Gas chromatography of the trimethylsilyl derivatives of shikimic acid and biochemically related compounds

Sugars and other polyhydroxy compounds can be analyzed by gas chromatography after conversion to the trimethylsilyl derivatives. SWEELY *et al*[1]. have described methods for preparing the trimethylsilyl derivatives of a large number of carbohydrates, and the conditions for their separation. More recently, LEE AND BALLOU[2] separated the trimethylsilyl ethers of some isomers of inositol by gas chromatography.

The present report outlines the conditions for the preparation and separation of the trimethylsilyl derivatives of shikimic acid, 5-dehydroshikimic acid, 5-dehydroquinic acid and quinic acid by gas chromatography.

Experimental

Shikimic and quinic acids were obtained commercially. The 5-dehydroshikimic and 5-dehydroquinic acids were prepared by dehydrogenation of shikimic acid and quinic acid, respectively, by the methods of HASLAM *et al*[3].

The trimethylsilyl derivatives were prepared according to the method of SWEELEY *et al.*[1] except that dry acetone was used instead of pyridine. Samples of 2 mg of each of the acids were dissolved in 0.5 ml of dry acetone. To each of the samples was then added 0.2 ml of hexamethyldisilazane and 0.1 ml of trimethylchlorosilane and the mixture shaken for 30 sec on a vortex mixer and left for 10 min. About 1 μl of the mixture was injected into the gas chromatograph.

The apparatus was an F & M model 810 with flame ionization detectors. The column was a 3/16 in. × 24 in. stainless steel column, and the following packings were used: 4% QF-1 on Chromosorb P, 2% SE-30 on Anachrom ABS, and 10% XE-60 on Anachrom ABS. Helium was the carrier gas and was used at a flowrate of 70 ml per min. The unit was run isothermally at the temperature indicated in Table I, and the injection temperature was 185°.

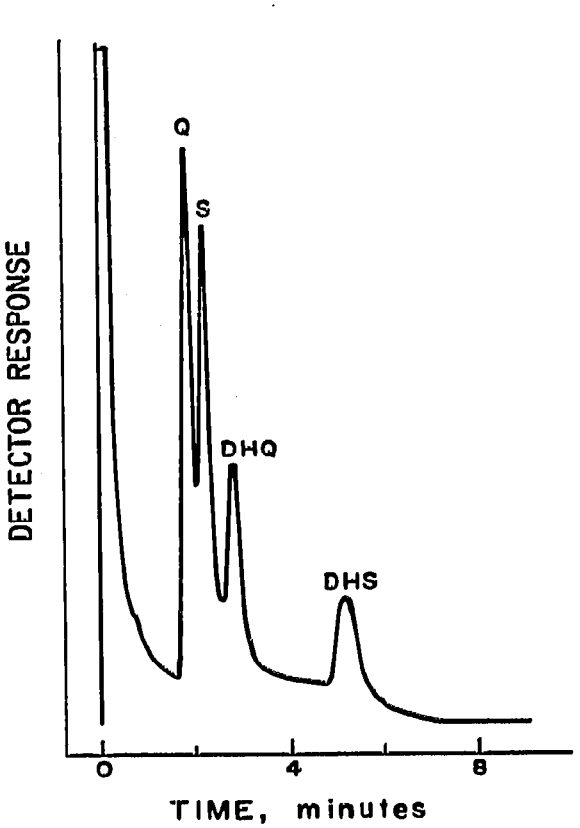
Results and discussion

The retention times of the trimethylsilyl derivatives of shikimic acid, quinic acid, 5-dehydroshikimic acid, and 5-dehydroquinic acid on three separate columns are shown in Table I, and the gas chromatographic separation of the derivatives is

TABLE I

RETENTION TIME IN MINUTES OF THE TRIMETHYLSILYL DERIVATIVES OF SHIKIMIC ACID AND BIOCHEMICALLY RELATED COMPOUNDS

Compound	*Columns and temperature*		
	4% QF-1–Chromosorb P	*2% SE-30–Anachrom ABS*	*10% XE-60–Anachrom ABS*
	150°	*160°*	*170°*
Quinic acid	1.8	3.0	2.1
Shikimic acid	2.1	2.2	2.7
5-Dehydroquinic acid	2.7	2.1	4.6
5-Dehydroshikimic acid	4.9	2.2	6.9



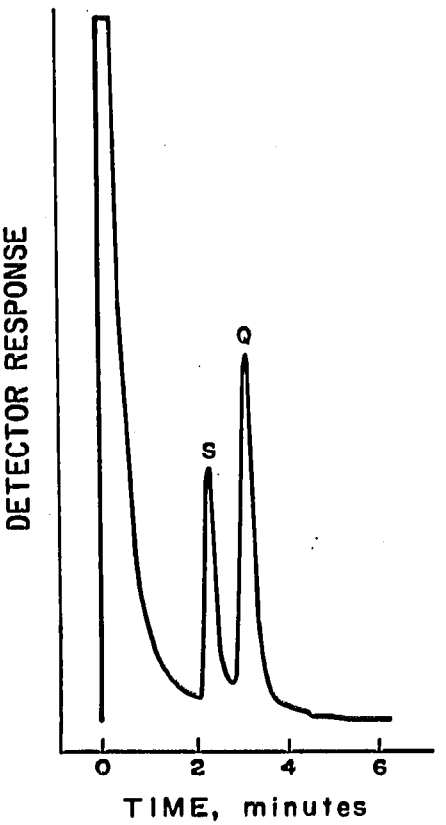


Fig. 1. The separation of the trimethylsilyl derivatives of quinic acid (Q), shikimic acid (S), 5-dehydroquinic acid (DHQ), and 5-dehydroshikimic acid (DHS) by gas chromatography. The column was packed with 4% QF-1 on Chromosorb P and the temperature was 150°.

Fig. 2. Analysis of the trimethylsilyl derivatives of quinic acid (Q) and shikimic acid (S) by gas chromatography. The column was 2% SE-30 on Anachrom ABS.

illustrated in Fig. 1. The results indicate that two of the columns can be used to separate the four compounds. The SE-30 on Anachrom ABS did not separate all the compounds, but it was the better column for the separation of quinic and shikimic acids alone (Fig. 2).

The QF-1 column was used successfully to analyze all four acids in enzyme reaction mixtures where one of the acids was used as substrate[4]. The method was used for qualitative analyses but the separation is adequate to allow for the quantitative determination of any two acids by selecting the proper column.

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Received July 11th, 1966