Fast Atom Bombardment Mass Spectrometry of Coumaric Acids

Chris G. de Koster*,**, Gerard J. Niemann*, and Wigger Heerma**

Botanisch Laboratorium* and Laboratorium voor Analytische Chemie**, Rijksuniversiteit Utrecht, The Netherlands

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o-, *m*- and *p*-Coumaric Acid, Fast Atom Bombardment Mass Spectrometry (FAB), FAB with Collisional Activation (FAB-CA) and with Metastable Ion (FAB-MI) Data

The technique of positive fast atom bombardment mass spectrometry combined with collisional activation and metastable ion data has been shown to provide molecular mass and useful fragmentation information for discrimination between the coumaric acid isomers. In addition to the occurrence of an *ortho*-effect, also *para* and *meta* positions could be distinguished.

Introduction

Introduction of fast atom bombardment (FAB) as a standard ionization technique has facilitated the acquisition of useful mass spectra of underivatized compounds to a great extent. Hithertoo information on underivatized material with sufficient volatility could be obtained from electron-impact (EI) or field-desorption (FD) spectra. FAB ionization produces ions during a relatively long period (10–20 min), and thus allows the recording of metastable ion (MI) and collisional activation (CA) spectra of selected ions. FAB has the advantage over EI that it can also be used for non-volatile naturally occurring plant glycosides without necessitating derivatization.

In connection with our investigations of phenylpropanoids of higher plants [1–4] positive ion FAB mass spectra and the combined techniques FAB-CA and FAB-MI were applied on the study of flavonols [5] and of some cinnamic acid derivatives. The results of the latter are presented here; the fragmentation patterns were compared with earlier data obtained by EI mass spectrometry. In case of cinnamic acid derivatives a first study of FAB spectra has been published [6], but to our knowledge the considerable potential of FAB-MI and FAB-CA has never before been applied in this field.

Reprint requests to G. J. Niemann, Botanisch Laboratorium, Lange Nieuwstraat 106, 3312 PN Utrecht, The Netherlands.

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Results and Discussion

Table I shows the FAB mass data of *o*-, *m*- and *p*-coumaric acid (OC, MC and PC). The samples were dissolved in glycerol for measurement; a 1:1 adduct ion of the protonated cinnamic acid with glycerol at *m/z* 257 with a relatively large intensity was found for all three acids. Loss of water results in *m/z* 239. Protonated molecular ions (*m/z* 165) constitute the base peak for the three acids, whereas the molecular ion (*m/z* 164) is relatively abundant for MC and PC, but not for OC. All compounds give fragment ions resulting from loss of H_2O (*m/z* 147), ketene (*m/z* 123) and formic acid (*m/z* 119). In general, the spectra of MC and PC are nearly identical, apart for some difference in the intensity for the loss of H_2O. The FAB spectrum of OC differs somewhat more, especially in the loss of formic acid (*m/z* 119 and 118) and the loss of H_2O and CO_2 (*m/z* 103).

In comparison, the EI spectra [7–9] show a pronounced ortho-effect, when OC is compared with PC. This results in an EI spectrum for OC with *m/z* 118 as base peak and a very low molecular ion peak. Loss of CH_2CO (*m/z* 123, 122) is almost absent in the EI spectra of both OC and PC.

The FAB-CA and FAB-MI spectra of the *m/z* 165 ion are presented in Tables II and III. Again, unlike the EI spectra, the presence of the *ortho* hydroxyl group does not result in pronounced loss of formic acid (*m/z* 119/118). On the other hand, both in CA and MI spectra an enhanced loss of CH_2CO is apparent. In addition, the FAB-CA of OC is characterized by loss of H_2O and CO_2 (*m/z* 103), an effect which is to a lesser extent visible in the FAB mass spectrum (Table I). A comparable effect was not al-

Table I. Positive-ion FAB mass spectral data of *o*-, *m*- and *p*-coumaric acid (OC, MC and PC).

Fragment ion	*m/z*	Rel. Int. OC	MC	PC
$[M+H+glycerol]^+$	257	443	559	302
$[M+H+glycerol-H_2O]^+$	239	57	147	70
$[M+H]^+$	165	1000	1000	1000
M^+	164	114	324	349
$[M+H-H_2O]^+$	147	486	648	372
$[M-H_2O]^+$	146	143	30	23
$[M+H-CH_2CO]^+$	123	143	118	116
$[M+H-HCOOH]^+$	119	71	118	116
$[M-HCOOH]^+$	118	157	59	47
$[M+H-H_2O-CO_2]^+$	103	143	59	70

Table II. FAB-CA mass spectra of the *m/z* 165 ion of *o*-, *m*- and *p*-coumaric acid (OC, MC and PC).

Fragment ion	*m/z*	Rel. Int. OC	MC	PC
M^+	164	98	155	182
$[M+H-H_2O]^+$	147	1000	1000	1000
$[M+H-2H_2O]^+$	129	–	15	–
$[M+H-CH_2CO]^+$	123	228	48	34
$[M+H-HCOOH]^+$	119	43	60	120
$[M-HCOOH]^+$	118	65	38	–
$[M+H-H_2O-CO_2]^+$	103	120	–	–
$\left[M+H-\begin{matrix}H\\H\end{matrix}\!\!\diagdown\!C=C\!\!\diagup\!\begin{matrix}H\\COOH\end{matrix}\right]^+$	93	–	14	10
$[C_7H_7]^+$	91	76	48	41
$[C_6H_5]^+$	77	33	12	10
$[C_5H_5]^+$	65	22	15	31

Table III. FAB-MI mass spectra of the *m/z* 165 ion of *o*-, *m*- and *p*-coumaric acid (OC, MC and PC).

Fragment ion	*m/z*	Rel. Int. OC	MC	PC
M^+	164	83	126	240
$[M+H-H_2O]^+$	147	1000	1000	1000
$[M+H-2H_2O]^+$	129	–	10	–
$[M+H-CH_2CO]^+$	123	283	26	40
$[M+H-CO_2]^+$	121	29	–	–
$\left[M+H-\begin{matrix}H\\H\end{matrix}\!\!\diagdown\!C=C\!\!\diagup\!\begin{matrix}H\\COOH\end{matrix}\right]^+$	93	–	13	–

ways found in the EI spectra [7–9]. MC and PC, as expected, differ less in their FAB-CA and -MI spectra from each other, than from OC. Nevertheless, also MC and PC can be distinguished. A small, but consistent, fragment ion $C_9H_5O^+$ (*m/z* 129, loss of 2 H_2O) is present in both the CA and MI spectrum of MC and not in those of OC and PC. Further characterization of MC can be found in the occurrence of the fragment ion $C_6H_5O^+$ (*m/z* 93) in the FAB-MI spectrum (Table III), an ion which is absent in the corresponding spectra of the *ortho* and *para* isomer. EI spectra of MC and PC are practically indistinguishable [8, 9].

Formation of a 1:1 adduct with glycerol also enabled the use of FAB-CA and FAB-MI of the *m/z* 257 ion, shown in Tables IV and V. Losses of H_2O (*m/z* 239), glycerol (*m/z* 165) and of both molecules (*m/z* 147) are responsible for the main fragment ions. The protonated molecular ion of the adduct apparently is relatively stable; formation of the protonated molecular ion of the coumaric acid is dependent on the position of the hydroxyl group. Thus, in addition to the FAB or FAB-CA and -MI spectra of the *m/z* 165 ion, the spectra of the *m/z* 257 adduct allow an easier differentiation of the three coumaric acids based on the formation of the protonated molecular ion by loss of glycerol (Tables IV and V).

Table V. FAB-MI mass spectra of the *m/z* 257 ion of the glycerol adduct of *o*-, *m*- and *p*-coumaric acid (GOC, GMC and GPC).

Fragment ion	*m/z*	Rel. Int. GOC	GMC	GPC
$[M+H+\text{glycerol}]^+$	257	1000	1000	1000
$[M+H+\text{glycerol}-H_2O]^+$	239	216	118	197
$[M+H+\text{ glycerol}-\text{glycerol}]^+$	165	76	20	216

In general it appears that the FAB spectra of this type of compounds give abundant protonated molecular ions, a phenomenon which was also observed for chlorogenic acids [6] and flavonols [5]. In this respect FAB differs from EI and use of the FAB technique thus can afford useful additional information, especially when mixtures of natural compounds are under investigation. Combination with CA and MI gives a valuable extension. In the present investigation it led to the possibility to distinguish among others MC from PC. The formation of a 1:1 adduct

Table IV. FAB-CA mass spectra of the *m/z* 257 ion of the glycerol adduct of *o*-, *m*- and *p*-coumaric acid (GOC, GMC and GPC).

Fragment ion	*m/z*	Rel. Int. GOC	GMC	GPC
$[M+H+\text{glycerol}]^+$	257	1000	1000	1000
$[M+H+\text{glycerol}-H_2O]^+$	239	145	129	189
$[M+H+\text{glycerol}-\text{glycerol}]^+$	165	283	580	926
$[M+H+\text{glycerol}-\text{glycerol}-H_2O]^+$	147	65	290	217

with glycerol introduces an extra source of information.

Experimental

Mass spectrometry

Spectra were obtained with a VG Analytical ZAB-2f mass spectrometer. The samples (estimated amounts of 10–20 μg) were dissolved in glycerol and acidified with acetic acid. The procedure was described extensively before [5].

Materials

The coumaric acids were commercially available from Fluka AG.

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