CHROM. 9184

Note

Gas chromatography of chloromethylsilylated phenolic acids*

H. MORITA and W. G. MONTGOMERY

Soil Research Institute, Agriculture Canada, Ottawa, Ontario (Canada)

(Received January 27th, 1976)

The purported advantages of chloromethylsilylation coupled with the use of the electron capture detector for the gas chromatography (GC) of hydroxystilbene[1] prompted us to inquire whether these advantages can be realized with phenolic acids. The importance of GC for the analysis of phenolic acids in microgram quantities has been well documented[2,3].

MATERIALS AND METHODS

A Perkin-Elmer Model 3920 gas chromatograph was fitted with two 7 ft. × 1/8 in. stainless-steel columns packed with 3% UCW-98 on 100–120 mesh Chromosorb W HP. Dual flame ionization and ^{63}Ni electron capture detectors (operating in a constant current mode) were used. The operating conditions were: injection port temperature, 200°; detector temperature, 300°; carrier gas (argon–methane) flow-rate, 40 ml/min; column temperature, 200°; attenuation, × 20.

The mass spectra were obtained with a Finnigan 3100 gas chromatograph-quadrupole mass spectrometer. The operating conditions were: separator temperature, 250°; transfer line temperature, 250°; analyzer temperature, 90°; electron energy voltage, 70 eV; multiplier voltage, −1.40 kV.

The chloromethylsilyl derivatives were prepared according to the published procedure[1]. Milligram amounts of the phenolic acids were dissolved in pyridine (600 μl), di(chloromethyl)tetramethyldisilazane (200 μl) (Pierce, Rockford, Ill., U.S.A.), and chloromethyldimethylchlorosilane (100 μl). After 30 min, the reaction mixture was diluted with appropriate amounts of hexane before analysis. 0.1- to 0.2-μl aliquots of the hexane solutions were injected into the gas chromatograph.

RESULTS AND DISCUSSION

The gas chromatogram of four chloromethylsilylated phenolic acids is shown in Fig. 1. Under the experimental conditions used here, the detection limit of the derivatives (defined as the amount affording a chromatographic peak height twice the noise level) was about 4 ng. Moreover, in our hands, 4-hydroxystilbene had a detection limit of 0.5 ng compared to 8 ng previously reported[1]. On the other hand, the
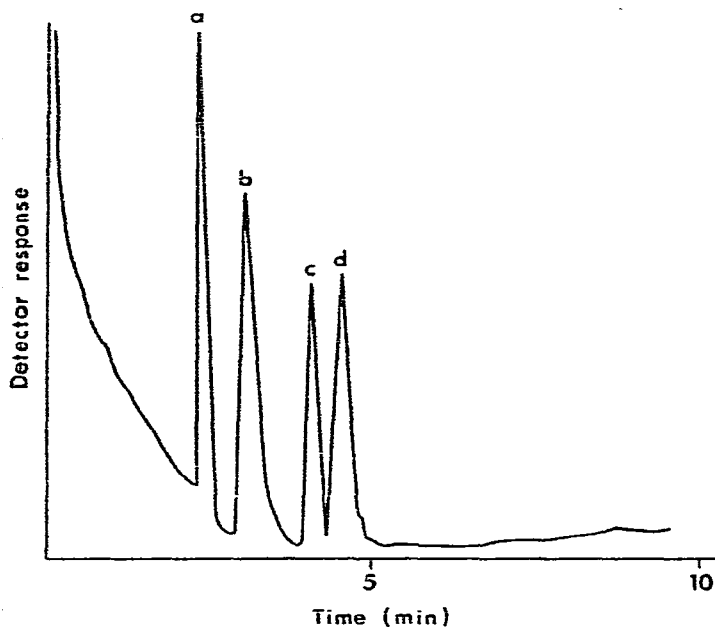
---

* Contribution No. 585 (SRI).

Fig. 1. Gas chromatogram of chloromethylsilylated phenolic acids. a = 2-Hydroxybenzoic acid; b = 4-hydroxybenzoic acid; c = 3-methoxy-4-hydroxybenzoic acid; d = 3,4-dihydroxybenzoic acid.

analysis of the chloromethylsilylated phenolic acids with the flame ionization detector afforded a detection limit of 70 ng. Clearly, there is an advantage to the use of an electron capture detector in conjunction with chloromethylsilylation.

Despite the advantage, however, chloromethylsilylation was found to have certain difficulties. Attempts to analyze unsaturated phenolic acids such as 4-hydroxycinnamic acid or 4-hydroxy-3-methoxycinnamic acid as well as the trihydroxybenzoic acids gave erratic results, because these acids frequently gave more than one derivative. Presumably the high column temperature (greater than 220°) required for the analysis caused thermal decomposition of the derivatives. Indeed, the relative instability of the chloromethylsilyl compounds (compared to the trimethylsilyl analogues) was observed even with 4-hydroxybenzoic acid, which exhibited multiple chromatographic peaks after a reaction time of 4 h at ambient temperature. Thus, it appears that GC with chloromethylsilyl derivatives could be impractical for polyphenols such as tannins or flavonoid compounds.

Considering the relative instability of the chloromethylsilyl derivatives, it is logical to question whether the chromatographic peaks shown in Fig. 1 are, in fact, the chloromethylsilyl derivatives. Then too, some phenolic acids (e.g., 2-hydroxybenzoic acid) are known to be difficult to derivatize fully with the conventional silylating reagents, hexamethyldisilazane and trimethylchlorosilane[4].

Evidence based on GC–mass spectrometry of the 4-hydroxybenzoic, 2-hydroxybenzoic, and 3,4-dihydroxybenzoic acid derivatives shows that the chromatographic peaks are, indeed, due to the chloromethylsilyl derivatives. The mass spectra are reproduced in Figs. 2–4.
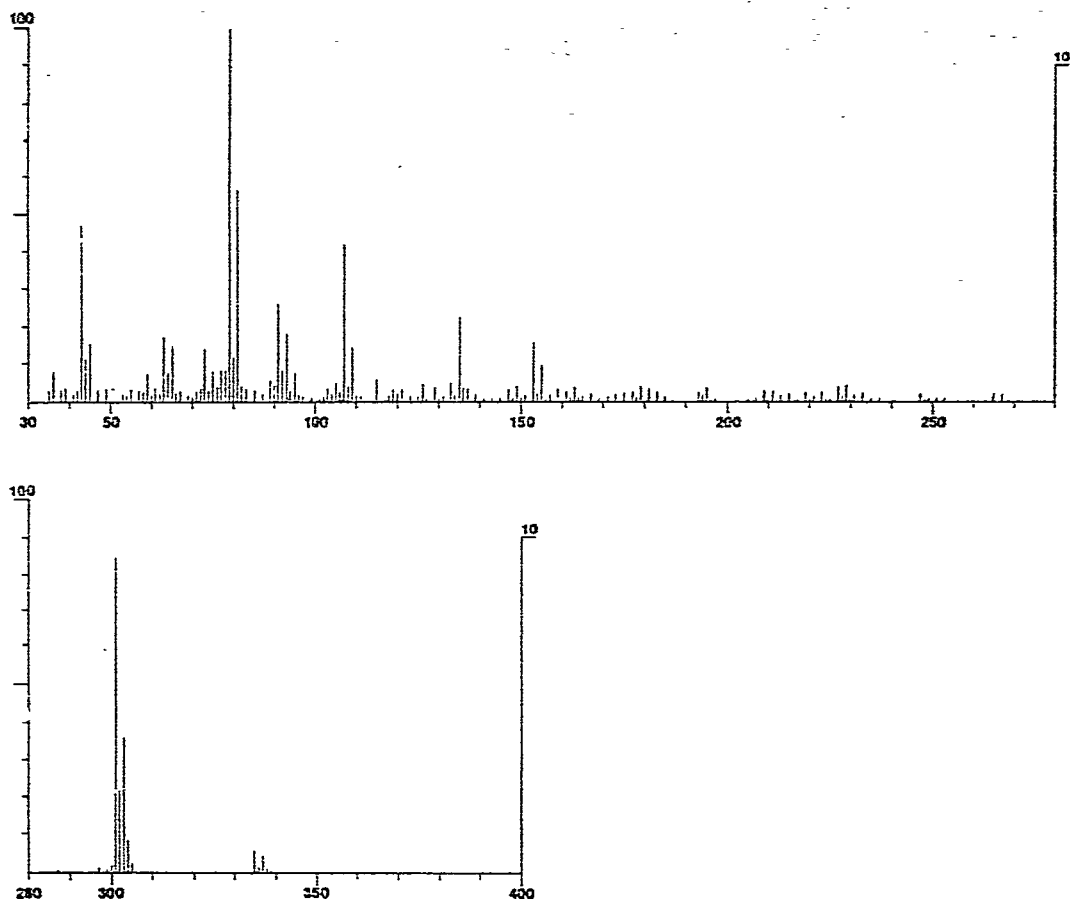
Fig. 2. Mass spectrum of chloromethylsilylated 4-hydroxybenzoic acid.

The mass spectral fragmentation patterns of the three phenolic acid derivatives exhibit certain notable features. The spectrum of the 4-hydroxybenzoic acid derivative (Fig. 2), for example, shows the molecular ion m/e 350. The prominent ion at m/e 301 can be attributed to the loss of the chloromethine moiety from the molecular ion, *i.e.*, $M^+ - ClCH_2$. This loss is equivalent to the loss of the methyl group observed in the trimethylsilyl analogue[2].

The mass spectrum of the chloromethylsilylated 2-hydroxybenzoic acid (Fig. 3) does not show the molecular ion. As in the case of the 4-hydroxy isomer, the prominent peak at m/e 301 can be attributed to the loss of a chloromethine function. Likewise, the peak at m/e 335 can be ascribed to the ejection of a methyl group, *i.e.*, $M^+ - CH_3$. The relative abundance of the m/e 335, 337, and 339 ions signifies the isotopic cluster from two chlorines and is another evidence that two chloromethylsilyl groups are incorporated in the derivative.

The molecular ion (m/e 472) is also lacking in the mass spectrum of the tri-substituted 3,4-dihydroxybenzoic acid derivative (Fig. 4). The ion, m/e 389, can arise
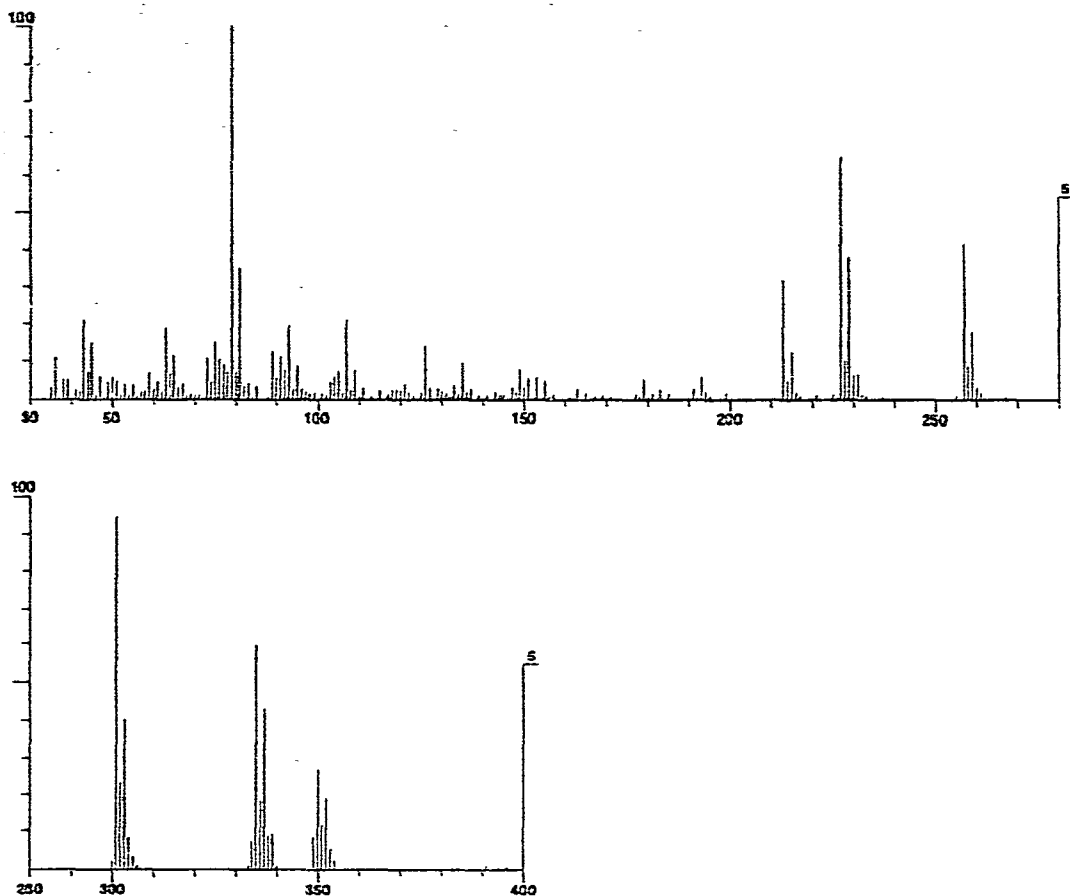
Fig. 3. Mass spectrum of chloromethylsilylated 2-hydroxybenzoic acid.

from the loss of the chloromethine and chlorine moieties from the molecular ion, i.e., $M^+ - (ClCH_2 - Cl)$. The prominent fragment at m/e 342 can be assigned to the loss of the methyl and chlorine functions from the ion m/e 342.

The prominent peak at m/e 258 is noteworthy in that it corresponds to the loss of two chloromethylsilyl groups from the molecular ion. This loss has its counterpart in the mass spectrum of the trimethylsilylated analogue, where the base peak is due to the removal of two trimethylsilyl groups[2].

As an aside, the mass spectrum of the vanillic acid derivative showed no unusual features. The fragmentation pattern was similar to the trimethylsilyl analogue[2], the prominent features being the molecular ion (m/e 380) and the $M^+ - ClCH_2$ ion (m/e 331).

Thus the mass spectra of the GC peaks can be rationalized on the basis of the chloromethylsilylated ether and ester derivatives.

The utility of the electron capture detector over the flame ionization detector for the GC of phenolic compounds has prompted efforts to seek alternatives to silyl-
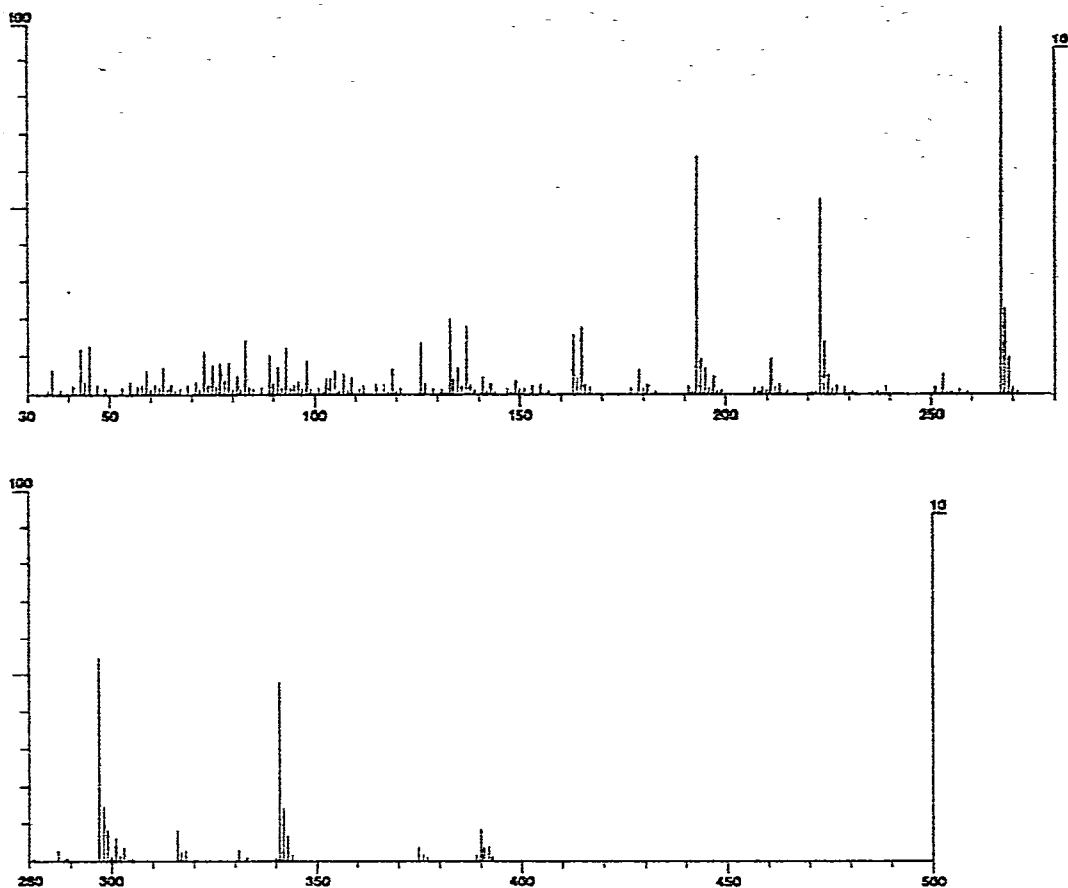
Fig. 4. Mass spectrum of chloromethylsilylated 3,4-dihydroxybenzoic acid.

ation. Pentafluorobenzylation is a case in point[5]. Analysis with this procedure, however, requires the isolation of the derivative. Chloromethylsilylation (as with trimethylsilylation) in this respect is more convenient in that the reaction mixture can be injected directly into the gas chromatograph.

The results described in this report show that chloromethylsilylation can be used effectively with certain phenolic compounds, particularly where the selectivity and sensitivity of the electron capture detector can be exploited[5]. Derivatization by this method, however, suffers from the limitation arising from the potential thermal instability of the products.

ACKNOWLEDGEMENT

REFERENCES

1 J. E. Sinsheimer and R. V. Smith, *J. Pharm. Sci.*, 56 (1967) 1280.
2 H. Morita, *J. Chromatogr.*, 71 (1972) 149.
3 A. E. Pierce, *Silylation of Organic Compounds*, Pierce, Rockford, Ill., 1968.
4 E. R. Blakely, *Anal. Biochem.*, 15 (1966) 350.
5 F. K. Kawahara, *Environ. Sci. Technol.*, 5 (1971) 235.