Phytochemistry, Vol. 24, No. 2, pp. 325–328, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00
Pergamon Press Ltd.

APPLICATION OF FAST ATOM BOMBARDMENT MASS SPECTROMETRY TO CHLOROGENIC ACIDS

AKIYO SAKUSHIMA,* SUEO HISADA, SANSEI NISHIBE and HANS BRANDENBERGER†

Faculty of Pharmaceutical Sciences, Higashi Nippon Gakuen University, Ishikari-Tobetsu, Hokkaido, 061-02, Japan; † Department of Forensic Chemistry, University of Zürich, CH-8028 Zürich, Switzerland

(*Revised received* 2 *July* 1984)

Key Word Index—Chlorogenic acids; chlorogenic acid; 3′-*O*-methylchlorogenic acid; neochlorogenic acid; 4,5-dicaffeoyl quinic acid; 1,5-dicaffeoyl quinic acid; electron impact mass spectrometry; positive-ion fast atom bombardment mass spectrometry; negative-ion fast atom bombardment mass spectrometry.

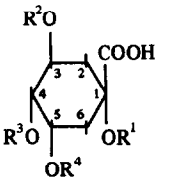
Abstract—The technique of positive- and negative-ion fast atom bombardment mass spectrometry has been shown to be capable of producing molecular mass and useful fragmentation information for the structural elucidation of chlorogenic acids. The mass spectra of chlorogenic acid and the related compounds 3′-*O*-methylchlorogenic acid, neochlorogenic acid, 4,5-dicaffeoyl quinic acid and 1,5-dicaffeoyl quinic acid are compared with those obtained by electron impact mass spectrometry.

INTRODUCTION

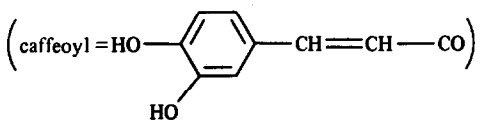
The application of electron impact (EI) mass spectrometry to the determination of the molecular masses and structures of phenolic glycosides has been limited [1]. Recently, the new technique of positive- and negative-ion fast atom bombardment (FAB) mass spectrometry has been applied successfully to investigations of polar molecules as well as thermolabile and involatile compounds [2, 3].

In continuation of our interest in the components of some pharmaceutically important chlorogenic acids [4–6], we have investigated the scope and limitation of EI and positive- and negative-ion FAB mass spectrometry for the characterization of chlorogenic acid (**1**) and the related acids 3′-*O*-methylchlorogenic (**2**), neochlorogenic (**3**), 4,5-dicaffeoyl quinic (**4**) and 1,5-dicaffeoyl quinic (**5**).

As far as we know, no mass spectra have been reported except for one example of a high-resolution field-desorption mass spectrum of **1** [7].



1 $R^1 = R^3 = R^4 = H$, R^2 = caffeoyl
2 $R^1 = R^3 = R^4 = H$, R^2 = caffeoyl, 3′-O-methyl ether
3 $R^1 = R^2 = R^3 = H$, R^4 = caffeoyl
4 $R^1 = R^2 = H$, $R^3 = R^4$ = caffeoyl
5 $R^2 = R^3 = H$, $R^1 = R^4$ = caffeoyl



RESULTS AND DISCUSSION

Table 1 shows the EI mass spectral data of compounds **1–5**. No molecular ions $[M]^+$; for which M is the M_r of chlorogenic acid, are observed in these spectra except for that of **1** at *m/z* 354 and **2** at *m/z* 368. These results indicate that the lactone is easily formed during heating of the sample, since these compounds possess a free hydroxyl group at the C-3 position of the quinic acid moiety [8]. Thus, the absence or presence of a $[M]^+$ in the EI mass spectrum makes it possible to distinguish between chlorogenic acid and neochlorogenic acid types. The significant peak is present at *m/z* 336 (350 for **2**) corresponding to $[M-18]^{+\cdot}$ or $[M-180]^{+\cdot}$ ions. The $[M-180]^{+\cdot}$ ion is the peak corresponding to $[M-\text{caffeic acid}]^{+\cdot}$ or $[M-H_2O-\text{caffeoyl}]^+$ ion with hydrogen transfer. The $[M-180]^{+\cdot}$ ion is the peak of the $[M-H_2O-\text{caffeoyl}]^+$ ion with hydrogen transfer, since the *m/z* 162 ion is an abundant one. The *m/z* 498 ion $[M-H_2O]^{+\cdot}$ in the spectra of **4** and **5** are of low relative intensity (< 1.5%), but these ions are valuable peaks for structural elucidation. The other significant ions are *m/z* 180 (194 for **2**) corresponding to caffeic acid and *m/z* 163 (177 for **2**) corresponding to caffeoyl.

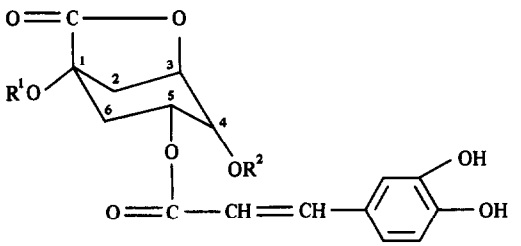
Thus, despite the structural differences between the compounds, their EI mass spectra show similar fragmentation patterns giving insufficient information for structural elucidation except for the distinction between chlorogenic acid and neochlorogenic acid types.

In the positive-ion FAB mass spectra (Table 2), protonated molecular ions $[M+H]^+$ are observed with abundant intensity, and the caffeoyl ion at *m/z* 163 (177 for **2**) is

*To whom correspondence should be addressed.

Table 1. EI mass spectral data of chlorogenic acids 1–5

Fragment ion	*m/z* (rel. int.)				
	1	2	3	4	5
$[M]^{+\cdot}$	354 (4.8)	368 (6.7)	—	—	—
$[M-H_2O]^{+\cdot}$	336 (12.4)	350 (22.2)	336 (22.6)	498 (0.4)	498 (1.3)
[M – caffeic acid]$^{+\cdot}$	—	—	—	336 (26.0)	336 (25.2)
[Methylcaffeic acid]$^{+\cdot}$	—	194 (66.3)	—	—	—
[Methylcaffeoyl]$^+$	—	177 (100.0)	—	—	—
[Methylcaffeoyl – H]$^+$	—	166 (1.8)	—	—	—
[Caffeic acid]$^{+\cdot}$	180 (67.4)	—	180 (43.5)	180 (44.6)	180 (80.2)
[Caffeoyl]$^+$	163 (100.0)	—	163 (100.0)	163 (100.0)	163 (100.0)
[Caffeoyl – H]$^+$	162 (17.7)	—	162 (21.5)	162 (33.0)	162 (19.8)



3′ $R^1 = R^2 = H$
4′ $R^1 = H$, R^2 = caffeoyl
5′ R^1 = caffeoyl, $R^2 = H$

Fig. 1. Structures (3′, 4′ and 5′) of ions due to the loss of water from the molecule of compounds 3, 4 and 5.

the base peak in every case; the *m/z* 163 ion is also characteristic in the positive-ion FAB mass spectra. Compounds 1 and 2 give significant ions at *m/z* 181 corresponding to the [caffeic acid + H]$^+$ ion for 1 and at *m/z* 195 corresponding to the [3-*O*-methylcaffeic acid + H]$^+$ ion for 2. Compounds 3 and 4 give significant ions at *m/z* 337 for 3 and at *m/z* 499 for 4, corresponding to the loss of water from the $[M+H]^+$ ions, respectively (Fig. 1). Water may be formed from the quinic acid moiety in chlorogenic acid. This ion is also observed in the EI mass spectra and the result indicates that the loss of water from the quinic acid moiety also occurs during the soft ionization without heating of sample. On the other hand, the ions of the quinic acid moiety are absent from the mass spectra of 1, 3, 4 and 5. Thus, it is established that the positive-ion FAB technique gives information on molecular mass and valuable fragment ions due to the caffeoyl

Table 2. Positive-ion FAB mass spectral data of chlorogenic acids 1–5

Fragment ion	*m/z* (rel. int.)				
	1	2	3	4	5
$[M+H]^+$	355 (17.1)	369 (18.3)	355 (25.6)	517 (4.0)	517 (10.0)
$[M+H-H_2O]^+$	—	—	337 (10.1)	499 (3.1)	—
[Methylcaffeic acid + H]$^+$	—	195 (23.4)	—	—	—
[Methylcaffeoyl]$^+$	—	177 (100.0)	—	—	—
[Caffeic acid + H]$^+$	181 (17.0)	—	—	—	—
[Caffeoyl]$^+$	163 (100.0)	—	163 (100.0)	163 (100.0)	163 (100.0)

Table 3. Negative ion FAB mass spectral data of chlorogenic acids 1–5

Fragment ion	m/z (rel. int.)				
	1	2	3	4	5
$[M-H]^-$	353 (17.7)	367 (14.8)	353 (51.5)	515 (69.6)	515 (32.7)
$[M-H-H_2O]^-$	—	—	335 (8.8)	497 (13.7)	—
[M – caffeoyl]$^-$	—	—	—	353 (47.3)	353 (100.0)
[M – H – caffeic acid]$^-$	—	—	—	335 (41.4)	335 (31.3)
[Quinic acid – H]$^-$	191 (100.0)	191 (100.0)	191 (100.0)	191 (75.8)	—
[Quinic acid – H – H_2O]$^-$	173 (8.6)	173 (25.2)	173 (53.1)	173 (100.0)	—

moiety, but insufficient information for structural elucidation as compared with the EI mass spectra.

In the negative-ion FAB mass spectra (Table 3), each compound gave reasonably intense $[M-H]^-$ ions. The [quinic acid – H]$^-$ ion at *m/z* 191 is the base peak in the case of compounds **1**–**4**, and the ions are characteristic of the mass spectra of negative-ion FAB. Compounds **1** and **2** give a significant ion at *m/z* 173 and 179 for **1** and at *m/z* 173 and 193 for **2**, which correspond to [quinic acid – H]$^-$ and [quinic acid – H_2O – H]$^-$ ions. In the spectra of compounds **3** and **4**, the $[M-H_2O-H]^-$ ions, from the dehydration of the quinic acid moiety, are observed at *m/z* 335 and 497. In particular, the successive loss of the caffeoyl moiety from $[M-H]^-$, the *m/z* 353 ion for **4** and **5**, is a significant peak, since it corresponds to the elimination of the caffeoyl moiety from the molecule. On the other hand, the peak corresponding to the [caffeoyl]$^-$ ion is absent in the negative-ion FAB mass spectrum in every case. This result indicates that the charge on the caffeoyl group in chlorogenic acids is always positive. Compound **5** gives a simple spectrum in which the fragment ions at *m/z* 173 and 191 characteristic of the negative-ion FAB mass spectra of **3** and **4** are not observed.

Conclusion

In most cases, it is difficult to obtain the molecular mass from the EI mass spectra of chlorogenic acids, but the spectra do give some information for structural elucidation. On the other hand, the negative-ion FAB mass spectra give information on the quinic acid moiety, as shown in Fig. 2, as well as on a quasi-molecular $[M-H]^-$ ion. In contrast, the positive-ion FAB mass spectra give information on caffeoyl groups together with a quasi-molecular $[M+H]^+$. Thus, a combination of the information obtained from both positive- and negative-ion mass spectra has been shown to be important in studying this class of compounds by FAB and EI mass spectrometry.

EXPERIMENTAL

Mass spectrometry. The spectra were recorded, after FAB ionization, using equipment designed according to the specifications of Dr. R. Rhyage and Margith Jansson from the Laboratory of Mass Spectrometry at the Karolinska Institute in Stockholm. The collision gas used was Ar (ion gun condition: 7 kV and 1.5 mA) and the matrix used was glycerol.

Material. Compounds **1** and **3** were obtained from the Chinese Crude Drug 'Jin yin huā' [2]. Compounds **2**, **4** and **5** were obtained from coffee bean [8]. Samples were prepared by dissolving the chlorogenic acids (50–100 μg) in MeOH and then mixing the soln with glycerol (1 μl) on the target. Stable ion currents were produced for several min, and in a number of cases both positive- and negative-ion mass spectra were recorded from the same sample loading and glycerol as background. The mass spectrometer was scanned over a mass range of 5–800 mu. Background subtraction of glycerol ions was carried out.

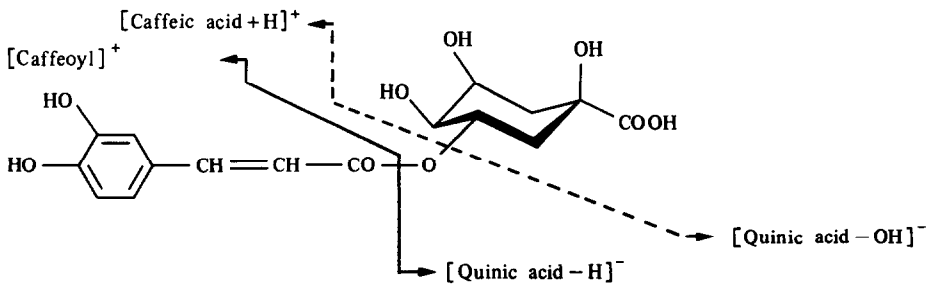


Fig. 2. Scheme of FAB mass spectral fragmentation.

Acknowledgements—This research was supported by grants from the special research foundation 82PA-2 of Higashi Nippon Gakuen University.

REFERENCES

1. Waller, G. R. (1972) *Biochemical Application of Mass Spectrometry*. Wiley-Interscience, New York.
2. Williams, D. H., Bradley, C., Bojesen, G., Santikarm, S. and Taylor, L. C. E. (1981) *J. Am. Chem. Soc.* **103**, 5700.
3. Barber, M., Bordoli, R. S., Sedgwich, R. D. and Tyler, A. N. (1981) *Nature (London)* **293**, 270.
4. Okabe, K., Agata, I., Hisada, S., Nishibe, S., Baba, H. and Akisada, T. (1981) *Abstr. of Paper*, 101*st Annu. Meeting Pharm. Soc. Jpn., Kumamoto*, p. 511.
5. Sakushima, A., Nishibe, S. and Hisada, S. (1978) *Yakugaku Zasshi* **98**, 1395.
6. Sakushima, A., Hisada, S, Nishibe, S. and Ogihara, Y. (1980) *Shoyakugaku Zasshi* **34**, 92.
7. Schulten, H. R. and Games, D. G. (1974) *Biomed. Mass Spectrom.* **1**, 120.
8. Corse, J., Layton, L. L. and Patterson, D. D. (1970) *J. Sci. Food Agric.* **21**, 164.
9. Saito, N., Timberlake, C. F., Tucknott, O. G. and Lewis, I. A. S. (1983) *Phytochemistry* **22**, 1007.