Journal of Pharmaceutical and Biomedical Analysis 53 (2010) 462–474

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Characterization of steroidal saponins in crude extracts from *Dioscorea zingiberensis* C. H. Wright by ultra-performance liquid chromatography/electrospray ionization quadrupole time-of-flight tandem mass spectrometry

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ARTICLE INFO

Article history:
Received 4 December 2009
Received in revised form 17 May 2010
Accepted 19 May 2010
Available online 27 May 2010

Keywords:
Steroidal saponin
Dioscorea zingiberensis C. H. Wright
UPLC
Q-TOF-MS/MS
Structural elucidation

ABSTRACT

Steroidal saponins are the major bioactive constituents of *Dioscorea zingiberensis* C. H. Wright (*D. zingiberensis*). In this work, ultra-performance liquid chromatography/electrospray ionization quadrupole time-of-flight tandem mass spectrometry (UPLC/Q-TOF-MS/MS) was applied to the separation and characterization of steroidal saponins in crude extracts from *D. zingiberensis*. The results showed that fragment ions from glycosidic and cross-ring cleavages gave a wealth of structural information related to aglycone skeletons, sugar types and the sequence of sugar units. According to the summarized fragmentation patterns, identification of steroidal saponins from *D. zingiberensis* could be fulfilled, even when reference standards were unavailable. As a result, a total of thirty-one saponins with five aglycone skeletons, including fourteen new trace saponins, were identified or tentatively elucidated in crude extracts from *D. zingiberensis* based on their retention times, the mass spectrometric fragmentation patterns, and MS and MS/MS data.

1. Introduction

A steroidal saponin molecule consists of an aglycone and several glycosyl moieties. Steroidal saponins are mainly present in *Liliaceae*, *Dioscoreaceae*, *Agavaceae* and *Smilacaceae* and can be classified into spirostanol, isospirostanol, furostanol, pseudospirostanol and cholestanol saponins according to their skeletons [1]. The medicinal plant *Dioscorea zingiberensis* C. H. Wright (*D. zingiberensis*) is widely distributed in Shanxi, Hunan, Hubei and Sichuan provinces of China. Its rhizome has been known as a traditional Chinese medicine (TCM) for a long time, and used as a folk treatment for cough, anthrax, rheumatic heart disease, rheumarthritis, tumefaction and sprain [2]. The water-soluble steroidal saponins from *D. zingiberensis* are the main bioactive components, which have been used in China for many years for the treatment of coronary heart disease [3]. Furthermore, dioscin, one of steroidal saponins from *D. zingiberensis*, whose cytotoxic activity against the cancer cell line K562 *in vitro* has been reported [4]. Many steroidal saponins have been isolated and identified from *D. zingiberensis* [5–10], and the five main steroidal aglycones are shown in Fig. 1a–e. Moreover, the common sugars present in steroidal saponins from *Dioscoreaceae* are hexose (glucose) and 6-deoxyhexose (rhamnose), and generally, glucosyls are connected with the hydroxyl groups at C-3 and/or C-26 positions of steroidal aglycones.

Up to now, characterization of steroidal saponins from *D. zingiberensis* mainly depends on the NMR spectra of the pure compounds obtained by preparative isolation and purification [6–10]. But this method wastes time and energy, especially for some trace compounds, which are very difficult to be purified and characterized. LC–MS has proven to be a very convenient and efficient technique for identification of steroidal saponins in plant extracts in recent years [11–15]. Ion trap (IT) MS allows MS^n for structural elucidation of steroidal saponins, but this analyzer provides nominal mass accuracy and may not well confirm the detailed identities of the product ions at most times [12–15]. The application of Q-TOF-MS can yield empirical chemical formula based on the accurate masses of molecular ions and detailed fragmentation information, which removes ambiguities out of the interpretation, confirms the identities of the fragment ions and facilitates structural elucidation [16–21]. Recently, ultra-performance liquid chromatography

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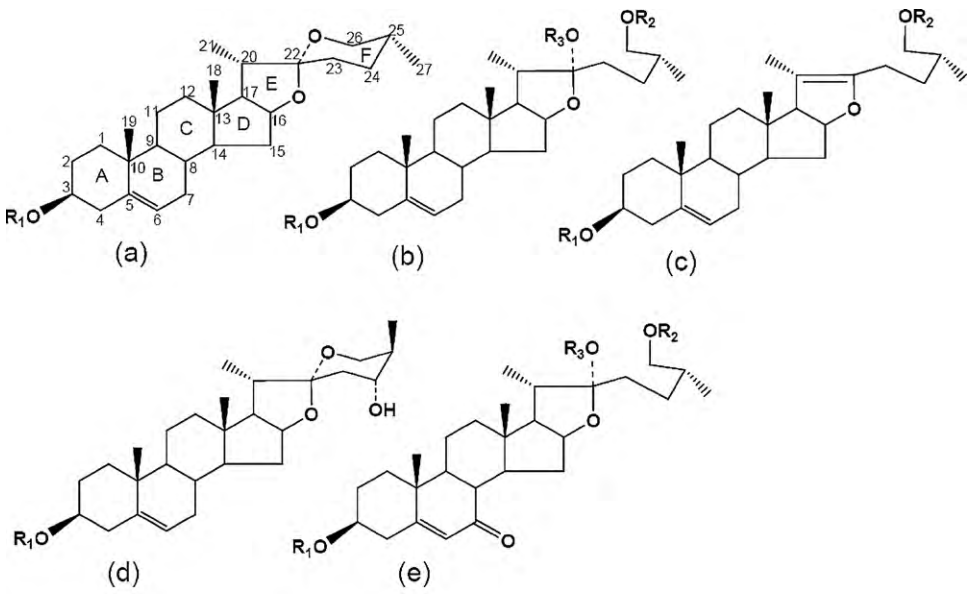
doi:10.1016/j.jpba.2010.05.019



Compounds	R_1	R_2	R_3	MW
[a]zingiberensis saponin	Glc(1→3)Glc(1→4)[Rha(1→2)]-Glc	--	--	1046.5298
[a]deltonin	Glc(1→4)[Rha(1→2)]-Glc	--	--	884.4769
[a]dioscin	Rha(1→4)[Rha(1→2)]-Glc	--		868.4821
[a]prosapogenin A of dioscin	Rha(1→2)-Glc	--	--	722.4241
[a]diosgenin diglucoside	Glc(1→4)-Glc	--	--	738.4191
[b]protobioside	Rha(1→2)-Glc	Glc	H	902.4875
[b]funkioside	H	Glc	H	594.3768
[b]peak 5	Glc(1→4)-Glc	Glc	H	918.4824
[b]peak 7	Glc	Glc	H	756.4296
[c]zingiberenin G	Glc(1→3)Glc(1→2)[Rha(1→4)]-Glc	Glc	--	1208.5829
[d]zingiberogenin	H	--	--	430.3083
[d]zingiberenin A_2	Rha(1→2)-Glc	--	--	738.4191
[e]zingiberenin H	Glc(1→3)Glc(1→4)[Rha(1→2)]-Glc	Glc	H	1240.5724

Glc=β-D-glucopyranosyl, Rha=α-L-rhamnopyranosyl

Fig. 1. Structures of the steroidal saponins from *D. zingiberensis*.

(UPLC) has been introduced as a rapid and efficient tool for complex sample analysis [16,18–21]. Generally, the column is packed with particles of less than 2 μm size and operated at high pressure up to 600 bar, which results in high resolution and superior peak capacity in short analysis time. The combination of UPLC and Q-TOF-MS/MS offers high chromatographic resolution with accurate mass measurement for both MS and MS/MS experiments, then, significant advantages for rapid screening target compounds in complex matrices are achieved.

In this work, the structural characteristics of the steroidal saponins in the ethanolic extracts from the dried rhizomes of *D. zingiberensis* have been investigated by UPLC/Q-TOF-MS/MS in both negative and positive ion modes. The fragmentation patterns of reference standards were investigated and the steroidal saponins in the extracts were identified or tentatively characterized according to the retention times, MS and MS/MS data. This UPLC/Q-TOF-MS/MS method has been successfully used to characterize of steroidal saponins in the crude extracts from *D. zingiberensis*. It also provides an excellent approach for rapid screening of steroidal saponins in plant extracts.

2. Experimental

2.1. Reagents and materials

Acetonitrile (HPLC grade) was purchased from Fisher Scientific Co. (Loughborough, UK). Formic acid (HPLC grade) was purchased from Acros Co. Ltd. (NJ, USA). Water (18.2 MΩ) was purified on a Milli-Q system (Millipore, Billerica, USA). Other reagents were commercially available of analytical purity. The dried rhizomes of *D. zingiberensis* were purchased from a drug store in Zaoyang City (Hubei Province, China). Standards of zingiberensis saponin, deltonin, dioscin, prosapogenin A of dioscin and diosgenin diglucoside were isolated and purified from *D. zingiberensis* in our laboratory. Their structures were confirmed by UV, ESI-MS and ^{1}H, ^{13}C NMR and comparison with the literature [4,6,10,22].

2.2. UPLC/Q-TOF-MS/MS analysis

UPLC/Q-TOF-MS/MS analysis was performed on a Waters ACQUITY^{TM} UPLC coupled with a Q-TOF Premier, a quadrupole and