Notes

CHROM. 6135

Identification of phenolic acids by gas chromatography–mass spectrometry*

The microanalysis of phenolic acids is an important aspect in the biochemistry of many natural products. Such analysis has been greatly facilitated by chromatographic and spectroscopic techniques. This report describes the use of combined gas chromatography–mass spectrometry (GC–MS) to overcome a limitation encountered in the GC identification of some naturally occurring phenolic acids.

The GC of phenolic acids has been reported before[1–6]. However, the analysis of the mixtures of phenolic acids used in this study has been limited.

Materials and methods

For GC a Pye Series 105 preparative gas chromatograph equipped with a flame ionization detector was used. The operating conditions were essentially as follows: A glass column of 9 ft. × 1/4 in. O.D. glass with a packing consisting of 3 % OV-1 or 3 % UCW-98 on 100–120 mesh Chromosorb W HP. The retention times obtained with the two stationary phases were similar. The choice of the stationary phase depended upon the amount of column bleeding which varied with the lot samples. The conditioning of the column has been described in a trade literature bulletin[7]. The column temperature was programmed from 100° at 6°/min; the carrier gas used was nitrogen, at a flow-rate of 40 ml/min; samples used were 1 μl of a 1 % (w/v) silylated solution.

For GC–MS, a Bell and Howell 21-490 mass spectrometer was interfaced with a Pye gas chromatograph. The essential elements of the GC–MS technique have been described[8]. The GC–MS analyses were performed on the silylated acids individually.

The trimethylsilyl derivatives were prepared by adding, at room temperature, 100 μl of the reagent N,O-bis(trimethylsilyl)acetamide (BSA, Pierce Chemical Co.) for each milligram of the test substance. *n*-Docosane was used as the internal standard. Occasionally 10 μl of trimethylchlorosilane was added to the reaction mixture to promote derivatization. The phenolic acids used in this study were recrystallized.

Results and discussion

The performance of the gas chromatograph under the conditions cited is illustrated in Fig. 1. The six phenolic acids used here are of importance in chemotaxonomy[9].

In addition to the retention times in the gas chromatogram, the identity of the six phenolic acids is confirmed by the mass spectra of the silylated derivatives. The characteristic ion fragments in the spectra are presented in Table I. The ions listed in the table were chosen on the basis of a spectroscopic fragmentation scheme proposed for silylated vanillic acid[6].

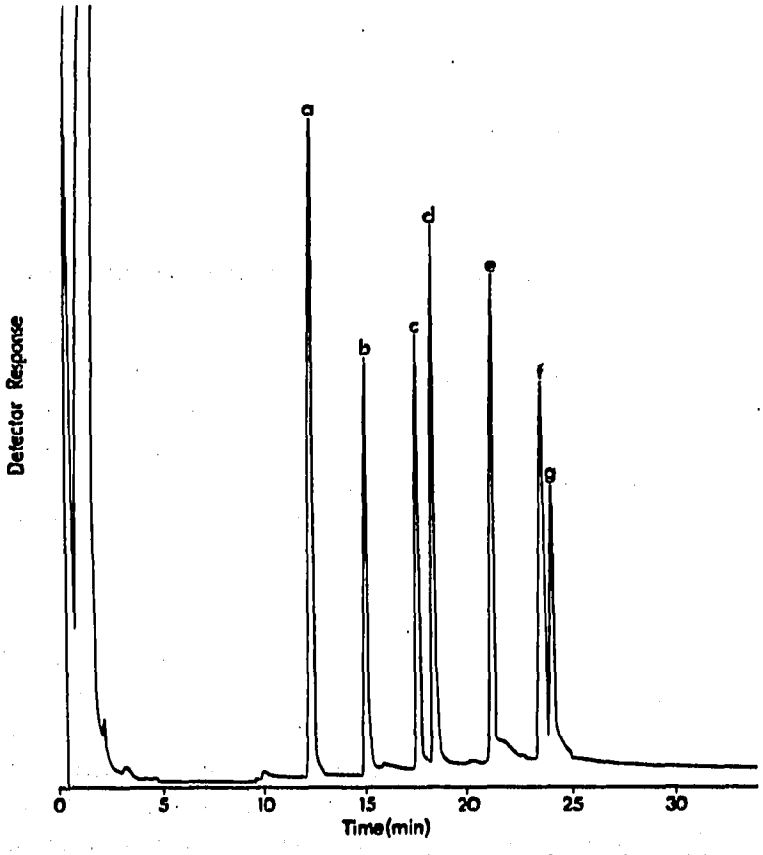


Fig. 1. Gas chromatogram of silylated phenolic acids using 3% UCW-98 on Chromosorb W HP. Retention times (min) with temperature programming of 6°/min starting from 100° are: (a) *p*-hydroxybenzoic, 6.0; (b) vanillic, 8.0; (c) syringic, 10.0; (d) *p*-coumaric, 10.8; (e) ferulic, 13.3; (f) sinapic, 16.2; (g) *n*-docosane, 26.2.

TABLE I

MASS SPECTRA OF SILYLATED PHENOLIC ACIDS BY GC–MS

Characteristic ions	*Relative abundances of the fragment ions from the silylated acids*					
	p-hydroxy-benzoic	*vanillic*	*syringic*	*p-coumaric*	*ferulic*	*sinapic*
M	14.4	43.1	33.3	16.7	26.0	50.0
M-15	54.9	64.7	43.9	22.9	14.8	25.7
M-30	0	31.4	39.4	0	16.4	52.1
M-30-15	0	54.9	33.3	0	9.0	15.0
M-15-44	39.0	38.2	13.2	14.4	5.3	5.1
M-89	43.9	52.9	25.8	37.8	17.8	15.0
M-89-30	0	30.4	17.3	0	12.7	17.9
M-89-30-28	8.5	17.6	8.6	3.4	9.2	6.7
M-186	2.7	23.5	6.8	0	0	0
m/e 147	12.7	19.6	7.8	9.7	42.5	9.3
m/e 73	100	100	100	100	100	100

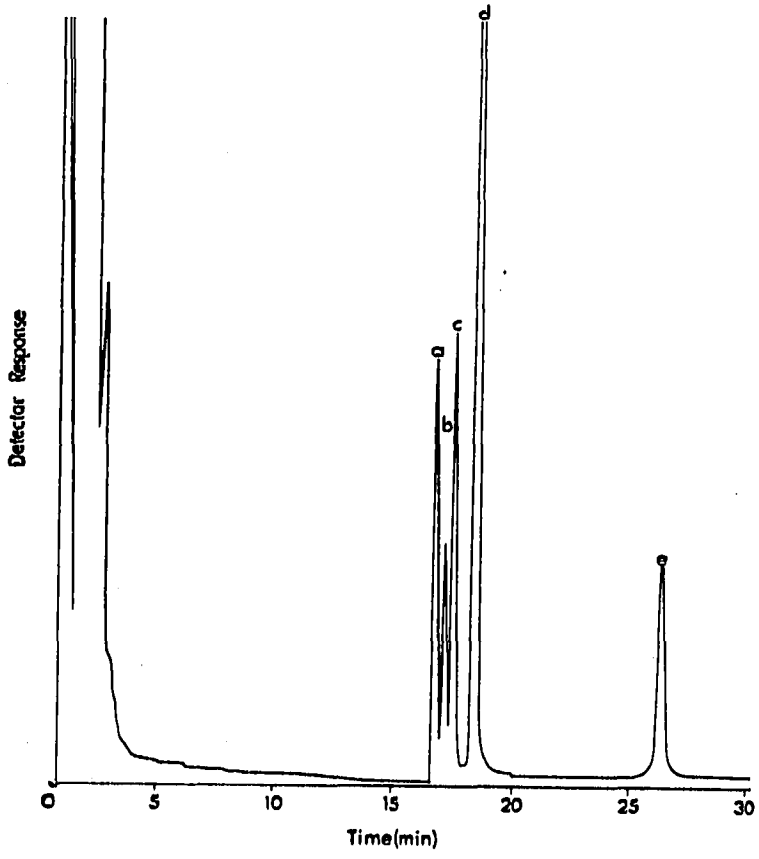


Fig. 2. Gas chromatogram of silylated dihydroxybenzoic acids using 3% OV-1 on Chromosorb W HP. Retention times (min) with temperature programming of 6°/min starting from 100° are: (a) 2,3-dihydroxybenzoic, 17.2; (b) 2,6-dihydroxybenzoic, 17.4; (c) 2,5-dihydroxybenzoic, 17.7; (d) 2,4-, 3,4-, and 3,5-dihydroxybenzoic, 18.7; (e) *n*-docosane, 26.7.

TABLE II

MASS SPECTRA OF SILYLATED DIHYDROXYBENZOIC ACIDS BY GC–MS

m/e of characteristic fragment ions	*Relative abundances of the fragment ions from the silylated benzoic acids with:*					
	2,3-$(OH)_2$	*2,4-$(OH)_2$*	*2,5-$(OH)_2$*	*2,6-$(OH)_2$*	*3,4-$(OH)_2$*	*3,5-$(OH)_2$*
370 (M)	0.5	0	1.6	1.1	22.1	88.0
355 (M-15)	45.5	44.0	38.6	68.0	15.0	82.7
311	0	0	0	0	7.1	24.0
281	0.9	6.1	2.4	0	7.1	20.0
269	4.2	0	0	27.6	0	0
193	20.8	1.9	1.3	4.3	100	4.5
147	1.7	22.7	15.8	35.4	11.4	32.0
137	19.2	0	0	0	7.6	2.5
133	5.4	7.9	6.1	9.5	4.3	17.3
73	100	100	100	100	92.1	100

A more striking example of the value of mass spectrometry is provided by the GC–MS analysis of the dihydroxybenzoic acids. The gas chromatogram of a silylated mixture of the six isomers is illustrated in Fig. 2. It is instructive to note that, under the experimental conditions, silylated 3,4-, 3,5-, and 2,4-dihydroxybenzoic acids have identical retention times. Therefore GC alone cannot be used for the identification of these acids. It is possible, however, to differentiate the acids by other chromatographic techniques[10].

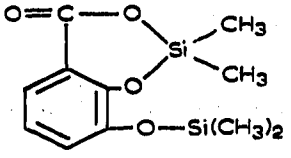
The diagnostic features in the mass spectra of the silylated dihydroxybenzoic acids, obtained by the GC–MS analysis of the individual acids, are shown in Table II. Certain aspects of the spectra deserve comment.

First, the spectrum of the 2,3-dihydroxybenzoic acid derivative demonstrates the efficacy of the silylation procedure used in this study. The difficulty in completely silylating the acid has been noted before[2]. Although the molecular ion (*m/e* 370) in the spectrum is not abundant, the prominent M-15 ion (*m/e* 355) leaves little doubt that the GC peak is due to the fully silylated acid.

Second, the mass spectra readily differentiate the three isomeric acid derivatives that are gas chromatographically identical. Among the characteristic features of the relevant spectra are: (a) the lack of the molecular ion in the 2,4-dihydroxy isomer, (b) the abundance of the *m/e* 193 (M-177) ions in the 3,4-dihydroxy derivative, (c) the prominent molecular ion as well as the *m/e* 311 ion in the 3,5-dihydroxy isomer, and (d) mixtures containing the three isomers may be amenable, in some instances, to qualitative analysis by GC–MS. This has been confirmed experimentally.

Third, except for two isomers, the mass spectra of silylated dihydroxybenzoic acids are characterized by the paucity of the molecular ions of even mass numbers. In fact, the spectrum due to the 2,4-dihydroxy derivative is devoid of this ion. The M-15 ion fragment is, however, prominent in all six isomers.

Additionally, the mass spectra of the silylated derivatives possessing the salicylic acid function (*i.e.*, the 2,6-dihydroxy and the 2,3-dihydroxy acids) manifest distinct *m/e* 269 (M-101) fragment ions. A structure which can be proposed tentatively, pending formal evidence, is protonated (I) for the fragment from silylated 2,3-dihydroxybenzoic acid.



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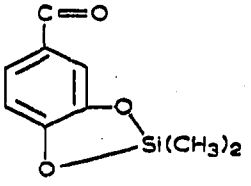

II

Similarly a cyclic silyl derivative can be envisaged for the silylated derivatives possessing the catechol moiety (*i.e.*, the two protocatechuic acids) which display abundant *m/e* 193 (M-177) fragment ions. A structure which corresponds to the mass number is (II) for the fragment from silylated 3,4-dihydroxybenzoic acid.

It is perhaps relevant to note that the M-177 ions observed in the dihydroxybenzoic acid derivatives were also encountered in the spectra of the silylated tri-

gallic acid derivative to 2.8 for the 2,4,6-trihydroxy isomer. As with the dihydroxybenzoic acid derivatives, the M-15 ion fragment was a very prominent feature in the mass spectra of the trihydroxy derivatives.

The experimental results reported here point out the effectiveness and potential of GC–MS for the micro analysis of phenolic compounds.

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