research worker with a new parameter by which means the elution order of a variety of materials can be changed virtually at will.

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Gas chromatographic analysis of hydroxydichlorophenoxyacetic acids*

Recently, certain hydroxydichlorophenoxyacetic acids have been reported to be metabolites of 2,4-dichlorophenoxyacetic acid (2,4-D) in plants[1] and microorganisms[2] in a reaction which has been named the "NIH shift" by later workers[3]. We found higher diazoalkanes useful in the preparation of derivatives for gas chromatography of phenolic compounds because they have greater phenol-alkylation activity than diazomethane and their activity is further increased by boron trifluoride catalysis[4]. An important feature of the above procedure is that derivatives may be made from each of several diazoalkanes and that set chosen which gives the best resolution by GLC. The higher diazoalkanes, therefore, offer advantages over conventional procedures for phenolic acids in the GLC analysis of hydroxydichlorophenoxyacetic acids.

Experimental apparatus

An F & M gas chromatograph, Model 400, equipped with a flame ionization detector and a 2 m × 5 mm I.D. glass column, packed with SE-30 (15 %, w/w) on Chromosorb W (60–80 mesh), was operated isothermally at 190°. The injection port and detector were maintained at 235° and 240°, respectively, and the helium carrier flow was 60 ml/min.

Materials

Diazoalkanes were prepared in ethyl ether without distillation[4]. The following acids were prepared: 6-hydroxy-2,4-dichlorophenoxyacetic acid (6-OH-2,4-D)[5];

* Adapted from Ph. D. thesis of senior author.

5-hydroxy-2,4-dichlorophenoxyacetic acid (5-OH-2,4-D)[6]; and 4-hydroxy-2,5-dichlorophenoxyacetic acid (4-OH-2,5-D)[2]. POWELL[1] supplied 4-hydroxy-2,3-dichlorophenoxyacetic acid (4-OH-2,3-D).

Methods

One-milliliter portions of methanolic solutions containing the phenolic acids (2.5 mg/ml) were treated with 3 ml of diazomethane or 1-diazopropane in ethyl ether. Sufficient diazoalkane was added to maintain a yellow color. After 30 min, 0.05 ml of 0.7 % methanolic boron trifluoride was added and the solution kept yellow by the addition of diazoalkane for an additional 30 min. The samples were then concentrated under a stream of dry nitrogen and adjusted to 1.0 ml with methanol for injection into the gas chromatograph. The smallest detectable quantity was 100 ng for 2,4-D and 250 ng for the hydroxydichlorophenoxyacetic acids.

Results and discussion

The sensitivity of detection of 6-OH-2,4-D was sometimes lower than the other hydroxydichlorophenoxyacetic acids possibly because this compound lactonizes at room temperature[7]. This compound should be hydrolyzed immediately before making up standard solutions in order to minimize lactonization.

The preparation of volatile derivatives is necessary for the analysis of hydroxy-acids by gas chromatography. Solutions of diazoalkanes in ether are useful in alkylating phenolic and carboxylic hydroxyls. This method will rapidly alkylate both types of hydroxyl groups at room temperature.

By making two sets of derivatives with different diazoalkanes, it is possible to separate the previously mentioned hydroxydichlorophenoxyacetic acids. Use of only one of the reagents did not produce derivatives of all four compounds which would separate under the conditions used during the course of this work as shown in Table I. The derivatives of 5-hydroxy-2,4- and 4-hydroxy-2,5-dichlorophenoxyacetic acid when treated with diazomethane were inseparable. This was also true when 6-hydroxy-2,4- and 4-hydroxy-2,3-dichlorophenoxyacetic acids were treated with 1-diazopropane. Identification was possible by treating separate samples of the four hydroxy-acids with each of the diazoalkanes as the same two compounds were not inseparable in each set of derivatives.

TABLE I

RETENTION TIMES (min) OF DERIVATIVES OF STANDARDS

Standard	*Methyl ester*		*Propyl ester*
2,4-D	4.0		6.5
	Methyl ester, ether	*Methyl ester, propyl ether*	*Propyl ester, ether*
6-OH-2,4-D	6.7	9.4	16.4
5-OH-2,4-D	8.1	10.9	19.8
4-OH-2,3-D	10.2	13.5	16.0
4-OH-2,5-D	8.0	11.4	21.5

The hydroxydichlorophenoxyacetic acids described here may be prepurified from plant material by the TLC system of THOMAS *et al*[1] for GLC. Some phenoxyacetic acids have been found to form methyl esters under relatively mild conditions[8]; we have found these phenolic acids to form methyl esters in methanol in the absence of any catalyst. When these phenolic acids are eluted from the TLC plate with methanol and the volume then reduced, each forms appreciable amounts of the methyl ester. Subsequent treatment with 1-diazopropane results in a mixture of propyl and methyl esters of the propyl ethers giving split peaks and poorer separation, as is indicated by the retention times of the methyl ester propyl ethers in Table I. This difficulty may be avoided by eluting with acetone. Recovery from TLC was greater than 90 % for all of these phenolic acids except 6-OH-2,4-D. Only 75 % of this compound was recovered, possibly due to facile lactonization.

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