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Two Novel Tetrairidoid Glucosides from *Dipsacus asper*

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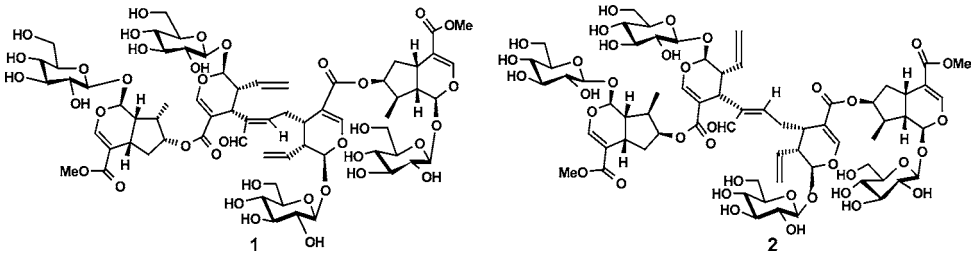
ABSTRACT



Two new iridoid glucoside tetramers, dipsanosides A (1) and B (2), the first-reported iridoid tetramers with four glucosides, were isolated from *Dipsacus asper.* Their structures were determined by analysis of 1D and 2D NMR data as well as by comparison with model compounds. Their cytotoxicities were tested, but neither of them showed obvious activity.

Dipsacus asper Wall., a perennial plant widespread in China, has been used in traditional Chinese medicine for hundreds of years as a tonic for refreshment, as a fissiparism promoter of the osseous cells, and as an embryo security agent, etc.[1] Many phytochemical studies have been carried out on this plant.[2−5]

In our preliminary screening, the *n*-butanol part of the ethanol extract of *D. asper* showed significant cytotoxicity, which has not been reported thus far. A bioassay-guided fractionation of the *n*-BuOH residue on macroporous resin D_{101} with EtOH−H_2O gradients (100% H_2O to 100% EtOH, 20% stepwise) furnished six fractions, among which the second and third portions were highly cytotoxic ($IC_{50} \sim 5$ μg/mL; see Table 3 in the Supporting Information) against several tumor cell lines, including human lung carcinoma A549 and colon cancer HCT-8. Further repeated chromatography of the cytotoxic portion 3 by Sephadex LH-20, RP-18, and preparative TLC and HPLC afforded two novel tetrairidoid glucosides, dipsanosides A (**1**) and B (**2**). To the best of our knowledge, this is the first isolation of iridoid glycoside tetramers. They are the largest and most complex iridoid glucosides known to date. Their cytotoxicities were tested, but none of them showed any significant activity.

The structures of **1** and **2** were determined by analysis of their 1D and 2D NMR data and by comparison with the model compound cantleyoside (**3**). Compound **1**, a white amorphous powder, showed IR bands at 3399 cm^{-1} for hydroxyl groups and 1693 and 1637 cm^{-1} for the α,β-unsaturated ester carbonyl groups. Its maximum UV absorption at 235.8 nm (log ϵ 3.264) was also due to the α,β-unsaturated carbonyl group. The positive ESI-MS of **1** exhibited a pseudomolecular ion peak at *m*/*z* 1497

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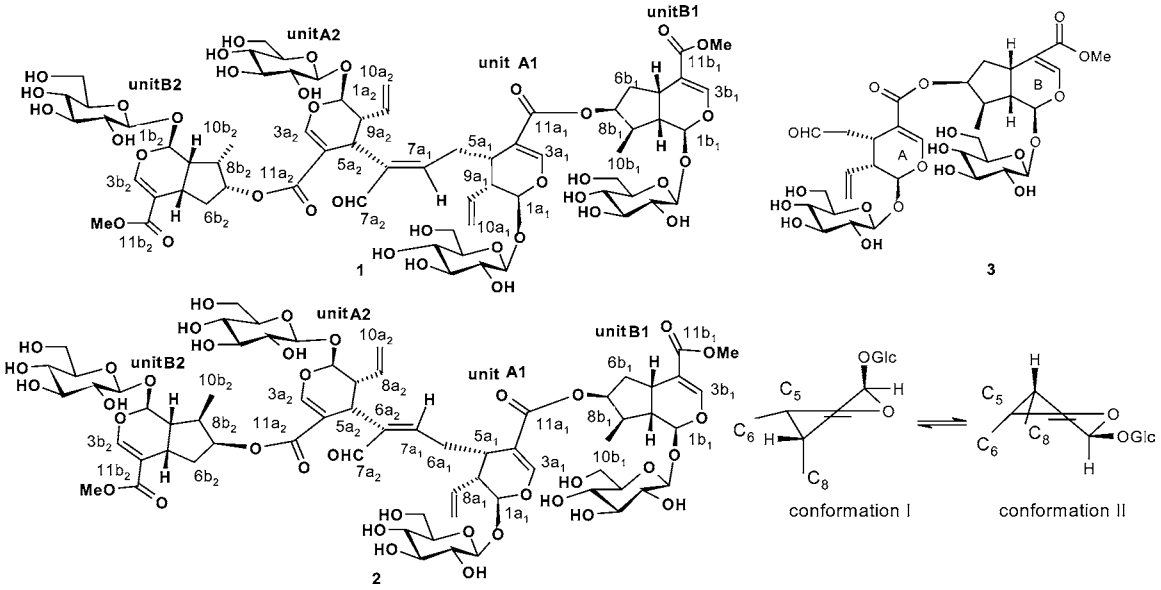

Figure 1. The structures of compounds **1**−**3** and the confirmation equilibrium of the pyran ring.

([M + Na]$^+$), and the high resolution (HR)-ESI-MS gave the peak at *m*/*z* 1497.5038, corresponding to the formula $C_{66}H_{90}O_{37}Na$ (calcd 1497.5059). Acid hydrolysis of **1** gave D-glucose, which was identified by HPLC and TLC analysis. In the ^{1}H NMR spectrum for the iridoid glycosides, two characteristic peaks, the H-3 olefinic proton of the enol and H-1 on glycosylated carbon, were observed. Four pairs of such peaks appeared in the ^{1}H NMR spectrum of **1** (500 MHz, CD_3OD), including H-3 olefinic protons at δ 7.49 (1H, s), 7.42 (1H, s), 7.39 (1H, s), 7.38 (1H, s) ppm and H-1 protons at δ 5.52 (d, 4.0 Hz), 5.43 (d, 5.5 Hz), 5.20 (d, 5.5 Hz), 5.12 (d, 4.0 Hz), clearly demonstrating it to be an iridoid glycoside tetramer. Moreover, the ^{13}C NMR spectrum (CD_3-OD, Table 1 in the Supporting Information) of **1** displayed signals due to four α,*β*-unsaturated ester groups at δ 169.4 × 2 and 168.2 × 2, and 154.1, 153.2, 152.7, 151.9, 113.1, 112.6, 111.1, and 109.8 for double bonds, besides four *β*-glucopyranosyl anomeric carbons at δ 100.4, 101.2 × 2, and 99.6. This further suggested the existence of four α,*β*-unsaturated esters attached to enol ethers in iridoid pyran rings. Iridoids represent a large group of cyclopentano[*c*]-pyran monoterpenoids. However, many of them exist in secoiridoid form, characterized by a cyclopentano[*c*]pyran system cleaved between C-7 and C-8. So, two sets of terminal carbon−carbon double bond signals at δc 135.5, 135.4, 120.6, and 119.4, characteristic for two secoiridoid glucoside units, and two methyls at δc 14.3 and 14.2 and δ_H 1.03 (d, 6.5 Hz) and 0.95 (d, 6.5 Hz), always observable in iridoid glycosides, suggested **1** was an iridoid glucoside tetramer consisting of two secoiridoid glucoside units (unit A) and two iridoid glucoside units (unit B). For unit B, two sets of carbon signals in the ^{13}C NMR spectrum at δ 33.6, 40.4, 78.6, 41.4, and 47.1, and at δ 33.0, 40.3, 77.8, 41.1, and 46.9, along with the corresponding protons correlated to each other and also to H-1 in the ^{1}H−^{1}H COSY spectrum, suggested the existence of two loganin[12] segments esterified at C-7. For unit A, the NMR spectra exhibited signals of an aldehyde [δ_H 9.26 (s), δ_C 197.2] and a trisubstituted carbon−carbon double bond [δ_H 6.71 (dd, 6.5, 7.0 Hz), δ_C 143.7, 156.1], indicating that two units of secologanin were linked, as shown in Figure 1. HMBC supported such a linkage by showing long-range correlations from H-7a_1 to C-7a_2, and H-7a_2 to C-7a_1. In addition, both H-7a_1 and H-6a_1 had a long-range correlation with C-6a_2 (for other important HMBC correlations, see Figure 2). The stereochemistry of the double bond conjugated to the aldehyde was assigned with NOE experiment. Irradiation of H-7a_2 led to the enhancement of H-7a_1 and vice versa. Thus the double bond bridging two cantleyoside-like segments was determined to be in *Z*-configuration.

In the literature, all of the reported compounds have the *β*-configuration at C-1 of the loganin and secologanin moieties.[6−11] For the loganin part (unit B), the bicyclic fused ring system is rather rigid and can be easily analyzed by NOE experiments and coupling constants. On irradiation of H-9b_1, NOE enhancement for H-5b_1 was observed. On irradiation of H-6$b_{1\beta}$, NOE enhancements for H-7b_1 and H-6$b_{1\alpha}$ were observed. On irradiation of H-8b_1, NOE enhancement for H-7b_1 was observed. These NOE results suggested that H-9b_1 and H-5b_1 had a *cis* relationship as did H-7b_1 and H-8b_1. The remaining two sets of protons were in a *trans* relationship. H-1b_1 and H-9b_1 were determined to

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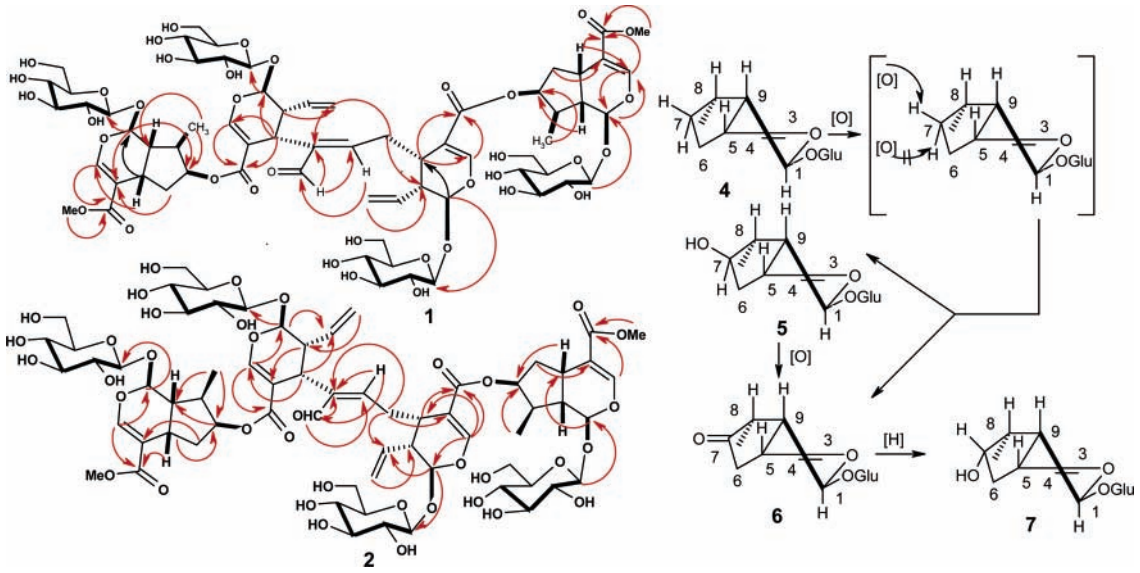

Figure 2. The key HMBC correlations of compounds **1** and **2**, and the putative biogenesis of unit B2 in compound **1**.

be in *trans*, similar to the known compounds,[6−9] due to the value of $J_{1b_1,9b_1}$ (4.0 Hz). With the assumption that C-1 had β-configuration, stereochemistries for all positions in unit B1 were assigned. On irradiation of H-9b$_2$, NOE enhancements for H-8b$_2$ and H-5b$_2$ were observed. On irradiation of H-8b$_2$, NOE enhancements for H-7b$_2$, H-9b$_2$, and H-5b$_2$ were observed. On irradiation of H-6b$_{2\beta}$, NOE enhancements for H-7b$_2$ and H-6b$_{2\alpha}$ were observed. These NOEs revealed that the stereochemistry for unit B2 was different from that of unit B1, in that H-9b$_2$, H-5b$_2$, H-8b$_2$, and H-7b$_2$ were in a *cis* relationship. The value of $J_{1b_2,9b_2}$ (5.5 Hz) suggested that H-9b$_2$ and H-1b$_2$ were in a *trans* relationship. Thus, the stereochemistry for the unit B2 was determined.

For the secologanin moiety (unit A), there is only one pyran ring, and its conformation was deduced from the value of $J_{1a,9a}$. The pyran ring should have two more stable half-chair conformers: one with their dihedral angle about 60° between the protons H-1a and H-9a (conformer I) and another 180° (conformer II) (see Figure 1). The calculated value of $J_{1a,9a}$ should be 2.5 Hz in conformer I, while it should be 9.7 Hz in conformer II.[10] The measured values for $J_{1a_1,9a_1}$ and $J_{1a_2,9a_2}$ in **1** were 4.0 and 5.5 Hz, respectively, suggesting the pyran ring should be in an equilibrium between conformers I and II. The NOE experiments supported the correctness of the above stereostructure. On irradiation of H-9a$_1$, the enhancement of H-5a$_1$ together with the magnitude $J_{9a_1,5a_1}$ (6.0 Hz) (no resolved coupling constants for these protons in CD_3OD; these coupling constants were read from the ^{1}H NMR spectrum in pyridine-d_5) indicated H-9a$_1$ should be *cis* to H-5a$_1$. On irradiation of H-8a$_1$, the enhancement of H-1a$_1$ suggested H-8a$_1$ should be *cis* to H-1a$_1$, and H-1a$_1$ should be *trans* to H-9a$_1$. When H-9a$_2$ was irradiated, enhancement of H-5a$_2$ together with the magnitude of $J_{5a_2,9a_2}$ (5.5 Hz) suggested H-9a$_2$ should be *cis* to H-5a$_2$. When H-8a$_2$ was irradiated, the enhancement of H-1a$_2$ suggested H-8a$_2$ should be *cis* to H-1a$_2$, similar to the secologanin part in the known compounds.[6−9] Accordingly, the structure of **1**, named dipsanoside A, was determined, as shown in Figure 1.

Compound **2** was obtained as a white amorphous powder. Its molecular formula was established as $C_{66}H_{90}O_{37}$ by HR-ESI-MS, indicating it was an isomer of compound **1**. The spectral data of **2** (500 MHz, CD_3OD) were similar to those of **1**, except for the obvious differences for the α,β-unsaturated aldehyde part, that is, the carbon signals at δ_C 192.1 (C-7a$_2$), 150.8 (C-7a$_1$), 141.1 (C-6a$_2$), and 21.4 (C-6a$_1$) in ^{13}C NMR spectrum of **2** were shifted upfield, when comparing with the corresponding carbon signals of **1** [δ_C 197.2 (C-7a$_2$), 156.1 (C-7a$_1$), 143.7 (C-6a$_2$), 29.6 (C-6a$_1$)]. The chemical shifts of H-7a$_2$ (δ_H 10.00) and H-7a$_1$ (δ_H 6.30) were markedly different from those [δ_H 9.26 (H-7a$_2$), 6.71 (H-7a$_1$)] in compound **1**. Furthermore, the fact that no NOEs between H-7a$_1$ and the aldehyde proton (H-7a$_2$) suggested that H-7a$_1$ and the aldehyde group were in a *trans* relationship, which meant that the configuration of the olefinic linker was *E*. For the stereochemistry of H-1a, 5a, and 9a in units A1 and A2, the magnitudes of $J_{1a_1,9a_1}$ (5.8 Hz), $J_{5a_1,9a_1}$ (4.6 Hz), $J_{1a_2,9a_2}$ (5.1 Hz), and $J_{5a_2,9a_2}$ (4.4 Hz) together with NOEs between H-5a$_1$ and H-9a$_1$, H-8a$_1$ and H-1a$_1$, H-5a$_2$ and H-9a$_2$, and H-8a$_2$ and H-1a$_2$ suggested that H-5a and H-9a were *cis* and H-9a and H-1a were *trans*, and H-8a and H-1a were *cis*-related. For units B1 and B2, it was found that both segments were loganin-type iridoids. So the structure of tetramer **2**, named dipsanoside B, was elucidated as a condensation product of two identical parts, that is, cantleyoside (**3**).

The stereochemistry for unit B2 in compound **1** was similar but different from loganin, in that both the C-7 hydroxyl and C-8 methyl groups had α configurations rather than β. This kind of substitution pattern is rarely reported even though the structures/substructures of loganin, 7-*epi*-loganin, and 8-*epi*-loganin are found in many iridoid

monomers and oligomers.[13,14] After checking and confirming the stereochemistry of compound **1**, we postulated its biogenetic pathway as shown in Figure 2. The biogenic precursor **4** is easily oxygenated at β-orientation to furnish 8-*epi*-loganin (**5**), while its direct oxygenation into compound **7**, the C-7 epimer of **5**, is hampered by steric hindrance from the 8α-Me. We reasoned that the β-OH in **7** can be alternatively derived from **4** through oxidation and reduction steps, with compound **6** as intermediate. So, the genesis of **7** will need additional biosynthesis steps, which may explain the reason **7** is rarely found in plants, compared to 8-*epi*-loganin.

Since it is the first isolation of iridoid glycoside tetramers, the possibility of **1** and **2** as artificial natural products under stringent extraction conditions should be excluded, especially for **2**, a condensation product of two molecules of **3** which was also isolated from the same extracts. The EtOH extracts of *D. asper* were freshly prepared with cold and boiling EtOH, respectively. Iridoid glycosides **1** and **2** could be detected by HPLC in both extracts. Moreover, **3** was kept in the boiling EtOH and MeOH for 24 h, and no derivatives were detected. Therefore, we concluded that **1** and **2** are not artifacts during the extraction and isolation procedures.

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Supporting Information Available: MS, HRMS, 1D, and 2D NMR spectra, spectral data of compounds **1** and **2**, and the experimental part. This material is available free of charge via the Internet at http://pubs.acs.org.

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