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Short communication

Analysis of iridoid glucosides in *Hedyotis diffusa* by high-performance liquid chromatography/electrospray ionization tandem mass spectrometry

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ABSTRACT

An HPLC-DAD-ESI-MS/MS method was developed for analysis of iridoid glucosides (IGs) from *Hedyotis diffusa* Willd. The optimized separation condition was achieved with the Complex Sample Analysis Software System (CSASS) software, under which the whole analytes were achieved complete resolution especially for some isomeric IGs. Based on the UV and fragmentations, eleven IGs were detected. According to the fragmentation patterns of the three standard IGs, especially those of the isomeric standards, seven IGs including three pairs of isomers were unambiguous/tentatively identified. For the isomeric IGs with methyl ester or carboxyl group at C-4, the extents of the losses of CH_3OH and/or H_2O from their molecular and/or the aglycone adducts are useful for the differentiation of the stereoisomers in positive ion (PI) mode, which depends on the stereochemistry of the hydroxyl group on the cyclopentanoid unit.

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1. Introduction

Hedyotis diffusa Willd has been known as a traditional Chinese medicine (TCM) for a long time, and used as a treatment for cancer and inflammations such as appendicitis, urethritis, and bronchitis, due to its antibacterial activity [1–4]. Recently, this herb has gained increasingly attention to further effectively utilize its usage as a treatment for tumours [5]. Up to now, three major classes of compounds, including iridoid glucosides (IGs), flavonoids, and anthraquinones, have been reported as bioactive compounds from this herb [6,7].

IGs are one of the most important classes of natural products with bioactivities of anti-oxidation [8], neuroprotective activity [7], anti-inflammatory and immunomodulatory [9], etc., and some of them have entered clinical trials [10]. Up to now, the characterization of IGs from *H. diffusa* is almost using the NMR spectra of the pure compounds obtained by preparative isolation and purification [5,7,11,12]. But this method wastes time and energy, and some of the minor compounds cannot be purified. Recently, LC–MS has become a very convenient technique for the identification of plant secondary metabolites. The method has advantages of higher sensitivity and providing molecular weight (MW) and structural information on-line. It, however, has not been greatly used for the analysis of IGs from *H. diffusa*.

In this work, we studied the application of HPLC-DAD-ESI-MS/MS for the analysis of IGs from *H. diffusa*. As some of IGs are isomeric, it is very important to achieve completely spectral resolution of them for acquiring apart their UV and mass spectra. The optimized elution curve was achieved with five different gradient programs using the CSASS software (Complex Sample Analysis Software System, Dalian Institute of Chemical Physics, Chinese Academy of Science, Dalian, China). Referred to the fragmentation patterns of the isolated standards, the structures of the IGs detected from *H. diffusa* were discussed.

2. Experimental

2.1. Chemicals and reagents

HPLC-grade acetonitrile and methanol were purchased from Fisher Scientific (Loughborough, Great Britain). Water used for liquid extraction was deioned water and Milli-Q water was used for LC/MS analysis. Formic acid (HPLC grade) was purchased from TEDIA (USA). Deacetylasperuloside acid methyl ester (DAME), scandoside methyl ester (SME) and scandoside (SD) were used as standards, isolated from the extract of *H. diffusa* by our research group, and identified by ^{1}H-NMR, ^{13}C-NMR, MS, UV spectra and comparison with those reported in the literatures [8,13,14]. Macro-

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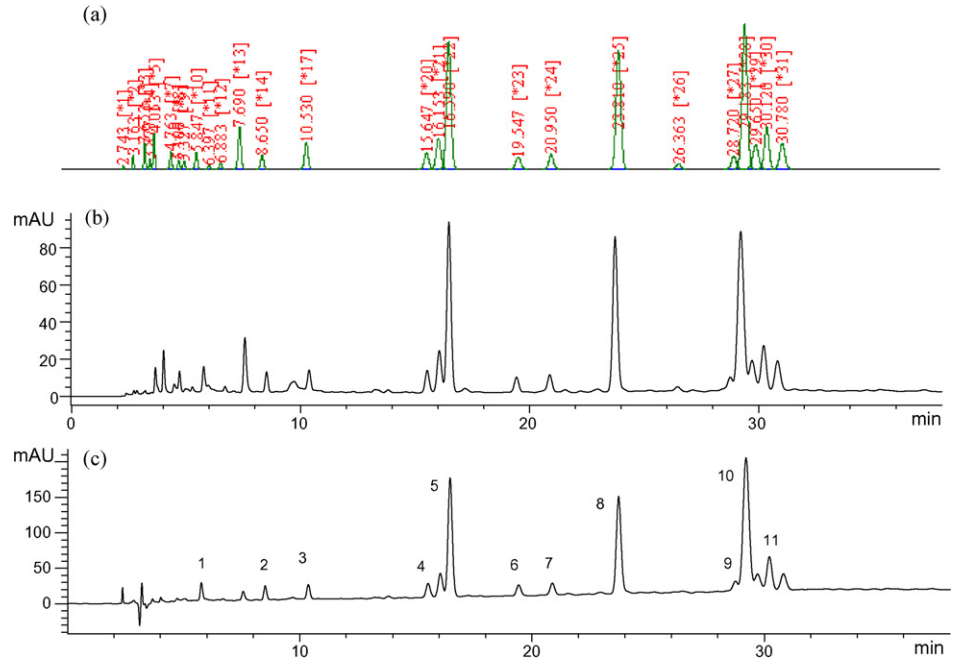


Fig. 1. Chromatograms of the extract of *H. diffusa* (a) simulated, at 254 nm; experimental: (b) at 254 nm, (c) at 235 nm.

porous resins D4020 used were purchased from Chemical Plant of Nankai University (China).

2.2. Materials

The aerial parts of *H. diffusa* (Willd.) were collected from Hengdong County, Hunan province (China) in May 2005. The herb was authenticated by Institute of Traditional Chinese Medicine, China Academy of Chinese Medical Sciences. The voucher specimens were deposited at Dalian Institute of Chemical Physics, Chinese Academy of Sciences, China.

The CSASS software was applied for optimizing the chromatographic condition.

2.3. Sample preparation

Dried and powdered samples (2 kg) of *H. diffusa* were extracted with tenfold water under reflux for twice, and each reflux time was 2 h. After filtration of the aqueous fraction, the filtrate was concentrated. An aliquot (50 g) of the extract was dissolved in 70% EtOH (v/v, 500 mL). The supernate was filtered and evaporated under reduced pressure. The residual aqueous solution was partitioned with EtOAC, *n*-BuOH, 70% EtOH in turns. The 70% EtOH fractions were subjected to macroporous resin D4020 and successfully eluted with H_2O and different concentrations of EtOH. The 35% EtOH fraction was concentrated to dryness under reduced pressure. In this work, an aliquot (5 mg) of the dry sample of 35% EtOH fraction was dissolved in 10 mL of water and filtered through a membrane filter (0.22 μm) prior to HPLC/MS analysis.

2.4. HPLC-DAD-ESI-MS analysis

2.4.1. Mass spectrometry analysis

All MS experiments were conducted on a MSD Trap XCT mass spectrometer equipped with electrospray ionization (ESI) interface (Agilent Technologies, USA). Both the auxiliary and sheath gases were nitrogen with a flow rate of 8 L/min. The dry gas temperature was set at 325 °C, and the nebulizer pressure was set at 35 psi. Spectra were recorded in positive and negative ion (PI and NI) modes at a spray voltage of +4.5 and −4.5 kV, respectively. The mass scan range was between *m*/*z* 100 and 1000.

2.4.2. Chromatographic conditions

HPLC-DAD measurements were carried out using Agilent 1100 liquid chromatography (Agilent Technologies), consisting of an HPLC quaternary pump, an autosampler, a column oven, and a diode array detector. Chromatograms were monitored at wavelengths of 235 and 254 nm, and UV spectra were recorded between 200 and 400 nm. Separation was performed on a C18 column (250 mm × 4.6 mm i.d., 5 μm, Chrom expert company, USA) and the column temperature was set at 30 °C. On the basis of the eluent system with optimized ionization efficiency, the linear gradient consisted of A (0.1% (v/v) formic acid in aqueous phase) and B (0.1% (v/v) formic acid in methanol) with a flow rate of 1.0 mL/min. Five linear gradient program conditions were investigated in (a)–(e) to obtain the optimized separation with the CSASS software.

(a) 5–25% B in 50 min, then to 95% B in 10 min;
(b) 7–25% B in 50 min, then to 95% B in 10 min;
(c) 8–25% B in 50 min, then to 95% B in 10 min;
(d) 9–25% B in 50 min, then to 95% B in 10 min;
(e) 10–25% B in 50 min, then to 95% B in 10 min.

2.5. Preparation the IGs from the extract of H. diffusa

In order to validate the structures of the IGs tentatively identified from the extract of *H. diffusa*, the isolation of the IGs was performed by AutoPurification System (Waters, USA) using a four-channel sample manager with four XTerra MS C18 columns (250 mm × 19 mm i.d., 5 μm, Waters, USA). The elution system consisted of A (0.1% (v/v) formic acid in aqueous phase) and B (0.1% (v/v) formic acid in methanol) with a flow rate of 16.4 mL/min. The linear gradient program was: 3% B, holding for 10 min, then to 10% B in 20 min, holding for 20 min, and finally to 95% B in 3 min. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker DRX-400 spectrometer.

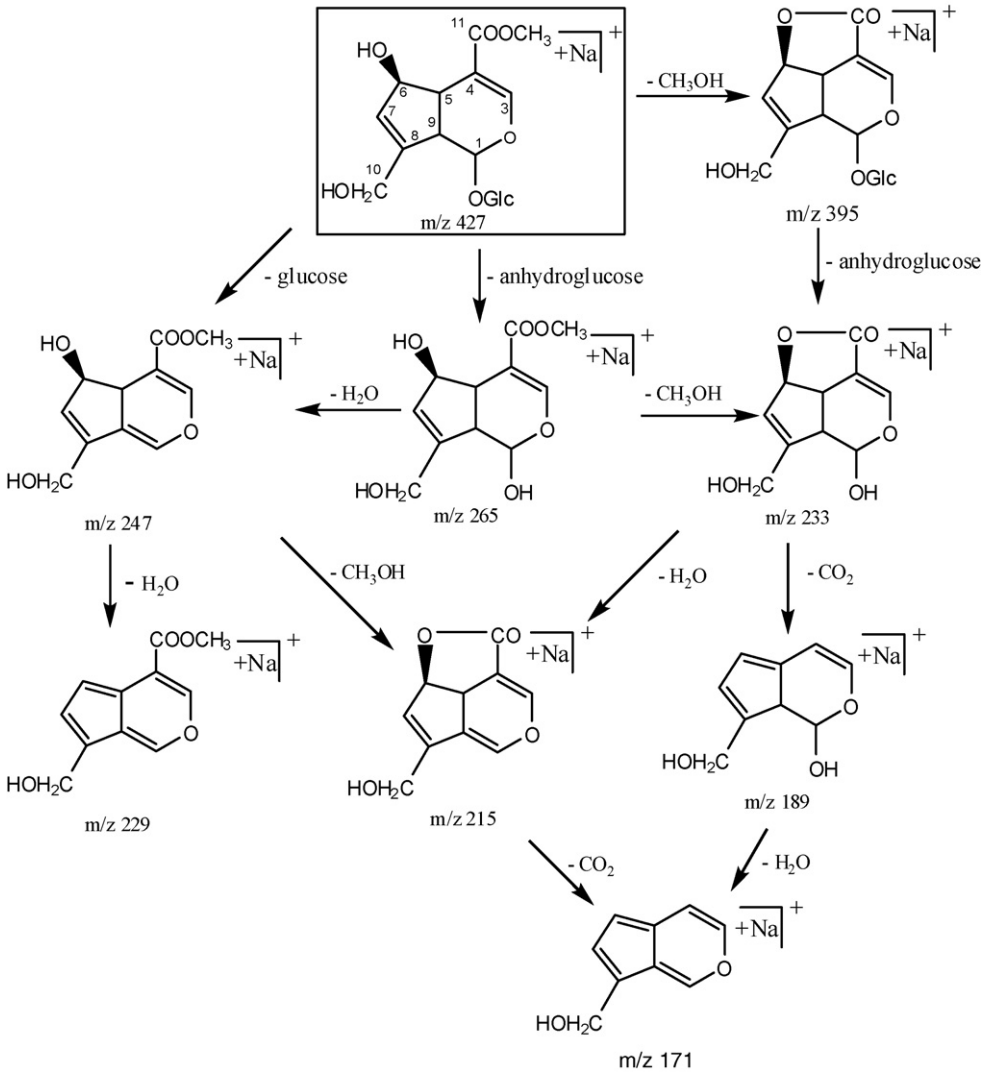


Fig. 2. The proposed fragmentation pathway of SME in PI mode.

3. Results and discussion

3.1. Optimization of LC/MS conditions

To enhance the ionization efficiency, organic solvent types (acetonitrile versus methanol), additives (acetic acid versus formic acid) in different concentrations and ratios were tested. The results showed that the system of CH_3OH/H_2O with 0.1% formic acid as additive gave sufficient ionization for most of the analytes in both ionization modes, which was selected as the optimized eluent system.

The CSASS software is a tool to develop rapidly the RP–LC methods for the separation of complex mixture. With CSASS, it is possible to predict retention behavior [15] and optimize chromatographic separation [16]. To obtain the optimal resolution for target analytes in the extract of *H. difussa*, five linear HPLC-gradient experiments (Section 2.4.2a–e) without intersection were performed to simulate the optimized separation condition with the CSASS software. 254 nm was selected as detection wavelength for simulating the optimum chromatogram, because more peaks can be observed and peak overlapping might be avoided during simulation at this wavelength.

The optimized program was: 0–20 min, 7–15% B; 20–30 min, 15–19% B; 30–35 min, 19% B; 35–45 min, 19–95% B. Under the optimized condition, 31 peaks were almost completely separated except for some minor components. This ensured the veracity of UV and MS spectra information of the target compounds (11 peaks, see Fig. 1c). Furthermore, the simulated and experimental chromatograms were consistent very well (Fig. 1). The relative standard deviation (R.S.D.) of the retention times between the experimental and simulated chromatograms for each of the detected peaks ranged from −1.66% to 0.33%.

The UV spectra of peaks 1–11 exhibited the characteristic of IGs with maximum absorbance near 235 nm due to the α,β-unsaturated acid, ester or lactone at C-4 in their structures [17]. In addition, these UV spectra did not show any absorption band at 270–290 nm, indicating no carbonyl on the cyclopentanoid moiety of the compounds [17].

3.2. MS fragmentation patterns of SME, DAME and SD

The diagnostic fragmentation pathway for IGs was obtained by using LC–MS/MS in PI mode analysis of the three representative standards of the species, SME, DAME and SD. SME and DAME are

epimers with MW of 404 Da, while SD has MW of 390 Da. The precursors and fragments of SD, SME and DAME were shown in Table 1. In the first-step MS spectra, the sodiated molecule $[M+Na]^+$ and the characteristic cluster ions $[2M+Na]^+$ were formed as predominant ions, by which the MWs were determined.

As precursor, the sodiated SME (*m*/*z* 427) produced ten primary fragments in MS/MS spectrum. Upon MS/MS, the glucosidic bond was easily cleaved to generate an ion at *m*/*z* 265 ($\Delta m = 162$) as the base peak. Meanwhile, the product ions at *m*/*z* 395 and 247 were due to the neutral molecular loss of a CH_3OH and glucose, respectively. The *m*/*z* 233 ion was due to the successive loss of an anhydroglucose and CH_3OH, and the product ion at *m*/*z* 229 was due to the successive loss of a glucose and H_2O. The ions at *m*/*z* 203 $[glucose+Na]^+$ and *m*/*z* 185 $[anhydroglucose+Na]^+$ were both corresponding to the loss of the aglycone moiety, which was the mutual product ions of sodiated molecules of IGs [18,19]. The fragmentation pathway of SME in PI mode was shown in Fig. 2.

As epimer of SME, DAME showed similar fragmentation (Table 1). However, due to the opposite configuration of the hydroxyl group at C-6, the relative abundances of the same fragment ions are obviously different, by which the isomeric pair can be readily differentiated. The distinct difference in MS/MS data of the isomeric pair was in the extent of the losses of CH_3OH and H_2O (Table 1). In the case of SME, the loss of CH_3OH, corresponding to $[M+Na-CH_3OH]^+$ ion at *m*/*z* 395 and $[M+Na-anhydroglucose-CH_3OH]^+$ ion at *m*/*z* 233, represented more significantly than those of DAME. It was most probably correlative to the configuration of the OH group at C-6. In the structure of SME, the OH group at C-6 is above the plane and easier to form the *cis*-junction of the two rings with the carbomethoxy group at C-4 by losing a molecule CH_3OH than that of DAME with the OH group at C-6 below the plane [20]. Similarly, the easier loss of a molecular H_2O, corresponding to $[M+Na-glucose-H_2O]^+$ at *m*/*z* 229, also occurs easily for the sodiated SME than that for the sodiated DAME. It was deduced that the OH group at C-6 also participated the H_2O elimination process, which is in agreement with what observed by desorption chemical ionization mass spectrometry [21]. Thus, the CH_3OH and H_2O losses were more pronounced in the case of the 6β-epimer than that of the 6α-epimer. Therefore, the MS/MS spectra of the $[M+Na]^+$ ion and the relative abundances of diagnostic ions may be an effective tool to differentiate homogeneous isomers.

As precursor, the sodiated SD ion (at *m*/*z* 413) produced eight primary fragments in MS/MS (Table 1), which were similar with those of SME and DAME. However, the $[M+Na-anhydroglucose]^+$ ion at *m*/*z* 251 exhibited a very low tendency for it was instable and tended to form the lactone-type configuration by losing a molecular H_2O. Comparison the structure of SD with those of SME and DAME, the only difference was the substituent group at C-4. The former was a COOH group, and the latter was a $COOCH_3$ group at C-4. The relatively distinct difference represented that the sodiated SD was more tended to form a lactone at C-4 by the loss of H_2O than that of SME and DAME by the elimination of CH_3OH. The fragmentation pathway of SD was shown in Fig. 3.

3.3. Analysis of the H. diffusa extract

The LC/MS profile of the *H. diffusa* extract showed 11 peaks, which were identified as IGs for having characteristic UV spectra and MS fragmentation behaviors as reference compounds and literatures reported (Table 2). Among them, four pairs of isomers were observed: peaks 1 and 3, peaks 4 and 6, peaks 5 and 8, and peaks 10 and 11. Peaks 3, 5 and 8 were identified as SD, SME and DAME, respectively based on their retention time and mass spectra information. Peaks 1, 2, 10 and 11 were tentatively identified. The proposed structures of these compounds were shown in Fig. 4.

Table 1
Positive ESI-MS data for the standards of SME, DAME and SD

Compound	$[M+Na]^+$ (*m*/*z*) (% base peak)	$[M+2Na]^+$ (*m*/*z*) (% base peak)	MS/MS fragments of $[M+Na]^+$ (*m*/*z*) (% base peak)										
			$[M+Na-H_2O]^+$	$[M+Na-CH_3OH]^+$	$[M+Na-H_2O-CO_2]^+$	$[M+Na-Agl]^+$	$[M+Na-Agl-H_2O]^+$	$[M+Na-Agl-CH_3OH]^+$	$[M+Na-glucose-H_2O]^+$	$[M+Na-glucose-CH_3OH]^+$	$[Agl+Na-H_2O-CO_2]^+$	$[glucose+Na]^+$	$[Agl+Na-CH_3-OH-CO_2]^+$	$[Aglc+Na]^+$
SME	427 (26.1)	831 (100)	409 (3.3)	395 (23.8)	–	265 (100)	247 (71.9)	233 (66.2)	229 (18.6)	215 (21.7)	–	203 (32.7)	189 (10.1)	185 (12.1)
DAME	427 (89.5)	831 (100)	–	395 (2.2)	–	265 (100)	247 (7.3)	233 (12.6)	229 (0.9)	215 (7.4)	–	203 (2.5)	189 (1.9)	185 (1.2)
SD	413 (100)	803 (86.0)	395 (100)	–	351 (2.4)	251 (0.7)	233 (89.3)	–	215 (5.1)	–	189 (1.1)	203 (6.8)	–	185 (3.7)

Agl: aglycone; Aglc: anhydroglucose.

Table 2
Positive ESI-MS data of IGs from *H. diffusa*

Peak	$[M+Na]^+$ (m/z) (% base peak)	$[2M+Na]^+$ (m/z) (% base peak)	MS/MS fragments of $[M+Na]^+$ (m/z) (% base peak)													
			$[M+Na-H_2O]^+$	$[M+Na-CH_3OH]^+$	$[M+Na-H_2O-CH_3COOH]^+$	$[M+Na-CO_2]^+$	$[M+Na-H_2O-CO_2]^+$	$[M+Na-Aglc]^+$	$[M+Na-H_2O-Aglc]^+$	$[M+Na-Aglc-CH_3OH]^+$	$[M+Na-Aglc-CO_2]^+$	$[M+Na-glucose-H_2O]^+$	$[M+Na-glucose-CH_3OH]^+$	$[glucose+Na]^+$	$[Agl+Na-CH_3OH-CO_2]^+$	$[Aglc+Na]^+$
1 (DAA)	413 (65.4)	803 (100)	395 (59.1)	–	–	369 (0.7)	351 (0.5)	251 (1.2)	233 (100)	–	–	215 (11.2)	–	203 (3.3)	–	185 (12.4)
2 (DA)	395 (81.5)	767 (100)	–	–	–	–	333 (0.6)	233 (100)	215 (7.1)	–	189 (0.9)	–	–	203 (6.1)	–	185 (3.0)
3 (SD)[a]	413 (100)	803 (86.0)	395 (100)	–	–	369 (0.1)	351 (2.4)	251 (0.7)	233 (89.3)	–	–	215 (5.1)	–	203 (6.8)	–	185 (3.7)
4	427 (100)	831 (0.8)	409 (21.5)	395 (8.8)	–	–	–	265 (100)	247 (35.9)	233 (70.4)	–	229 (8.6)	215 (34.0)	203 (20.5)	189 (14.5)	185 (17.8)
5 (SME)[a]	427 (26.1)	831 (100)	409 (3.3)	395 (23.8)	–	–	–	265 (100)	247 (71.9)	233 (66.2)	–	229 (18.6)	215 (21.7)	203 (32.7)	189 (10.1)	185 (12.1)
6	427 (100)	831 (20.3)	409 (100)	395 (7.3)	–	–	–	265 (8.0)	247 (5.1)	233 (27.8)	–	229 (1.0)	215 (3.6)	203 (9.3)	189 (0.8)	185 (5.6)
7	559	–	541 (25.1)	–	–	–	–	397 (90.2)	379 (100)	–	353 (45.9)	361 (0.9)	–	203 (6.2)	–	185 (0.9)
8 (DAME)[a]	427 (89.5)	831 (100)	409 (0.3)	395 (2.2)	–	–	–	265 (100)	247 (7.3)	233 (12.6)	–	229 (0.9)	215 (7.4)	203 (2.5)	189 (1.9)	185 (1.2)
9	443 (100)	863 (43.6)	425 (3.0)	411 (1.5)	–	–	381(100)	281 (31.0)	263 (5.0)	249 (7.2)	–	–	231 (1.1)	203 (46.8)	–	185 (7.5)
10 (AA)[a]	455 (100)	887 (17.7)	437 (16.7)	–	377 (0.4)	411 (3.3)	393(0.1)	293 (1.5)	275 (100)	–	249 (9.8)	257 (0.2)	–	203 (6.6)	–	185 (3.1)
11 (ASD)[a]	455 (100)	887 (45.6)	437 (21.8)	–	377 (1.4)	411 (1.6)	393(0.9)	293 (3.2)	275 (100)	–	249 (0.2)	257 (2.3)	–	203 (2.2)	–	185 (6.5)

Agl: aglycone; Aglc: anhydroglucose; DAA: deacetylasperuloside acid; DA: deacetylasperuloside; AA: aseruloside acid; ASD: 10-acetyl scandoside.
[a] Positively identified.

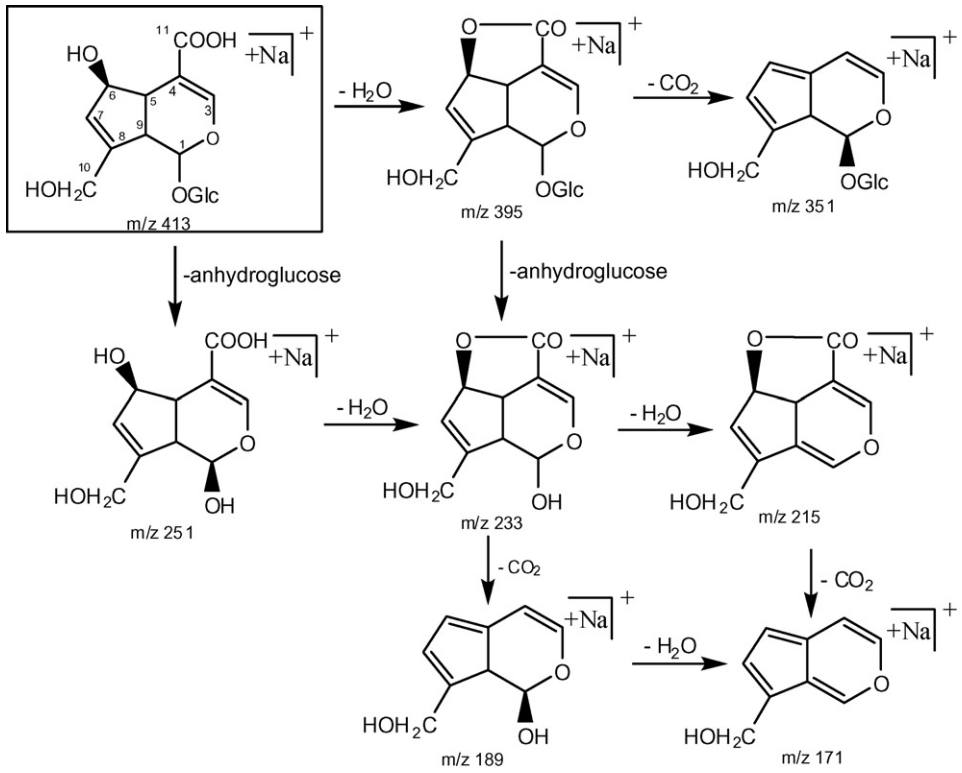
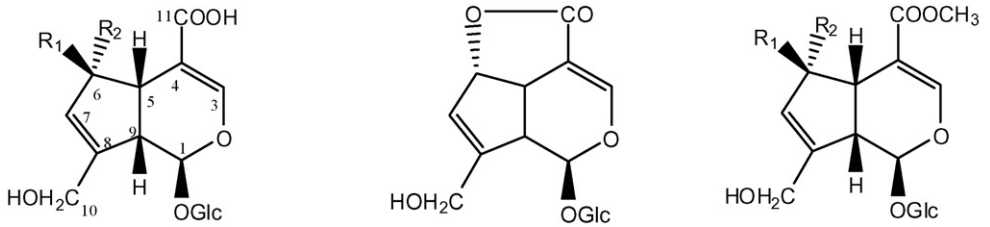


Fig. 3. The proposed fragmentation pathway of SD in PI mode.

Peak 1 was deduced as an isomer of the standard of SD for they have identical MS and MS/MS data (Table 2). The relative abundance of the product ion $[M+Na–H_2O]^+$ at *m*/*z* 395 is different between those of peaks 1 and 3 (SD). It is probably because the OH group at C-6 of peak 1 is below the plane, which resulted in the more difficult loss of H_2O to form a lactone than SD. Thus, it was deduced that peak 1 might be an αOH-epimer of SD, i.e. peaks 1 and 3 are probably 6α-OH and 6β-OH epimers, respectively. Based on the literature [22], deacetylasperuloside acid has been isolated from *H. diffusa* and its structure was just accorded with our deducted structure of peak 1.

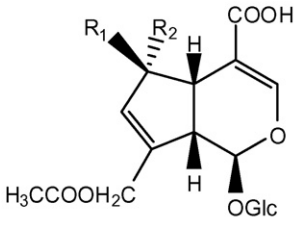
The isomers of peak 10 and 11 have almost identical fragmentation patterns (Table 2), which reflected the similarities of their



1: R_1=H, R_2=OH
3 (**SD**): R_1=OH, R_2=H

2

5 (**SME**): R_1=OH, R_2=H
8 (**DAME**): R_1=H, R_2=OH



10: R_1=H, R_2=OH
11: R_1=OH, R_2=H

Fig. 4. Structures of the standards and tentatively identified analyte compounds.

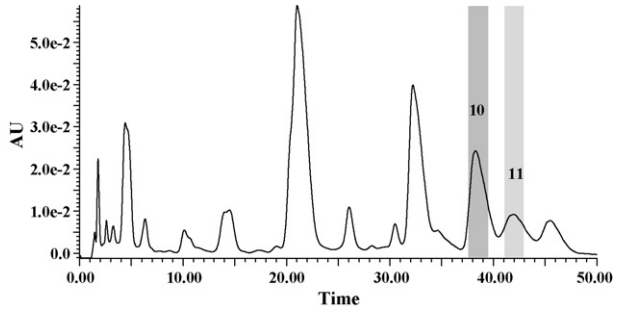


Fig. 5. Chromatogram of the *H. diffusa* extract performed by Autopurification System.

structures. Based on the differences of the relative abundances of diagnostic ion $[M+Na-H_2O]^+$ at *m*/*z* 437, as well as the sequence of retention time, peaks 10 and 11 were presumed as α- and β-epimers, respectively. On the basis of the previous publications, peaks 10 and 11 were tentatively identified as aseruloside acid and 10-acetyl scandoside, respectively, and both have been isolated from *H. diffusa* [22,23].

The sodiated ion of peak 2 was *m*/*z* 395, so its MW was confirmed as 372 Da. The fragmentation of peak 2 was very similar with peaks 1 and 3. Considering the difference of 18 Da between their MWs, it was presumed that peak 2 was the lactone-type IG, resulting from peaks 1 or 3 by the neutral loss of H_2O. Deacetylasperuloside has been isolated from *H. Chrysotricha* [14], the same genus plant with *H. diffusa*. Thus, peak 2 was deduced as deacetylasperuloside.

3.4. Preparation the IGs from the extract of H. diffusa

The preparative isolation chromatogram by AutoPurification System was shown in Fig. 5. The pure compounds of peaks 10 and 11 were obtained with marked parts of grey-white print and identified with Q-TOF MS, UV, ^1H and ^{13}C NMR analysis, but the pure compounds of the other unidentified peaks could not be obtained due to their minor contents or unefficient resolution. The spectral data of peaks 10 and 11 were as followed.

Compound 10: white amorphous powder; UV(MeOH–H_2O)λ_{max} 236.2 nm; ^1H NMR (D_2O, 400MHz): δ7.75 (1H, s, H-3), 6.12 (1H, s, H-7), 5.02 (1H, d, *J* = 9.0 Hz, H-1), 4.91 (1H, m, H-10), 3.13 (1H, t, *J* = 6.5 Hz, H-9), 2.77 (1H, t, *J* = 8.2 Hz, H-5), 2.16 (3H, s, Ac-CH_3); ^{13}C-NMR (D_2O, 100 MHz): δ176.66 (MeCO–), 173.49 (C-11), 158.05 (C-3), 146.68 (C-8), 133.68 (C-7), 109.13 (C-4), 102.98 (C-1), 101.56 (C-1′), 78.82 (C-5′), 78.31 (C-3′), 76.66 (C-6), 75.46 (C-2′), 72.12 (C-4′), 65.62 (C-10), 63.42(C-6'), 47.09 (C-9), 42.78 (C-5), 20.89 (–CH_3). HRESIMS (PI mode) *m*/*z* 455.1157 $[M+Na]^+$ (calcd for $C_{18}H_{24}O_{12}$ Na, 455.1165).

Compound 11: white amorphous powder; UV(MeOH–H_2O) λ_{max} 236.2 nm; ^1H NMR (D_2O, 400 MHz): δ7.55 (1H, s, H-3), 5.95 (1H, s, H-7), 5.42 (1H, d, *J* = 5.4 Hz, H-1), 4.82 (1H, s, H-10), 4.66 (1H, s, H-6), 3.08 (1H, dd, *J* = 3.3, 3.4 Hz, H-9), 2.16 (3H, s, Ac-CH_3); ^{13}C-NMR (D_2O, 100 MHz): δ176.58 (MeCO–), 173.66 (C-11), 155.74 (C-3), 143.37 (C-8), 134.33 (C-7), 111.83 (C-4), 101.22 (C-1), 98.97 (C-1′), 82.79 (C-5′), 78.83 (C-3′), 78.19 (C-6), 75.25 (C-2′), 72.09 (C-4′), 64.84 (C-10), 63.28 (C-6′), 48.52 (C-9), 45.01 (C-5), 22.88 (–CH_3). HRESIMS (PI mode) *m*/*z* 455.1155 $[M+Na]^+$ (calcd for $C_{18}H_{24}O_{12}$Na, 455.1165).

Compared above data with the literatures [14,24], peak 10 was identified as aseruloside acid and peak 11 was identified as 10-acetyl scandoside, which were consistent with our structural deduction by the MS fragmentation information.

4. Conclusions

For the first time, we present a systematic HPLC-DAD-ESI-MS/MS investigation for the IGs from *H. diffusa*. The optimized separation condition was obtained with the CSASS software, under which the complete resolution of analytes was achieved. It is very important for the isomers to avoid the chromatograms overlapped damaging the veracity of UV and mass spectra information. Based on the UV and mass spectra of the standards SME, DAME and SD, eleven IGs were screened out, including four pairs of isomers. Considering the fragmentation patterns of the three standards, especially those of the isomeric standards, seven IGs including three pairs of isomers were unambiguous/tentatively identified. The fragmentation rule was validated by the isolated standards of IGs and was considered to be suitable for the rapid characterization of IGs in crude and partially purified samples.

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References

[1] R. Ahmad, A.M. Ali, D.A. Israf, N.H. Ismail, K. Shaari, N.H. Lajis, Life Sci. 76 (2005) 1953–1964.
[2] S.H. Kim, B.Z. Ahn, S.Y. Ryu, Phytother. Res. 12 (1998) 553–556.
[3] C.C. Lin, L.T. Ng, J.J. Yang, Y.F. Hsu, Am. J. Chin. Med. 30 (2002) 225–234.
[4] B.E. Shan, Y. Yoshida, T. Sugiura, U. Yamashita, Int. J. Immunopharmacol. 21 (1999) 149–159.
[5] S. Gupta, D. Zhang, J. Yi, J. Shao, J. Herb. Pharmacother. 4 (2004) 21–33.
[6] C.M. Lu, J.J. Yang, P.Y. Wang, C.C. Lin, Planta Med. 66 (2000) 374–377.
[7] Y. Kim, E.J. Park, J. Kim, Y.B. Kim, S.R. Kim, Y.C. Kim, J. Nat. Prod. 64 (2001) 75–78.
[8] D.H. Kim, H.J. Lee, Y.J. Oh, M.J. Kim, S.H. Kim, T.S. Jeong, N.I. Baek, Arch. Pharm. Res. 28 (2005) 1156–1160.
[9] E.L. Ghisalberti, Phytomedicine 5 (1998) 147–163.
[10] S. Mandal, R. Jain, S. Mukhopadhyay, Indian J. Pharm. Sci. 60 (1998) 123–127.
[11] Y. Nishihama, K. Masuda, M. Yamaki, S. Takagi, K. Sakina, Planta Med. 43 (1981) 28–33.
[12] H. Wu, X. Tao, Q. Chen, X. Lao, J. Nat. Prod. 54 (1991) 254–256.
[13] M. Ono, N. Ishimatsu, C. Masuoka, H. Yoshimttsu, R. Tsuchihashi, M. Okawa, J. Kinjo, T. Ikeda, T. Nohara, Chem. Pharm. Bull. 55 (2007) 632–634.
[14] J.N. Peng, X.Z. Feng, G.Y. Li, X.T. Liang, Acta Pharmacol. Sin. 32 (1997) 908–913 (in Chinese).
[15] D.Y. Zhou, Q. Xu, X.Y. Xue, F.F. Zhang, X.M. Liang, J. Pharm. Biomed. Anal. 42 (2006) 441–448.
[16] Y. Jin, X.Y. Xue, Y.F. Liu, Y.S. Xiao, J. Zhang, H. Shi, F.F. Zhang, X.M. Liang, J. Chromatogr. A 1183 (2008) 76–86.
[17] C.H. Xiao (Ed.), Traditional Chinese Medicinal Chemistry, Shanghai Science and Technology Press, Shanghai, 1996, pp. 444.
[18] K.P. Madhusudanan, K. Raj, A.P. Bhaduri, J. Mass Spectrom. 35 (2000) 901–911.
[19] N.E. Es-Safi, L. Kerhosa, P.H. Ducrot, Rapid Commun. Mass Spectrom. 21 (2007) 1165–1175.
[20] K.P. Madhusudanan, V.T. Mathad, K. Shefali, A.P. Raj, Bhaduri, J. Mass Spectrom. 35 (2000) 321–329.
[21] P.A. Demirev, N. Handjieva, H. Saadi, S.S. Popov, O.S. Reshetova, B.V. Rozynov, Org. Mass Spectrom. 26 (1991) 151–153.
[22] S. Takagi, M. Yamaki, Y. Nishihama, K. Ishiguro, Shoyakugaku Zasshi 36 (1982) 366–369.
[23] Y.B. Yang, X.Q. Yang, Z.T. Ding, J. Yunnan Univ. 29 (2007) 187–189.
[24] H. Inouye, S. Inouye, N. Shimokaw, Chem. Pharm. Bull. 17 (1969) 1942–1947.