

THE USE OF CHROMOTROPIC ACID FOR THE QUANTITATIVE
DETERMINATION OF 2,4-DICHLOROPHENOXY-
ACETIC ACID¹

DUANE LETOURNEAU AND NORMAN KROG

(WITH TWO FIGURES)

Received April 7, 1952

FREED (7) described a qualitative test for 2,4-dichlorophenoxyacetic acid (2,4-D) based on the formation of a purple color developed when 2,4-D and chromotropic acid were heated in concentrated H₂SO₄. However, Freed was not successful in adapting this test for quantitative determinations. The present investigation was undertaken to develop a quantitative method for the determination of 2,4-D based on Freed's reaction and to obtain information concerning its specificity.

Technical grade, 2,4-D was recrystallized twice from benzene, M.P. 138-139. Chromotropic acid (Eastman practical grade) was recrystallized by the method of BRICKER and JOHNSON (3). The sulphuric acid was a C.P. reagent of sp. gr. 1.84. The sulphuric acid-chromotropic acid reagent was made by dissolving 400 mg. of chromotropic acid in 200 ml. of concentrated H₂SO₄. Standard 2,4-D solutions were prepared by dissolving 35 mg. of 2,4-D in sufficient benzene to make one liter and diluting with benzene to 2.5 to 35 γ /ml. One milliliter of the standard or unknown was pipetted into a cuvette (12 \times 75 mm.) and evaporated by the microvacuum distillation method of JUDKINS (8) and 2 ml. of the sulphuric acid-chromotropic acid reagent were added to each cuvette. A blank with reagents was included. The cuvettes were heated in a glycerine bath at 150 to 155° C for two minutes. After cooling, the optical density of the solution was measured in a Coleman spectrophotometer at a wave length of 580 m μ . with the instrument set at 100% transmission with the reagent blank.

Preliminary experiments showed that the colored solutions resulting from this procedure gave the highest optical density or absorption at wave lengths of 580 m μ . (fig. 1), and therefore all measurements were made in this region.

An accurate control of the amount of chromotropic acid was found to be important for the successful application of the procedure (table I) and was accomplished by dissolving chromotropic acid in concentrated H₂SO₄. A chromotropic acid solution in concentrated H₂SO₄, 2 mg. per ml., is stable for at least three weeks. The intensity of the color produced by this solution increases with increments of 2,4-D according to Beer's Law (fig. 2).

¹ Paper no. 2817 of the Scientific Journal Series of the Minnesota Agricultural Experiment Station.

Chromotropic acid has been widely used as an analytical reagent in organic and biological chemistry following EGGRIWE'S discovery (6) that it reacts with formaldehyde to give a red to purple color when heated in 72% H_2SO_4 solution. Among the aldehydes, the test was specific for formaldehyde and negative results were obtained with a variety of organic acids, ketones, sugars, and alcohols.

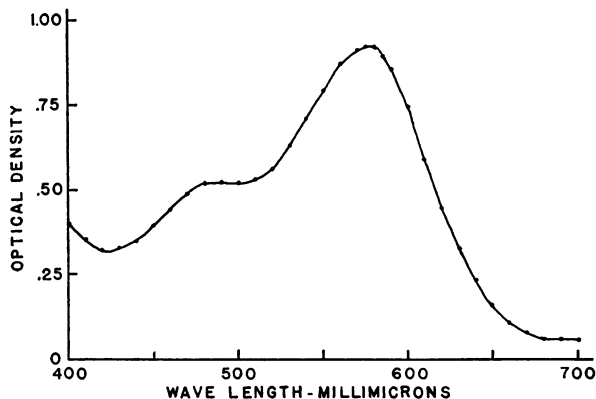


FIG. 1. Absorption spectrum of 2,4-D when reacted with chromotropic acid in concentrated sulphuric acid at 150 to 155° C for two minutes.

The reaction between chromotropic acid and formaldehyde has been applied to the qualitative and quantitative estimation of compounds which can be hydrolyzed, reduced or oxidized to yield formaldehyde. Formic acid (6), methanol (1), serine (2), diacetyl (13), methylal, pentaerythritol diformal, pentaerythritol monoformal, piperonal, n-propyl formal, and triox-

TABLE I

OPTICAL DENSITY RESULTING FROM THE REACTION OF VARIOUS CONCENTRATIONS OF CHROMOTROPIC ACID WITH 35% OF 2,4-D IN 2 ML. OF H_2SO_4 .

Chromotropic acid	Optical density
<i>mg.</i>	
0.5	0.88
1.0	1.08
2.0	1.18
3.0	1.25
4.0	1.27
5.0	1.27

ane (3) have been determined qualitatively and/or quantitatively by means of this reaction. MACFAYDEN (10) and BOOS (1) have made quantitative determinations of formaldehyde with this reaction by reading extinction values at 580 mμ. BRICKER and VAIL (5) showed that chromotropic acid reacts with or retains the formaldehyde when a solution is evaporated to dryness and certain interfering organic compounds were removed. A num-

ber of organic compounds have been found to interfere with the reaction (3, 4). FREED (7) listed a number of other compounds which gave a color reaction with chromotropic acid.

The coincidence of the absorption maxima for the formaldehyde and 2,4-D color reactions as well as similarities in conditions under which the reactions are carried out suggests that the end products may be identical in each case, with formaldehyde ultimately reacting with the chromotropic acid. To obtain some information on the nature of the group or groupings involved in the 2,4-D reaction, qualitative tests were made with a number of compounds.

For preliminary tests, a few crystals of solid or 0.05 ml. of liquid compounds were treated with the reagents used for the quantitative determination of 2,4-D. Compounds which gave wine-purple colors were then ex-

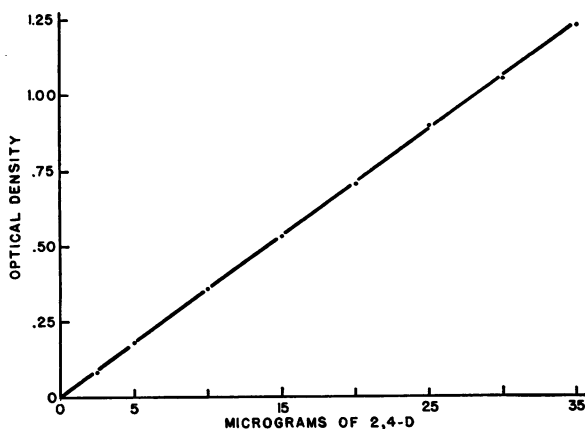


FIG. 2. Optical density of various concentrations of 2,4-D when reacted with 4 mg. of chromotropic acid in 2 ml. of concentrated sulphuric acid at 150 to 155° C for two minutes.

amined in a suitable concentration and absorption spectra were recorded of 11 of these compounds. Those that gave wine-purple colors were 2,4-D, phenoxyacetic acid, *o*-chlorophenoxyacetic acid, sodium 2,4-dichlorophenoxyacetate · H₂O, isopropyl ester of 2,4-D, monoethylamine salt of 2,4-D, monoisopropylamine salt of 2,4-D, 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), β -naphthoxyacetic acid, methoxyacetic acid, glycolic acid, formaldehyde, trioxane, paraformaldehyde, methylal, piperonal, diacetyl, indoleacetic acid, and skatole. The absorption spectra of the solutions produced by these compounds were qualitatively similar to the spectrum of 2,4-D. All had absorption maxima at 490 and 580 m μ .

Compounds tested that did not produce a wine-purple color were phenylacetic acid, α -phenoxypropionic acid, phenoxyacetamide, anisole, benzoic acid, *o*-chlorobenzoic acid, phenol, toluene, trans cinnamic acid, 2,4-dichlorophenol, α -naphthaleneacetic acid, α -naphthaleneacetamide, formic acid, ox-

alic acid, citric acid, monochloroacetic acid, acetic acid, methyl alcohol, succinic acid, glycine, diethyl ether, indole, tryptophan, β -3-indolepropionic acid, and γ -3-indolebutyric acid.

Although it may be suggested, on the basis of the similarity of the absorption spectra, that formaldehyde is responsible for the color produced in all cases, definite proof of this fact has not been established. If it is assumed that one molecule of formaldehyde is formed for every molecule of 2,4-D, the molecular extinction coefficients of the colored compound obtained from formaldehyde or from 2,4-D should be the same and should hold for the other compounds which react to yield one molecule of formaldehyde. Molecular extinction coefficients based on an average of two to four determinations were calculated. The length of the light path was 1.0 cm. Since the exact concentration of the available formaldehyde was not

TABLE II
MOLECULAR EXTINCTION COEFFICIENTS AT 580 MILLIMICRONS OF COMPOUNDS WHICH PRODUCED WINE-PURPLE COLORS WITH CHROMOTROPIC ACID IN CONCENTRATED SULPHURIC ACID.

Compounds	Concentration	Optical density	Molecular extinction coefficient
	<i>mg./ml.</i>		
Formaldehyde	.00175	.97	16,600
2,4-dichlorophenoxyacetic acid (2,4-D)	.0125	.92	16,300
Glycolic acid	.0038	.80	16,000
Sodium 2,4-dichlorophenoxyacetate · H ₂ O	.0125	.77	16,100
Monoethylamine salt of 2,4-D	.0125	.75	16,000
Monoisopropylamine salt of 2,4-D	.0125	.71	15,900
2,4,5-Trichlorophenoxyacetic acid	.0125	.79	16,100
Isopropyl ester of 2,4-D	.0125	.57	12,000
Phenoxyacetic acid	.0125	.95	11,500
o-Chlorophenoxyacetic acid	.0125	.76	11,300
Indoleacetic acid	.0125	.66	9,300
β -Naphthoxyacetic acid	.0125	.45	7,300

known, the calculation of its extinction coefficient was based on its polymer trioxane which yields 3 molecules of formaldehyde upon hydrolysis.

The results of the calculations are summarized in table II. The values obtained are not absolute since they were determined with round rather than square cuvettes. The relative values are valid for purposes of comparison, however, and close agreement is found in the case of seven of the substances examined. The values for the other compounds are lower than theoretically expected if the assumption is correct that one mole of formaldehyde is produced for one mole of substance. The lower values could be due to impurities, interfering substances, side reactions or other causes.

Chromotropic acid has been used extensively in qualitative and quantitative inorganic analysis. Reactions of chromotropic acid are known with titanium salts, chromic acid, chromates, dichromates, mercuric salts, ferric

salts, uranyl salts, neutral tungsten, silver, nitrate and nitrite ions (9, 14, 15). Since chromotropic acid reacts with many inorganic ions in aqueous solution, the reactivity of various ions in concentrated sulphuric acid was also investigated. Nitrate, nitrite, chromate, and dichromate reacted to give colors in the visible range under the conditions of the test. The following ions did not react to give colored reaction products with chromotropic acid in sulphuric acid: Fe^{2+} , Fe^{3+} , Cl^- , Hg^{2+} , SO_4^{2-} , PO_4^{3-} , K^+ , Mg^{2+} , Ca^{2+} , Na^+ , NH_4^+ , Ag^+ , UO_2^{2+} . It was not determined, however, if these ions interfere with color formation.

Since the completion of this work, SLEPECKY and BECK (12) have referred in an abstract to their procedure for a quantitative adaptation of Freed's method of detection of 2,4-D. MARQUARDT and LUCE (11) published the details of their procedure for determination of 2,4-D in milk. While the procedure reported here has not been used for the determination of 2,4-D in materials of plant or animal origin, it appears to have the potential advantages of greater sensitivity and greater stability of reagents when compared with the method of Marquardt and Luce.

DIVISION OF PLANT PATHOLOGY AND AGRICULTURAL BOTANY
UNIVERSITY FARM
ST. PAUL, MINNESOTA

LITERATURE CITED

1. BOOS, R. N. Quantitative colorimetric microdetermination of methanol with chromotropic acid reagent. *Anal. Chem.* **20**: 964-965. 1948.
2. BOYD, M. J. and LOGAN, M. A. Colorimetric determination of serine. *Jour. Biol. Chem.* **146**: 279-287. 1942.
3. BRICKER, C. E. and JOHNSON, H. R. Spectrophotometric method for determining formaldehyde. *Ind. Eng. Chem. Anal. Ed.* **17**: 400-402. 1945.
4. BRICKER, C. E. and ROBERTS, K. H. Determination of end unsaturation in organic chemistry. *Anal. Chem.* **21**: 1331-1334. 1949.
5. BRICKER, C. E. and VAIL, W. A. Microdetermination of formaldehyde with chromotropic acid. *Anal. Chem.* **22**: 720-722. 1950.
6. EEGRIWE, E. Reaktionen und Reagenzien zum nachweis Organischer Verbindungen. *Zeit anal. Chemie* **110**: 22-25. 1937.
7. FREED, V. H. Qualitative reaction for 2,4-dichlorophenoxyacetic acid. *Science* **107**: 98-99. 1948.
8. JUDKINS, W. P. The extraction of auxin from tomato fruit. *Amer. Jour. Bot.* **32**: 242-249. 1945.
9. KOENIG, P. Über ein organisches Chromreagens. *Chem. Zeit.* **35**: 277-278. 1911.
10. MACFAYDEN, D. A. Estimation of formaldehyde in biological mixtures. *Jour. Biol. Chem.* **158**: 107-133. 1945.
11. MARQUARDT, R. P. and LUCE, E. N. Determination of small amounts of 2,4-dichlorophenoxyacetic acid in milk. *Anal. Chem.* **23**: 1484-1486. 1951.

12. SLEPECKY, R. A. and BECK, J. V. The effect of 2,4-dichlorophenoxy-acetic acid on nitrification in soil. *Proc. Soc. Amer. Bact.* 1950.
13. SPECK, J. C., JR. Spectrophotometric determinations of diacetyl. *Anal. Chem.* **20**: 647-648. 1948.
14. TANANEFF, N. A. and PANTSCHENKO, G. A. Nachweis des Titans und Urans mit Hilfe der Tupfenanalyse. *Zeit. anorg. allgemeine Chemie.* **150**: 163-166. 1926.
15. VAGI, S. Neue Reaktionen der Nitrate und Nitrite. *Zeit. anal. Chemie* **66**: 14-16. 1925.