

## SEPARATION OF ALKYL AND TRIMETHYLSILYL DERIVATIVES OF DICAMBA, 3,6-DICHLORO-5-HYDROXY-*o*-ANISIC ACID, AND 3,6-DICHLOROGENTISIC ACID BY THIN-LAYER AND GAS-LIQUID CHROMATOGRAPHY\*

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(Received May 19th, 1967)

The herbicide 3,6-dichloro-*o*-anisic acid (dicamba) is reported to be metabolized by some higher plants to 3,6-dichlorosalicylic acid (DCSA) and 3,6-dichloro-5-hydroxy-*o*-anisic acid (5-HO-dicamba)<sup>1</sup>. The evidence for the production of 5-HO-dicamba consisted largely of identical GLC retention times, I.R. spectra, and mass spectra of methylated derivatives of the metabolite and authentic 5-HO-dicamba. The derivatives were prepared by means of diazomethane. Since 5-HO-dicamba and 3,6-dichlorogentisic acid (DCGA) could be expected to yield the common derivative methyl 3,6-dichloro-2,5-dimethoxybenzoate from diazomethane, it seemed possible that DCGA might also be a metabolite of dicamba. Our inability to separate 5-HO-dicamba and DCGA by any one of thirty TLC solvent systems did not dispel our interest<sup>2</sup>. Gas chromatographic separations of other phenolic acids and their methyl ethers have been accomplished by acetylating the phenolic hydroxyl before esterifying by means of diazomethane<sup>3</sup>. Discrete derivatives have also been prepared in one step by simultaneous overnight reaction of phenol and carboxyl with trimethylsilylating reagents<sup>4</sup> or higher diazoalkanes<sup>5</sup>. The overnight reaction period for certain phenols has been reduced to 30 min by means of boron trifluoride catalysis of the higher diazoalkanes<sup>6</sup>. A method for the separation of dicamba and these suspected metabolites from plant materials by TLC and their estimation as alkyl derivatives by GLC is described here.

### EXPERIMENTAL

#### *Equipment*

A Perkin-Elmer 202 spectrophotometer was used. The gas chromatograph was a Barber-Colman 100 equipped with a hydrogen flame ionization detector and glass column, 2 m by 4 mm I.D., packed with 1.5% SE-30 polyester on 90-100 mesh Anakrom ABS (Analabs Inc., Hamden, Conn., U.S.A.). The column was operated isothermally at various temperatures with flow rates of argon or nitrogen as tabulated. The injection heater and detector were maintained at a temperature 25° above that of the column.

\* Florida Agricultural Experiment Stations Journal Series No. 2692.

\*\* Adapted from Ph. D. Thesis of senior author.

*Alkylation*

Diazoalkanes were prepared without distillation<sup>5</sup>. The sample (10 mg) was dissolved in 1 ml anhydrous methanol and treated with ethereal diazoalkane catalyzed with approximately 0.007 % boron trifluoride<sup>6</sup> for 30 or 60 min. The alkylated solutions were injected into the gas chromatograph without further treatment. The unreacted phenol remaining after alkylation was estimated as previously described<sup>6</sup> by phenolate difference spectra at appropriate wavelengths as listed in Table I. The percent alkylation was calculated by difference.

TABLE I

PHENOLIC ALKYLATION YIELDS BY DIAZOALKANES CATALYZED WITH 0.007 % BORON TRIFLUORIDE<sup>a</sup>

Sample	Wavelengths		Reagent			
	Uncorrected reading (m $\mu$ )	Points on spectrum defining baseline (m $\mu$ )	Diazoethane		Diazo-n-propane	
			30 min (%)	60 min (%)	30 min (%)	60 min (%)
DCSA	303	278 318	99.9	—	—	—
5-HO-Dicamba	312	294 344	99.9	—	—	—
DCGA	266	225 301	<90	94.8	99.9	99.9

<sup>a</sup> Phenolate difference spectrum method<sup>5</sup>. Averages of two determinations.

*Trimethylsilylation*

Preliminary attempts to prepare volatile derivatives by BLAKLEY's method<sup>4</sup> in reagent dioxane used without further purification were unsuccessful and were abandoned. Trimethylsilyl derivatives were then prepared by the method of LACH AND SAWARDEKER<sup>7</sup>.

*Thin-layer chromatography*

The samples (2.5 mg) were added to plant extracts and subjected to thin-layer chromatography as described by THOMAS *et al.*<sup>8</sup> for hydroxydichlorophenoxyacetic acids. Dicamba and DCSA were not resolved, nor were 5-HO-dicamba and DCGA, but there was adequate separation between the two bands containing these two pairs of compounds. Most plant material was left near the origin. These four compounds were detected conveniently by U.V. light. The silica gel between  $R_F$  0.2 and 0.7 was removed, eluted once with sufficient methanol to cover the silica gel to a depth of 4 mm in a fritted funnel, and then eluted three times with the same volume of acetone. The acetone eluates were combined and evaporated under a stream of nitrogen before being redissolved in the methanol extract. The percent recovery was determined by gas chromatography of the butyl derivatives.

## RESULTS

*Alkylation*

The alkylation yields of the suspected phenolic metabolites of dicamba are listed in Table I. Treatment with diazoethane for 30 min gave essentially quantitative

yields for DCSA and 5-HO-dicamba, but treatment for 1 h was required for DCGA. Diazo-*n*-propane gave satisfactory yields from DCGA in 30 min.

The retention times of alkyl derivatives of the four acids appear in Table II. Dicamba and DCSA yielded derivatives with identical retention times when treated

TABLE II

RETENTION TIMES OF ALKYL DERIVATIVES OF DICAMBA AND SUSPECTED METABOLITES

Compound	Alkylating reagent			
	Diazomethane 150° <sup>a</sup> (min)	Diazoethane 150° <sup>a</sup> (min)	Diazo- <i>n</i> -propane 165° <sup>b</sup> (min)	Diazo- <i>n</i> -butane 165° <sup>a</sup> (min)
Dicamba	1.1	1.4	2.8	2.6
DCSA	1.1	1.7	4.0	3.7
5-HO-dicamba	2.6	3.8	8.6	8.2
DCGA	2.6	4.5	16.5	13.3

<sup>a</sup> Argon carrier at 170 ml per min, 22 p.s.i. drop.

<sup>b</sup> Argon carrier at 120 ml per min, 18 p.s.i. drop.

with diazomethane, as previously reported<sup>9</sup>. DCGA and 5-HO-dicamba also gave derivatives with identical retention times. The higher diazoalkanes yielded discrete derivatives which gave single symmetrical peaks with ample separation. These derivatives were stable in the freezer for six months. The threshold of detection was considered to be the amount of sample which when converted to a derivative gave a peak height twice the noise level. These values for the butyl derivatives were determined with 100 ml of nitrogen carrier per minute and a pressure drop of 16 p.s.i. The threshold of detection for dicamba and DCSA was 50 ng at 175°, and for 5-HO-dicamba and DCGA, 20 ng at 210°.

### Trimethylsilylation

No attempt was made to estimate the conversion of phenolic hydroxyls to trimethylsilyl ethers because of the instability of the product. The thresholds of detection for dicamba and DCSA were approximately 2000 ng at a column temperature of 140° with an argon carrier flow of 150 ml per min through a pressure drop of 20 p.s.i. Retention times for the derivatives appear in Table III.

TABLE III

RETENTION TIMES OF TRIMETHYLSILYL DERIVATIVES OF DICAMBA AND SUSPECTED METABOLITES<sup>a</sup>

Compound	Column temperature	
	165° (min)	140° (min)
Dicamba	— <sup>b</sup>	2.4
DCSA	— <sup>b</sup>	3.5
5-HO-dicamba	4.1	>20
DCGA	6.4	>20

<sup>a</sup> Column flow of 150 ml argon per min, pressure of 20 p.s.i.

<sup>b</sup> Coincident with solvent peak.

*Thin-layer chromatography*

The recovery data given in Table IV are the averages of four determinations. The recovery of dicamba is adequate for quantitative residue determinations, while the recoveries of the other three acids are sufficient for their detection as metabolites.

TABLE IV

RECOVERY OF DICAMBA AND SUSPECTED METABOLITES FROM TLC PREPURIFICATION<sup>a</sup>

<i>Compound</i>	<i>Recovery (%)</i>
Dicamba	98
DCSA	87
5-HO-dicamba	96
DCGA	85

<sup>a</sup> Assayed as butyl derivatives.

## DISCUSSION

It has been reported that diazoethane is less active than diazo-*n*-propane<sup>6,10</sup> and diazo-*n*-butane, the latter two being of similar activity<sup>6</sup>. Consideration of the alkylation yields from DCGA from this standpoint suggests that although diazoethane requires 60 min for adequate yields, the higher homologs would require but 30 min. Previous results with diazoisobutane<sup>5</sup> suggest that the "Rule of Six"<sup>11</sup> may apply to the diazoalkane itself in alkylating these highly sterically hindered aromatic acids. For this reason our attention was restricted to C<sub>2</sub> to C<sub>4</sub> normal diazoalkanes in this work. The latter gave stable, discrete derivatives from the phenolic acid DCGA and its methyl ether 5-HO-dicamba in one step under mild conditions. An important feature of the procedure is that a set of derivatives may be made from each of several diazoalkanes and that set chosen which gives the best resolution from interfering substances in GLC. The majority of interfering plant constituents was removed by prepurification by TLC, using Silica Gel G (Merck) in ethyl ether-ligroine-formic acid (50:50:2).

Trimethylsilylation also gave discrete derivatives with retention times that allowed separation under the conditions of this work. The separation was so great that 5-HO-dicamba and DCGA were more conveniently chromatographed at a higher temperature than dicamba and DCSA, where derivatives of the latter two compounds coincided with the solvent peak. The percentages of yields of the trimethylsilyl ethers were not assayed because of the instability of the product. The apparent threshold of detection was forty times larger than for the butyl derivatives of dicamba and DCSA, suggesting poor yields or poor sensitivity by the flame ionization detector for these trimethylsilyl derivatives.

## ACKNOWLEDGEMENTS

We are indebted to Mr. A. W. BURGESS for preparation of diazoalkane precursors and to Dr. WARREN ZICK of Velsicol Chemical Company for providing dicamba and analogs. This work was supported in part by a Caleb Hollingsworth Memorial Grant from the American Cancer Society.

## SUMMARY

Procedures for the simultaneous assay of dicamba and three suspected phenolic acid metabolites are described. The four compounds are separated from most plant products by means of thin-layer chromatography. They are then converted quantitatively in 30 or 60 min to alkyl ether alkyl esters by means of *n*-dialkylamines catalyzed by boron trifluoride. This treatment yields discrete derivatives which are easily separated on the gas chromatograph.

Trimethylsilyl derivatives were also easily separated, but forty times as much material was required for equivalent response by the flame ionization detector of the gas chromatograph.

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