Gas–liquid chromatography of trimethylsilyl derivatives of N-arylglucosylamines

In recent years increased emphasis has been placed on the identification of metabolites of pesticides in plants. A number of pesticides containing a free or potential phenolic or amino group have been shown to be the precursors of glucosides found in pesticide-treated plants[1–5]. Glucosides may be isolated by column, thin-layer, or paper chromatography; however, it is difficult to remove all contaminants when isolating small amounts of polar substances from plant extracts by these methods. Gas–liquid chromatography of a suitable derivative would appear to be a more convenient procedure for isolating and identifying glucosides in many cases. The gas–liquid chromatography of trimethylsilyl (TMS) derivatives of sugars has become very useful[6,7]. After the present study began, FURUYA[8] reported that the O-glucosides of many naturally occurring phenolic compounds can be made into suitable derivatives for gas chromatography by converting them into their TMS derivatives. Presumably, this technique would work as well for the gas chromatography of O-glucosides formed from pesticides or pesticide breakdown products. We wish to report the use of TMS derivatives of N-arylglucosylamines as suitable for gas chromatography and useful for the separation and identification of N-arylglucosylamines found in herbicide-treated plants. Infrared comparison of the TMS derivatives is a further aid to positive identification.

Experimental

Preparation of N-arylglucosylamines. N-(3-Carboxyphenyl)-D-glucosylamine (I) prepared from D-glucose and 3-aminobenzoic acid by the method of MICHEEL AND SCHLEPINGHOFF[9] melted at 112° (dec.).

N-(3-Carboxy-2,5-dichlorophenyl)-D-glucosylamine (II) was prepared from D-glucose and 3-amino-2,5-dichlorobenzoic acid, and was isolated by TLC as described by SWANSON *et al.*[2].

N-(4-Chlorophenyl)-D-glucosylamine (III) was prepared by heating glucose (2.5 g) and 4-chloroaniline (2.5 g) in methanol (15 ml) containing acetic acid (0.2 ml) until solution was complete, and filtering the solid after cooling. The product melted at 155–156° after crystallization from methanol[9,10].

N-(3,4-Dichlorophenyl)-D-glucosylamine (IV) was prepared from 3,4-dichloroaniline (2.2 g) and D-glucose (2.5 g), as described above and melted at 151–154° after crystallization from methanol.

Analysis, calculated for $C_{12}H_{15}Cl_2NO_5$: C 44.5, H 4.7, Cl 21.8; found: C 44.2, H 4.8, Cl 21.8.

Preparation of methyl esters. Approximately 10 mg of synthetic N-arylglucosylamine or 200 μg of glucosylamine of plant origin was dissolved in methanol (0.5 ml) and the solution treated with diazomethane entrained in a slow stream of nitrogen[11]. After the yellow color persisted for a few seconds, the solution was evaporated to dryness under a stream of dry nitrogen.

Trimethylsilylation of N-arylglucosylamines. One ml of a solution of pyridine–hexamethyldisilizane–trimethylchlorosilane (10:1.5:1) was added to 10 mg of the N-arylglucosylamine or its methyl ester. Samples of plant origin containing approximately 200 μg of N-arylglucosylamine were dried under nitrogen and treated with

200 μl of the hexamethyldisilizane–trimethylchlorosilane solution. After 10 min the mixture was injected into the gas chromatograph. Samples appeared to be unchanged after 24 h. For peak identification, one μl of sample was injected into the gas chromatograph. For infrared analysis 20–30 μl samples were injected.

Gas chromatography. An F&M Model 400* gas chromatograph equipped with a ten-to-one exit splitter, flame ionization detector and a Packard Model 850* fraction collector were used for analysis and collection of samples for infrared comparisons. The column used was 3 ft. of 3/8 in. O.D. aluminum tubing packed with 60–80 mesh Chromosorb W/AW coated with 5 % Carbowax 20*M*. Nitrogen was used as carrier gas at a flow rate of 60 ml/min.

Infrared spectra. A Perkin-Elmer Model 337* spectrophotometer equipped with a beam condenser was used to record infrared spectra. A modification of a method reported by GIUFFRIDA[12] was used for collecting samples. Tubes were cut from 9 mm O.D. × 2 mm I.D. tubing after softening about 1 cm of tubing and blowing a bubble out to about 6 mm I.D. The tube was then cut through the enlarged section of the tube to a length of 44 mm. A clip was made from aluminum wire to support a small wad of cotton on which was placed about 5 mg of KBr. Similar tubes were made from 1 mm I.D. tubing for use with smaller amounts of KBr.

TABLE I

RETENTION TIMES OF TMS N-ARYLGLUCOSYLAMINES

	Column temperature (°C)	*Retention time (min)*
α-D-Glucose	150	3.1
β-D-Glucose	150	5.5
N-(4-Chlorophenyl)-D-glucosylamine	190	11.5
N-(3,4-Dichlorophenyl)-D-glucosylamine	195	20.4
3,4-Dichloroaniline complex from rice plants	195	20.5
N-(3-Carboxyphenyl)-D-glucosylamine	205	13.2
N-(3-Carboxy-2,5-dichlorophenyl)-D-glucosylamine	210	17.7
2,5-Dichloro-3-aminobenzoic acid complex from tomato roots	210	17.8

Results

Gas chromatography of the trimethylsilyl (TMS) derivatives of four N-arylglucosylamines was successfully carried out on a 5 % Carbowax 20*M* column. Results are summarized in Table I, with D-glucose anomers included for comparison.

Retention times for TMS derivatives of IV and a complex of 3,4-dichloroaniline found in rice plants[13] treated with 3,4-dichloropropionanilide (propanil) are compared in Table I, and the infrared comparison spectra are shown in Fig. 1.

Before a TMS derivative of I or II could be chromatographed, it was necessary to prepare the methyl ester. Diazomethane appeared to be the reagent of choice for methylation even though side reactions were possible. To see if reactions other than esterification would occur under the conditions used for preparation of methyl esters, diazomethane was bubbled through methanol solutions of α-D-glucose and III prior to

* Use of trade names is for purposes of identification of equipment and does not constitute endorsement by the U.S. Department of Agriculture.

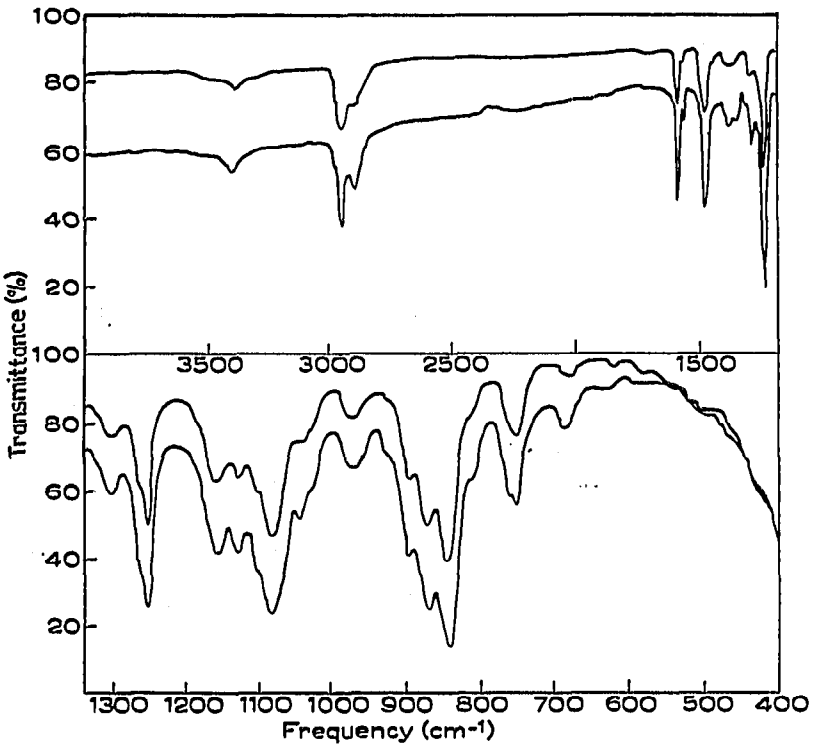

Fig. 1. Infrared spectral comparison of TMS derivatives of synthetic N-(3,4-dichlorophenyl)-D-glucosylamine (lower) and N-(3,4-dichlorophenyl)-D-glucosylamine from rice plants (upper).

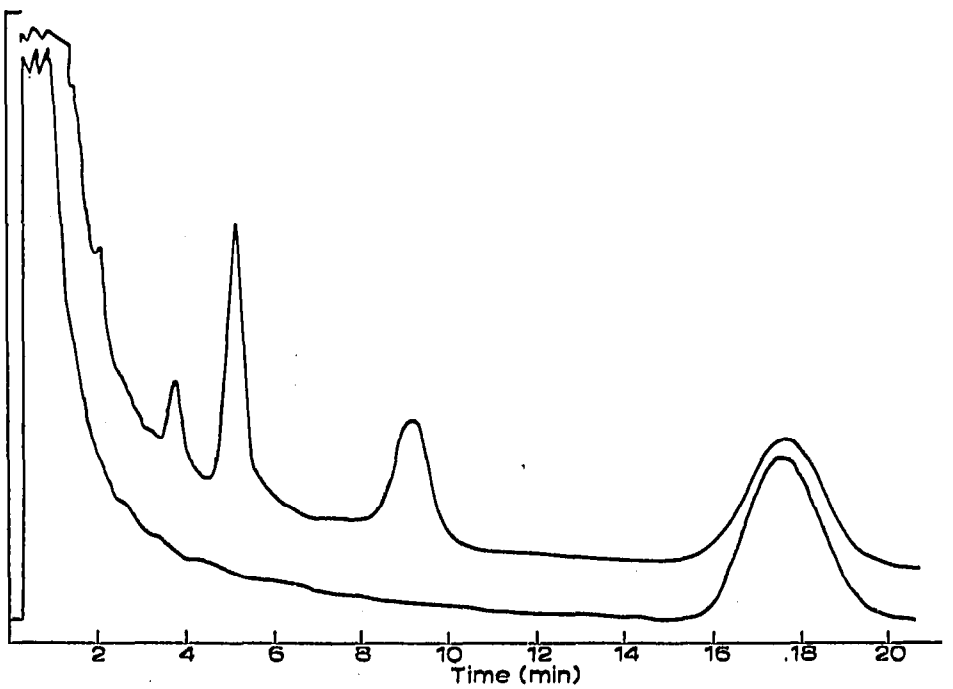

Fig. 2. Chromatogram obtained by injecting TMS methyl ester of synthetic N-(3-carboxy-2,5-dichlorophenyl)-D-glucosylamine (lower) and N-(3-carboxy-2,5-dichlorophenyl)-D-glucosylamine from tomato plants (upper).

the preparation of the TMS derivatives. Retention times were the same for TMS D-glucose samples pre-treated with diazomethane in methanol and α- and β-D-glucose. The retention time for the TMS derivative of III was also unchanged by pre-treatment of III with diazomethane.

A chromatogram of the TMS derivative of the methyl ester of II is shown in Fig. 2. Also shown in Fig. 2 is a chromatogram of a tomato root extract. Tomato plants treated with 2,5-dichloro-3-aminobenzoic acid (amiben) by root application have been shown to contain a glucoside previously described as II[1,2]. Glucosylamine II from amiben-treated tomato roots was partially purified by TLC[2], methylated, then converted to the TMS derivative and the sample injected into the gas chromatograph. The infrared spectral comparison for synthetic and plant samples is shown in Fig. 3.

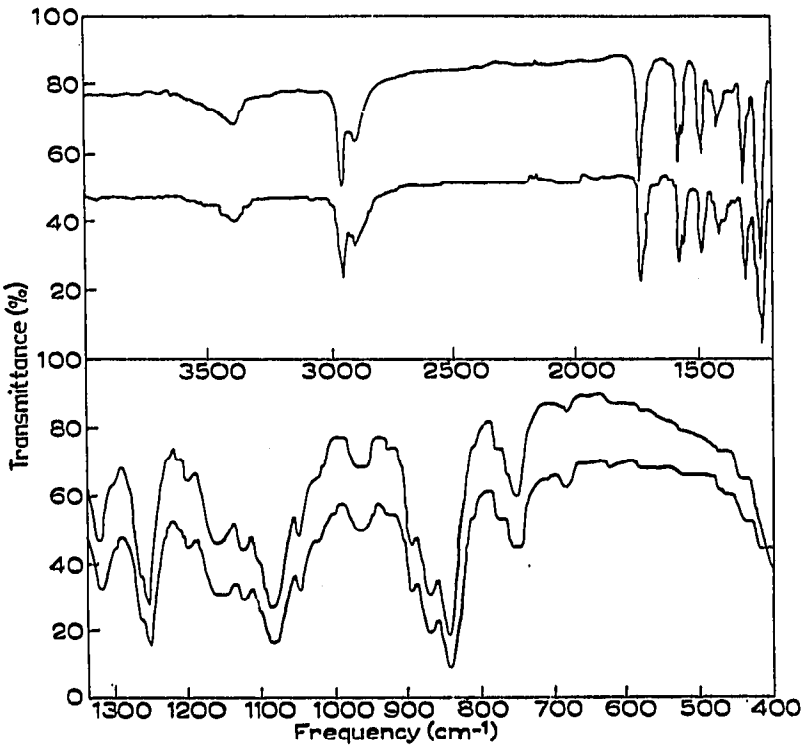

Fig. 3. Infrared spectral comparison of TMS methyl ester of synthetic N-(3-carboxy-2,5-dichlorophenyl)-D-glucosylamine (lower) and N-(3-carboxy-2,5-dichlorophenyl)-D-glucosylamine from tomato plants (upper).

The results show that gas chromatography of TMS derivatives is useful for the identification of N-arylglucosylamines found in pesticide-treated plants. Infrared spectral comparison of the effluent from the gas chromatograph is a further aid in identification; however, similarity of the infrared spectra of the TMS derivatives between 1300 and 400 cm^{-1} makes the identification of the aromatic amino moiety difficult as strong absorption bands due to the trimethylsilyl groups apparently mask most of the aromatic absorption bands. In this laboratory, the aromatic amines have been identified by gas chromatography after hydrolysis of the N-arylglucosylamines[2,13].

The amiben used in these studies was supplied by Amchem Products, Inc., Ambler, Pa.

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Received February 13th, 1967

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The effect of water on the retention times of alcohols and esters

The commonly used techniques for isolating volatiles from fruits and other plant products—distillation, freeze-drying, and trapping head-space vapours—yield aqueous solutions containing only traces of volatiles. It is often desirable, particularly in preliminary studies, to analyze these dilute solutions without further concentration.

We attempted to obtain tentative identifications of the volatile compounds in a dilute aqueous distillate from apples by gas chromatography, utilizing an FFAP (Wilkens-Varian Aerograph) column and the water-insensitive hydrogen flame detector. When increasing volumes (2.5 to 10 μl) of distillate were injected onto the column, there was a noticeable variation in the chromatograms, in that some peaks appeared or disappeared and some relative peak areas were altered. Since apple volatiles consist primarily of esters and alcohols[1], we have studied the effect of injecting increasing volumes of water on the retention times of several esters and alcohols.

Table I shows typical retention data for a mixture of esters. The retention times of esters eluted before the water front (*i.e.* esters up to butyl acetate) are clearly unaffected by the presence of variable quantities of water. In contrast, esters that are eluted after the water front have a shorter retention time when water is present, and for the higher homologs at least, the effect becomes greater as the proportion of water is increased. Similar results are obtained on FFAP at 60°, on SE-30, QF-1 and Carbowax 1540 at 100°, and on Carbowax 20M at 120°, though the effect is less pronounced with the Carbowax columns. Studies with a few aldehydes and 2-alkanones