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High-energy and low-energy collision-induced dissociation of protonated flavonoids generated by MALDI and by electrospray ionization

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Abstract

Product ion mass spectra of a series of nine protonated flavonoids have been observed by electrospray ionization combined with quadrupole/time-of-flight (ESI QTOF), and matrix-assisted laser desorption ionization combined either with quadrupole ion trap (MALDI QIT) tandem mass spectrometry or time-of-flight tandem mass spectrometry (MALDI TOF ReTOF). The compounds examined are 3,6-, 3,2′-, and 3,3′-dihydoxyflavone, apigenin (5,7,4′-trihydroxyflavone), luteolin (5,7,3′,4′-tetrahydroxyflavone), apigenin-7-*O*-glucoside, hesperidin (5,7,3′-trihydroxy-4′-methoxyflavanone), daidzen (7,4′-dihydroxyisoflavone), and rutin (quercitin-3-*O*-rutinoside) where quercitin is 3,5,7,3′,4′-pentahydroxyflavone; sodiated rutin was examined also. The center-of-mass energies in ESI QTOF and MALDI QIT are similar (1–4 eV) and their product ion mass spectra are virtually identical. In the MALDI TOF ReTOF instrument, center-of-mass energies range from 126–309 eV for sodiated rutin to protonated dihydroxyflavones, respectively. Due to the high center-of-mass energies available with the MALDI TOF ReTOF instrument, some useful structural information may be obtained; however, with increasing precursor mass/charge ratio, product ion mass spectra become simplified so as to be of limited structural value. Electronic excitation of the protonated (and sodiated) species examined here offers an explanation for the very simple product ion mass spectra observed particularly for glycosylated flavonoids.

Keywords: Center-of-mass energy; Collision-induced dissociation; Matrix-assisted laser desorption ionization (MALDI); Electrospray ionization; Flavonoid

1. Introduction

Matrix-assisted laser desorption ionization (MALDI) is a complex series of thermodynamic and physicochemical processes that lead, in turn, to the act of ionization [1–5]. Electrospray ionization (ESI) is realized as a series of thermodynamic processes that lead also, in turn, to the act of ionization [6–11]. Relatively little mass spectrometric information is available on the behavior of compounds of low molecular weight (MW < 1000) in both MALDI and ESI. In MALDI, the protonated analyte molecule $[M+H]^+$ is the product of gas-phase reactions between matrix ions and analyte molecules, with photoradical matrix ions initiating the reactions [12–14]. Cationized molecules are formed similarly [14]. In ESI, protonated and cationized molecules are formed in solution and are observed readily upon nebulization of the analyte solution.

The comparative study undertaken here is concerned with the tandem mass spectrometric examination of flavonoid molecules protonated (and, in one case, sodiated) within the processes of each of MALDI and ESI. Low energy (10–30 eV) collision-induced dissociation (CID) was employed for tandem mass spectrometric (MS/MS) examination of protonated molecules using both a quadrupole/time-of-flight instrument equipped with an electrospray source and a quadrupole ion trap (QIT)/time-of-flight instrument equipped with a MALDI source. High energy (20,000 eV) CID was employed for MS/MS examination of protonated molecules using a time-of-flight combined with a curved field reflectron (CFR) instrument equipped with a MALDI source. It was anticipated that product ion mass spectra obtained at low collision energy for a given protonated flavonoid molecule would be similar despite the different modes

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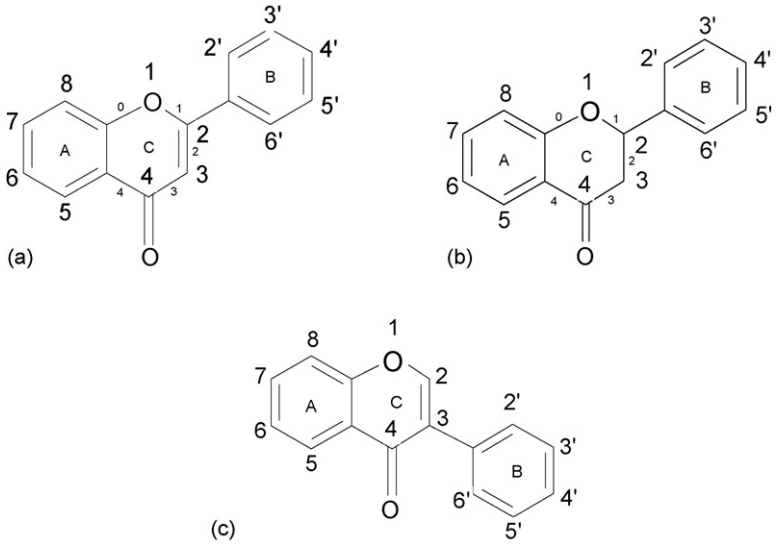


Scheme 1. Structures and numbering schemes: (a) flavones; (b) flavanones; and (c) isoflavones.

used for kinetic excitation in the quadrupole/time-of-flight and QIT/time-of-flight instruments. Furthermore, it was anticipated that product ion mass spectra obtained at high collision energy would yield more structural information than that obtained at low collision energy.

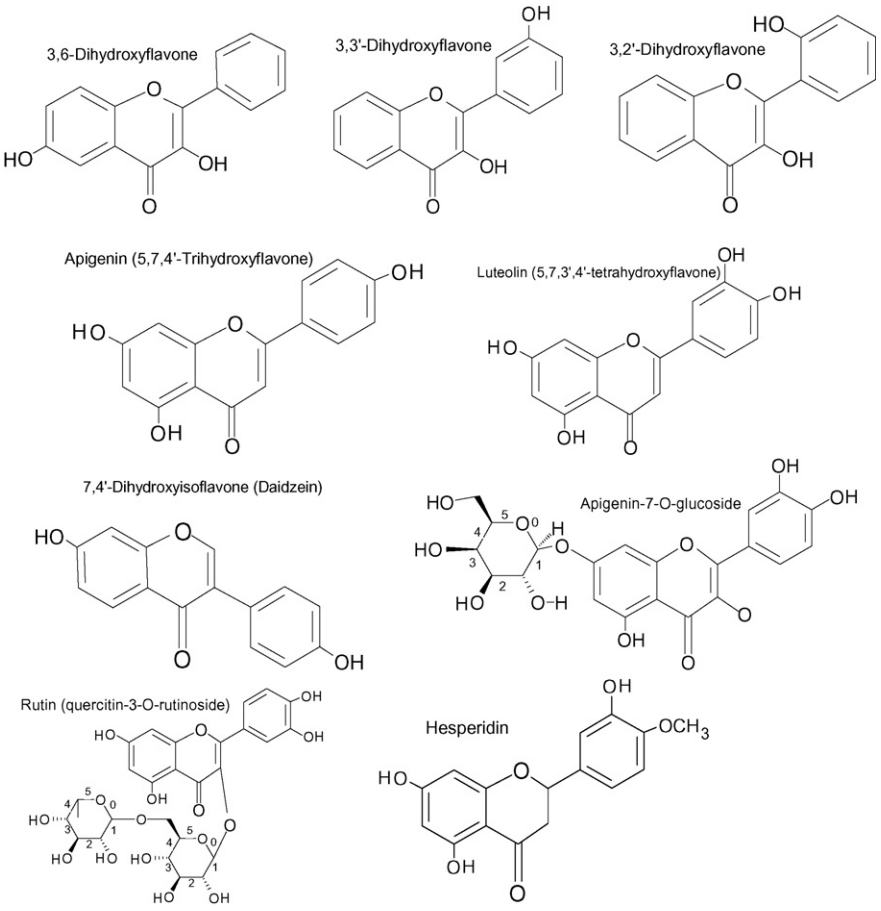
A series of flavonoid compounds that covered a range of molecular weights was selected for experimentation; flavonoids were chosen in part because there are no reports of examination of flavonoids using MALDI and, in part, because of a fundamental interest of one of the authors (REM) in the behavior of flavonoids under a variety of mass spectrometric conditions. The structures and numbering schemes for flavones, flavanones, and isoflavones are shown in Scheme 1. There was no thought, a priori, that MALDI/mass spectrometry may either supplant or complement liquid chromatography/mass spectrometry for the analysis of flavonoids from plants, but there is more to the study of flavonoids than such analyses.

The compounds selected for examination in this study comprised three dihydoxyflavones (3,6-, 3,2′-, and 3,3′-dihydoxyflavone), a 5,7,4′-trihydroxyflavone known as apigenin, a 5,7,3′,4′-tetrahydroxyflavone known as luteolin, a monoglycoside of apigenin (apigenin-7-*O*-glucoside), a 5,7,3′-trihydroxy-4′-methoxyflavanone known as hesperidin, a 7,4′-dihydroxyisoflavone known as daidzen, and a 3,5,7,3′,4′-pentahydroxyflavone diglycoside (quercitin-3-*O*-rutinoside) known as rutin. The structures of the above flavonoids are shown in Scheme 2.

The ubiquitous class of phytochemicals known as the flavonoids [15] are synthesized, along with secondary metabolites, by plants for protection against pathogens and herbivores; thus, flavonoids are found in petals, the foliage of trees and bushes, and are distributed widely in the edible parts of plants. The flavonoids (that is, flavones, flavanones, flavonols, and isoflavones, see Scheme 1) were reviewed extensively in 1994 [16]. The basic structure of a flavone is that of a C_{15} phenylbenzopyrone skeleton where two benzene rings (A and B) are linked through a heterocyclic pyrone (with a double bond) ring (C) in the middle as shown in Scheme 1. Cuyckens and Claeys have reviewed recently the role of mass spectrometry in the structural analysis of flavonoids [17]. ESI/MS/MS has been employed for analysis of 6′-*O*-malonylated β-D-glucosides in plants [18], and for the investigation of gas phase apigenin anionic clusters [19], Na^+-bound clusters of quercetin [20], 14 flavonoids [21], flavonoid aglycons [22], characterization of flavonoid-*O*-diglycosides [23,24], isoorientin, orientin, and vitexin [25], and kaempferol [26], genistein-7-*O*-glucoside [27], and flavonoid glycosides [28] at high mass resolution.

The two basic processes of CID are those of collision of the projectile ion with the target neutral and dissociation of the projectile ion. The observation of a product ion mass spectrum is made possible through collision of a mass-selected projectile ion with a target neutral species when the energy in the center-of-mass system becomes available for conversion from translational (or kinetic) energy to internal (or vibrational) energy in the projectile ion. A vibrationally excited projectile ion may undergo dissociation subsequently to yield product ions that can be observed as a product ion mass spectrum; the mechanics of dissociation are those of unimolecular fragmentation and are well understood [29,30]. As set forth in the quasi-equilibrium theory [31], such decompositions of the ion are governed by the internal energy of the ion and not by its history.

Historically, comparisons of CID of mass-selected ions have been studied at the electron-volt energy regime using quadrupole instruments [32–34] and in the kiloelectron-volt regime using sector instruments [32–35]. Normally, the latter regime has been limited to kinetic energies of 7–8 keV due to instrumental restrictions. The principal objective in carrying out studies in the kiloelectron-volt regime beyond 7–8 keV is to obtain structural or analytical information through the observation of ions formed via high-energy processes. To the extent that CID [36], neutralization [37], charge transfer [38], charge inversion



Scheme 2. Flavonoid structures.

[39], and charge stripping [40] have been studied for polyatomic ions at high collision energy, it would appear that the trends observed are similar to those observed, and rationalized theoretically, for simple atomic and diatomic ion systems [37,41–43].

The energy in the center-of-mass system, E_{cm}, is related to the laboratory energy, E_{lab}, as in Eq. (1) [44]:

$$E_{cm} = E_{lab}\left(\frac{m_T}{m_P + m_T}\right) \quad (1)$$

where m_T and m_P are the target and projectile masses, respectively. Normally, $m_T \ll m_P$ such that $E_{cm} \ll E_{lab}$. All of the ESI QTOF product ion mass spectra were observed with the precursor ion at a constant E_{lab} of 30 eV in collision with argon, such that $E_{cm} = 4.1$ eV for protonated 3,6-dihydroxyflavone (m/z 255). However, the beam of protonated molecules is attenuated in the collision cell by some 30–50% that indicates multiple collisions for the protonated molecules thus, after the first collision, there can be a range of center-of-mass energies in subsequent collisions; the maximum center-of-mass energy is the calculated value.

In a QIT, a distribution of ion kinetic energies is established during resonant excitation and is maintained due to continued resonant excitation; it is somewhat difficult to identify readily a mean ion kinetic energy in the laboratory system. By virtue of the high dissociation efficiency of the QIT, virtually all of the mass-selected ions are fragmented and product ions are trapped; normally, less than 10% of mass-selected ions are lost. Because the product ion mass spectra observed using the MALDI QIT are remarkably similar to those obtained with the ESI-TOF instrument, it is reasonable to assume that, within the QIT, the E_{cm} for m/z 255 $\approx$ 4 eV also. When it is assumed that $E_{cm} = 4$ eV for m/z 255 in the QIT wherein argon was used as collision gas, a mean value for E_{lab} within the QIT is obtained as 29.5 eV. Computations of ion trajectories within the QIT carried out using a simulation program (M. Sudakov, personal communication) developed at Shimadzu Research Laboratory, Manchester, UK, yielded a maximum kinetic energy, $(E_{lab})_{max}$, of 15 eV for m/z 255 during dipolar resonant excitation. Thus, the maximum center-of-mass energy, $(E_{cm})_{max}$, for the QIT is $\leq$2 eV. For the MALDI TOF ReTOF instrument, wherein laser-desorbed protonated 3,6-dihydroxyflavone molecules, for example, are accelerated through a potential of 20,000 V, the E_{lab} value is 20,000 eV and the E_{cm} value for m/z 255 in collision with helium is 309 eV.

This relatively enormous center-of-mass energy is not encountered normally in tandem mass spectrometry, thus the manifestation in CID of an E_{cm} of the order of 300 eV is largely unknown except for the $CH_4^{\bullet+}$ system. The total CID cross-section for $CH_4^{\bullet+}$ increases up to a kinetic energy of 25 keV

[41] and decreases at kinetic energies greater than 50 keV [45]. The abundance of CH^+ ions increases to 17% of total ion abundance at 25 keV [41,45] and to 23% at 80 keV [45].

2. Experimental

2.1. Materials

All chemicals are analytical grade. The 3,6-, 3,2′-, and 3,3′-dihydroxyflavones, apigenin (5,7,4′-trihydroxyflavone), luteolin (5,7,3′,4′-tetrahydroxyflavone), hesperidin (5,7,3′-trihydroxy-4′-methoxyflavanone), daidzen (7,4′-dihydroxyisoflavone), naringin (naringenin-7-*O*-rhamnoglucoside), and rutin (quercitin-3-*O*-rutinoside) where quercitin is 3,5,7,3′,4′-pentahydroxyflavone were purchased from Indofine Chemical Company Inc. (Hillsborough, NJ). Apigenin-7-*O*-glucoside was obtained from the Roth Co., Germany. The structures of the compounds examined are shown in Scheme 2. All chemicals were used as supplied, without further purification. The MALDI matrices α-cyano-4-hydroxy cinnamic acid (CHCA) and 2,5-dihydroxy benzoic acid (DHB) were supplied by LaserBio Labs (LBL, France). Trifluoroacetic acid (TFA) was supplied by Sigma (Poole, UK). The organic solvents acetone, methanol, acetonitrile (ACN) were obtained from Rathburn (Scotland). The water (Milli Q) was from Millipore (USA).

2.2. Methods

For ESI using the Q-TOF 2™ mass spectrometer, the analyte solutions were prepared using methanol and water (1:1) at a concentration of 80–200 μg mL^{-1}. The solutions were infused to the ESI source using a Harvard Apparatus Model 11 syringe pump (Harvard Apparatus, Holliston, MA) at a flow rate of 10 μL min^{-1}.

For MALDI experiments, save for 3,2′-dihydroxyflavone, and 3,3′-dihydroxyflavone, the flavonoids were dissolved in water at a concentration of approximately 1–2 mg mL^{-1}; 3,2′-dihydroxy-flavone was dissolved in acetonitrile containing 0.1% TFA while 3,3′-dihydroxyflavone was dissolved in methanol. All samples were sonicated in order to help the dissolution. The matrix solution consisted of either CHCA or DHB (12 mg mL^{-1}) dissolved in 50/50 ACN/0.1% TFA in water (v/v) depending on the experiment. For MALDI-MS analysis, 0.8 μL of the sample solution was mixed on target with 0.8 μL of the CHCA or DHB matrix solution.

2.3. Electrospray ionization mass spectrometry

ESI-MS and MS/MS experiments were performed on a Q-TOF II™ (quadrupole mass filter-time-of-flight) mass spectrometer (Micromass, Manchester, UK) equipped with a Z-spray™ ES source; this instrument is referred to as the ESI QTOF tandem mass spectrometer. The ES source potential on the capillary was 3.0 kV. The sampling cone voltage was varied from 20–140 V for ES mass spectra. The quadrupole mass filter to the TOF analyzer was set with LM and HM resolution of 15.0 (arbitrary units), which is equivalent to a 1.0 Da mass window for transmission of precursor ions. The source block and desolvation temperatures were set at 80 and 150 °C, respectively. Collision-induced dissociation (CID) of mass-selected ions was performed in an RF-only quadrupole collision cell. Ultra high purity argon was used as the collision gas at 10 psi inlet pressure for CID experiments. Signal detection was performed with a reflector, microchannel plate detector and time-to-digital converter. Mass calibration was carried out using a NaI/CsI standard solution from *m/z* 50–1000. Data acquisition and processing were carried out using software MassLynx NT version 3.5 supplied with the instrument. The MS survey range was *m/z* 50–1000 and the duration of each scan was 1.0 s with an interscan delay of 0.1 s. Mass spectra were accumulated over a period of 60 s or more for both single analyzer profiles and CID experiments. For each of the ion species examined, the lock mass in each product ion mass spectrum was the calculated monoisotopic mass/charge ratio of the precursor ion. Product ions were identified within a mass accuracy of ±1.5 mDa.

2.4. MALDI mass spectrometry

2.4.1. MALDI-QIT-TOF instrument

Positive ion MALDI mass spectra were acquired on an AXIMA quadrupole ion trap/time-of-flight (QIT-TOF) (Shimadzu Biotech, Manchester, UK); this instrument is referred to as the MALDI QIT tandem mass spectrometer. Ions produced by laser (UV light at 337 nm) desorption from the flavonoids were confined within the QIT for which the RF frequency was 500 kHz. Ions of *m/z* 255, for example, protonated dihydroxyflavone, were confined initially at an RF drive potential of 300 $V_{0\text{-peak}}$ and cooled collisionally with helium. The RF drive potential was ramped down to 255 $V_{0\text{-peak}}$ at which the axial secular frequency of *m/z* 255 is ~70 kHz. Isolation of protonated molecules was performed using the Filtered Noise Field (FNF) broadband excitation waveform. The RF drive potential was ramped down further to 200 $V_{0\text{-peak}}$, for which the low-mass cut-off (LMCO) is 86 Da; under these conditions, precursor ions of *m/z* 255 oscillate with an axial secular of 54.22 kHz. The isolated ions were irradiated with a sinusoidal dipole excitation waveform oscillating at 54.4 kHz. Ultra high purity argon was used as the collision gas for CID experiments. Ions were activated in a pressure transient environment generated by multiple Ar gas pulses; ion ejection is delayed by 50–60 ms relative to the final gas pulse. Product ion mass spectra were accumulated.

2.4.2. MALDI-TOF-CFR instrument

Positive ion mass spectra in the MS and MS/MS modes of operation were acquired on an Axima TOF/ReTOF instrument (Shimadzu Biotech, Manchester, UK) [46]. ReTOF refers to the curved field reflectron (CFR) that allows the efficient collection of all fragment ions, including those produced from metastable decay as well as collision-induced dissociation occurring in the collision cell (CID mode). This instrument is referred to as the MALDI TOF ReTOF tandem mass spectrometer. Analyte ions are produced by pulses of light (337 nm, 3 ns pulse width)

generated by a nitrogen laser (Spectra Physics, UK) with a maximum pulse rate of 10 Hz. The pulsed extraction ion source typically accelerates the ion beam to 20 keV. CID of selected precursor ions using helium as collision gas generates fragment ions. The laser power is the only parameter that can be varied in the fragmentation process. The pressure of the gas remained unchanged for all product ion mass spectra.

3. Results

3.1. CID of three protonated dihydroxyflavonoids isomers: 3,6-, 3,2′-, and 3,3′-dihydroxyflavone

Three product ion mass spectra of protonated 3,6-dihydroxyflavone (*m/z* 255) are shown in Fig. 1 where the scales

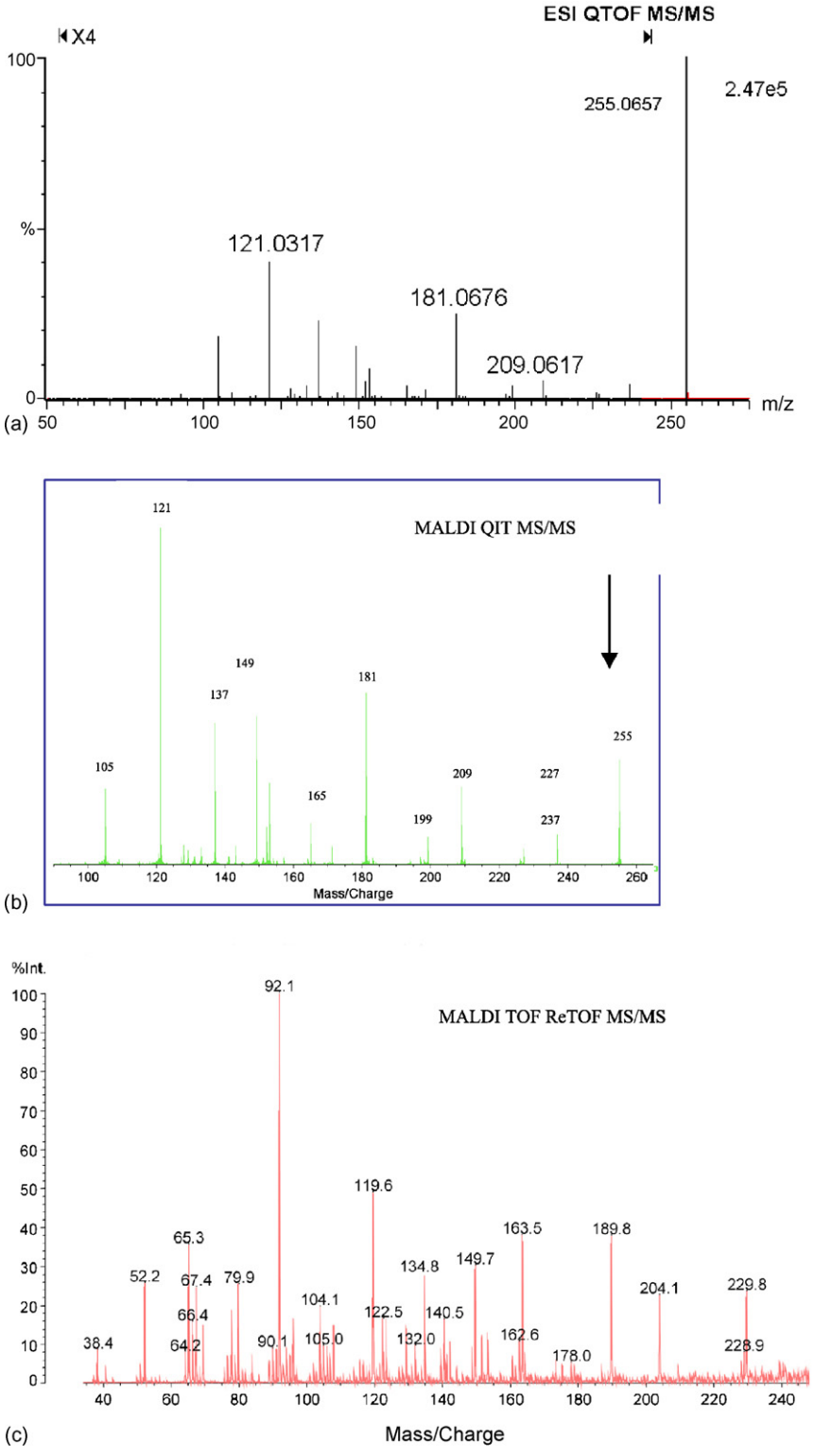


Fig. 1. Product ion mass spectra of protonated 3,6-dihydroxyflavone, *m/z* 255. (a) Observed with ESI QTOF; (b) MALDI QIT; (c) MALDI TOF ReTOF.

on the abscissas have been aligned approximately; the top mass spectrum was obtained with the ESI QTOF instrument while the center and bottom mass spectra were obtained using MALDI QIT and MALDI TOF ReTOF instruments, respectively. Upon comparison of the three product ion mass spectra, two conclusions become clear immediately: first, there is a remarkably high level of agreement with respect to product ion mass/charge ratio and relative ion signal intensity between the ESI QTOF and MALDI QIT product ion mass spectra and, second, the fragment ions in the product ion mass spectrum obtained with the MALDI TOF ReTOF instrument are mutually exclusive with those obtained with ESI QTOF and MALDI QIT. That is to say, for protonated 3,6-dihydroxyflavone, not a single fragment ion species in the product ion mass spectra observed with ESI QTOF and MALDI QIT is common with those of the product ion mass spectrum obtained with the MALDI TOF ReTOF instrument. These conclusions may be drawn readily from an examination of Table 1 wherein the relative ion signal intensities are given. The same conclusions may be drawn from the product ion mass spectra of protonated 3,2′- and 3,3′-dihydroxyflavones; the relative ion signal intensities of these product ion mass spectra are shown in Tables 2 and 3, respectively. Thus, despite the different sources of protonated molecules, the ESI QTOF and MALDI QIT instruments yield almost identical product ion mass spectra not only for the dihydroxyflavonoid isomers examined but for all of the flavonoid molecules examined.

It should be noted that the product ion mass spectra observed with MALDI TOF ReTOF for protonated 3,2′-dihydroxyflavone, as shown in Table 2, were obtained at laser powers of 63 and 67. A higher ion signal intensity was observed at a power of 67; however, this observation could be due to a better laser position or improved ionization efficiency [47]. Despite the difference in laser power, the same product ions were observed for each mass spectrum and the ion signal intensities differed modestly.

The primary fragmentations observed in product ion mass spectra of protonated dihydroxyflavonoid molecules formed by ESI are due to either cross-ring cleavage of the C-ring (retro-Diels-Alder reaction) or to cleavage of a C-ring bond followed by loss of either a small neutral molecule or, on relatively rare occasions, a radical. A systematic ion nomenclature for fragmentation, such as $^{1,3}B^+$, of flavonoid aglycons has been proposed [48] as shown in Scheme 3 and is used here; this nomenclature is conceptually similar to that introduced for the description of carbohydrate fragmentations in product ion mass spectra of glycoconjugates [49].

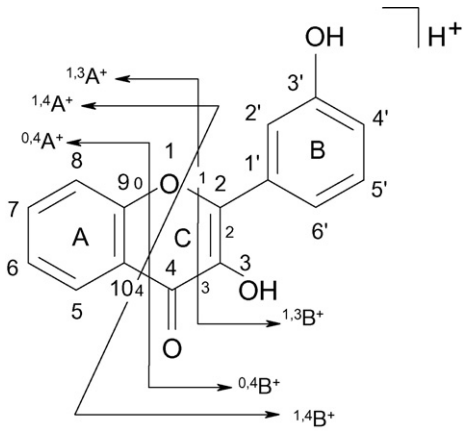
Table 1
Identification and relative abundances (in parentheses) of product ions observed from protonated molecules of 3,6-dihydroxyflavone formed by ESI QTOF (at a collision energy, 30 eV), MALDI QIT, and by MALDI TOF ReTOF (at power 57)

Compound	3,6 (ESI QTOF)	3,6 (MALDI QIT)	3,6 (MALDI TOF ReTOF)
$[M+H]^+$	*m/z* 255	*m/z* 255	*m/z* 255
$[M+H\text{-}H_2O]^+$	237 (10)	237 (8)	–
$[M+H\text{-}C_2H^\bullet]^+$	–	–	230 (23)
$[M+H\text{-}CO]^+$	227 (4)	227 (4)	–
$[M+H\text{-}CHO^\bullet]^+$	226 (5)	226 (2)	–
$[M+H\text{-}(CO+H_2O)]^+$	209 (12)	209 (22)	–
$[M+H\text{-}C_4H_3^\bullet]^+$	–	–	204 (22)
$[M+H\text{-}2CO]^+$	199 (9)	199 (7)	–
$[M+H\text{-}C_5H_5^\bullet]^+$	–	–	190 (37)
$[M+H\text{-}(2CO+H_2O)]^+$	181 (62)	181 (49)	–
$[M+H\text{-}3CO]^+$	171 (5)	171 (5)	–
$[M+H\text{-}C_7H_7^\bullet]^+$	–	–	164 (38)
$[M+H\text{-}(3CO+H_2O)]^+$	153 (22)	153 (22)	–
$[M+H\text{-}(2CO+H_2O+CHO^\bullet)]^+$	152 (13)	152 (6)	–
$C_6H_4O^{\bullet+}$	–	–	92 (100)
$^{0,2}A^+ - H^\bullet$	–	–	150 (32)
$^{0,2}A^+$	151 (1)	151 (1)	–
$^{0,3}A^+$	121 (100)	121 (100)	–
$^{0,3}A^+ - H^\bullet$	–	–	120 (49)
$^{0,2}B^+$	105 (45)	105 (20)	–
$^{0,3}B^+$	–	–	135 (28)
$^{0,3}B^+ - 2H$	133 (9)	133 (6)	–
$^{0,4}B^+ + 2H$	165 (8)	165 (11)	–
$^{1,3}A^+$	137 (57)	137 (41)	–
$^{1,4}A^+$	109 (5)	109 (2)	–
$^{1,4}B^+ + 2H$	149 (38)	149 (58)	–
$C_6H_8^{\bullet+}$	–	–	80 (26)
$C_6H_6^{\bullet+}$	–	–	78 (20)
$C_5H_7^+$	–	–	67 (24)
$C_5H_5^+$	–	–	65 (36)
$C_4H_4^{\bullet+}$	–	–	52 (24)
$C_3H_2^{\bullet+}$	–	–	38 (8)

Table 2
Identification and relative abundances (in parentheses) of product ions observed from protonated molecules of 3,2′-dihydroxyflavone formed by ESI QTOF (at a collision energy, 30 eV), MALDI QIT, and by MALDI TOF ReTOF at powers 63 and 67

Compound	3,2′ (ESI QTOF)	3,2′ (MALDI QIT)	3,2′ (MALDI TOF ReTOF) Power 63	3,2′ (MALDI TOF ReTOF) Power 67
$[M+H]^+$	*m/z* 255	*m/z* 255	*m/z* 255	*m/z* 255
$[M+H\text{-}O]^+$	–	–	239 (74)	239 (55)
$[M+H\text{-}H_2O]^+$	237 (12)	237 (29)	–	–
$[M+H\text{-}CO]^+$	227 (14)	227 (4)	–	–
$[M+H\text{-}CHO^\bullet]^+$	226 (8)	226 (1)	–	–
$[M+H\text{-}C_3H_4]^+$	–	–	215 (9)	215 (8)
$[M+H\text{-}C_4H_3^\bullet]^+$	–	–	204 (18)	204 (14)
$[M+H\text{-}(CO+H_2O)]^+$	209 (72)	209 (100)	–	–
$[M+H\text{-}2CO]^+$	199 (54)	199 (68)	–	–
$[M+H\text{-}C_5H_5^\bullet]^+$	–	–	190 (26)	190 (23)
$[M+H\text{-}(2CO+H_2O)]^+$	181 (76)	181 (60)	–	–
$[M+H\text{-}3CO]^+$	171 (34)	171 (15)	–	–
$[M+H\text{-}C_7H_7^\bullet]^+$	–	–	164 (28)	164 (28)
$[M+H\text{-}4CO]^+$	143 (8)	143 (3)	–	–
$[M+H\text{-}(2CO+H_2O+CHO^\bullet)]^+$	152 (16)	152 (9)	–	–
$[M+H\text{-}109\ Da]^{\bullet+}$	–	–	146 (16)	146 (14)
$[M+H\text{-}147\ Da]^{\bullet+}$	–	–	108 (28)	108 (32)
$C_6H_4O^{\bullet+}$	–	–	92 (47)	92 (51)
$C_5H_4O^{\bullet+}$	–	–	80 (56)	80 (60)
$^{0,2}A^+ + 2H$	–	–	135 (100)	135 (100)
$^{0,2}B^+ - H^\bullet$	–	–	120 (23)	120 (24)
$^{1,2}A^+ + 2H$	153 (56)	153 (27)	–	–
$^{1,3}A^+$	121 (76)	121 (22)	–	–
$^{1,4}B^+ + 2H$	165 (44)	165 (68)	–	–
$^{0,2}A^+ - 2H$ and/or $^{1,3}B^+ - 2H$	133 (100)	133 (64)	–	–
$^{0,3}A^+$ and/or $^{1,2}B^+$	105 (8)	–	–	–
$^{1,2}A^+$ and/or $^{0,3}B^+$	151 (8)	151 (1)	–	–
$C_6H_8^{\bullet+}$	–	–	80 (56)	80 (60)
$C_6H_6^{\bullet+}$	–	–	78 (20)	78 (19)
$C_5H_7^+$	–	–	67 (14)	67 (11)
$C_5H_5^+$	–	–	65 (26)	65 (26)
$C_4H_4^{\bullet+}$	–	–	52 (33)	52 (33)
$C_3H_2^{\bullet+}$	–	–	38 (4)	38 (3)

Thus, product ion mass spectra of protonated molecules (normally of odd mass/charge ratio) obtained by ESI are dominated by product ions of odd mass/charge ratio due to losses of neutral moieties that are almost invariably of even molecular weight.



Scheme 3. Structure of protonated 3,3′-dihydroxyflavone, nomenclature and examples of cross-ring cleavages observed.

For protonated 3,6-dihydroxyflavone obtained by MALDI TOF ReTOF (Table 1), seven of the eight major species are of even mass/charge ratio and are radical cations. Product ions of *m/z* 230, 204, 190, and 164 are formed by loss of the radicals $C_2H^\bullet$, $C_4H_3^\bullet$, $C_5H_5^\bullet$, and $C_7H_7^\bullet$, respectively. The base peak, *m/z* 92, is identified as $C_6H_4O^{\bullet+}$. Two product ions, *m/z* 150 and 120, arise from cross-ring cleavages and are identified as $^{0,2}A^+ - H^\bullet$ and $^{0,3}A^+ - H^\bullet$, respectively; while such cross-ring cleavages are observed in product ion mass spectra from protonated molecules formed by ESI, the losses of single hydrogen atoms to form radicals are rare. The sole product ion of odd mass/charge ratio is *m/z* 135 that is identified as the $^{0,3}B^+$ species. A number of hydrocarbon product ions of low mass/charge ratio were observed as shown in Table 1; of these, *m/z* 52 is complementary to *m/z* 204.

The base peak in each of the product ions mass spectra from protonated 3,2′- (Table 2) and 3,3′-dihydroxyflavone (Table 3) formed by MALDI TOF ReTOF is *m/z* 135 that is identified in each case as the $^{0,2}A^+ + 2H^\bullet$ species. Of the remaining product ions from protonated 3,2′-dihydroxyflavone, only two species, of *m/z* 239 and *m/z* 215, are not radicals; these species are iden-

Table 3
Identification and relative abundances (in parentheses) of product ions observed from protonated molecules of 3,3′-dihydroxyflavone formed by ESI QTOF (at a collision energy, 30 eV), MALDI QIT, and by MALDI TOF ReTOF at power 56

Compound	3,3′ (ESI QTOF)	3,3′ (MALDI QIT)	3,3′ (MALDI TOF ReTOF)
$[M+H]^+$	*m/z* 255	*m/z* 255	*m/z* 255
$[M+H\text{-}O^\bullet]^+$	–	–	239 (18)
$[M+H\text{-}H_2O]^+$	237 (6)	237 (7)	–
$[M+H\text{-}CO]^+$	227 (13)	227 (11)	–
$[M+H\text{-}CHO^\bullet]^+$	226 (6)	226 (7)	–
$[M+H\text{-}(CO+H_2O)]^+$	209 (20)	209 (93)	–
$[M+H\text{-}C_4H_3^\bullet]^+$	–	–	204 (29)
$[M+H\text{-}2CO]^+$	199 (15)	199 (39)	–
$[M+H\text{-}62\ Da]^+$	–	193 (7)	–
$[M+H\text{-}C_5H_5^\bullet]^+$	–	–	190 (46)
$[M+H\text{-}(2CO+H_2O)]^+$	181 (83)	181 (100)	–
$[M+H\text{-}3CO]^+$	171 (34)	171 (15)	–
$[M+H\text{-}C_7H_7^\bullet]^+$	–	–	164 (44)
$[M+H\text{-}(2CO+H_2O+CHO^\bullet)]^+$	152 (23)	152 (25)	–
$[M+H\text{-}4CO]^+$	–	143 (3)	–
$[M+H\text{-}147\ Da]^+$	–	–	108 (48)
$C_6H_4O^{\bullet+}$	–	–	92 (100)
$C_5H_4O^{\bullet+}$	–	–	80 (78)
$^{0,2}A^+$	133 (39)	133 (84)	–
$^{0,2}A^+ + 2H$	–	–	135 (100)
$^{0,2}B^+ - H^\bullet$	–	–	120 (30)
$^{0,3}B^+$	153 (39)	153 (63)	–
$^{1,3}A^+$	121 (100)	121 (65)	–
$^{1,4}A^+$	93 (11)	–	–
$^{1,2}B^+ - 2H$	105 (13)	105 (10)	–
$^{1,4}B^+ + 2H$	165 (15)	165 (63)	–
$C_6H_8^{\bullet+}$	–	–	80 (25)
$C_6H_6^{\bullet+}$	–	–	78 (19)
$C_5H_7^+$	–	–	67 (25)
$C_5H_5^+$	–	–	65 (36)
$C_4H_4^{\bullet+}$	–	–	52 (26)
$C_3H_2^{\bullet+}$	–	–	38 (10)

tified as $[M+H\text{-}O]^+$ and $[M+H\text{-}C_3H_4]^+$, respectively. Of the product ions observed from protonated 3,3′-dihydroxyflavone, only *m/z* 239, $[M+H\text{-}O]^+$, was a non-radical.

For the MALDI TOF ReTOF instrument, laser-desorbed protonated 3,6-dihydroxyflavone molecules are accelerated through a potential of 20,000 V such that the E_{lab} value is 20,000 eV and the E_{cm} value for *m/z* 255 in collision with helium is 309 eV. This relatively enormous center-of-mass energy is manifested in the observation of product ions, such as the base peak in Fig. 1(c), *m/z* 92 $(C_7H_8)^+$, that would be expected to have a high appearance energy from the protonated molecule. The major product ions in Fig. 1(c) may be due to the loss of hydrocarbon radicals.

Clearly, while the protonated molecule was mass selected in each case for CID, the center-of-mass energy (309 eV) of the protonated species formed by MALDI in the MALDI TOF ReTOF instrument and accelerated to 20,000 eV subsequently is much greater than those of the same species formed by either ESI and accelerated to 30 eV in the ESI QTOF (E_{cm} = 4.1 eV) or MALDI and cooled collisionally in the MALDI QIT ($(E_{cm})_{max} \leq 2$ eV) and resonantly excited subsequently. Thus, Fig. 1 shows product ion mass spectra from two markedly different center-of-mass energy ranges and, as may be expected, the product ions formed in the two center-of-mass energy ranges differ substantially.

3.2. CID of a protonated dihydroxyisoflavonoid

Daidzen is 7,4′-dihydroxyisoflavone where the inclusion of "iso" indicates that the phenyl (B) ring is attached at the C(3) position of the C-ring (Schemes 1 and 2) rather than at the C(2) position as for flavones. The product ion mass spectra of protonated daidzen, *m/z* 255, as observed from ESI QTOF and MALDI TOF ReTOF are shown in Fig. 2. Note that because the mass/charge ratio is unchanged from that of the dihydroxyflavonoids discussed above, the center-of-mass energies are unchanged. The upper mass spectrum, obtained under conditions of high mass resolution with the QTOF instrument, shows that C-ring opening followed by successive fragmentations, can account for the majority of product ions observed. The base peak at *m/z* 199 is due to losses of two CO molecules whereas product ions of *m/z* 237, 227, 209, 181, and 153 correspond to losses of H_2O, CO, (H_2O+CO), $(2CO+H_2O)$, and $(3CO+H_2O)$, respectively. The retro-Diels-Alder reaction leads to the formation of *m/z* 137 as the $^{1,3}A^+$ species. The product mass spectrum obtained by MALDI QIT, and not shown here, is again very

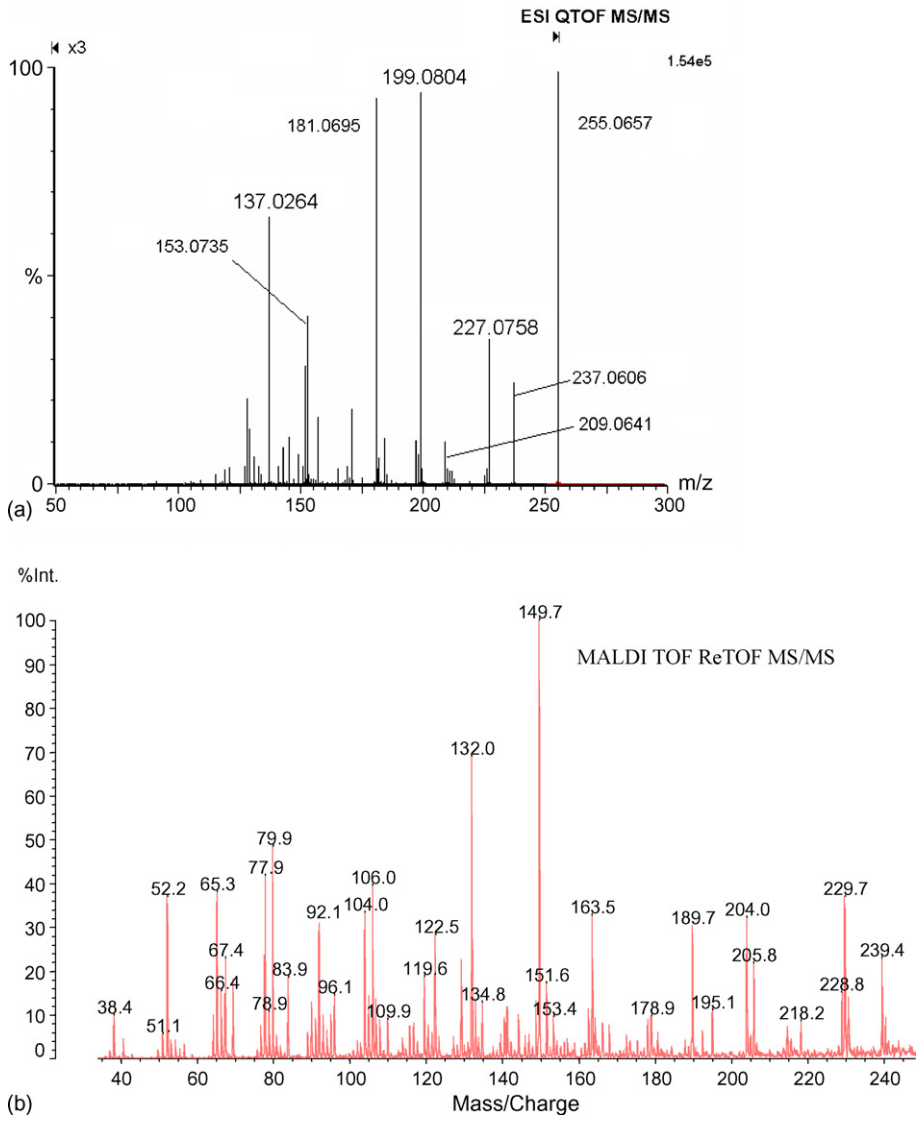


Fig. 2. Product ion mass spectra of protonated 7,4′-dihydroxyisoflavone (daidzein), *m/z* 255. (a) Observed with ESI QTOF; (b) MALDI TOF ReTOF.

similar to the ESI QTOF mass spectrum, Fig. 2(a), in that the base peak is common to both and that the majority of product ions are observed in each mass spectrum. The product ion mass spectrum obtained by MALDI TOF ReTOF and shown in Fig. 2(b) differs greatly from that in Fig. 2(a). The base peak, *m/z* 149.7, may be the $^{2,3}A^+$ ion with the loss of the B-ring moiety C_7H_5O. The origin of the second most intense product ion, *m/z* 132, is less clear but it may be due to H_2O loss from *m/z* 149.7. Minor losses requiring relatively high energy bond scission are indicated by the observation of *m/z* 239 (oxygen atom loss), *m/z* 229.7 (possibly C_2H_2 loss), and *m/z* 204 ($C_4H_3^\bullet$ loss); these species were observed in Fig. 1(c) also. The high center-of-mass energy in the MALDI TOF ReTOF instrument is manifested in the observation of a series of hydrocarbon ions of the form $C_3H_x^+$ (x = 1–2), $C_4H_x^+$ (x = 2–6), $C_5H_x^+$ (x = 4–7), and $C_6H_x^+$ (x = 4–9). Because the elemental composition of protonated daidzein is $C_{15}H_{11}O_4^+$, that is, the C:H ratio is <1, the formation of product ions having C:H ratios of >1 requires substantial rearrangements of the precursor ion.

3.3. CID of a protonated methoxylated trihydroxyflavone, hesperidin

Hesperidin (5,7,3′-trihydroxy-4′-methoxyflavanone) differs from apigenin (5,7,3′-trihydroxyflavone) in two respects; the former is methoxylated at the 4′-position whereas apigenin is not and the C(2)–C(3) ethylenic bond in apigenin has been hydrogenated in hesperidin. The product ion mass spectra of protonated hesperidin, *m/z* 303, as observed from ESI QTOF and MALDI TOF ReTOF are shown in Fig. 3. The base peak *m/z* 153 in the product ion upper mass spectrum, obtained by ESI under conditions of E_{cm} = 3.5 eV and of high mass resolution with a QTOF instrument, is the $^{1,3}A^+$ species whereas the *m/z* 177 product ion is the $^{1,4}B^+ - 2H$ species formed by the loss of the A-ring moiety $C_6H_6O_3$ with $\Delta m = -3.2$ mDa. Product ions of *m/z* 145 and 117 are possibly formed by 0,3 C-ring scission; the resulting $^{0,3}B^+$ ion (*m/z* 167) is not observed as it dissociates immediately by loss of H_2O and of ($H_2O + CO$) to yield *m/z* 145 and 117, respectively.

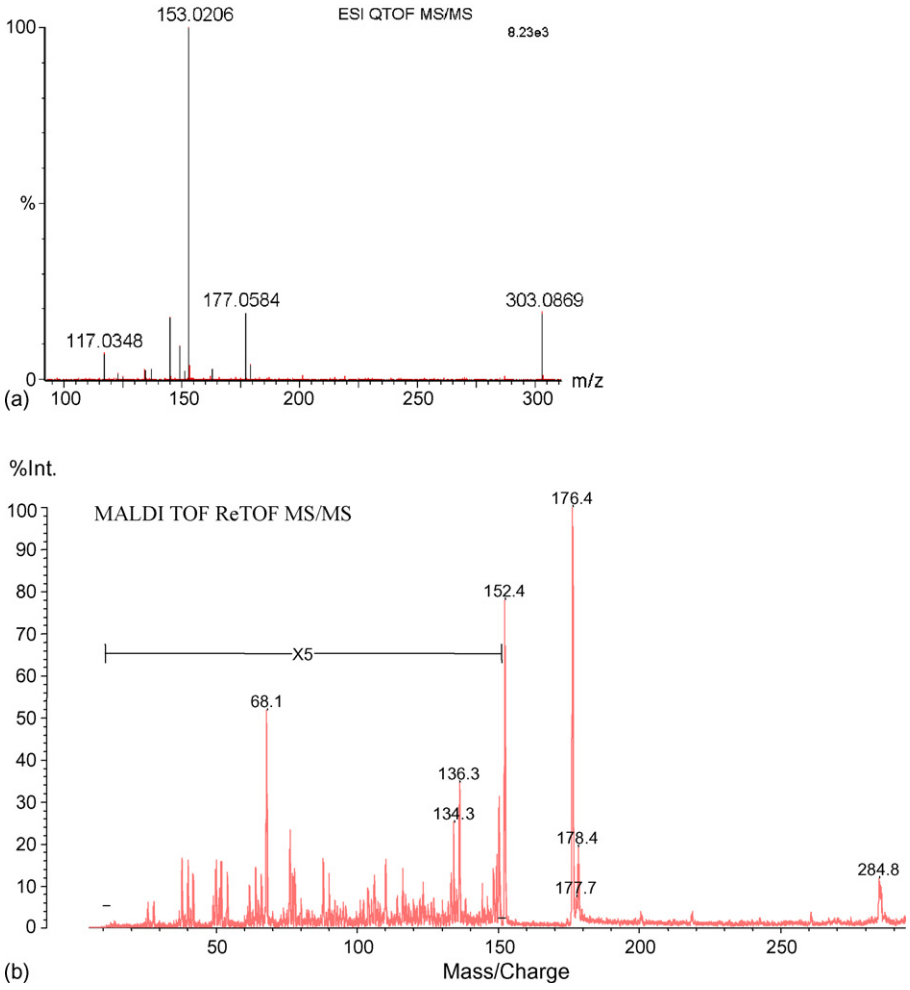


Fig. 3. Product ion mass spectra of protonated hesperidin (5,7,3′-trihydroxy-4′-methoxyflavanone) *m/z* 303. (a) Observed with ESI QTOF; (b) MALDI TOF ReTOF.

The product ion mass spectrum obtained by MALDI TOF ReTOF and shown in Fig. 3(b), for which $E_{cm} = 261$ eV, is striking in that the two major product ions, *m/z* 176 and 152, are radical cations and correspond to the two major product ions observed under ESI conditions less a hydrogen atom in each case. On this basis, it is proposed that the elemental compositions for *m/z* 176 and 152 are $C_{10}H_8O_3^{\bullet+}$ and $C_7H_4O_4^{\bullet+}$, respectively. In each product ion mass spectrum is observed at low ion signal intensity *m/z* 285 due to the loss of H_2O. Hesperidin affords the first example, thus far, where product ions formed through collisions of high center-of-mass energy ions have resembled those formed through collisions of low center-of-mass energy ions.

The high center-of-mass energy in the MALDI TOF ReTOF instrument is manifested once more in the observation of a series of hydrocarbon ions of the form $C_3H_x^+$ ($x = 1$–6), $C_4H_x^+$ ($x = 1$–6), $C_5H_x^+$ ($x = 1$–8), and $C_6H_x^+$ ($x = 2$–8). The elemental composition of protonated hesperidin is $C_{16}H_{14}O_6^+$; as the C:H ratio in the precursor ion is <1, formation of the above series of hydrocarbon product ions having C:H ratios of >1 requires substantial rearrangements of the precursor ion. Such rearrangements are possible in CID processes where $E_{cm} = 261$ eV.

3.4. CID of a protonated trihydroxyflavone, apigenin

The product ion mass spectra of protonated apigenin, 5,7,3′-trihydroxyflavone, *m/z* 271, as observed from ESI QTOF and MALDI TOF ReTOF are shown in Fig. 4. The upper product ion mass spectrum, that was obtained under conditions of $E_{cm} = 3.9$ eV and of high mass resolution with a QTOF instrument, affords a classic tandem mass spectrometric example of a protonated (or deprotonated) flavonoid. In such cases, product ions are formed by cross-ring cleavages of the C-ring (Scheme 3) and by opening of the C-ring followed by successive fragmentations in which small, stable molecules are lost. In Fig. 4(a), the two major peaks at *m/z* 153 and 119 are identified as $^{1,3}A^+$ and $^{1,3}B^+$, respectively; they are complementary products of retro-Diels-Alder reactions (cross-ring cleavage). The product ions of *m/z* 163 and 145 are identified as the $^{0,4}B^+$ and $^{0,4}B^+ - H_2O$ species, respectively. The remaining product ions of *m/z* 253, 243, 229, 225, 203, 197, 187, and 169 are due, respectively, to C-ring opening followed by losses of H_2O, CO, C_2H_2O, ($H_2O + CO$), ($C_2H_2O + C_2H_2$), ($H_2O + 2CO$), 3CO, and ($H_2O + 3CO$). The product ion mass spectrum obtained by MALDI QIT (not shown) is, again, remarkably similar to that

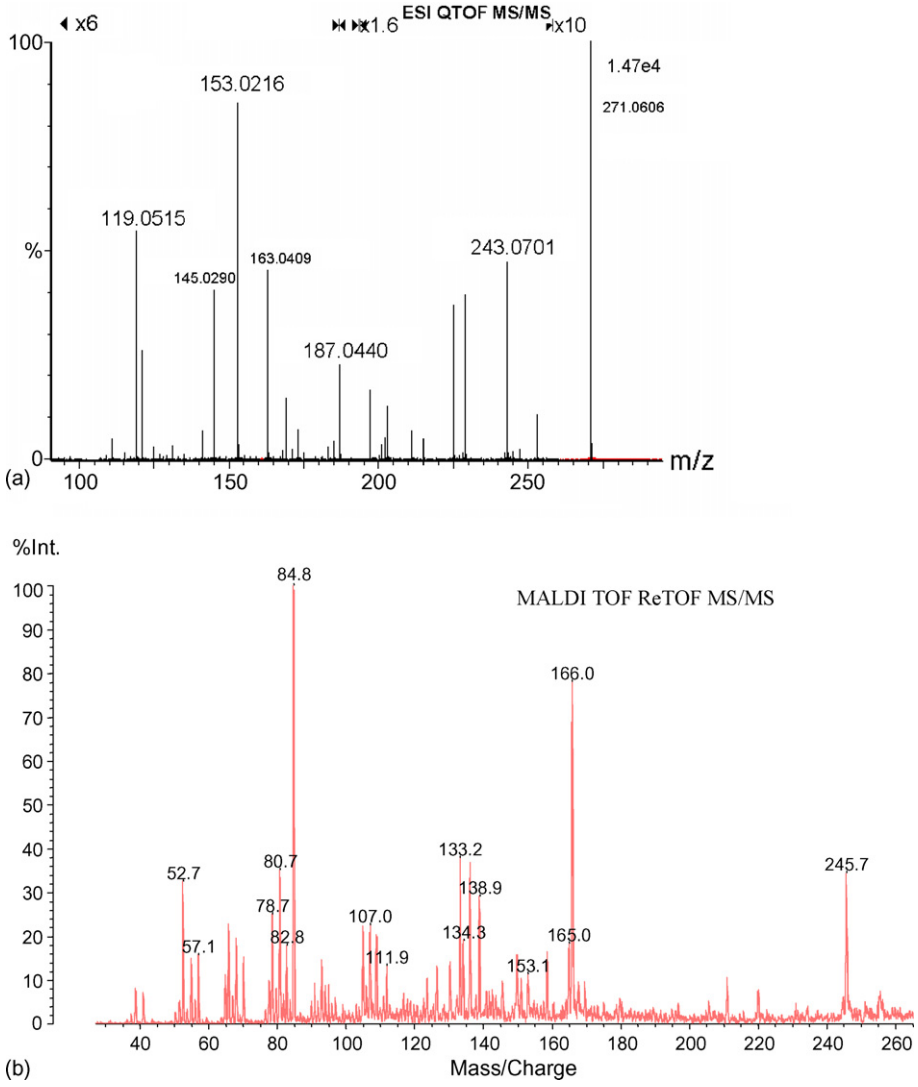


Fig. 4. Product ion mass spectra of protonated apigenin (5,7,3′-trihydroxyflavanone) *m/z* 271. (a) Observed with ESI QTOF; (b) MALDI TOF ReTOF.

of Fig. 4(a) with respect to both product ion species and relative ion signal intensities.

The product ion mass spectrum obtained by MALDI TOF ReTOF at $E_{cm} = 291$ eV and shown in Fig. 4(b) differs markedly from that of Fig. 4(a). The ion of *m/z* 245 corresponds to the loss of an oxygen atom from protonated apigenin while that of *m/z* 166 corresponds to the loss of 105 Da from the precursor ion. It is noted that the *m/z* 149.7 species in Fig. 2(b), and identified tentatively as the $^{2,3}A^+$ ion, corresponds also to the loss of 105 Da from its precursor ion. However, 2,3 (or 1,2) cleavage in a flavone involves scission of an ethylenic bond plus a single C–C bond while 2,3 cleavage in daidzein involves scission of but two single C–C bonds. Thus, the identification of *m/z* 166 as the $^{0,4}B^+ - 3H^\bullet$ ion, formed by the loss of the A-ring fragment $C_6HO_2^\bullet$, may be of greater probability than as the $^{1,2}A^+$ ion formed by the loss of the B-ring fragment $C_7H_5O^\bullet$. The identity of *m/z* 84.8 is not clear; however, as C_7H^+ and $C_5H_9O^+$ are unlikely elemental compositions, it is possible that the elemental composition is $C_4H_5O_2^+$. Once more a series of hydrocarbon ions of the form $C_3H_x^+$ ($x = 1$–5), $C_4H_x^+$ ($x = 3$–9), $C_5H_x^+$ ($x = 4$–10), and $C_6H_x^+$ ($x = 5$–11) was observed.

3.5. CID of a protonated tetrahydroxyflavone, luteolin

The product ion mass spectra of protonated luteolin, 5,7,3′,4′-tetrahydroxyflavone, *m/z* 287, as observed from ESI QTOF and MALDI TOF ReTOF are shown in Fig. 5. The ESI QTOF product ion mass spectrum shown in Fig. 5(a), $E_{cm} = 3.7$ eV, is very similar to that of MALDI QIT (not shown) and the common base peak, *m/z* 153, in each is identified as $^{1,3}A^+$. The remaining product ions correspond to C-ring opening followed by fragmentations as for protonated apigenin; that is, *m/z* 241, 213, and 179 are due, respectively, to C-ring opening followed by losses of ($H_2O + CO$), ($H_2O + 2CO$), and ($H_2O + 2CO + CO_2$). Once more, the product ion mass spectrum obtained by MALDI TOF ReTOF with $E_{cm} = 275$ eV and shown in Fig. 5(b) differs enormously from that in Fig. 5(a). The base peak, *m/z* 167, may be the hydrogenated form of *m/z* 166 shown in Fig. 4(b); formed

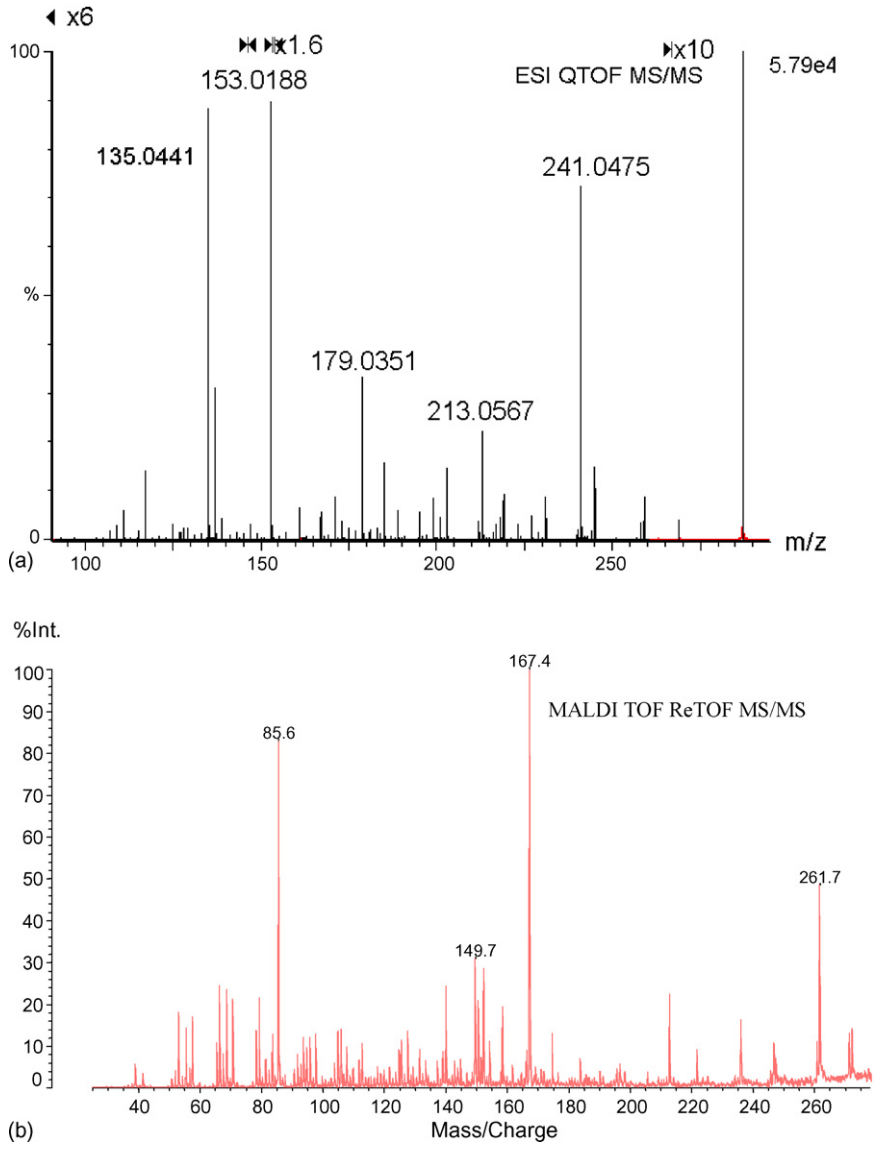


Fig. 5. Product ion mass spectra of protonated luteolin (5,7,3′,4′-tetrahydroxy-flavanone) *m/z* 287. (a) Observed with ESI QTOF; (b) MALDI TOF ReTOF.

by the loss of 120 Da from the precursor ion, *m/z* 167 may be the $^{1,2}A^+$ ion formed by the loss of the B-ring fragment $C_7H_4O_2$. The second most intense product ion, *m/z* 85 that was observed in Fig. 4(b), may have the elemental composition of $C_4H_5O_2^+$. The product ion of *m/z* 261.7 that is probably due to the loss of C_2H_2 from the precursor ion, is one of seven product ion species of $m/z > 200$. Each of these product ions corresponds to the loss of a neutral hydrocarbon moiety and is complementary to the one of the hydrocarbon ions in the series of product ions of $m/z < 100$. The product ions of *m/z* 272, 271, 261.7, 246–248, 236, 222, and 213 correspond to neutral losses of $CH_3^\bullet$, O/CH_4, C_2H_2, $C_3H_5^\bullet$–$C_3H_3^\bullet$, $C_4H_3^\bullet$, $C_5H_5^\bullet$, and C_6H_2, respectively.

3.6. CID of a protonated glucosylated trihydroxyflavone, apigenin-7-O-glucoside

The product ion mass spectra of protonated apigenin-7-*O*-glucoside, *m/z* 433, as observed from ESI QTOF and MALDI TOF ReTOF and shown in Fig. 6, are relatively simple. In Fig. 6(a) obtained by ESI QTOF with $E_{cm} = 2.5$ eV, the base peak of *m/z* 271 is the Y^+ species that corresponds to scission of the glucose moiety of 162 Da. Cross-ring cleavage of the C-ring, following loss of the glucose moiety, leads to formation of the $^{1,3}A^+$ species of *m/z* 153. There are two noteworthy points here: first, there are no fragmentary losses from the glucose moiety that would produce ions of $271 < m/z < 433$; second, the product ions of $m/z < 271$ resemble closely those of protonated apigenin shown in Fig. 4(a). The product ion mass spectrum of protonated apigenin-7-*O*-glucoside, *m/z* 433, as observed from MALDI TOF ReTOF with $E_{cm} = 183$ eV and shown in Fig. 6(b), consists essentially of a single peak, that of *m/z* 289; a facile identification of this species would be the $Y^+ + H_2O$ species. Under conditions of higher laser power, the product ions shown in the inset to Fig. 6(b) were observed. The minor product ion of *m/z* 273.8 may correspond to the loss of an oxygen atom from *m/z* 289 in which case *m/z* 273.8 may be the $Y^+ + 2H^\bullet$ species. The

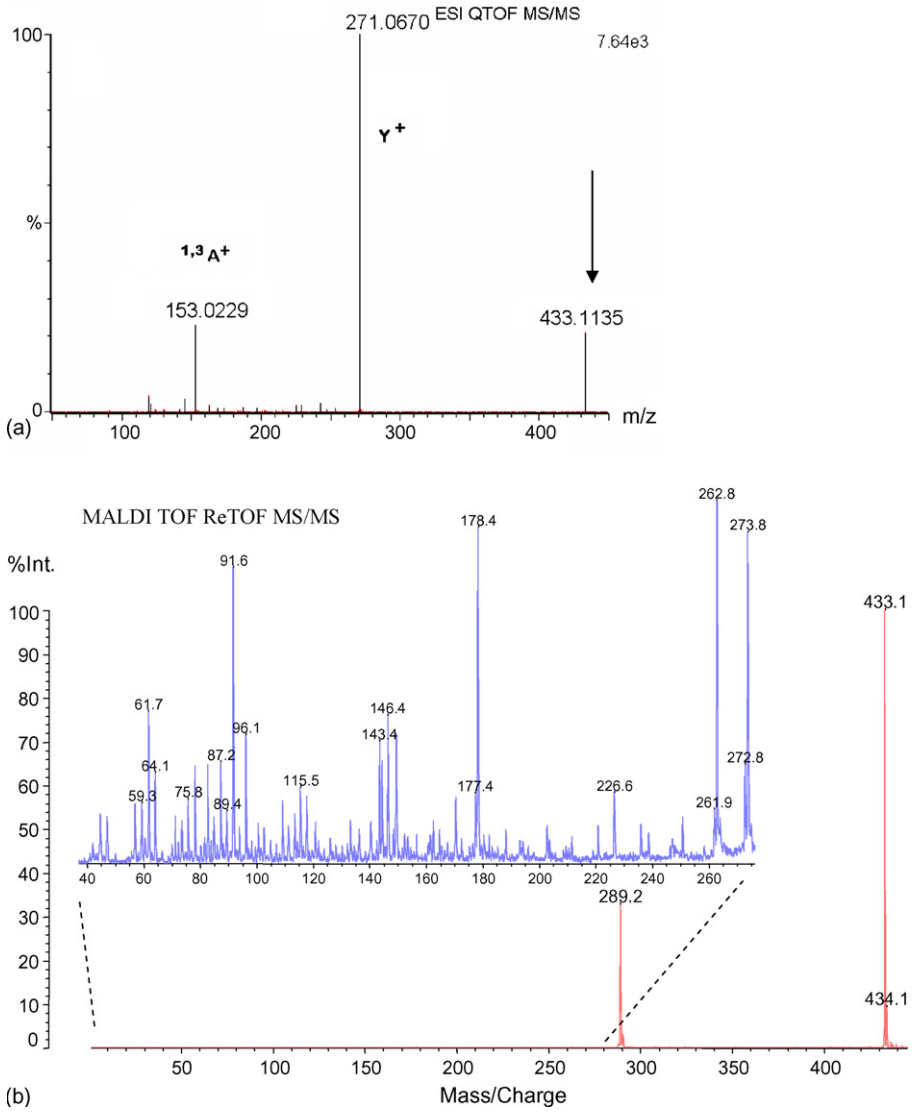
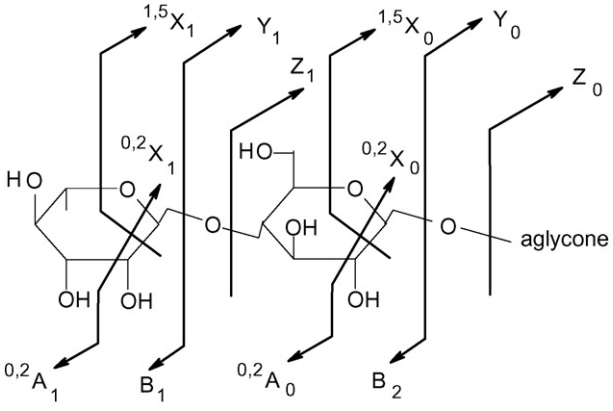


Fig. 6. Product ion mass spectra of protonated apigenin-7-*O*-glucoside, *m/z* 433. (a) Observed with ESI QTOF; (b) MALDI TOF ReTOF, the inset was observed at a higher laser power.

base peak of the inset, *m/z* 262.8, does not appear to be related to the Y^+ ion and thus it may be due to the direct loss of 171 Da from the precursor ion.

3.7. CID of a protonated di-O-glycosylated pentahydroxyflavone, rutin (quercitin-3-O-rutinoside)

Rutin (quercitin-3-*O*-rutinoside) is a pentahydroxyflavone (quercitin) to which a diglycoside (glucose-rhamnose) has been substituted in the 3-position such that there is an -*O*- linkage between the flavonoid and the diglycoside. The nomenclature for carbohydrate ions was proposed by Domon and Costello [49] and modified for application to flavonoid polyglycosides by Claeys and co-workers [50]; the modified nomenclature is shown in Scheme 4. In solution, flavonoids are found normally in protonated form and, occasionally, in sodiated form; the latter form can be obtained readily upon the addition of sodium ions. Thus, an opportunity is afforded here to investigate both



Scheme 4. Carbohydrate ion nomenclature.

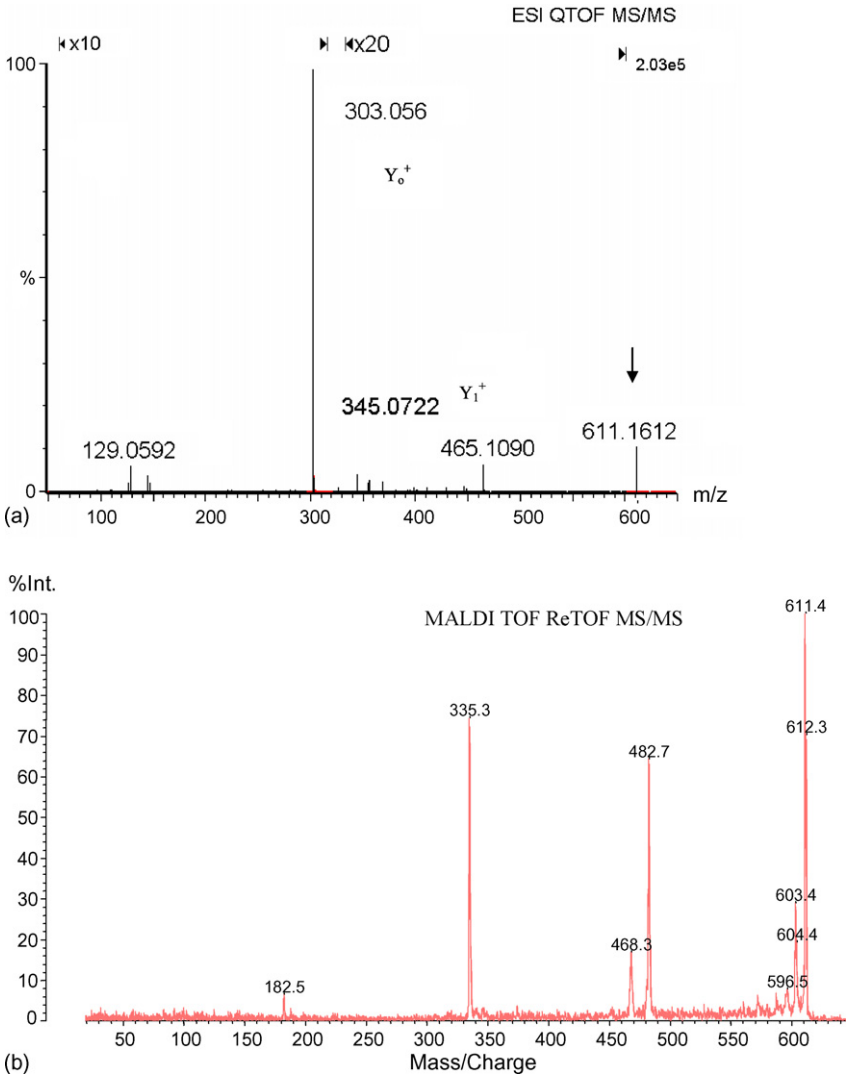


Fig. 7. Product ion mass spectra of protonated rutin, *m/z* 611. (a) Observed with ESI QTOF; (b) MALDI TOF ReTOF.

protonated and sodiated rutin by the above mass spectrometric instruments.

The product ion mass spectra of protonated rutin, *m/z* 611, as observed from ESI QTOF and MALDI TOF ReTOF and shown in Fig. 7, are relatively simple. In Fig. 7(a) obtained by ESI QTOF with $E_{cm} = 1.8$ eV, the base peak, *m/z* 303, is the Y_0^+ species that corresponds to loss of the diglycoside (308 Da); the Y_1^+ species observed at *m/z* 465 corresponds to the loss of a single glycoside (146 Da). The absence of product ions in the range $465 \leq m/z \leq 611$ indicates that fragmentation of the rhamnose moiety does not occur with $E_{cm} = 1.8$ eV; however, fragmentation of the glucose moiety is observed in the range $303 \leq m/z \leq 465$. The product ion mass spectrum shown in Fig. 7(b) as observed from MALDI TOF ReTOF with $E_{cm} = 130$ eV is relatively simple. The center-of-mass energy has been dissipated in fragmentations of the diglycoside moiety. The base peak, *m/z* 335, of Fig. 7(b) may correspond to the $Y_0^+ + CH_3OH$ species. The radical cations in Fig. 7(b) at *m/z* 482 and 468 correspond to losses of 129 and 143 Da, respectively. It is proposed that *m/z* 482 is due to loss of the rhamnose (Rha) moiety less $OH^\bullet$, that is, loss of $(Rha - OH^\bullet)$ and *m/z* 468 is due to the loss of $(Rha - 3H^\bullet)$. The remaining features of interest at *m/z* 182.5 and 603.4 may be due to ring-opened and hydrogenated glycoside radical cation and the loss of $8H^\bullet$, respectively.

The product ion mass spectra of sodiated rutin, *m/z* 633, as observed from ESI QTOF and MALDI TOF ReTOF and shown in Fig. 8, are again relatively simple. In Fig. 8(a), obtained by ESI QTOF with $E_{cm} = 1.8$ eV, the base peak of *m/z* 331 is the sodiated diglycoside (with the loss of 302 Da) and is accompanied by ion signals of relatively low intensity due to the Y_0^+ and Y_1^+ species. Again, the MALDI QIT product ion mass spectrum (not shown) resembles closely that observed with the ESI QTOF instrument. The base peak in the relatively simple product ion mass spectrum obtained by MALDI TOF ReTOF, with $E_{cm} = 126$ eV and shown in Fig. 8(b), is *m/z* 363 that appears to be sodiated diglycoside plus CH_3OH, $(Digly + CH_3OH + Na^+)$. Thus, the base peaks of *m/z* 331 and 363 in Fig. 7 are analogous to the base peaks of *m/z* 303 and 335 in Fig. 6. Product ions of low ion signal intensity in Fig. 7(b) are due to losses of $O^\bullet$ (*m/z* 617), 104 Da (*m/z* 529.6),

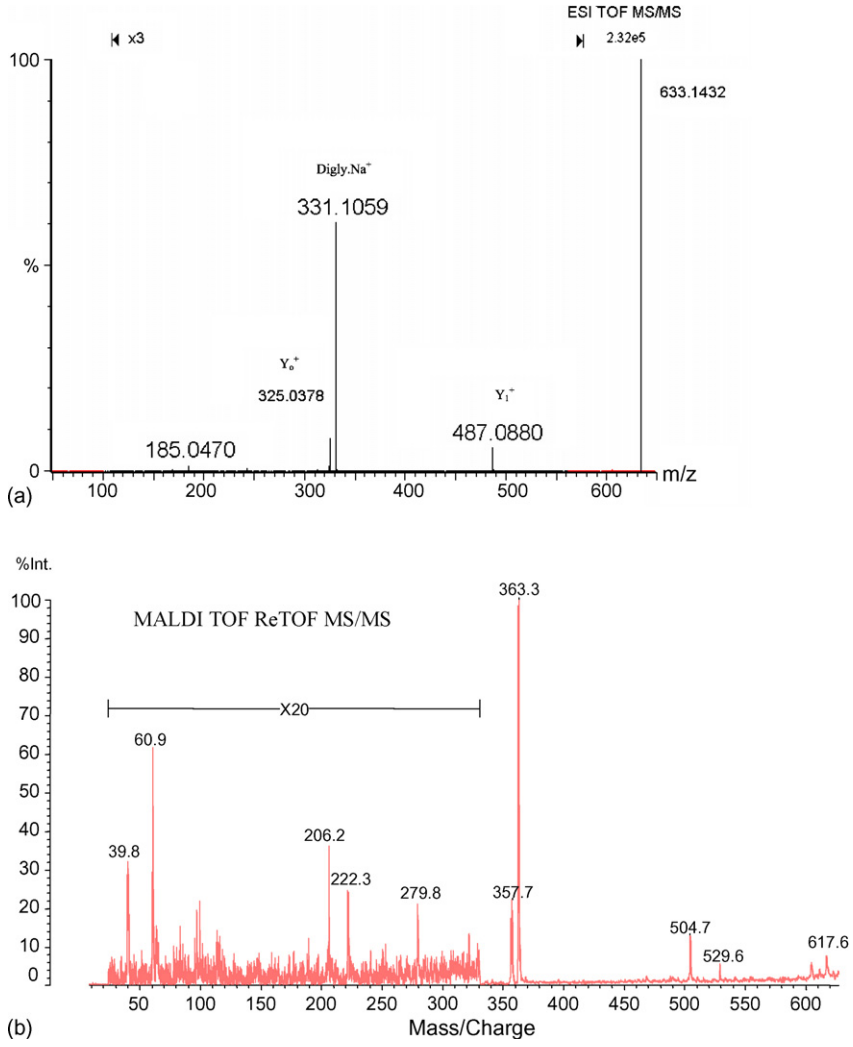


Fig. 8. Product ion mass spectra of sodiated rutin, *m/z* 633. (a) Observed with ESI QTOF; (b) MALDI TOF ReTOF.

129 Da (*m/z* 504.7), and 276 Da (*m/z* 357). The product ions of *m/z* 504.7 and 357.7 in Fig. 8(b) are the sodiated analogues of *m/z* 482.7 and 335.3 in Fig. 7(b).

4. Conclusions

Product ion mass spectra of non-glycosylated flavonoids observed with MALDI TOF ReTOF at high center-of-mass energies do not reflect the competing fragmentation processes observed at low (1–4 eV) center-of-mass energies, that is, cross-ring cleavage of the C-ring and scission of the C-ring followed by successive neutral losses. Despite the low C:H ratio (<1) in non-glycosylated flavonoids, CID at high E_{cm} values tends to produce hydrocarbon ions of low mass/charge ratio with C:H > 1 together with product ions corresponding to the loss of specific hydrocarbon neutrals. The identification of product ions in the range *m/z* 100–160 is difficult particularly as the mass resolution with which these ions were observed does not permit unambiguous determination of elemental composition.

As the mass of the projectile or precursor ion is increased, with concomitant reduction in E_{cm} to 261 eV, product ion mass spectra observed with MALDI TOF ReTOF become much more simple in that the mass spectra show two or three dominant product ions. For protonated hesperidin, Fig. 3, the two major product ions were radical cross-ring cleavage species. For protonated luteolin, Fig. 5, the two major product ions corresponded to hydrogenated species of the two major product ions observed with protonated apigenin, Fig. 4. This result is somewhat unexpected because there is no simple fragmentation process common to protonated luteolin and apigenin that can account for these observations. CID of protonated apigenin-7-*O*-glucoside yields a product ion mass spectrum that is dominated by the base peak of *m/z* 289.2, proposed as the $Y^+ + H_2O$ species. It is, indeed, curious that with E_{cm} = 183 eV, a single fragmentation pathway would be so dominant under these energy-rich conditions. In the CID of protonated rutin, the center-of-mass energy has been dissipated in fragmentations of the diglycoside moiety to yield only two major product ions that correspond to the $Y_0^+ + CH_3OH$ and $Y_1^+ + OH^\bullet$ species of *m/z* 335.3 and 482.7, respectively. In the CID of sodiated rutin, the center-of-mass energy has been dissipated in the formation of, essentially, a single product ion, *m/z* 363,

that appears to be sodiated diglycoside plus CH_3OH, that is, $[Digly + CH_3OH + Na]^+$.

Naïve expectations of the manifestation of increasing center-of-mass energy in CID are increases in both the number of product ion species and their ion signal intensities. Such expectations are based on the increase in vibrational energy that is expected to be deposited in the projectile ion with increasing center-of-mass energy. Thus, the simplification of product ion mass spectra observed here must be due to another influence that leads to excitation of the projectile ion with increasing center-of-mass energy but with limited enhancement of projectile ion vibrational energy. In this case, transfer of electronic energy may be considered. Vij et al. [51] reported that dissociation of the *p*-pentazolylphenolate anion by low-energy collisions wrought fragmentation of the pentazolate ring while high-energy collisions led to neat scission within the anion with charge transfer to the pentazolate ring to yield N_5^- (and no fragmentation of the pentazolate ring). It has been proposed by Belau et al. [52] that this result is accounted for by considering the electronic structure of the system. Electronic excitation of the protonated species examined here may well offer an explanation for the very simple product ion mass spectra observed here, particularly in Figs. 6–8.

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References

[1] M. Karas, D. Bachmann, F. Hillenkamp, Anal. Chem. 57 (1985) 2935.
[2] M. Karas, F. Hillenkamp, Anal. Chem. 60 (1988) 2299.
[3] K. Dreisewerd, Chem. Rev. 103 (2003) 395.
[4] K. Dreisewerd, S. Berkenkamp, A. Leisner, A. Rohlfing, C. Menzel, Int. J. Mass Spectrom. 226 (2003) 189.
[5] K.H. Tanaka, H. Wake, Y. Ido, S. Akita, Y. Yoshida, I. Yoshida, Rapid Commun. Mass Spectrom. 8 (1988) 2.
[6] M. Yamashita, J.B. Fenn, Phys. Chem. 88 (1988) 4451.
[7] M. Yamashita, J.B. Fenn, Phys. Chem. 88 (1988) 4671.
[8] J.A. Loo, H.R. Udseth, R.D. Smith, Anal. Biochem. 179 (1989) 404.
[9] R.D. Smith, J.A. Loo, C.G. Edmonds, C.J. Barinaga, H.R. Udseth, Anal. Chem. 62 (1990) 882.
[10] M.G. Ikonomou, A.T. Blades, P. Kebarle, Anal. Chem. 62 (1990) 957.
[11] M. Mann, Org. Mass Spectrom. 25 (1990) 575.
[12] H. Ehring, M. Karas, F. Hillenkamp, Org. Mass Spectrom. 27 (1992) 472.
[13] J.-C. Tabet, R.J. Cotter, Anal. Chem. 56 (1984) 1662.
[14] B.H. Wang, K. Dreisewerd, U. Bahr, M. Karas, F. Hillenkamp, J. Am. Soc. Mass Spectrom. 4 (1993) 393.
[15] B.A. Bohm, Introduction to the Flavonoids, Harwood Academic, 1998.
[16] J.B. Harborne (Ed.), The Flavonoids: Advances in Research since 1986, Chapman and Hall, London, 1994.
[17] F. Cuyckens, M. Claeys, J. Mass Spectrom. 39 (2004) 1 (erratum 39 (2004) 461).
[18] B. Boss, E. Richling, P. Schreier, in: Schreier P. (Ed.), Nat. Prod. Anal. [Symp.], Meeting Date 1997, 1998, p. 187.
[19] T.R. Croley, R.J. Hughes, C.D. Metcalfe, R.E. March, Rapid Commun. Mass Spectrom. 14 (16) (2000) 1494.
[20] T.R. Croley, R.J. Hughes, C. Hao, C.D. Metcalfe, R.E. March, Rapid Commun. Mass Spectrom. 14 (23) (2000) 2154.
[21] R.J. Hughes, T.R. Croley, C.D. Metcalfe, R.E. March, Int. J. Mass Spectrom. 210/211 (2001) 371.
[22] N. Fabre, I. Rustan, E. de Hoffmann, J. Quetin-Leclercq, J. Am. Soc. Mass Spectrom. 12 (2001) 707.
[23] F. Cuyckens, R. Rozenberg, E. de Hoffmann, M. Claeys, J. Mass Spectrom. 36 (2001) 1203.
[24] Y.-L. Ma, F. Cuyckens, H. Van den Heuvel, M. Claeys, Phytochem. Anal. 12 (2001) 159.
[25] M. McCullagh, C.A.M. Periera, J.H. Yariwake, 19th Montreux Symposium, Montreux, Switzerland, 6–8 November, 2002.
[26] R.E. March, X. Miao, Int. J. Mass Spectrom. 231 (2004) 157.
[27] R.E. March, X.-S. Miao, C.D. Metcalfe, M. Stobiecki, L. Marczak, Int. J. Mass Spectrom. 232 (2004) 171.
[28] R.E. March, E.G. Lewars, C.J. Stadey, X.-S. Miao, X. Zhao, C.D. Metcalfe, Int. J. Mass Spectrom. 248 (1–2) (2006) 61.
[29] P.J. Robinson, K.A. Holbrook, Unimolecular Reactions, John Wiley & Sons, New York, 1972.
[30] W. Forst, Theory of Unimolecular Reactions, Academic Press, New York, 1973.
[31] H.M. Rosenstock, M.B. Wallenstein, A.L. Wahrhaftig, H. Eyring, Proc. Natl. Acad. Sci. 38 (1952) 667.
[32] J.D. Ciupek, D. Zakett, R.G. Cooks, K.V. Wood, Anal. Chem. 54 (1982) 2215.
[33] S.A. McLuckey, R.G. Cooks, in: F.W. McLafferty (Ed.), Tandem Mass Spectrometry, John Wiley & Sons, New York, 1983 (Chapter 15).
[34] H.I. Kenttämaa, R.G. Cooks, Int. J. Mass Spectrom. Ion Proc. 64 (1985) 79.
[35] R.G. Cooks, J.H. Beynon, J.F. Litton, Org. Mass Spectrom. 10 (1975) 503.
[36] P.J. Todd, F.W. McLafferty, in: F.W. McLafferty (Ed.), Tandem Mass Spectrometry, John Wiley & Sons, New York, 1983 (Chapter 7).
[37] M.S. Kim, F.W. McLafferty, J. Am. Chem. Soc. 100 (1978) 3280.
[38] F.W. McLafferty, P.J. Todd, D.C. McGilvery, M.A. Baldwin, J. Am. Chem. Soc. 102 (1980) 3360.
[39] J.E. Szulejko, I. Howe, J.H. Beynon, Int. J. Mass Spectrom. Ion Phys. 37 (1981) 27.
[40] T. Ast, C.J. Proctor, C.J. Porter, J.H. Beynon, Int. J. Mass Spectrom. Ion Phys. 40 (1981) 111.
[41] P.J. Todd, F.W. McLafferty, Int. J. Mass Spectrom. Ion Phys. 38 (1981) 371.
[42] P.J. Todd, Ph.D. Thesis, Cornell University, 1980.
[43] J.A. Laramee, D. Cameron, R.G. Cooks, J. Am. Chem. Soc. 103 (1981) 12.
[44] (a) G.R. Fowles. Analytical Mechanics, Holt, Rhinehart and Winston, Toronto, 1962, p. 148;
(b) R.D. Levine, R.B. Berstein, Molecular Reaction Dynamics and Chemical Reactivity, Oxford University Press, New York, 1987, p. 36.
[45] S.E. Kupriyanov, A.A. Perov, Z. Tech. Fiz. 33 (1963) 823.
[46] O. Belgacem, A. Bowdler, I. Brookhouse, F.L. Brancia, E. Raptakis, Rapid Commun. Mass Spectrom. 20 (2006) 1653.
[47] I. Fournier, C. Marinach, J.-C. Tabet, G. Bolbach, J. Am. Soc. Mass Spectrom. 14 (2003) 893.
[48] Y.L. Ma, Q.M. Li, H. Van den Heuvel, M. Claeys, Rapid Commun. Mass Spectrom. 11 (1997) 1357.
[49] B. Domon, C. Costello, Glycoconjugate J. 5 (1988) 397.
[50] F. Cuyckens, Y.L. Ma, G. Pocsfalvi, M. Claeys, Analusis 28 (2000) 888.
[51] A. Vij, J.G. Pavlovich, W.W. Wilson, V. Bij, K.O. Kriste, Angew. Chem. Int. Ed. 41 (2002) 3051.
[52] L. Belau, Y. Haas, S. Zilberg, J. Phys. Chem. A 108 (2004) 11715.