Analyses of the Iridoid Glucoside Dimers in *Paederia scandens* Using HPLC–ESI–MS/MS

Chen-Ceng Lu, Jian-Hua Wang, Dong-Mei Fang, Zhi-Jun Wu and Guo-Lin Zhang

**ABSTRACT:**

Introduction – Many dimers consisting of structurally similar monomers are difficult to be identified even using NMR. Rapid structural identification of iridoid glycoside dimers, especially isomeric dimers in a complicated matrix, remains desirable. Objective – To develop a rapid, sensitive analytical method for structural elucidation of trace iridoid glycoside isomers in a complicated extract.

Methods – Three isomeric iridoid glucoside dimers, paederoscandoside, saprosmoside E and saprosmoside D, were isolated and further analysed by electrospray ionisation quadrupole time-of-flight tandem mass spectrometry (ESI–QTOF–MS/MS) in positive-ion mode. Energy-resolved mass spectrometry (ERMS) was used to provide information on the relative intensity of ions versus collision energy. The crude extract of *Paederia scandens* was analysed by HPLC–ESI–MS/MS.

Results – The relative abundance of product ions in the MS/MS spectra from ammonium adduct ions varied greatly for the three isomers. The energy-resolved experiments enhanced differences among the three isomers. A total of 13 iridoid glucoside dimers (three groups of isomers) in the extract of *P. scandens* were identified or tentatively characterised by using HPLC–ESI–QTOF based on the tandem mass spectra of references.

Conclusion – Linkage sites between different hydroxyl groups on the sugar and carboxyl groups for the three groups of isomers are confirmed. The reason for fragmentation differences might be that cleavage of the glycosidic bond accompanies the active H in vicinal hydroxyl rearrangement. The MS method is a useful tool for the analyses of isomers, especially trace isomers in a complicated extract. Copyright © 2013 John Wiley & Sons, Ltd.

Keywords: ESI–QTOF–MS/MS; fragmentation; iridoid glucoside dimmer; isomer; Paederia scandens

**Introduction**

Iridoids are secondary metabolites of terrestrial and marine flora and fauna, and are found in plenty of plant families, usually as glycosides (Dinda *et al.*, 2007). Iridoid glycosides have various biological activities, including anti-tumour, anti-inflammatory, hepatoprotective, anti-tussive, analgesic, anti-oxidant and treatment of skin diseases (Kapadia *et al.*, 1996; Konoshima *et al.*, 2000; Wang and Huang, 2005; Chen *et al.*, 2008). Structural elucidation of the natural products is the key to study their bioactivity. However, some iridoid glycosides, especially dimmers, are difficult to identify (Quang *et al.*, 2002). Nuclear magnetic resonance (NMR) spectroscopy can offer more structural information for the identification of iridoid glycosides (Ling *et al.*, 2002; Otsuka, 2002; Quang *et al.*, 2002). However, low sensitivity limits the application of NMR coupled with high-performance liquid chromatography (HPLC) for analysis of trace compounds in complex matrices. In addition, some oligomers consisting of structurally similar monomers are difficult to be identified even using NMR (Karchesy *et al.*, 1986; Li and Deinzer, 2007; Wu *et al.*, 2008). The reason is that the $^1$H- and $^{13}$C-NMR spectra might overlap and influence the quality of corresponding spectra. Thus, a rapid and sensitive method for the confirmation of iridoid glycosides, especially dimmers, in a complicated mixture remains desirable.

Mass spectrometry (MS) has been an important physicochemical method for the identification of trace natural products in extracts due to its rapidity, sensitivity, and low levels of sample consumption. A series of iridoid glycosides was studied by ESI–MS/MS (Es-Saft *et al.*, 2007; Ren *et al.*, 2007; Zhou *et al.*, 2007; Li *et al.*, 2008; Cao *et al.*, 2012; Lu *et al.*, 2013). In this study, HPLC–ESI–MS/MS was applied for the phytochemical analysis of the extract of *Paederia scandens*. A total of 13 iridoid glucoside dimers (Fig. 1) containing three groups of isomers were identified or tentatively characterised based on MS/MS spectra of references.

**Experimental**

**Materials and reagents**

*Paederia scandens* (Lour) Merrill was purchased from Weite drugstore in Chengdu. It is a climbing plant in the madder family, Rubiaceae. The plant was identified by Jian Gu at Chengdu Institute of Biology, Chinese Academy of Sciences (Chengdu, China). Dried powder of the stem of *P. scandens* was

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first extracted by 70% acetone–water, and then concentrated under reduced pressure to evaporate the acetone; the aqueous phase was further extracted by n-butanol after washing with dichloromethane. The n-butanol extraction phase was concentrated and dried by rotary evaporator to obtain the crude extract. The crude extract was purified by gel chromatography, silica gel chromatography and HPLC preparative chromatography respectively to obtain three iridoid glucoside dimers, that is, paederoscandoside, saprosmoside E and saprosmoside D. Paederoscandoside and saprosmoside E were characterised by 1H- and 13C-NMR spectra, and saprosmoside D by the 1H-NMR spectrum according to reported references (Ling et al., 2002; Otsuka, 2002; Quang et al., 2002). Ultrapure water was prepared using an ultrapure water system from Aquapro (Chongqing, China). HPLC grade methanol and acetonitrile were purchased from Fisher Scientific (Pittsburgh, PA, USA). Analytical reagent grade ammonium acetate was purchased from Bodi (Tianjin, China).

Mass spectrometry
High-resolution experiments were performed on a Bruker microTOF-Q mass spectrometer (Bremen, Germany) with an electrospray ionisation (ESI) source in positive-ion mode. The capillary voltage was maintained at $-4500\text{V}$ with the end plate offset at $-500\text{V}$. Nitrogen was used as the drying and nebulising gases at a flow rate of $4.0\text{L/min}$ and a pressure of $0.3\text{bar}$, respectively. Dry gas temperature was maintained at $180\text{°C}$. Argon was introduced into the collisional cell as the collision gas. The sample introduction rate was $180\mu\text{L/h}$. Samples were dissolved in methanol and ammonium acetate was added into solutions to improve the intensity of $[\text{M} + \text{NH}_4]^+$. In the MS/MS mode, collision energies of $8–16\text{eV}$ and isolation width of $3\text{amu}$ were used for collision-induced dissociation (CID). The mass data were processed with Bruker Compass DataAnalysis 4.0.

High performance liquid chromatography
The LC analyses were performed on an Agilent 1100 HPLC system (Palo Alto, CA, USA) equipped with an autosampler, a quaternary pump, a thermostated column compartment, and a diode-array detector (DAD). A Phenomenex Kinetex C$_{18}$-column ($2.1 \times 100\text{mm}, 2.6\mu\text{m}$) (Torrance, CA, USA) was used for separation and was maintained at $25\text{°C}$. Mobile phase A (water containing 0.1% ammonium acetate), mobile phase B (acetonitrile) and mobile phase C (methanol) were in gradient mode ($T_{\text{min}}$ A:B:C $T_0$ 95:50, $T_5$ 92:80, $T_{10}$ 75:12:13, $T_{20}$ 55:20:25, $T_{40}$ 40:60:0). The injection volume was $10\mu\text{L}$ and the flow rate was set at 0.2 mL/min.

Method optimisations of HPLC
Methanol–ammonium acetate aqueous solution or acetonitrile–ammonium acetate aqueous solution as the mobile phase on the separation were investigated. The iridoid glucoside dimers cannot be separated...
by methanol–water or acetonitrile–water. When the three-phase system, methanol–acetonitrile–0.1% ammonium acetate aqueous solution was used as the mobile phase, the dimers were clearly separated and their retention times were appropriate.

Results and Discussion

Fragmentation of precursor ion [M + NH₄]⁺ for references

Paederoscandoside, saprosmoside E and saprosmoside D are isomers. The main difference is in the linkage sites of their two moieties. The paederosidic acid moiety is linked to the glucose of paederoside moiety at 6-OH, 2-OH, 3-OH in paederoscandoside, saprosmoside E and saprosmoside D, respectively (Otsuka, 2002; Quang et al., 2002; Ling et al., 2002).

The ammonium adduct ions, [M + NH₄]⁺ at m/z 910, were selected as the precursor ion for the product ion scans. The CID mass spectra of the three dimers are shown in Fig. 2. Interestingly, the MS/MS spectra of the three isomers are different, although their structures are very similar. For saprosmoside D, the fragmentation experiment provided abundant product ions. The accurate masses and elemental compositions of major product ions from saprosmoside D are shown in Table 1. In contrast, only a product ion at m/z 603 was clearly observed in the MS/MS spectra of saprosmoside E. Other product ions are negligible. For paederoscandoside, the main product ion is the ion at m/z 603. The product ions at m/z 713, 267 and 175 show low relative abundance. Thus, the relative abundance of most product ions varied greatly, although only the linkage sites are different for the three references. Energy-resolved experiments (Kurimoto et al., 2006; Sun et al., 2010) were performed. Charts of relative intensities of precursor and product ions versus collision energies are provided (Supporting information) that further indicate the differences of their CID MS/MS spectra.

Analyses of dimers in the extract of P. scandens by HPLC–ESI–MS/MS

The extract of P. scandens was investigated using HPLC–ESI–QTOF and a total of 13 iridoid glucoside dimers (three groups of isomers) were identified or tentatively characterised based on tandem mass spectra of references. Linkage sites between different hydroxyl groups on the sugar and carboxyl groups are confirmed. Possible causes for the fragmentation differences of compounds 1–4 were elucidated.

Structural analysis of compounds 1–4

Compounds 1–4 are isomers. The [M + NH₄]⁺ ions at m/z 910 for compounds 1–4 are observed and corresponding molecular formula is confirmed as C₃₆H₄₄O₂₂S₂ by accurate masses. Compounds 1–3 are confirmed as paederoscandoside, saprosmoside D and saprosmoside E respectively, according to MS/MS spectra and retention time (Fig. 2 and Supporting information). The major

Figure 2. The MS/MS spectra of the ions at m/z 910 (collision energy: 10 eV) for: (a) paederoscandoside, (b) saprosmoside D, (c) saprosmoside E and (d) paederoside B.
Product ions in the MS/MS spectrum of compound 4 are similar to that of compound 2, only the relative abundance is different. This indicates that the structure of 4 is similar to that of saprosmoside D and also condensed from paederoside and paederosidic acid. Compound 4 should be paederoside B (Zou et al., 2006), the paederosidic acid moiety linked to the glucose of the paederoside moiety at 4-OH. Interestingly, the product ion at \( m/z \) 603 shows high abundance in the MS/MS spectra of compounds 1–3, but is very low for compound 4.

### Structural analysis of compounds 5–8

The accurate masses of the \([M + NH_4]^+\) ions for compounds 5–8 provide the molecular formula \( C_{36}H_{46}O_{23}S_2 \), only one H2O molecule different from compounds 1–4. Similar profiles of product ions from compounds 5–8 (Fig. 3) to those from compounds 1–4 were observed. These indicate that compounds 5–8 are isomers and are condensed by two paederosidic acid moieties. The MS/MS spectra of compounds 5 and 1 are very

### Table 1. Elemental constituents of major product ions from \([M + NH_4]^+\) for saprosmoside D (collision energy: 10 eV)

<table>
<thead>
<tr>
<th>Ions</th>
<th>Calculated</th>
<th>Observed</th>
<th>Formula</th>
<th>Error (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>([M + NH_4]^+)</td>
<td>910.2104</td>
<td>910.2142</td>
<td>( C_{36}H_{48}NO_{22}S_2 )</td>
<td>-4.2</td>
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<tr>
<td>([M + H – H_2O]^+)</td>
<td>875.1733</td>
<td>875.1779</td>
<td>( C_{20}H_{23}O_{17}S_2 )</td>
<td>-5.3</td>
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<tr>
<td>([M + H – C_6H_{10}O_5 – H_2O]^+)</td>
<td>713.1205</td>
<td>713.1224</td>
<td>( C_{20}H_{23}O_{17}S_2 )</td>
<td>-2.8</td>
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<tr>
<td>([M + H – C_6H_{10}O_5 – H_2O – CH_3SCOOH]^+)</td>
<td>621.1273</td>
<td>621.1291</td>
<td>( C_{20}H_{23}O_{17}S_2 )</td>
<td>-3.0</td>
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<tr>
<td>([M + H – 2H_2O – CH_3SCOOH]^+)</td>
<td>603.1167</td>
<td>603.1181</td>
<td>( C_{20}H_{23}O_{17}S_2 )</td>
<td>-2.3</td>
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<tr>
<td>([M + H – C_6H_{12}O_6S – H_2O]^+)</td>
<td>591.1378</td>
<td>591.1400</td>
<td>( C_{20}H_{23}O_{17}S_2 )</td>
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<tr>
<td>([M + H – C_6H_{12}O_6S – C_6H_{12}O_6]^+)</td>
<td>429.0850</td>
<td>429.0840</td>
<td>( C_{10}H_{21}O_{10}S )</td>
<td>2.2</td>
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<td>([M + H – C_6H_{12}O_6S – C_6H_{12}O_6 – H_2O]^+)</td>
<td>411.0744</td>
<td>411.0753</td>
<td>( C_{10}H_{21}O_{10}S )</td>
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<td>([M + H – C_6H_{12}O_6S – C_6H_{12}O_6 – CH_3SCOOH]^+)</td>
<td>337.0918</td>
<td>337.0916</td>
<td>( C_{10}H_{25}O_{9}S )</td>
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<td>([M + H – C_6H_{12}O_6S – C_6H_{12}O_6 – CH_3SCOOH – H_2O]^+)</td>
<td>319.0812</td>
<td>319.0813</td>
<td>( C_{10}H_{25}O_{9}S )</td>
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<tr>
<td>([M + H – C_6H_{12}O_6S – C_6H_{12}O_6 – CH_3SCOOH – H_2O]^+)</td>
<td>285.0427</td>
<td>285.0428</td>
<td>( C_{12}H_{19}O_{8}S )</td>
<td>0.4</td>
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<tr>
<td>([M + H – C_6H_{12}O_6S – C_6H_{12}O_6 – CH_3SCOOH – H_2O]^+)</td>
<td>267.0322</td>
<td>267.0330</td>
<td>( C_{12}H_{19}O_{8}S )</td>
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<tr>
<td>([M + H – C_6H_{12}O_6S – C_6H_{12}O_6 – CH_3SCOOH – H_2O]^+)</td>
<td>193.0495</td>
<td>193.0494</td>
<td>( C_{12}H_{19}O_{8}S )</td>
<td>0.9</td>
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<td>([M + H – C_6H_{12}O_6S – C_6H_{12}O_6 – CH_3SCOOH – H_2O]^+)</td>
<td>175.0390</td>
<td>175.0395</td>
<td>( C_{12}H_{19}O_{8}S )</td>
<td>-3.0</td>
</tr>
</tbody>
</table>

Figure 3. MS/MS spectra of the ions at \( m/z \) 928 (collision energy: 10 eV) for: (a) compound 5, (b) saprosmoside F, (c) dimer of paederosidic acid and (d) compound 8.
similar, which indicates that the linkage site of two moieties for compounds 5 and 1 is the same (6-OH). Similarly, the linkage site of two moieties for compounds 6 and 4, 7 and 2, or 8 and 3 should be the same by comparing Figs 2 and 3. Compounds 5, 6, 7 and 8 are condensed from two paederosidic acid moieties at 6-OH, 4-OH, 3-OH and 2-OH, respectively. Compound 6 is saprosmoside F (Ling et al., 2002) and 7 is a dimer of paederosidic acid (Quang et al., 2002).

**Structural analysis of compounds 9–13**

Compounds 9–13 are isomers and the accurate masses of the [M + NH4]⁺ ions for compounds 9–13 correspond to the molecular formula C36H46O23S, only one S atom is different from compounds 1–4. Compound 11 was reported in our previous work and condensed from paederoside and asperulosidic acid (Wu et al., 2013). However, the linkage site cannot be confirmed.

It was found that the asperulosidic acid moiety is linked to the glucose of the paederoside moiety at 3-OH affording compound 11 by comparing Figs 4c and 2b. The higher relative abundance of the product ion at m/z 603 than that of m/z 571 indicates that the loss of CH₃COOH of asperulosidic acid is ready to occur compared with the loss of CH₃SCOOH of paederoside. If the compound is condensed from paederosidic acid and asperuloside, the loss of CH₃SCOOH would occur readily (Wu et al., 2013). In other words, relative abundance of the product ion at m/z 571 will be higher than that of m/z 603. By comparing Figs 4 and 2, compound 9 and compound 12 are condensed by the carboxyl group of paederosidic acid and the glucose of the asperuloside moiety at 6-OH and 2-OH, respectively. The asperulosidic acid moiety is linked to the glucose of the paederoside moiety at 6-OH in compound 10. The MS/MS spectrum of compound 13 (Fig. 4) is very similar to that of 11. The two compounds might be stereoisomers.

**Figure 4.** MS/MS spectra of the ions at m/z 878 (collision energy: 10 eV) for: (a) compound 9, (b) compound 10, (c) compound 11, (d) compound 12, and (e) compound 13.
Possible causes for fragmentation differences of compounds 1–4

The cleavage of glycosidic bond and the losses of H₂O and CH₃SCOOH are the main fragmentation patterns for isomers 1–4. Cleavage of the first glycosidic bond is ready to occur for every compound. Thus, the product ion at m/z 713 can be clearly observed (Scheme 1). Cleavage of the second glycosidic bond occurs readily for compound 2 rather than 3. The product ion at m/z 429 resulting from m/z 713 is formed by cleavage of the second glycosidic bond (Scheme 1). The reason is that cleavage of the glycosidic bond accompanies the active H on hydroxyl rearrangement (Es-Safi et al., 2005; Wu et al., 2009). However, the 2-OH is esterified for compound 3. The cleavage of an ester bond linking the two moieties, other than a glycosidic bond, is a main fragmentation pattern for compound 4, affording the product ion at m/z 429 (Supporting information). It further produces the ions at m/z 285 and m/z 175 by the cleavage of glycosidic bond. The higher relative abundance of m/z 175 in the MS/MS spectrum of compound 4 is consistent with the fact that the loss of sugar by cleavage of the glycosidic bond occurs more readily.

Supporting Information

Supporting information can be found in the online version of this article.

Acknowledgements

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