Determination of 2,2-Dichloropropionic Acid (Dalapon) in Sugar Cane

G. N. SMITH, M. E. GETZENDANER, and A. H. KUTSCHINSKI
The Dow Chemical Co., Midland, Mich.

The analytical method used to determine residues of 2,2-dichloropropionic acid (dalapon) in juice from sugar cane treated with the herbicide is described. The compound is ether-extracted, isolated chromatographically, and determined spectrophotometrically by hydrolysis to pyruvic acid and conversion to its 2,4-dinitrophenyl hydrazone. Application of the procedure to artificially fortified samples results in a recovery of about 78% with a 0.1-p.p.m. sensitivity. No residual dalapon was detected when sugar cane from fields treated with 5 to 7 pounds of the herbicide per acre was analyzed. Complete safety in the use of this material is thus assured.

THE COMPOUND, 2,2-dichloropropionic acid (dalapon) (*3–5*), is a new herbicide now finding wide application in the control of noxious grasses and weeds in fields of sugar cane. The present investigations were undertaken to develop a method to determine trace quantities of this herbicide in sugar cane in order to ascertain whether it accumulates in the plant and, if so, to what extent.

The method is based on the conversion of dichloropropionic acid to pyruvic acid, which is then estimated colorimetrically as its 2,4-dinitrophenylhydrazone (*2*). It is thus necessary to separate the dichloropropionic acid from naturally occurring α-keto acids and other carbonyl compounds which produce a color by this method. It is also necessary to remove those compounds which would be converted to carbonyl compounds during the hydrolysis of dalapon to pyruvic acid.

A three-step isolation procedure is used. The organic acids and other ether-soluble components are removed from an acidified water extract of the plant material by continuous extraction with ether. Acidic components are then extracted from the ether with aqueous sodium hydroxide.

The carbonyl compounds present in the aqueous phase are removed by converting them to their corresponding 2,4-dinitrophenylhydrazones. The hydrazones are separated from other components by partition between water and benzyl alcohol on a reversed-phase chromatographic column. Hydrazones remain on the column while dichloropropionic acid and other constituents pass through. As each component in the aqueous phase passes through the column at a specific rate, additional separation can be accomplished by collecting only that fraction of the eluate known to contain dichloropropionic acid.

Amino acids and related compounds, which are present up to this point, are converted to carbonyl compounds by hydrolysis (*1*), so they must be removed. On treatment with copper sulfate and calcium hydroxide, copper complexes are formed which are carried down with the copper sulfate—calcium hydroxide precipitate, while the dichloropropionic acid remains in solution.

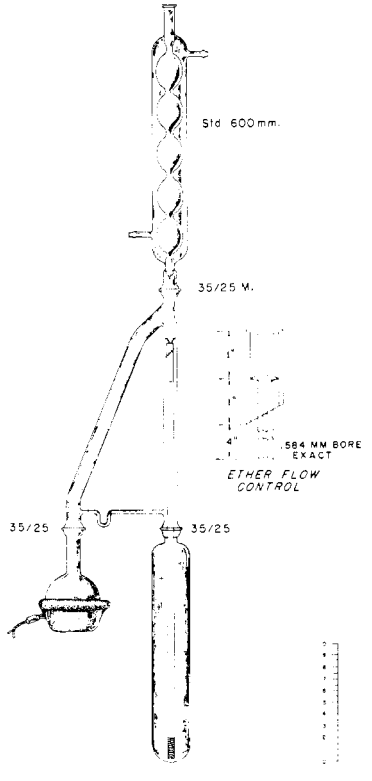

Figure 1. Continuous liquid-liquid extractor

Reagents

Diethyl ether, C.P. The ether should be tested with dinitrophenylhydrazine to be sure that it is free of interfering substances.
Hydrochloric acid, C.P.
Sodium hydroxide, 5*N* solution
2,4-Dinitrophenylhydrazine. Dissolve 200 mg. of 2,4-dinitrophenylhydrazine (Eastman White Label) in 100 ml. of 2*N* hydrochloric acid. Store in refrigerator while not in use.
Benzyl alcohol, C.P. (Eastman White Label, chloride free)
Siliconized Hy-Flow Super-Cel. Approximately 400 grams of Johns-Manville Hy-Flow Super-Cel are slurried with 1.5 liters of 60° to 70° C. Skelly Solve containing 10 ml. of dimethyl dichlorosilane (Dow Corning Corp.). After being shaken for several minutes, the suspension is filtered to remove the solvent, then washed with fresh solvent. After air drying to remove most of the solvent, the Super-Cel is dried overnight at 105° C. and stored in an air-tight bottle until used.
Hydrochloric acid, 0.1*N*, saturated with benzyl alcohol
Deionized water, saturated with benzyl alcohol
Copper sulfate, 20% solution
Calcium hydroxide, C.P.
Toluene, C.P.
Sodium carbonate, 10% solution, filtered to remove insoluble materials
Sodium bicarbonate, C.P.

Special Apparatus

Continuous liquid-liquid extractors (see Figure 1)
Chromatographic columns, 24 × 350 mm.
Centrifuge
Spectrophotometer, Beckman DU

Procedure

A 250-gram sample of raw sugar cane juice, expressed from cane in a commercial-type sugar mill, is mixed with 15 ml. of concentrated hydrochloric acid and sufficient deionized water to dilute the sample to approximately

1500 ml. The solution is then transferred to a continuous extraction apparatus (Figure 1) and sufficient diethyl ether is added to the system to permit continuous operation. Approximately 150 ml. of ether should remain in the distillation flask during the extraction. Boiling chips are added to prevent bumping. The extraction is continued for 8 hours at a constant rate of about 5 ml. per minute.

The ether extract is transferred to a 250-ml. separatory funnel and extracted once with 10 ml. of 5*N* sodium hydroxide solution and once with 5 ml. of deionized water. The combined aqueous extracts are acidified to Congo red paper with concentrated hydrochloric acid, treated with 15 ml. of 2,4-dinitrophenylhydrazine reagent, shaken, and allowed to stand at room temperature for 1 hour.

The sample is then transferred to a chromatographic column (prepared as described below) and allowed to pass slowly into the column, using 3 to 5 pounds per square inch air pressure. As the liquid level reaches the surface of the column, 75 ml. of 0.1*N* hydrochloric acid saturated with benzyl alcohol are added and allowed to pass through as above; the eluate is discarded. The acid solution is followed by approximately 100 ml. of deionized water saturated with benzyl alcohol. As soon as the water is introduced into the column, collection of the eluate in graduated receivers is started. In initial experiments the eluate was collected in 10-ml. fractions. Each fraction was then analyzed as described below. After some experience with the column a collection pattern of 25, 10, 25, and 10 ml. was used, with only the second, third, and fourth fractions being analyzed. In most cases the dichloropropionic acid was confined to the third fraction (25 ml.), the preceding and subsequent 10-ml. fractions being analyzed separately as a precaution.

For removal of amino acids, 0.5 ml. of 20% copper sulfate solution and 0.5 gram of calcium hydroxide are added to each of the solutions. Each is then shaken vigorously for 1 minute, and allowed to stand for 15 minutes, with occasional shaking. The resulting precipitate is centrifuged, the supernatant decanted, and the precipitate washed twice with 10 ml. of deionized water.

Each supernatant and its corresponding washings are combined in a 125-ml. Erlenmeyer flask, acidified with concentrated hydrochloric acid and buffered by saturating with solid sodium bicarbonate. The flask is capped with aluminum foil and autoclaved at 120° to 125° C. (15 pounds per square inch) for 50 minutes. After cooling, the solution is acidified to Congo red paper with concentrated hydrochloric acid, and quantitatively transferred to a 60-ml. separatory funnel, using a small amount of water for rinsing.

Two milliliters of 2,4-dinitrophenylhydrazine reagent are then added with thorough mixing and the solution allowed to stand 5 minutes at room temperature. Toluene, 10 ml., is added and the mixture is shaken vigorously for 1 minute. When the layers have separated, the water phase is transferred to another separatory funnel and extracted with a second 10-ml. portion of toluene. The water phase is then discarded and

Table I. Specificity of Analytical Method for 2,2-Dichloropropionic Acid

Compound	Equivalent 2,2-Dichloropropionic Acid Found, %
2-Monochloropropionic acid	1.2
3-Monochloropropionic acid	0.6
2,2-Dichloropropionic acid (dalapon)	100.0
2,3-Dichloropropionic acid	0.8
2,2,3-Trichloropropionic acid	1.8

Table II. Analysis of Sugar Cane Juice from Untreated Plots

Replicate Number	Blanks, Louisiana Cane: Absorbance[a]	Blanks, Louisiana Cane: 2,2-Dichloropropionic acid equivalent, p.p.m.	Blanks, Florida Cane: Absorbance[a]	Blanks, Florida Cane: 2,2-Dichloropropionic acid equivalent, p.p.m.
1	0.180	0.07	0.060	0.02
2	0.189	0.08	0.071	0.03
3	0.024	0.01	0.064	0.03
4	0.021	0.01	0.081	0.03
5	0.031	0.01	0.088	0.04
6	0.045	0.02	0.064	0.03
7	0.046	0.02	0.067	0.03
8	0.052	0.02	0.102	0.04
9	0.089	0.04	Av. 0.075	0.03
10	0.095	0.04		
11	0.139	0.06		
12	0.155	0.06		
13	0.124	0.05		
14	0.155	0.06		
15	0.157	0.06		
16	0.097	0.04		
17	0.115	0.05		
18	0.055	0.02		
19	0.101	0.04		
Av.	0.098	0.04		

[a] Corrected for reagent blank.

Table III. Recovery of 2,2-Dichloropropionic Acid from Fortified Sugar Cane Juice Samples (Louisiana)

P.P.M. 2,2-Dichloropropionic Acid Added	Rep. No.	Absorbance[a]	Recovery P.P.M. Gross	Recovery P.P.M. Net[b]	Recovery %
0.1	1	0.427	0.17	0.13	130
	2	0.386	0.15	0.11	110
	3	0.280	0.11	0.07	70
	4	0.234	0.09	0.05	50
	5	0.250	0.10	0.06	60
	6	0.227	0.09	0.05	50
0.2	1	0.523	0.21	0.17	85
	2	0.484	0.19	0.15	75
	3	0.505	0.20	0.16	80
	4	0.468	0.19	0.15	75
0.3	1	0.686	0.27	0.23	77
	2	0.754	0.30	0.26	87
0.5	1	0.985	0.39	0.35	70
	2	0.962	0.38	0.34	68
	3	1.078	0.43	0.39	78
	4	0.945	0.38	0.34	68
1.0	1	2.180[c]	0.87	0.83	83
	2	2.005[c]	0.80	0.76	76
	3	1.945[c]	0.78	0.74	74
	4	1.923[c]	0.77	0.73	73
				Av.	77

[a] Corrected for reagent blank.
[b] Corrected for average blank of 0.04 p.p.m.
[c] Calculated from absorbance of diluted aliquots.

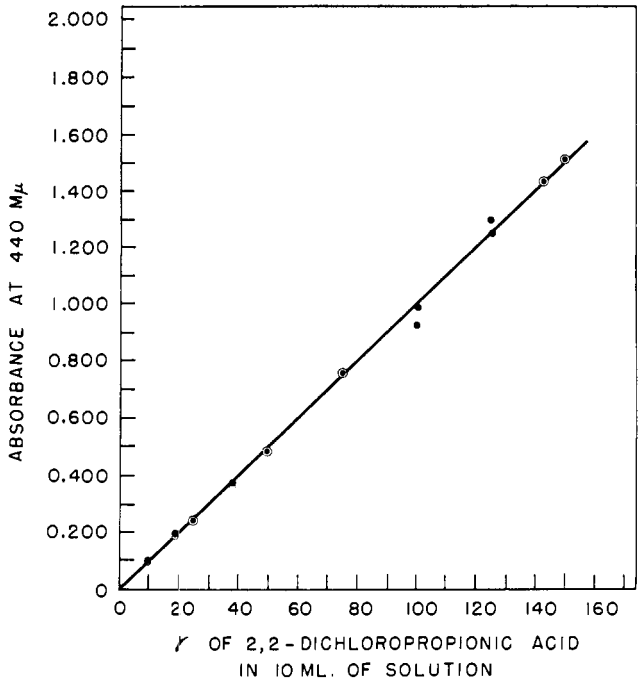

Figure 2. Standard curve for analysis of 2,2-dichloropropionic acid

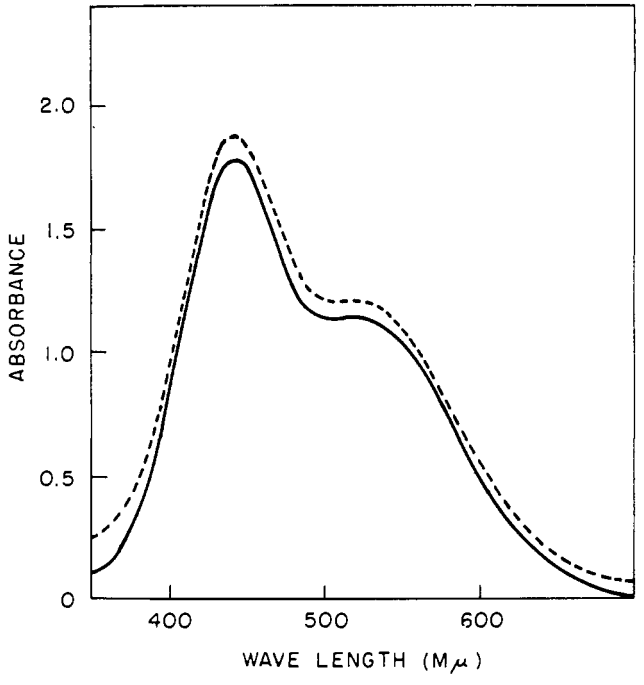

Figure 3. Spectral curves of alkaline solutions of 2,4-dinitrophenylhydrazones derived from 2,2-dichloropropionic acid and pyruvic acid

-- 2,2-Dichloropropionic acid
— Pyruvic acid

the toluene layer is added to the first toluene extract. The combined toluene layers are then extracted successively with 5 and 3 ml. of 10% sodium carbonate solution.

The carbonate solutions are combined in a 10-ml. volumetric flask and diluted to volume with 5*N* sodium hydroxide solution. The solution is filtered through a Reeve Angel 12.5 cm. fluted filter (No. 871). Fifteen minutes after the addition of sodium hydroxide, the absorbance of the solution is measured at 440 mμ, using a Beckman Model DU spectrophotometer. The reading is corrected for reagent blank, which is determined by running a distilled water sample through the complete procedure described above.

The amount of dichloropropionic acid corresponding to the corrected absorbance reading can then be estimated by reference to a standard curve.

Preparation of Chromatographic Column

Twenty grams of siliconized Super-Cel are thoroughly mixed with 16 ml. of benzyl alcohol by stirring the material in a beaker with a spatula. A thin slurry is prepared by the addition of distilled water saturated with benzyl alcohol. The slurry is transferred to a 500-ml. filtering flask and evacuated until largely free of air bubbles, then poured into a 24 × 350 mm. chromatographic column and packed uniformly, using 3 to 5 pounds per square inch air pressure and intermittent tamping. The tamper consists of a small brass disk, slightly smaller in diameter than the inside of the chromatographic tube, which is perforated with a series of small holes and attached to a long brass rod. In order to obtain a uniformly packed column the tube should be rotated during the tamping operation.

Preparation of Standard Curve

A standard solution was prepared by dissolving 100 mg. of redistilled 2,2-dichloropropionic acid (boiling point, 98–98.5° C. at 20 mm.) in 1 liter of distilled water, and diluting an aliquot of this solution tenfold with distilled water. Appropriate aliquots of the latter solution containing from 10 to 100 γ of 2,2-dichloropropionic acid were transferred to 125-ml. Erlenmeyer flasks and distilled water was added to each flask to give a final volume of approximately 25 ml. An excess of solid sodium bicarbonate was added and the flasks were capped with aluminum foil.

The procedure described above, from the hydrolysis step on, was then followed to obtain values shown in Figure 2.

Discussion and Experimental Results

The procedure outlined above appears satisfactory for the determination of trace amounts of 2,2-dichloropropionic acid. In order to determine the specificity of the method, a series of related chlorinated propionic acids was subjected to the analytical scheme. The results of these investigations are shown in Table I. Purity of the compounds used had previously been checked by infrared analysis. They were found to be 98±2% pure. Indications were obtained that one of the major impurities might be 2,2-dichloropropionic acid. The results obtained with the chemical method are, therefore, in agreement with those obtained by infrared analysis.

The 2,4 - dinitrophenylhydrazone which was obtained in the analysis of 2,2-dichloropropionic acid was compared with that of recrystallized pyruvic acid. The spectral curves of these compounds in alkaline solution, obtained with a Cary recording spectrophotometer, are shown in Figure 3.

In the present investigations, sugar cane from two geographical areas was analyzed for residual dalapon. One series of field tests was carried out by E. R. Stamper of the Louisiana Experiment Station and the other series by V. L. Guzman of the Everglades Experiment Station in Florida.

In the Louisiana experiments, stubble cane growing in the vicinity of Baton Rouge, La., was treated with water sprays of Dow dalapon, sodium salt 85%. The cane in one instance was sprayed once when the plants were 8 to 12 inches tall (April 1) and again when the plants were 18 to 24 inches tall (April 27). Other plots were sprayed only once when the cane was 18 to 24 inches high (April 27). All applications were made on the basis of treating only the row portion where the plants were growing, so that actually only

Table IV. Analyses of Sugar Cane Juice from Cane Treated with Dow Dalapon, Sodium Salt

Application Rate, Pounds per Acre							
Dow Dalapon Sodium Salt 85%		2,2-Dichloropropionic Acid					
Row basis	Over-all basis[a]	Row basis	Over-all basis[a]	Date of Application	Analysis No.	Absorbance	Gross 2,2-Dichloropropionic Acid Found, P.P.M.[c]
				Louisiana Experiments			
3.5	10.5	2.6	7.8	April 1 and 27	1	0.060	0.02
					2	0.060	0.02
					3	0.068	0.03
					4	0.092	0.04
					5	0.070	0.03
					6	0.109	0.04
					7	0.055	0.02
					8	0.061	0.02
4.7	14.1	3.5	10.4	April 27	1	0.051	0.02
					2	0.027	0.01
					3	0.049	0.02
					4	0.029	0.01
					5	0.016	0.01
					6	0.007	0.00
					7	0.031	0.01
					8	0.050	0.02
7.0	21.0	5.2	15.6	April 27	1	0.036	0.01
					2	0.045	0.02
					3	0.022	0.01
					4	0.022	0.01
					5	0.089	0.04
					6	0.053	0.02
				Florida Experiment			
5.8	17.4	4.3	12.9	March 26, 1954	1	0.060	0.02
					2	0.071	0.03
					3	0.064	0.03
					4	0.081	0.03
					5	0.088	0.04
					6	0.064	0.03
					7	0.067	0.03
					8	0.102	0.04

[a] Calculated on basis of treating 1/3 of area.
[b] Corrected for reagent blank.
[c] Net, when corrected for average blank of 0.03 p.p.m. and expressed in terms of method sensitivity, showed none detected.

1/3 of the area was treated. No attempt was made to direct the spray so as to avoid hitting the cane. Applications were made with three nozzles per row using a total spray volume of 15 gallons per acre.

The cane was harvested on October 30, 1954, and the juice was immediately expressed at the Audubon Sugar Factory, University of Louisiana. In this process, 100 pounds of cane yielded approximately 100 pounds of raw juice, as water was added during the extraction. The raw juice samples were frozen immediately and stored in this state until analyzed. The analyses were carried out in December 1954 and January and February 1955.

Most juice samples contain small quantities of interfering substances which pass completely through the isolation procedure and contribute to the final color obtained. Therefore, similar determinations must be conducted on representative untreated samples and the true residual dichloropropionic acid calculated by subtracting the blank found with the untreated samples from the apparent dichloropropionic acid residue found with the treated samples. Typical blanks and recovery data are shown in Tables II and III. The results of analyses for residual herbicide in cane from several treated Louisiana plots are shown in Table IV.

In the Florida experiments, first stubble cane growing in the Belle Glade area was treated with water sprays of Dow dalapon, sodium salt 85% on March 26, 1954 (Table IV). The cane was 6 inches tall at the time of treatment and growing rapidly. The spray was applied on a broadcast basis with no attempt made to prevent the spray from contacting the growing cane. After harvest on January 10, 1955, the juice was expressed as soon as possible at the U. S. Sugar Corp. mill at Clewiston, Fla., and stored frozen until analyzed in February 1955. The results of these analyses are shown in Table IV. Blanks are shown in Table II.

The data presented show that the method described can be used for the quantitative estimation of dichloropropionic acid in sugar cane juice in amounts as low as 0.1 p.p.m. The recoveries that can be expected by this method are in the range of 70 to 80% which is adequate to evaluate the residue problem of dichloropropionic acid in sugar cane juice.

From the data obtained by analysis of treated cane, no residual dichloropropionic acid in the cane was observed when the maximum recommended dosage of 3.7 pounds of acid equivalent per acre of sugar cane was applied on a row basis (1/3 of the area treated), or even when 1.4 times the maximum recommended (5.2 pounds of acid equivalent per acre) was used and the cane was harvested under normal conditions.

Literature Cited

(1) Barker, S. B., Summerson, W. H., *J. Biol. Chem.* **138,** 535 (1941).
(2) Friedemann, T. E., Haugen, C. E., *Ibid.*, **147,** 415 (1943).
(3) Guzman, V. L., *Everglades Station Memo. Rept.* **56-14,** University of Florida, 1956.
(4) Stamper, E. R., *Proc. 9th Ann. Southern Weed Conf.*, **1956,** 86–91.
(5) Stamper, E. R., Chilton, S. J. P., *Proc. 8th Ann. Southern Weed Conf.*, **1955,** 207–10.

Received for review December 12, 1956. Accepted March 13, 1957.