., *J. Agric. Food Chem.* **15**, 632 (1967).

Ander, P., Sonnessa, A. J., "Principles of Chemistry—An Introduction to Theoretical Concepts", Macmillan, New York, N.Y., 1965, p 571–574.

Beynon, K. I., Hutson, D. H., Wright, A. N., *Residue Rev.* **47**, 55 (1973).

Beynon, K. I., Wright, A. N., *J. Sci. Food Agric.* **20**, 251 (1969).

Cebrian, G. R., *An. R. Soc. Esp. Fis. Quim., Ser. B* **44**, 587 (1948); *Chem. Abstr.* **42**, 8176 (1948).

Gutenmann, W. H., St. John, L. E., Lisk, D. J., *J. Agric. Food Chem.* **19**, 1259 (1971).

Moelwyn-Hughes, E. A., "Physical Chemistry", Pergamon Press, London, 1964, p 1127.

Muhlmann, R., Schrader, G., *Z. Naturforsch.* **126**, 196 (1957).

Sheets, T. T., *Am. Assoc. Adv. Sci.* **85**, 311 (1967).

Silverstein, R. M., Bassler, G. C., "Spectrometric Identification of Organic Compounds", Wiley, New York, N.Y., 1968, p 9, 84.

Whetstone, R. R., Philips, D. D., Sun, Y. P., Ward, L. F., Shellenberger, T. E., *J. Agric. Food Chem.* **14**, 352 (1966).

Received for review October 19, 1976. Accepted February 7, 1977. Contribution No. 666.