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ScienceDirect
International Journal of Mass Spectrometry 262 (2007) 51–66
International Journal of Mass Spectrometry
www.elsevier.com/locate/ijms

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

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Received 25 June 2006; received in revised form 9 October 2006; accepted 9 October 2006
Available online 13 November 2006

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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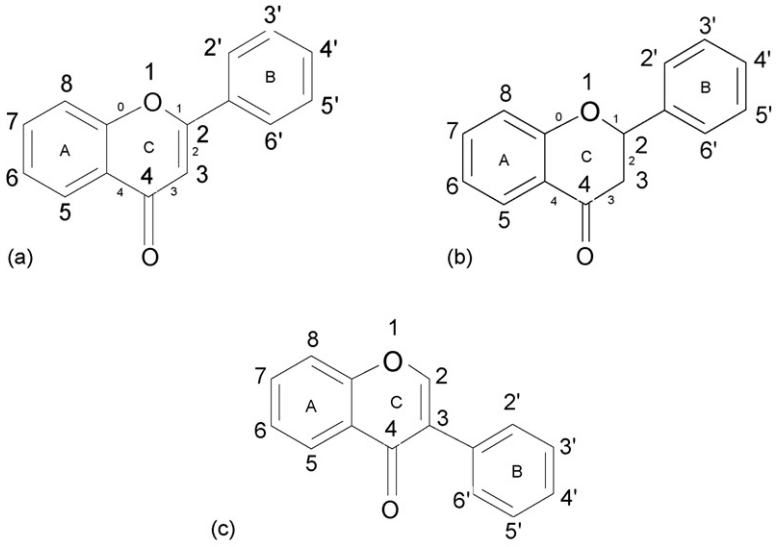
doi:10.1016/j.ijms.2006.10.008



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