Notes

CHROM. 3949

Gas chromatography and mass spectroscopy of plant phenolics and related compounds*

Gas–liquid chromatography (GLC) of trimethylsilyl ethers of sugars and other polyhydroxy compounds[1–3], phenolic acids[4–7], anthroquinones[8], phenols[9,10] and their glycosides have been reported by many workers. HORII *et al.*[11,12] have successfully separated some aromatic acids and Krebs-cycle acids as trimethylsilyl (TMS) derivatives. In addition, the results of BOLAN AND STEELE[13], STALLING *et al.*[14] suggest that GLC has a wide application for analysis of mixtures encountered in biological extracts.

When GLC is used in conjunction with mass spectroscopy the molecular weight of unknown TMS derivatives of a mixture can be determined. The application of these techniques is receiving considerable attention and this communication presents the results obtained with mixtures of TMS-ethers of 28 phenolic compounds and 4 phenolic glycosides.

Experimental

Materials. All compounds used were commercially available. Hexamethyldisilazane (HMDS) and trimethylchlorosilane (TMCS) were purchased from Applied Science Co. Reagent grade pyridine (Baker) which had been dried over 4A molecular sieve and distilled under N_2 atmosphere was used.

Trimethylsilylation. Five–six mg of a component was weighed for each mixture prepared and placed in a sealed vial under N_2 atmosphere. One ml of freshly prepared reagent containing HMDS, TMCS and pyridine (3:1:9) was injected into each vial. The reaction mixture was shaken vigorously for 30 sec and then allowed to stand at room temperature for at least 5 min. An aliquot from each mixture was injected directly into the gas–liquid chromatograph.

Gas–liquid chromatography. Analyses were performed on a Hewlett-Packard instrument (F & M, model 810-DR-12) equiped with dual glass columns, flame ionization and thermal conductivity detectors (only flame ionization was used). Glass columns with 1:1 effluent splitters were 4.0 ft. long (4 mm I.D.) and packed with Chromosorb Q (60–80 mesh) coated with 3% OV-1 (Applied Science Co.).

The carrier gas (He), H_2, and air flow rates were 50, 42, and 485 ml/min, respectively. The injection port and flame ionization detector temperatures were both 310°. These conditions were constant during the entire analyses. The column temperature was programmed as indicated in each figure.

Gas chromatography and mass spectroscopy. An LKB 9000 gas chromatograph–mass spectrometer (LKB-Produkter AB Stockholm-Brommal, Sweden) was used to determine the mass of the parent ion of each TMS ether derivative.

The TMS ether derivatives were chromatographed as above except thermal conductivity and 8.0 ft. columns were employed. The effluent of each component in a

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mixture entered directly into the mass spectrometer and mass spectra were obtained for each compound.

The mass spectrometer was operated at an electron energy of 20 eV, accelerator voltage of 3.5 kV, and an ion source temperature of 280°. A scan speed of 100 *m/e*/sec was used. The molecular separators were maintained at 275°.

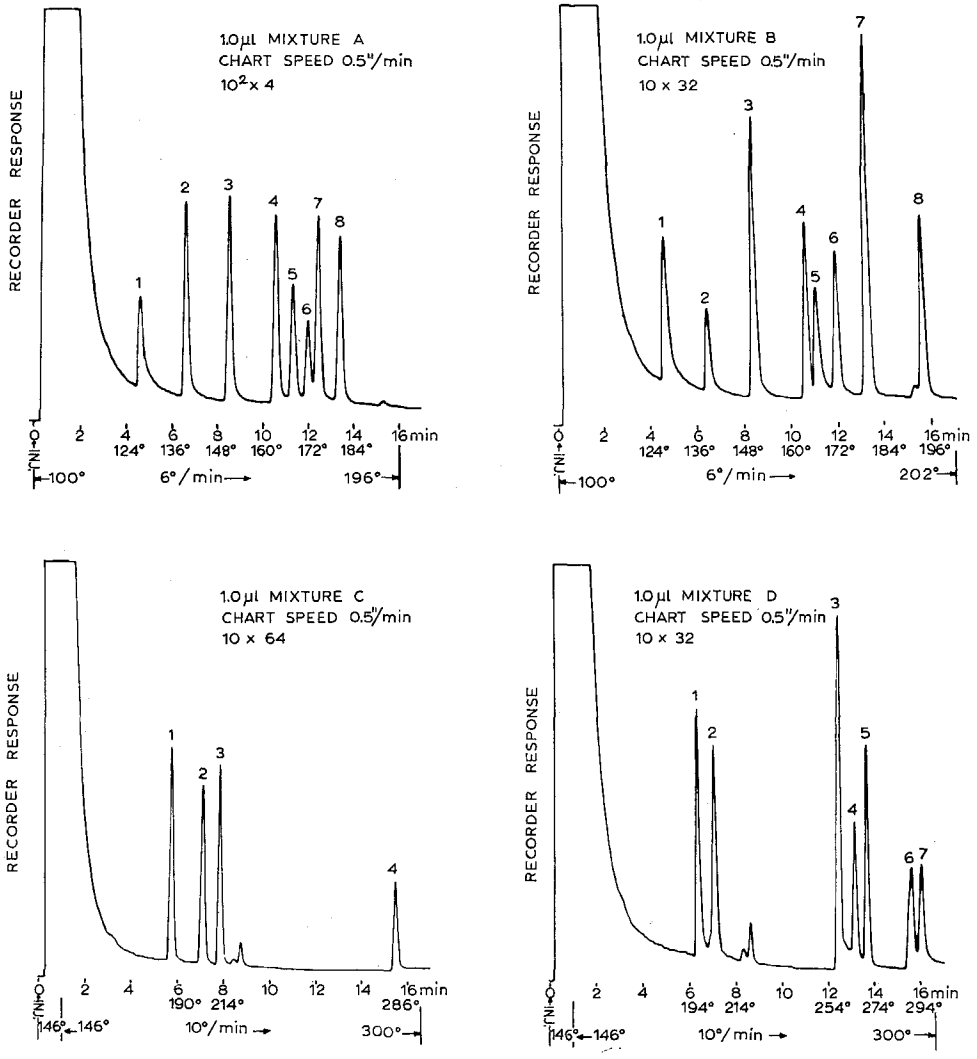

Fig. 1. Gas chromatographic separation of the TMS ether derivatives. The amount injected, chart speed, range and attenuation are described in the legends. Mixture A: 1 = coumarin, 2 = cinnamic acid, 3 = *p*-hydroxyphenylacetic acid, 4 = *p*-hydroxyphenylpropionic acid, 5 = *o*-coumaric acid, 6 = 3,4-dihydroxyphenylacetic acid, 7 = *m*-coumaric acid, 8 = *p*-coumaric acid. Mixture B: 1 = coumarin, 2 = vanillin, 3 = *p*-hydroxybenzoic acid, 4 = vanillic acid, 5 = umbelliferone, 6 = 3,4-dihydroxyphenylacetic acid, 7 = quinic acid, 8 = ferulic acid. Mixture C: 1 = quinic acid, 2 = *p*-hydroxyphenylpyruvic acid, 3 = caffeic acid, 4 = chlorogenic acid. Mixture D: 1 = scopoletin, 2 = esculetin, 3 = phloretin, 4 = naringenin, 5 = catechin, 6 = quercitin, 7 = myricetin. Mixture E: 1 = arbutin, 2 = esculin, 3 = phloridzin. Mixture F: 1 = chalcone, 2 = 2-hydroxychalcone, 3 = phloretin, 4 = phloridzin.

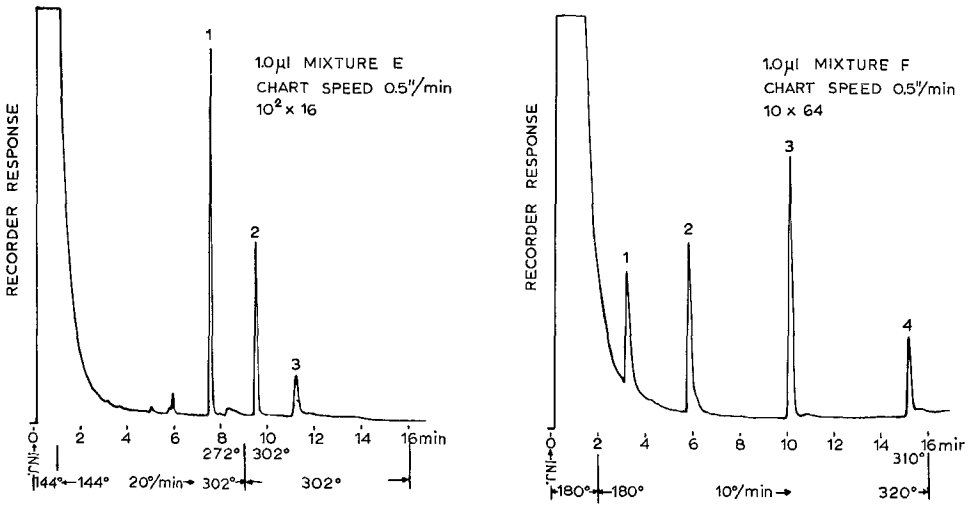

Fig. 1 *(continued)*.

TABLE I

PARENT ION OF THE TMS ETHER DERIVATIVES

TMS ether derivative	*Parent ion (m/e)*	*TMS ether derivative*	*Parent ion (m/e)*
Mixture A		*Mixture D*	
Coumarin	146	Scopoletin	264
Cinnamic acid	220	Esculetin	322
p-Hydroxyphenylacetic acid	296	Phloretin	562
p-Hydroxyphenylpropionic acid	310	Naringenin	488
o-Coumaric acid	308	Catechin	650
3,4-Dihydroxyphenylacetic acid	384	Quercitin	662
m-Coumaric acid	308	Myricetin	750
p-Coumaric acid	308		
		Mixture E	
Mixture B		Arbutin	632
Coumarin	146	Esculin	700
Vanillin	224	Phloridzin	940
p-Hydroxybenzoic acid	282		
Vanillic acid	312	*Mixture F*	
Umbelliferone	234	Chalcone	208
3,4-Dihydroxyphenylacetic acid	384	2-Hydroxychalcone	296
Quinic acid	552	Phloretin	562
Ferulic acid	338	Phloridzin	940
Mixture C		*Miscellaneous*	
Quinic acid	552	Pomiferin	638
p-Hydroxyphenylpyruvic acid	324	Solanidine	469
Caffeic acid	396		
Chlorogenic acid	786*		

* Did not give parent ion.

Results and discussion

The results of the gas chromatographic separation of the TMS ether derivatives are shown in mixtures A–F (Fig. 1). In mixtures A and B coumarin served as an internal standard since it does not have a hydroxyl group. All trimethylsilylated hydroxy-compounds were resolved from each other as sharp peaks. The retention time increased with the number of hydroxyl substituents or the molecular weight. The resolution of mixture A demonstrates that it is possible to separate closely related compounds and to resolve the isomers of coumaric acid.

Table I depicts the parent ion of the TMS ether derivatives. The observed *m/e* value agrees with the calculated molecular weight for each component. The TMS ether derivatives are thermally stable under the experimental conditions and each peak represents a completely trimethylsilylated compound.

The simultaneous use of gas chromatography and mass spectroscopy provides a powerful tool for the separation and identification of components in extracts of biological systems[1–3]. Mixture F contained known intermediates in the biosynthesis of the phenolic glycoside, phloridzin.

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