8,4′-Oxyneolignane glucosides from an aqueous extract of “ban lan gen” (*Isatis indigotica* root) and their absolute configurations

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**KEY WORDS**
Cruciferae; *Isatis indigotica*; Isatioxyneolignosides A–F; 8,4′-Oxyneolignane; Threo/erythro Isomers; $\Delta\delta_{C8-C7}$ value; Absolute configuration; Cotton effect

**Abstract** Three pairs of glycosidic 8,4′-oxyneolignane diastereoisomers, named isatioxyneolignosides A–F (1–6), were isolated from an aqueous extract of *Isatis indigotica* roots. Their structures and absolute configurations were elucidated by comprehensive spectroscopic data analysis and enzyme hydrolysis. The validity of $\Delta\delta_{C8-C7}$ values to distinguish threo and erythro aryl glycerol units and Cotton effects at 235±5 nm to determine absolute configurations at C-8 in 1–6 and their aglycones (1a–6a) are discussed.

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1. Introduction

“Ban lan gen”, the root of *Isatis indigotica* Fort. (Cruciferae), is an important traditional Chinese medicine for the treatment of fever and influenza. This herbal medicine has been pharmacologically and chemically investigated for more than half century. However, previous studies were predominated by extracting “ban lan gen” with ethanol or methanol, which alters a practical application by decocting with water. As part of a program to assess the chemical diversity and biological activity of traditional Chinese medicines, our investigation on an aqueous extract of “ban lan gen” led to discovery of various new constituents with different biological activities. Herein, we report isolation and structure characterization of six new 8,4'-oxyneolignanes (1–6, Fig. 1) from the same decoction. In addition, determination of the absolute configuration of 8,4'-oxyneolignane derivatives is discussed by analysis of NMR spectroscopic and circular dichroism (CD) data in combination with electronic CD (ECD) calculations.

2. Results and discussion

Compound 1, a white amorphous powder with [α]20D +5.2 (c 0.12, MeOH), showed IR absorption bands for hydroxyl (3390 cm⁻¹), conjugated carbonyl (1644 cm⁻¹), and aromatic ring (1600 and 1512 cm⁻¹) functionalities. The molecular formula C24H30O13 of 1 was determined by (+)-HR-ESI-MS at m/z 549.1594 [M + Na]⁺. The NMR spectroscopic data of 1 in DMSO-d6 (Tables 1 and 2) indicated the presence of two meta-para-disubstituted phenyls, two aromatic methoxy groups, an oxymethylene, and two oxy- methines, as well as a β-glucopyranosyl. However, in the NMR spectra, resonances for one of the two phenyls were significantly broadened (See Supplementary Information Figs. S46 and S47). Especially an expected coupling between two vicinal aromatic protons (J 1,6) was unresolved while the 13C NMR spectrum displayed two fewer carbon resonances (C-1' and C-7') than those expected from the molecular formula. Subsequently, the structure of 1 was further elucidated by acquisition and analysis of 2D NMR spectroscopic data.

The proton and proton-bearing carbon resonances in the NMR spectra of 1 were assigned unambiguously by interpretation of 1H-1H COSY and HSQC spectroscopic data. The HMBC spectrum of 1 showed two- and three-bond correlations from H-1' to C-4; from H-2 to C-1, C-3, C-4, and C-6; from H-5 to C-1, C-3, C-4, and C-6; from H-6 to C-2 and C-4; and from OCH3-3' to C-3 (Fig. 2). These correlations, together with the 1H-1H COSY cross-peaks of H-5/H-6/H-2 and H-1'/H-2'/H-3'/H-4'/H-5'/H-6' and chemical shifts of these proton and carbon resonances as well as the coupling constant values (7.8 Hz for J 1,2 and J 5,6), indicated the presence of a 4-β-glucopyranosylxylo-3-methoxyphenyl in 1. The HMBC correlations from both H-2 and H-6 to C-7; from H-7 to C-1, C-2, C-6, and C-8; from H-8 to C-1, C-7, and C-9; and from H-9 to C-7 and C-8; along with their chemical shifts and the 1H-1H COSY cross-peaks of H-7/H-8/H2-9, demonstrated that the 4-β-glucopyranosylxylo-3-methoxyphenyl connected to a glycerol unit to form a 4-β-glucopyranosylxylo-3-methoxyphenylglycerol moiety. In addition, the HMBC correlations from H-2' to C-4' and C-6'; from H-5' to C-3'; from H-6' to C-2' and C-4'; and from OCH3-3' to C-3', in combination with their chemical shifts and the 1H-1H COSY cross-peaks of H-5'/H-6', revealed that there was a 3'-methoxy-4'-oxyphenyl though these correlations had much less intensities and the proton and carbon resonances were broad in the spectra, along with the unobserved C-1' resonance and unresolved coupling (J 5,6). Meanwhile, the intensive HMBC correlation from H-8 to C-4' revealed that the 4-β-glucopyranosyloxy-3-methoxyphenylglycerol and 3'-methoxy-4'-oxyphenyl connected via an ether bond between C-8 and C-4'. The above deduction suggests that there must be a COOH unit at C-1′ in 1 to match requirement of the molecular formula and to explain the unobserved and broadened NMR resonances due to conjugation and/or dissociation of the COOH unit. Therefore, the gross structure of 1 was elucidated as guaiacylglycerol-8-vanillic acid 4-O-β-glucopyranoside, of which the aglycone with the threo or erythro configuration was reported and obtained also in this study. However, their absolute configurations were not determined in the literatures (according to nomenclature of lignans and neolignans recommended by IUPC, herein numberings 8 and 4 are used instead of 8' and 4' in the literatures).

Enzymatic hydrolysis of 1 with snailase afforded sugar and aglycone (1a). The sugar exhibited retention factor (Rf) on TLC, specific rotation [α]20D 51 or CD3OD. This suggested that 1 possessed the erythro configuration. The CD spectra of 1 and 1a were identical to those of an authentic D-glucose (see Experimental Section 4.3.7). The NMR spectroscopic data of 1a in CD2OD were different from those of threo guaiacylglycerol-8-vanillic acid in the same solvent, but highly consistent with those of the erythro isomer in acetone-d6 or CD2OD. This suggested that 1a possessed the erythro configuration. The CD spectra of 1 and 1a displayed negative Cotton effects at 229 and 236 nm (Fig. 3), respectively. Because the sign of Cotton effect at 235 ± 5 nm are validated for assignment of the C-8 configuration of the arylglycerol units in various 8,4′-oxyneolignans (positive for 8S and negative for 8R) 53-55, the 8R configuration was assigned for 1 and 1a. Therefore, the structure of compound 1 was determined as shown in Fig. 1 and designated as isatoxyneolignoside A.

Compound 2 was isolated as a white amorphous powder with [α]20D +31.4 (c 0.07, MeOH). The spectra of 2 were almost identical to those of 1, except that the CD spectrum of 2 displayed a mirrored curve to that of 1 (Fig. 3). This suggested that the aglycone (2a) in 2 was

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**Figure 1** The structures of compounds 1–6.
was the enantiomer of 1a since the specific rotation of 2 had the same negative sign as that of 1 and since L-glucose is rare in natural products. The suggestion was confirmed by 2D NMR spectroscopic data analysis and enzyme hydrolysis of 2. Particularly, from the hydrolysate of 2, L-glucose was isolated and identified by using the aforementioned protocol, while the 1H NMR spectroscopic data of the aglycone (2a) were identical to those of 1a, whereas the specific rotation and Cotton effect curve were reversed (Fig. 3).

Therefore, the structure of compound 2 was determined and assigned as isatioxyneolignoside B.

Compound 3, a white amorphous powder with \([\alpha]_D^{20} = -16.0^\circ\) (c 0.12, MeOH), displayed spectroscopic data similar to those of 1 and 2. The significant differences were two resonances of methoxy groups at \(\delta_{H} 3.71\) (OCH3-3) and 3.77 (OCH3-5) (separated by \(\Delta\delta_{H} 0.06\)) in the 1H NMR spectrum of 3 replaced the overlapped resonances of the methoxy groups around \(\delta_{H} 3.72\) (OCH3-3/5) in the spectra of 1 and 2, while the H-9b resonance in 3 was significantly shielded by \(\Delta\delta_{H} -0.35\). The differences indicated that 3 was a three stereoisomer of 1 and 2\(^{[24]}\). This was further proved by 2D NMR spectroscopic data analysis and enzyme hydrolysis of 3 that liberated L-glucose and an aglycone (3a). Especially the differences of the 1H NMR spectroscopic data between 3a and 1a (or 2a) were in good agreement with those between 3 and 1 (or 2).

The 1H NMR spectral data (Table 1) for compounds 1–6 were consistent with those between 2 and 1, suggesting that 4 is the 75S85 diastereoisomer of 3. Especially, the Cotton effect curves of 4 and the aglycone 4a almost mirrored to those of 3 and 3a (Fig. 4), respectively. Therefore, the structure of compound 4 was determined and designated as isatioxyneolignoside D.

Compound 5 was isolated as a white amorphous powder with \([\alpha]_D^{20} = +13.3^\circ\) (c 0.03, MeOH). The molecular formula of 5 was elucidated as C28H38O14 by (+)-HR-ESI-MS and NMR spectroscopic data. The NMR spectroscopic data of 5 in CD3OD showed signals assignable to syringylglycerol, sinapyl alcohol, and \(\beta\)- glucopyranosyl moieties. Analysis of 2D NMR spectroscopic data confirmed the presence of the three structural moieties and established connections among them. Especially, the HMBC correlation from H-8 to C-4’, together with their chemical shifts, revealed a typical structure of 8,4’-oxyneolignane for the aglycone (5a) as shown in Fig. 2, while the HMBC correlation from H-1’ to C-4 located the \(\beta\)-glucopyranosyl oxy at C-4 of the aglycone. Hydrolysis of 5 with snailase afforded sugar and 5a. The sugar was identified as L-glucose by using the same methods as described for 1–4, while 5a was identified as erythro syringylglycerol-8-O-4’-(sinapyl alcohol) ether (erythro-SGSE) by comparison of the measured 1H NMR spectroscopic data with that reported in the literature\[20\]. The 8R configuration was assigned by the negative Cotton effects at 236 and 241 nm\(^{[20-22]}\) in the CD spectra of 5 and 5a (Fig. 5). Therefore, the structure of compound 5 was determined and trivially named as isatioxyneolignoside E. A compound from an ethanol extract of *Indocalamus latifolius* leaves was recently reported to have the same structure as 5. However, the reported data does not support the claimed structure and the structure drawing is incorrect in the literature.
Compound 6 exhibited spectroscopic data almost identical to those of 5 (see Experimental section and Tables 1 and 2). However, the specific rotation value \([\alpha]_D^{20} = -11.5\) (c 0.05, MeOH) and CD curve of 6 were reversed (Fig. 5). This demonstrated that 6 was the 7R,8S stereoisomer of 5, which was confirmed by enzymatic hydrolysis of 6. From the hydrolysate, the sugar was isolated and identified as D-glucose, while the aglycone (6a) gave the same \(^1\)H NMR spectroscopic data as 5a (See Supplementary Information Figs. S117 and S130), but opposite specific rotation and CD data (Fig. 5). Thus, the structure of compound 6 was determined and named as isatioxyneolignoside F.

Our previous investigation demonstrated that in various 8,4'-oxyneolignane derivatives the difference of chemical shift values \((\Delta \delta_{C8-C7})\) for an erythro arylglycerol unit was consistently smaller than that for the threo diastereoisomer as compared the data acquired in a same solvent. Accordingly, the \(\Delta \delta_{C8-C7}\) values are validated to distinguish threo and erythro arylglycerol units in 8,4'-oxyneolignane derivatives. The validity of this rule for the threo and erythro guiancylglycerol-8-vanillic acids was confirmed by the reported \(^{13}\)C NMR spectroscopic data of 1a/2a and/or 3a/4a in CD3OD or acetone-\(d_6\) and those of the related analogues in different solvents. Application of the rule for

| No. | 1  | 2  | 3  | 4  | 5  | 6  |
|-----|----|----|----|----|----|----|
| 1   | 135.8 | 135.8 | 135.7 | 135.6 | 139.5 | 139.5 |
| 2   | 111.7 | 111.8 | 111.2 | 111.2 | 106.0 | 106.0 |
| 3   | 148.3 | 148.3 | 148.3 | 148.3 | 153.8 | 153.8 |
| 4   | 145.5 | 145.5 | 145.5 | 145.5 | 153.5 | 153.5 |
| 5   | 114.5 | 114.5 | 114.6 | 114.5 | 153.8 | 153.8 |
| 6   | 119.2 | 119.2 | 118.6 | 117.8 | 106.0 | 106.0 |
| 7   | 71.3  | 71.3  | 70.7  | 70.6  | 74.0  | 74.0  |
| 8   | 83.2  | 83.2  | 83.7  | 83.6  | 87.1  | 87.1  |
| 9   | 59.9  | 59.9  | 60.0  | 59.9  | 61.6  | 61.6  |
| 1'  |     |     |     |     |    |    |
| 2'  | 113.0 | 112.9 | 113.0 | 113.2 | 104.9 | 104.9 |
| 3'  | 148.6 | 148.6 | 148.4 | 148.3 | 154.5 | 154.5 |
| 4'  | 150.5 | 150.5 | 150.0 | 149.7 | 136.4 | 136.4 |
| 5'  | 113.7 | 113.8 | 113.7 | 113.7 | 154.5 | 154.5 |
| 6'  | 122.5 | 122.5 | 122.2 | 122.2 | 104.9 | 104.9 |
| 7'  |     |     |     |     | 131.3 | 131.3 |
| 8'  |     |     |     |     | 129.9 | 129.9 |
| 9'  |     |     |     |     | 63.6  | 63.6  |
| 1'' | 100.2 | 100.1 | 100.1 | 100.1 | 105.6 | 105.6 |
| 2'' | 73.2  | 73.2  | 73.2  | 73.2  | 75.7  | 75.7  |
| 3'' | 76.8  | 76.8  | 76.8  | 76.8  | 77.8  | 77.8  |
| 4'' | 69.6  | 69.6  | 69.6  | 69.6  | 71.3  | 71.3  |
| 5'' | 77.0  | 77.0  | 77.0  | 77.0  | 78.4  | 78.4  |
| 6'' | 60.6  | 60.6  | 60.6  | 60.6  | 62.6  | 62.6  |
| OCH3-3 | 55.5 | 55.5 | 55.4 | 55.4 | 57.0 | 57.0 |
| OCH3-5 |     |     |     |     | 57.0 | 57.0 |
| OCH3-3' | 55.5 | 55.5 | 55.4 | 55.4 | 56.7 | 56.7 |
| OCH3-5' |     |     |     |     | 56.7 | 56.7 |

\(^{13}\)C NMR data \((\delta)\) were measured at 150 MHz in DMSO-\(d_6\) for 1–4 and in CD3OD for 5–6, respectively. The assignments were based on \(^1\)H–\(^1\)H COSY, HSQC, and HMBC experiment.

Figure 2  Main \(^1\)H–\(^1\)H COSY (thick lines) and three-bond HMBC (arrows, from \(^1\)H to \(^13\)C) correlations of compounds 1–5.

Figure 3  (A) The overlaid experimental CD spectra of 1 (red) and 2 (blue) and the calculated ECD spectra of 1 (red dash) and 2 (blue dash).  (B) The overlaid experimental CD spectra of 1a (red) and 2a (blue) and the calculated ECD spectra of 1a (red dot) and 2a (blue dot).
The 13C NMR data of 1–4 in DMSO-d6 and 5 and 6 in CD3OD (Table 2) supported the assignment of the relative configurations for 1–6.

Theoretical calculations of the ECD spectra are increasingly applied for determination of absolute configurations of natural products. However, our previous ECD spectra calculations of β-D-glucosidic natural products showed that intensities, wavelengths, and signs of the Cotton effects in the calculated ECD spectra were varied significantly by the β-D-glucopyranosyl unit on the chromophores and that the β-D-glucopyranosyloxy possibly played a decisive role for the signs of the partial or whole Cotton effects. With the experimental CD spectra of 1–6 and enantiomeric pairs (1a/2a, 3a/4a, and 5a/6a) in hand, the theoretical ECD spectra were calculated to evaluate applicability of the calculated ECDs for determination of the absolute configurations of the arylglycerol units in the 8,4′-oxyneolignane derivatives. Comparison of the calculated ECD spectra between 1 and 2, 3 and 4, and 5 and 6 indicated that the β-D-glucopyranosyl indeed had significant influences on the intensities, wavelengths, and signs of the Cotton effects (See Supplementary Information Figs. S4, S16 and S28). This was confirmed by comparing the ECD spectra between the glucoside and aglycone pairs (1/1a, 2/2a, 3/3a, 4/4a, 5/5a, and 6/6a, See Supplementary Information Figs. S5, S6, S17, S18, S29 and S30). Furthermore, the experimental CD spectra of 1–6 and 1a–6a significantly differed the corresponding calculated ECD spectra in part or whole wavelength regions (See Supplementary Information Figs. 3–5, S7, S8, S10, S11, S19, S20, S22, S23, S31, S32, S34 and S35) except for those of 1 and 3. As compared with the experimental CD spectra, in short or/and long wavelength region(s) of the calculated ECD spectra of 2, 4–6, and 1a–6a the sign(s) of Cotton effect(s) was reversed. Especially, each experimental CD spectrum displayed a characteristic Cotton effect around 235 ± 5 nm, whereas this Cotton effect was indistinguishable in the calculated spectra of 1a–4a, 2, 5, or 6. This indicated that, under the experimental conditions relatively stable conformers of these compounds differed from that predicted by the theoretical calculations. Therefore, the theoretical ECD calculation is generally invalid to determine the absolute configuration of 8,4′-oxyneolignane derivatives. This result, together with our previous investigations supports that the Cotton effect around 235 ± 5 nm in the experimental CD spectrum.
spectrum remains to be characteristic and specific to assign the absolute configuration at C-8 of the aryl glycerol units in the aryl glycerols and neolignans (positive for 8S and negative for 8R).

3. Conclusions
Six new β-D-glucopyranosides having three pairs of enantiomeric 8,4′-oxyneolignane aglycones (1–6) were isolated from an aqueous extract of *Isatis indigotica* roots (ban lan gen). Analysis of the 13C NMR data of the diastereoisomers confirmed the rule using the ΔδC3,7,13 values to distinguish *threo* and *erythro* aryl glycerol units in various 8,4′-oxyneolignanes. Comparison of the experimental CD and calculated ECD spectra of these compounds indicates that the ECD calculation is generally invalid to determine their absolute configurations. However, the experimental CD spectra of 1–6 substantiated relationship of the signs of the Cotton effects around 235 nm; IR effects around 235 nm; IR

4. Experimental

4.1. General experimental procedures

Optical rotations were measured on a P-2000 polarimeter (JASCO, Tokyo, Japan). UV spectra were acquired on a V-650 spectrophotometer (JASCO, Tokyo, Japan). CD spectra were measured on a JASCO J-815 CD spectrometer (JASCO, Tokyo, Japan). IR spectra were obtained on a Nicolet 5700 FT-IR microscope instrument (FT-IR Microscope transmission, Thermo Electron Corporation, Madison, WI, USA). NMR spectra were recorded at 600 MHz for 1H NMR and 150 MHz for 13C NMR, respectively, on a SYS 600 instrument (Varian Associates Inc., Palo Alto, CA, USA) in CD3OD, DMSO-d6, or D2O with solvent peaks used as references. ESI-MS and HR-ESI-MS data were taken on an Agilent 1100 Series LC–MSD-Trap-SL and an Agilent 6520 Accurate-Mass Q-TOFQMS spectrometers (Agilent Technologies, Ltd., Santa Clara, CA, USA), respectively. Column chromatography (CC) was carried out on macroporous adsorbent resin (HPD-110, Cangzhou Bon Absorber Technology Co., Ltd., Cangzhou, China), CHF2P 20 P (Mitsubishi Chemical Inc., Tokyo, Japan), silica gel (200-300 mesh, Qingdao Marine Chemical Inc., Qingdao, China), Sephadex LH-20 (Pharmacia Biotech AB, Uppsala, Sweden), HW-40C (Mitsubishi Chemical Inc.), or reversed phase C-18 silica gel (W. R. Grace & Co., Maryland, USA). HPLC separation was performed on an instrument equipped with an Agilent ChemStation for LC system, an Agilent 1200 pump, and an Agilent 1100 single-wavelength absorbance detector (Agilent Technologies Ltd.) using a Grace semipreparative column (250 mm x 10 mm i.d.) packed with C18 reversed phase silica gel (5 μm, W. R. Grace & Co., Maryland, USA). TLC was carried out on glass precoated silica gel GF254 plates (Qingdao Marine Chemical Inc.). Spots were visualized under UV light or by spraying with 5% H2SO4 in 95% EtOH followed by heating. Unless otherwise noted, all chemicals were purchased from commercially available sources and were used without further purification.

4.2. Plant material

The *Isatis indigotica* roots (ban lan gen) were collected in December 2009 from Bozhou, Anhui Province, China. Plant identity was verified by Mr. Lin Ma (Institute of Materia Medica, Beijing, China). A voucher specimen (No. ID-S-2385) was deposited at the herbarium of Natural Medicinal Chemistry, Institute of Materia Medica.

4.3. Extraction and isolation

For the extraction and preliminary fractionation of the extract see Ref. 42. Subfraction B2-4 (120 g) was separated by CC over Sephadex LH-20, eluting with CHCl3-MeOH (1:1) to yield subfractions B2-4-1–B2-4-3, of which B2-4-1 (40 g) was re-chromatographed over Sephadex LH-20 eluting with H2O to yield B2-4-1-3–B2-4-1-13. Subfraction B2-4-1-6 (20 g) was separated by CC over Sephadex LH-20 (H2O) to yield B2-4-1-6-1–B2-4-1-6-5. Further fractionation of B2-4-1-6-1 (8 g) by flash CC over reversed phase C18 silica gel (0–40% MeOH in H2O, v/v) gave B2-4-1-6-1-1–B2-4-1-6-1-9, of which B2-4-1-6-1-3 (800 mg) was separated again by CC over Sephadex LH-20 (H2O) to yield B2-4-1-6-1-3-1 and B2-4-1-6-1-3-2. Isolation of B2-4-1-6-1-3-2 (100 mg) by RP-HPLC (22% MeOH in H2O with 0.2% acetic acid, v/v, 2.0 mL/min) afforded mixtures of 1 and 3 (4.0 mg, tR = 45 min) and 2 and 4 (3.5 mg, tR = 50 min). The two mixtures were separated by HPLC on a Chiral CD-PH column (8% MeCN in H2O with 0.2% acetic acid, v/v, 2.0 mL/min) to yield 1 (1.5 mg, tR = 45 min) and 3 (1.1 mg, tR = 52 min) from the former, and 2 (1.3 mg, tR = 65 min) and 4 (1.2 mg, tR = 85 min) from the latter.

Subfraction B2-4-2 (10 g) was subjected to MPLC over reversed phase C18 silica gel, eluting with a gradient of increasing MeOH (0%–60%) in H2O, to yield B2-4-2-1–B2-4-2-8. Separation of B2-4-2-3 (800 mg) by CC over HW-40C (H2O) gave B2-4-2-3-1–B2-4-2-3-10. Further isolation of B2-4-2-3-3 (60 mg) by flash CC over reversed phase C18 silica gel, eluted by a gradient of increasing MeOH (0%–100%) in H2O, afforded B2-4-2-3-3-1–B2-4-2-3-3-3, of which B2-4-2-3-3-2 (7 mg) was purified by RP-HPLC (25% MeOH in H2O, 2.0 mL/min) to yield 6 (1.5 mg, tR = 120 min), and B2-4-2-3-3-4 (4 mg) by RP-HPLC (35% MeOH in H2O, 2.0 mL/min) to yield 5 (1.5 mg, tR = 28 min).

4.3.1. Isatixoyneolignoside A (1)

White amorphous powder; [α]D20 20° = –5.2 (c 0.12, MeOH); UV (MeOH) λmax (logε) 203 (3.62), 255 (3.09), 284 (2.93, sh) nm; CD (MeOH) 229 (Δε = –2.18), 256 (Δε = +1.33), 291 (Δε = +0.51) nm; IR vmax 3390, 3010, 2921, 2850, 1644, 1600, 1554, 1512, 1468, 1417, 1382, 1268, 1222, 1185, 1118, 1076, 1030, 884, 782, 723, 647 cm–1; 1H NMR (DMSO-d6, 600 MHz) data; see Table 1; 13C NMR (DMSO-d6, 150 MHz) data; see Table 2; (+)-ESI-MS: m/z 549 [M+Na]+; (–)-ESI-MS: m/z 525 [M–H]+; (+)-HR-ESI-MS: m/z 549.1594 [M+Na]+ (Calcd. for C24H30O13Na, 549.1579).

4.3.2. Isatixoyneolignoside B (2)

White amorphous powder; [α]D20 20° = –31.4 (c 0.07, MeOH); UV (MeOH) λmax (logε) 203 (3.58), 256 (3.06), 283 (2.93, sh) nm; CD (MeOH) 213 (Δε = +2.30), 233 (Δε = +0.66), 269 (Δε = –1.55) nm; IR vmax 3395, 3186, 3011, 2922, 2830, 1646, 1601, 1548, 1512, 1469, 1419, 1381, 1325, 1300, 1269, 1218, 1187, 1119,
1076, 1030, 889, 816, 722, 647 cm⁻¹; 1H NMR (DMSO-d6, 600 MHz) data, see Table 1; 13C NMR (DMSO-d6, 150 MHz) data, see Table 2; (+)-ESI-MS: m/z 549 [M+Na]+; (-)-ESI-MS: m/z 549 [M–H]⁺; (+)-HR-ESI-MS: m/z 549.1575 [M+Na]+ (Calcd. for C23H30O7Na, M=549.1579).

4.3.3. Isat oxyloignoside C (3)

White amorphous powder (MeOH); [α]D 20  –16.0 (c 0.12, MeOH); UV (MeOH) λ max (logε) 203 (3.07), 254 (2.65), 282 (2.51, sh) nm; CD (MeOH) 215 (Δε +0.97), 235 (Δε –0.57) nm; IR ν max 3394, 3198, 3011, 2921, 2850, 1645, 1601, 1548, 1452, 1468, 1493, 1301, 1265, 1219, 1188, 1118, 1075, 1030, 933, 861, 784, 722, 647 cm⁻¹; 1H NMR (DMSO-d6, 600 MHz) data, see Table 1; 13C NMR (DMSO-d6, 150 MHz) data, see Table 2; (+)-ESI-MS: m/z 549 [M+Na]+; (-)-ESI-MS: m/z 525 [M–H]⁺; (+)-HR-ESI-MS: m/z 549.1577 [M+Na]+ (Calcd. for C23H30O7Na, M=549.1579).

4.3.4. Isat oxyloignoside D (4)

White amorphous powder: [α]D 20  –15.0 (c 0.07, MeOH); UV (MeOH) λ max (logε) 203 (3.60), 254 (2.94), 284 (2.72, sh) nm; CD (MeOH) 212 (Δε –2.95), 243 (Δε +0.34), 280 (Δε –0.26) nm; IR ν max 3395, 3190, 3011, 2922, 2850, 1646, 1601, 1553, 1512, 1468, 1419, 1383, 1267, 1220, 1187, 1118, 1075, 1030, 890, 860, 784, 722, 647 cm⁻¹; 1H NMR (DMSO-d6, 600 MHz) data, see Table 1; 13C NMR (DMSO-d6, 150 MHz) data, see Table 2; (+)-ESI-MS: m/z 549 [M+Na]+; (-)-ESI-MS: m/z 525 [M–H]⁺; (+)-HR-ESI-MS: m/z 549.1581 [M+Na]+ (Calcd. for C23H30O7Na, M=549.1579).

4.3.5. Isat oxyloignoside E (5)

White amorphous powder: [α]D 20  +13.3 (c 0.03, MeOH); UV (MeOH) λ max (logε) 202 (3.25), 223 (2.69, sh), 271 (2.23) nm; CD (MeOH) 216 (Δε +1.16), 236 (Δε –2.51), 273 (Δε +1.26) nm; IR ν max 3391, 3011, 2921, 2849, 1639, 1596, 1503, 1465, 1421, 1333, 1229, 1125, 1073, 921, 847, 803, 722, 628 cm⁻¹; 1H NMR (CD3OD, 600 MHz) data, see Table 1; 13C NMR (CD3OD, 150 MHz) data, see Table 2; (+)-ESI-MS: m/z 621 [M+Na]+; (-)-ESI-MS: m/z 633 [M+Cl]−; (+)-HR-ESI-MS: m/z 643.2268 [M+HCOO]− (Calcd. for C29H35O16, M=643.2244).

4.3.6. Isat oxyloignoside F (6)

White amorphous powder: [α]D 20  –11.5 (c 0.05, MeOH); UV (MeOH) λ max (logε) 202 (3.19), 222 (2.71, sh), 280 (2.27) nm; CD (MeOH) 212 (Δε –7.76), 240 (Δε +2.76), 270 (Δε –1.39) nm; IR ν max 3374, 2918, 2850, 1731, 1639, 1596, 1503, 1462, 1421, 1333, 1228, 1126, 1074, 919, 846, 803, 619 cm⁻¹; 1H NMR (CD3OD, 600 MHz) data, see Table 1; 13C NMR (CD3OD, 150 MHz) data, see Table 2; (+)-ESI-MS: m/z 621 [M+Na]+; (-)-ESI-MS: m/z 633 [M+Cl]−; (+)-HR-ESI-MS: m/z 643.2235 [M+HCOO]− (Calcd. for C29H35O16, M=643.2244).

4.3.7. Enzymatic hydrolysis of I–6

Compounds I–6 (1.0–1.5 mg) were separately hydrolyzed in H2O (3 mL) with snailase (3.0 mg, CODE S0100, Beijing Biodere Biotech Co., Ltd., Beijing, China) at 37 °C for 24 h. The hydrolysate was concentrated under reduced pressure and the residue was isolated by CC over silica gel eluting with CH3CN–H2O (8:1) to afford sugar and aglycone. The sugar (0.3–0.6 mg) showed a retention factor (Rf 0.38) on TLC (EtOAc–MeOH–AcOH–H2O, 12:3:3:2), with [α]D 20 values of +43.9–+48.0 (c 0.03–0.06, H2O), and 1H NMR (D2O) data in agreement with those of an authentic d-glucose (See Supplementary Information Figs. S53, S57, S86, S102, S118, S131, and S132).

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Appendix A. Supporting information

Supplemental data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.apsb.2017.09.006.

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