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International Journal of Mass Spectrometry 247 (2005) 93–100
International Journal of Mass Spectrometry
www.elsevier.com/locate/ijms

Application of ESI/MS, CID/MS and tandem MS/MS to the fragmentation study of eriodictyol 7-*O*-glucosyl-(1→2)-glucoside and luteolin 7-*O*-glucosyl-(1→2)-glucoside

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Received 27 July 2005; received in revised form 6 October 2005; accepted 6 October 2005
Available online 14 November 2005

Abstract

A mass spectrometric method based on the combined use of positive and negative electrospray ionization, collision-induced dissociation and tandem mass spectrometry has been applied to the structural characterization of the eriodictyol 7-*O*-glucosyl-(1→2)-glucoside and luteolin 7-*O*-glucosyl-(1→2)-glucoside. The low-energy product ion mass spectrum of $[M+H]^+$ and $[M-H]^-$ ions showed extensive fragmentation of the diglucose moiety, loss of the glycan residue, and fragmentation of the aglycon units that permit characterization of the interglycosidic linkage and the substituents in the A- and B-rings. Both glycosides were shown to yield the $^{0,2}X_0{}^{0,2}X_1$ ion which can be considered as characteristic of the 1→2 interglycosidic linkage in the glucoglucoside adducts, since it can not be formed in the case of other interglycosidic types. In the case of the eriodictyol diglucoside the 1, 3 fragmentation of the C-ring was observed before those involving the carbohydrates thus allowing the position determination of the diglucoside moiety on the A-ring. In the negative ion mode only the luteolin diglucoside was shown to undergo collision-induced homolytic and heterolytic cleavages of the *O*-glycosidic bond producing the aglycone radical–anion $[Y_0–H]^{\bullet-}$ and $Y_0{}^-$ product ions, while this was not observed in the case of eriodictyol glycoside. CID MS/MS analysis of the sodiated molecules gave complementary informations for the structural characterization of the studied compounds. The $B_2{}^+$ fragment which is useful for establishing that the terminal carbohydrate unit is linked to another carbohydrate and not directly to the aglycone was obtained as base peak. This result is of analytical value for the differentiation of *O*-diglycosyl and di-*O*-glycosyl flavonoids.

Keywords: Flavonoids; Glucosides; ESI/MS; MS/MS; CID/MS

1. Introduction

Flavonoids are polyphenolic natural products which are recognized as one of the largest and most widespread class of plant constituents occurring throughout the plant kingdom, and are also found in substantial levels in commonly consumed fruits, vegetables and beverages. Flavonoids have recently aroused considerable interest because of their potential beneficial biochemical and antioxidant effects on human health. Most of the experimental results demonstrate that flavonoid compounds have several biological activities including radical scavenging, anti-inflammatory, anti-mutagenic, anti-cancer, anti-HIV, anti-allergic, anti-platelet and anti-oxidant activities [1]. Flavonoids are grouped together into subclasses based on their basic chemical structures; the most common ones being flavones, flavonols, isoflavones, flavanones, anthocyanins and chalcones. Flavonoids can exist as free aglycones but most of them commonly occur as *C*- or *O*-glycosides. Disaccharides are also often found in association with flavonoids and occasionally tri- and even tetrasaccharides.

The structure determination of flavonoids plays an important role in many areas of science. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities. The structural characterization of flavonoids is generally performed by a combination of spectroscopic methods, including ultraviolet (UV), nuclear magnetic resonance (NMR) and mass spectrometry (MS). Among these methods

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doi:10.1016/j.ijms.2005.10.002

mass spectrometry (MS) is one of the important physicochemical methods applied to the structure determination of natural products due to its sensitivity, rapidity, and low levels of sample consumption [2–4]. With the development of soft ionization techniques, mass spectrometry has become a powerful analytical tool of flavonoids, a polar, non-volatile, and thermally labile classes of compounds [5–7]. Mass spectrometric methods such as electron ionization EI/MS [8], fast atom bombardment FAB/MS [9], atmospheric pressure chemical ionization APCI/MS [5,10,11] and electrospray ionization ESI/MS [12–14] have proved useful to determine flavonoids in herbs and other foods [8,12,14,15]. The coupling of liquid chromatography/mass spectrometry (LC/MS) with API/MS and ESI/MS/MS techniques has been demonstrated to be a powerful tool for the identification of flavonoids [16–18]. The combination of FAB with collision-induced dissociation (CID) and tandem mass spectrometric techniques has been shown to yield important structural information for the characterization of underivatized flavonoid aglycones and glycosides [9,19]. Furthermore, the low-energy CID MS/MS spectra of various flavonoid aglycones and glycosides in mixture were described in both LC/ESI/MS/MS [14,15] and LC/APCI/MS/MS [7,10]. It has been demonstrated that fragment ions provide important structural information for flavonoids and can be used to establish the distribution of the substituents between the A- and B-rings. A careful study of the fragmentation patterns in CID MS/MS can also be of a particular value in the structural elucidation of *O*- and *C*-glycosides [9].

Since the number of flavonoid compounds is steadily increasing, and in order to further increase the applicability of LC/ESI/MS/MS in the analysis of plant phenolics, it appeared important to investigate the fragmentation pathways of new flavonoids in order to correlate the structures with their fragmentation patterns, thus allowing a rapid identification and analysis of flavonoids. In the present study, LC/MS and MS/MS using ESI were applied for molecular mass and structural information of two flavonoid glycosides, namely eriodictyol 7-*O*-glucosyl-(1→2)-glucoside and luteolin 7-*O*-glucosyl-(1→2)-glucoside. Analyses were first conducted using ESI/MS in the negative and positive ion modes to obtain ionized molecular species. Then, tandem MS/MS spectra were obtained by low energy collision induced dissociation (CID) of the $[M+H]^+$ or $[M-H]^-$ ions, and were interpreted to propose plausible fragmentation pathways for each studied compound.

2. Experimental

2.1. Materials

The two studied flavonoid glycosides were isolated from an aqueous methanolic extract of *G. alypum* aerial parts. Fresh aerial parts were air-dried in shade at room temperature and the dried aerial parts were powdered. Hundred grams of the obtained powder were macerated during 48 h at room temperature with 500 mL of a 3:2 mixture of distilled water–methanol. The crude preparation was filtered and concentrated under reduced pressure to provide a crude extract which was stored at −20 °C until time of use. The resulting aqueous phase was extracted with hexane and the subsequent aqueous phase was subjected to a SPE column. Elution was performed successively by H_2O, MeOH 10%, MeOH 40%, MeOH 50% and MeOH 100%. The fractions were concentrated under reduced pressure, lyophilized, tested for their scavenging activity, and analysed through analytical HPLC. The MeOH 50% fraction, which was shown to be rich in natural antioxidant compounds, was explored first using semipreparative HPLC. After several successive injections, samples corresponding to the same chromatographic peaks were verified by analytical HPLC, concentrated under reduced pressure and lyophilised. This operation gave the studied purified compounds.

2.2. Mass spectrometry

LC/MS analyses were performed with a chromatographic system (Alliance) consisting of a Waters 2695 separations module equipped with an autosampler and a Waters 2487 dual lambda absorbance detector (Waters, Milford, MA, USA). The column was a 150 mm × 2.1 mm Interchrom UP5ODB#15E (Uptisphere 5 μm ODB) with a 10 mm × 2.1 mm precolumn from Interchim (Montluçon, France). Chromatography was carried out in isocratic mode with a 60/40 mixture of acetonitrile (RS-Plus quality for HPLC from Carlo Erba) and water with 0.2% acetic acid. The flow rate was 0.2 mL/min, the analyses were performed with the column and the samples kept at ambient temperature and 5.0–10 μL was injected for each analysis. The effluent from the UV detector was introduced into the mass spectrometer without any splitting of the flow. The HPLC system was coupled directly to a Quattro LC/MS/MS triple quadrupole mass spectrometer (Micromass, Manchester, UK) equipped with a pneumatically assisted electrospray ionisation source (ESI). Data acquisition and processing were performed using a MassLynx NT 3.5 data system. The electrospray source parameters were fixed as follows: electrospray capillary voltage 3.25 kV in positive mode and 3 kV in negative mode, source block temperature 120 °C, desolvation gas temperature 400 °C. Nitrogen was used as drying gas and nebulising gas at flow rates of approximately 50 and 450 L/h.

3. Results and discussion

The samples selected for this study were eriodictyol 7-*O*-glucosyl-(1→2)-glucoside **1** and luteolin 7-*O*-glucosyl-(1→2)-glucoside **2** (Fig. 1). These flavonoids have to our knowledge not been examined in previous mass spectrometric studies and are thus interesting from a phytochemical and analytical point of view. In addition, compounds **1** and **2** are diglycosides consisting of two glucoside units, which are different from the most previously reported flavonoids consisting of rhamnose and glucose moieties [4,9,20–28].

3.1. Nomenclature

The major diagnostic fragmentations for flavonoid identification are those involving the cleavage of two C–C bonds of the