Colorimetric Determination of O,O-Dialkyl 1-Hydroxyphosphonates Derived from Chloral

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A new series of organic phosphorus insecticides can be made by condensing chloral with a dialkyl phosphite. Chloral condensed with dimethyl phosphite yields O,O-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate, produced in Germany and tested here under the designation Bayer L 13/59. This and other dialkyl 1-hydroxyphosphonates were quantitatively estimated by heating the compound to split out chloroform, which was absorbed in aqueous pyridine and warmed with alkali to develop the red color first described by Fujiwara. The method is sensitive to 20 γ of L 13/59. The water solubilities of diethyl, dipropyl, and dibutyl esters have been estimated by this method.

ORGANIC PHOSPHORUS COMPOUNDS of possible use as insecticides can be made by condensing chloral with a dialkyl hydrogen phosphite (*2*):

$$(RO)_2POH + Cl_3CCHO \rightarrow (RO)_2P(=O)-CHOHCCl_3$$

The first member of the homologous series, *O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethylphosphonate, has recently attracted interest as an insecticide for DDT-resistant houseflies (*4*). This ester was originally produced in Leverkusen, Germany, and tested in the United States under the designation "Bayer L 13/59."

Abramov *et al.* (*1*) observed that dialkyl 1-hydroxyphosphonates are decomposed by the action of either alkali or heat, which cleaves the carbon-phosphorus bond and regenerates the starting materials:

$$(RO)_2P(=O)-C(R')(OH)(R'') \rightarrow (RO)_2POH + R'-C(=O)-R''$$

In seeking a colorimetric method for the estimation of Bayer L 13/59 and its homologs, alkali cleavage was attempted without success, but pyrolysis produced chloroform, which apparently was a breakdown product of chloral. The chloroform produced was determined colorimetrically by a modification of the pyridine-alkali Fujiwara test (*3*), which gives a bright red color in the presence of chloroform, chloral, and some other simple hydrocarbons containing three or more chlorine atoms. Griffon *et al.* (*5*) studied the Fujiwara test and its modifications and attempted to establish the proportions of water, sodium hydroxide, and pyridine, as well as the optimum time of heating in a boiling water bath for development of a red color sufficiently stable for quantitative determinations of chloral, chloroform, and trichloroethylene. The Griffon procedure was not satisfactory when tested on standard solutions of chloral hydrate and chloroform, but a modified procedure gave a reproducible red color which was stable for at least 30 minutes.

A temperature of approximately 550° C. was found necessary to break the carbon-phosphorus linkage of *O,O*-dialkyl 2,2,2-trichloro-1-hydroxyethylphosphonates to obtain reproducible colors obeying Beer's law over a wide range of concentrations. A simple microfurnace was constructed for this purpose (Figure 1).

The *O,O*-dialkyl 2,2,2-trichloro-1-hydroxyphosphonates were carefully purified by recrystallization from petroleum ether containing a little benzene. The dibutyl ester, a liquid not stable to heat, was purified by repeated washing of a benzene solution with water until the washings showed no traces of free chloral by the color test. The physical properties of the esters, the preparation of which was described by Barthel *et al.* (*2*), are given below:

Ester	M.P., ° C.
Dimethyl (L 13/59)	78–80
Diethyl	55–56
Dipropyl	68–70.5
Diisopropyl	105–106.5
Dibutyl	Liquid, $n_D^{25} = 1.4718$

Apparatus and Reagents

The microfurnace, absorption apparatus, and evaporator and capsule are shown in Figure 1.

The ice bath is made by mixing crushed ice and salt in a 1-pint Dewar flask in such proportion that the bath will register from −15° to −20° C.

Photoelectric colorimeter, Klett-Summerson or equivalent. No. 54 green filter (spectral range 500 to 570 mμ).

Colorimeter tubes, matched. Use corks wrapped with aluminum foil to fit tubes.

Pyridine-water mixture. Add 50 ml. of distilled water to 400 ml. of redistilled colorless pyridine.

Sodium hydroxide solution, 0.25*N*.

Benzene, redistilled.

Procedure

Adjustment of Microfurnace to Receive Sample. Turn on the heat of the microfurnace (Figure 1,*A*) at least 2 hours before samples are to be run and adjust the Variac setting (at about 25 volts) to obtain a steady temperature of approximately 550° C. inside the pyrolysis tube, 3 (Figure 1,*A*). The temperature is most conveniently measured by a thermocouple inserted into the tube; an appropriate thermometer will serve the same purpose.

Preparation of Standard Curve. Weigh accurately 50 mg. of the purified *O,O*-dialkyl 2,2,2-trichloro-1-hydroxyethylphosphonate in a 0.5-ml. cup or microbeaker. Drop the cup into a 100-ml. volumetric flask and make to the mark with redistilled benzene. After mixing, pipet 5 ml. of the solution into a second 100-ml. volumetric flask, then 20 ml. into a third similar flask. Make

each flask to the mark with redistilled benzene and mix well. Transfer, by pipet, appropriate aliquots to a series of capsules, 4 (Figure 1,*C*) to contain 25, 50, 75, etc., up to 300 γ of ester. Carefully evaporate the solvent in the capsules almost to dryness on the steam bath. Volumes of benzene 4 ml. or less can be placed directly in the capsule; larger volumes necessitate the use of the Kuderna-Danish type evaporator (Figure 1,*C*).

Remove the last trace of benzene from the capsule at room temperature with the aid of a fine stream of air. Pipet 6 ml. of the pyridine-water mixture into the absorption tube, 14 (Figure 1,*B*). After greasing joint 15, insert the delivery tube, 12, connect to the vacuum line, and regulate with a pinch clamp to give smooth but not too vigorous bubbling through the fritted-glass bubbler, 13. Place the absorption tube, 14, in the Dewar flask containing an ice-salt mixture (−15° to −20° C.), then securely clamp together the ball-and-socket joint, 11, using a light coating of silicone grease. Remove inlet cap 1 and insert capsule 4 (open end forward) into the hot pyrolysis tube, 3, and push the capsule in as far as possible with a glass rod. Replace the air-inlet cap, 1. Let the pyrolysis and absorption of the products in the receiver proceed for 1 hour. Then unclamp joint 11, place tube 14, with the vacuum line still connected, in a beaker to hold it while pipetting 3 ml. of the pyridine-water mixture through the socket joint into delivery tube 12. After all the mixture has run into the bulb of the absorption tube, as indicated by bubbling at bubbler 13, remove 12 by disconnecting joint 15.

To develop the color, pipet 1 ml. of 0.25*N* sodium hydroxide solution into the absorption tube, mix, cover loosely with a glass stopper, and place in a boiling water bath for 3 minutes. Then remove the tube from the bath and cool at once under tap water. Filter the colored turbid solution through a Whatman No. 41, 11-cm. filter paper directly into a colorimeter tube to obtain a clear bright-red solution which is stable for 30 minutes. Stopper the tube with a foil-covered cork and measure against a blank sample (treated in the same way) in the photometer. Prepare the standard curve by plotting the logarithm of the per cent transmittance (or the logarithmic photometer reading) against micrograms of ester (Figure 2).

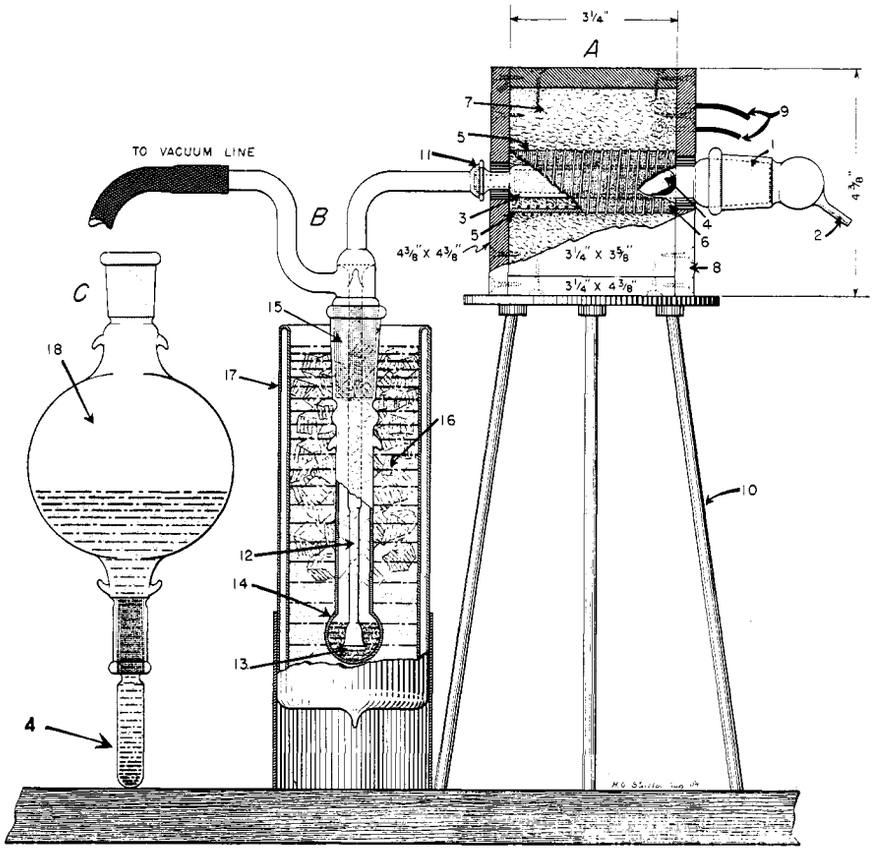
Figure 1. Apparatus for determining dialkyl trichlorohydroxyethylphosphonates

A. Microfurnace
1. Cap on inlet ⅎ 24/40
2. Cotton plug in air inlet
3. Pyrolysis tube, borosilicate glass, 22 mm. o.d.
4. Capsule for sample extractive
5. Heating wire, 25 feet No. 25 B&S gage Chromel A
6. Asbestos paper wrapping
7. Asbestos fiber packing
8. Transite housing
9. Connections to Variac
10. Tripod
11. Ball and socket joint 12/5, and clamp (not shown)

B. Absorption apparatus
12. Delivery tube
13. Fritted-glass bubbler
14. Absorption tube, 15 cm. × 15 mm. o.d., bulb 25 mm. o.d.
15. ⅎ 24/40 joint
16. Ice-salt mixture
17. Dewar flask, 1 pint

C. Evaporator (Kuderna-Danish type)
18. Reservoir for benzene extract of insecticide residues

4. Capsule for concentrated extract



Determination of Water Solubilities. Make a saturated solution of each ester by shaking a bottle containing an excess of the purified ester in distilled water on a shaking machine for several hours and holding overnight in a constant-temperature bath at 25° C. Carefully transfer a 2.0-ml. aliquot of the clear saturated solution by pipet to a 500-ml. volumetric flask. Add about 400 ml. of benzene and shake the flask vigorously to extract the ester from the 2 ml. of water. Make the flask to the mark with benzene and mix well. If the solution is turbid, warm it slightly on the steam bath with occasional shaking until the turbidity disappears. As in the preparation of the standard curve (above), transfer an appropriate aliquot of the clear benzene solution to the capsule, evaporate carefully, pyrolyze, develop the color, and read the micrograms of ester from the standard curve. The following values were obtained as an average of three or more determinations:

Ester $(RO)_2P(O)CHOHCCl_3$ R	Solubility in Water at 25° C., Grams/100 Ml.
CH_3	15.4
C_2H_5	13.1
C_3H_7	0.74
C_4H_9	0.18

Determination of Solubility in Organic Solvents. Because of its special interest as an insecticide, the solubility of the dimethyl ester (Bayer L 13/59) was run, by the same method, in several organic solvents:

Solvent	Solubility at 25° C., Grams/100 Ml.
n-Hexane	0.08
n-Pentane	0.10
Benzene	15.2
Diethyl ether	17.0
Chloroform	75.0

Discussion

Because Abramov *et al.* (*1*) reported that *O,O*-dialkyl 1-hydroxyphosphonates thermally dissociate to their starting materials—i.e., dialkyl phosphite and carbonyl compound—chloral was believed to be the pyrolysis product. Griffon *et al.* (*5*) showed that chloral

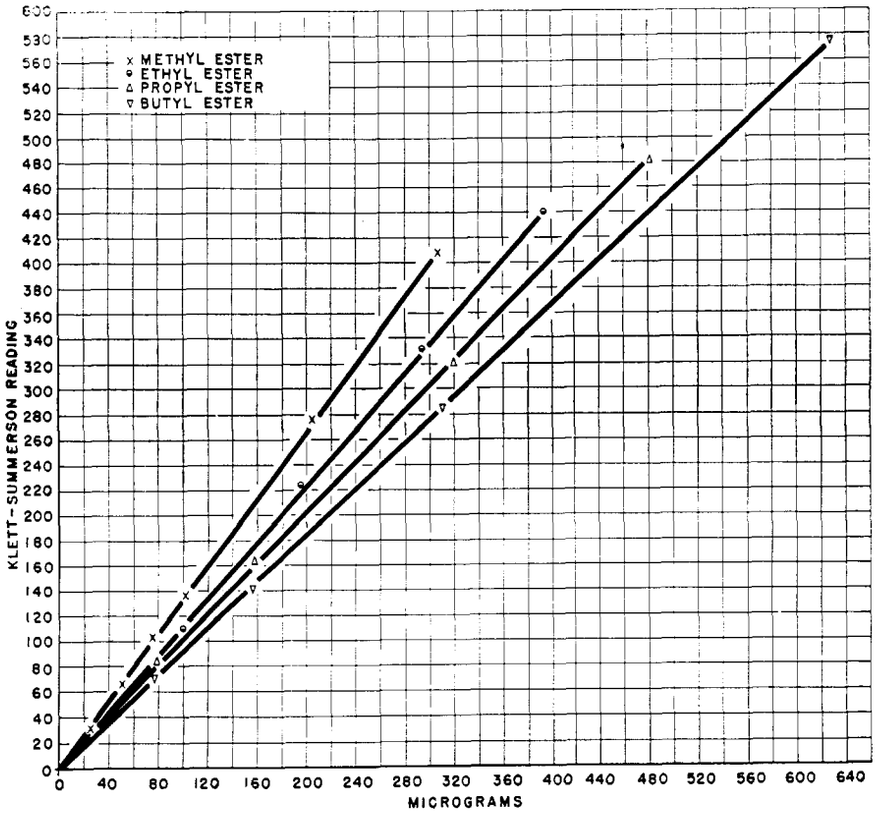


Figure 2. Standard curves for O,O-dialkyl-2,2,2-trichloro-1-hydroxyethylphosphonates

hydrate gave a color of higher specific absorbance than chloroform. The authors confirmed this. A plot of the colorimeter readings against equimolar concentrations of chloroform, chloral hydrate, and *O,O*-dialkyl 2,2,2-trichloro-1-hydroxyethylphosphonates is shown in Figure 3. The apparent molar conversion of the dimethyl ester to chloroform is about 94%. The apparent conversion of the diethyl, dipropyl, and dibutyl esters to chloroform is about 88%; nearly all fall on the same line. If the esters were converted by pyrolysis to chloral, a red color of greater absorbance would be expected.

The absorbance–wave-length curves (Figure 4) of the red Fujiwara color from chloral hydrate, chloroform, and pyrolyzed *O,O*-diethyl 2,2,2-trichloroethyl-1-hydroxyethylphosphonate were run on a Beckman Model B spectrophotometer. The curve for chloral hydrate differs slightly from the curves for chloroform and the pyrolyzed ester, which are nearly identical. This is further evidence that chloroform instead of chloral is actually being determined. The absorption maxima are at 360 and 530 mμ. Although the absorbance at 360 mμ is about twice that at 530 mμ, the latter wave length was selected for measurement because it was adapted to the Klett-Summerson colorimeter with a No. 54

Figure 3. Plot of colorimeter reading against different molar concentrations of dialkyl esters, chloroform, and chloral hydrate

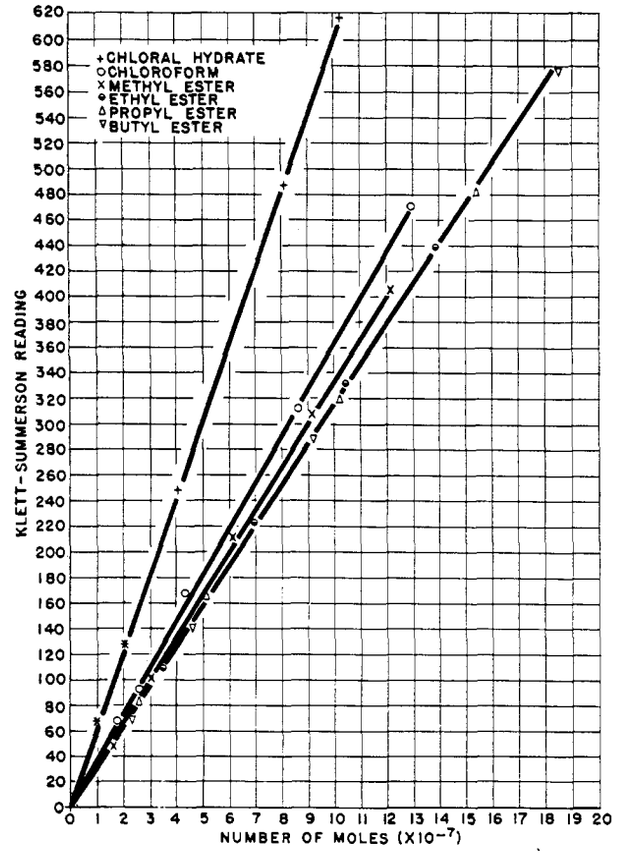
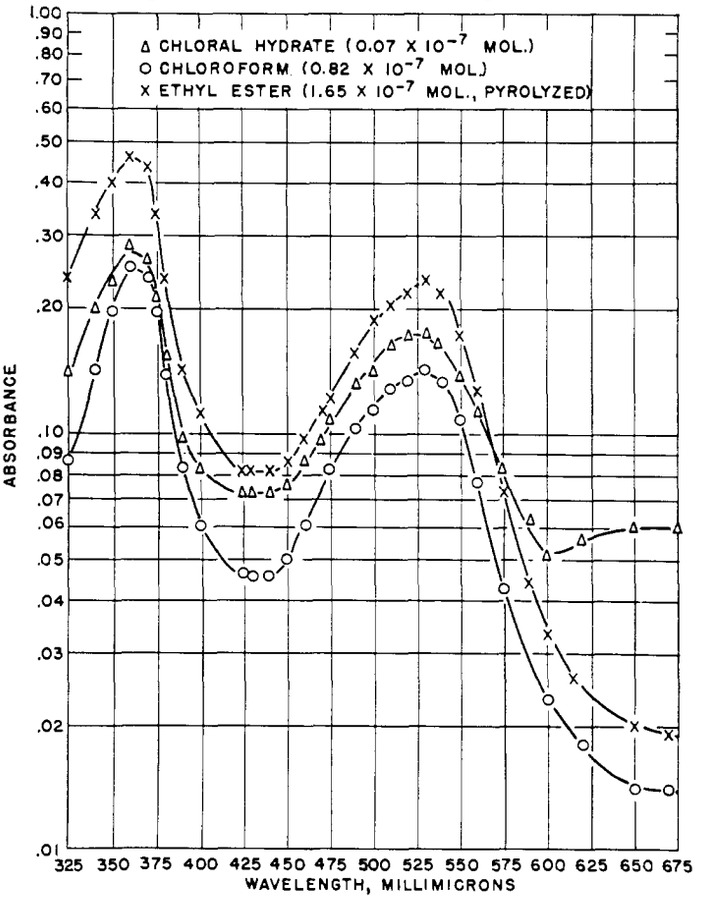


Figure 4. Absorbance–wave-length curve



filter. Advantage might be taken of the increased absorbance at 360 mμ and a Beckman Model B spectrophotometer used for the determinations. However, at the lower wave length difficulty might be encountered from impurities absorbing in the ultraviolet region.

The diisopropyl ester of 2,2,2-trichloro-1-hydroxyethylphosphonic acid behaved anomalously on pyrolysis. No reproducible red-color tests were obtained by pyrolysis of this homolog. Most interest has centered about the dimethyl ester (Bayer L 13/59) because of its use in controlling DDT-resistant houseflies in dairy barns. Attempts were made to determine micro quantities of the order of 1 p.p.m. of this ester in cow's milk, but interfering colors were obtained. Although no actual determinations were made of spray residues of this compound, the method is sensitive to 20 γ and hence could be used for this purpose.

A number of chlorine-containing insecticides were tested for their possible interfering effects:

Insecticide, 200 γ	Color	Equivalent to L 13/59, γ
DDT	None	..
Lindane	None	..
Chlordan	Reddish brown	24
Heptachlor	Reddish brown	41
Aldrin	Reddish brown	10
Dieldrin	Reddish brown	9
Endrin	Reddish brown	8
Toxaphene	Reddish brown	14

The trace of reddish brown color resulting from some of the insecticides could well be due to the impurities present in the materials tested.

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POTATO COMPOSITION

Survey of Major and Minor Sugar and Starch Components of the White Potato

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Quantitative analysis and qualitative paper chromatography of the ion-free alcohol-soluble sugars of plants have confirmed the fact that sucrose, fructose, and glucose comprise the major sugars of the white potato. Trace amounts of sugars chromatographically similar in behavior to ketoheptose, melibiose, melezitose, and raffinose, along with a non-moving fructan (fructosan) and a significant quantity of inositol, have been detected in such extracts. The generally accepted pattern of sugar change of potatoes stored at various temperatures was confirmed, and it was found that fructose, of the three major sugars, seemed to be most responsive to temperature changes. Neither the amylose-amylopectin ratio nor the phosphorus content of the starches isolated from the potatoes was affected by storage time and temperature. A study of various starch dispersion procedures has defined optimum conditions of solubilization of potato starch for starch-iodine "blue value" determinations.

The sugar and starch contents of white potatoes in relation to processing (*6*, *15*, *20*, *26*) and to carbohydrate metabolism (*2*, *4*) have been studied extensively since the pioneer work of Müller-Thurgau almost 75 years ago (*13*). Recently, however, chromatographic methods have been designated for the detection of sugar components of the alcohol-soluble, nonionic, nitrogen- and lipide-free carbohydrate fraction of vegetable tissue (*9*, *23*). Williams and Bevenue have reported evidence of the presence of ketoheptose and melibiose in such extracts from potato (*24*). The present paper is primarily concerned with the changes in the sugar and starch components, as revealed by both quantitative chemical and qualitative chromatographic procedures, of several varieties of potatoes subjected to different storage temperatures. The results presented are part of an extensive investigation of compositional factors influencing the browning of processed potato products. Subsequent publications will deal with phosphate and nitrogenous components and their relation to nonenzymatic browning of processed potatoes.

Materials and Methods

Potatoes Most of the results were obtained with two contrasting potato varieties: White Rose potatoes harvested in September 1952 in the