Larrealignans A and B, Novel Lignan Glycosides from the Aerial Parts of Larrea tridentata

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Two new lignan glycosides, named larrealignans A (1) and B (2), and a known lignan (3) were isolated from the aerial parts of Larrea tridentata (Zygophyllaceae). The structures of 1 and 2 were determined on the basis of spectroscopic analysis and the results of hydrolytic cleavage. The isolated compounds (1—3) and aglycones (1a, 2a) of 1 and 2 were evaluated for their cytotoxic activities against HL-60 human leukemia cells.

Key words lignan; lignan glycoside; Larrea tridentata; Zygophyllaceae; cytotoxic activity

Larrea tridentata (Sessee, and Moc. Ex DC. Coville, (Zygophyllaceae) is an evergreen shrub that grows in the desert area of the American continents. The aerial parts (leaves and stems) of this plant are called Chaparral and are an alternative herbal medicine used for the treatment of various cancers, tuberculosis, menstrual pains, and diabetes in the United States.1) Previously, we have reported 25 triterpene glycosides from the aerial parts of Larrea tridentata.2) Further phytochemical analysis of the MeOH extract of this plant has resulted in the isolation of two new lignan glycosides, named larrealignans A (1) and B (2), and a known lignan (3). In this paper, we describe the structural elucidation of 1 and 2 on the basis of spectroscopic analysis, including various two-dimensional (2D)-NMR spectroscopic studies, and the results of hydrolytic cleavage. The cytotoxic activities of the isolated compounds (1—3) and the aglycones (1a, 2a) of 1 and 2 against HL-60 human leukemia cells are also reported.

The aerial parts of Larrea tridentata (3.0 kg) were extracted with MeOH under reflux conditions. After removal of the solvent, the concentrated MeOH extract (940 g) was passed through a porous-polymer polystyrene resin (Diaion HP-20) column and successively eluted with MeOH in H2O (3 : 7), MeOH–H2O (1 : 1), MeOH, EtOH, and EtOAc. The MeOH eluate fraction (477 g) was subjected to column chromatography on silica gel and octadecylsilanized (ODS) silica gel, yielding compounds 1 (23.2 mg), 2 (14.5 mg), and 3 (11.2 mg). Compound 3 was identified by its physical and spectroscopic data as (E)-4,4'-dihydroxy-7,7'-dioxolign-8(8'-ene).3)

Larrealignan A (1) was obtained as an amorphous solid and showed an accurate [M+Na]+ ion at m/z 973.3557 in the high resolution (HR)-electrospray ionization (ESI)-time-of-flight (TOF)-MS, corresponding to the empirical molecular formula C42H62O24. The IR spectrum of 1 suggested the presence of hydroxy groups (3376 cm⁻¹) and aromatic rings (1613, 1509 cm⁻¹). The UV spectrum showed an absorption maximum due to substituted aromatic rings (273 nm). The 1H-NMR spectrum of 1 contained signals for two 1,3,4-trisubstituted aromatic rings (273 nm). The 1H-NMR spectrum of 1 contained signals for two 1,3,4-trisubstituted aromatic rings at δ 7.60 (1H, d, J = 8.2 Hz), 7.50 (1H, br s), and 6.81 (1H, br d, J = 8.2 Hz); 7.60 (1H, d, J = 8.2 Hz), 7.51 (1H, br s), and 6.81 (1H, br d, J = 8.2 Hz), two methyl groups at δ 4.06 (3H, d, J = 6.5 Hz) and 0.66 (3H, d, J = 6.5 Hz), and four anomic protons at δ 5.65 (1H, d, J = 7.9 Hz), 5.64 (1H, d, J = 7.3 Hz), 5.60 (1H, d, J = 7.6 Hz), and 5.59 (1H, d, J = 7.3 Hz). Enzymatic hydrolysis of 1 with β-d-glucosidase in an HOAc/NaOAc buffer (pH 5.0) at room temperature yielded meso-nordihydroguaiaretic acid (meso-NDGA) ([α]D + 0)4) and d-glucose. The above data suggest that 1 is a meso-NDGA tetraglucoside.

The linkage positions of the glucosyl moieties to the aglycone were solved by detailed analysis of the one-dimensional (1D) totally correlated spectroscopy (TOCSY) and 2D-NMR spectra. The 1H-NMR substructures of the individual glucosyl...
units were obtained by using selective irradiation of non-overlapping proton signals in a series of 1D-TOCSY experiments. Subsequent analysis of the $^1$H–$^1$H shift correlation spectroscopy (COSY) spectrum resulted in the sequential assignments of all the proton resonances due to the four glucosyl units (Table 1). Using the $^1$H-detected heteronuclear multiple-quantum coherence (HMQC) and $^1$H-detected heteronuclear single-quantum coherence (HSQC)-TOCSY spectra, the proton resonances were correlated with the corresponding one-bond coupled carbon signals, which allowed the carbon shifts to be completely assigned. The $^1$H- and $^{13}$C-NMR signals thus assigned were indicative of the presence of four terminal β-D-glucopyranosyl moieties (Glc$^\beta$/H11032, Glc$^\beta$/H11033, Glc$^\beta$/H11630, and Glc$^{\beta}$m$^\beta$) in 16,7 (Table 1). The β-orientations of the anomeric centers of all of the glucosyl moieties were supported by the relatively large $J$ values of their anomeric protons (7.3—7.9 Hz). In the $^1$H-detected heteronuclear multiple-bond connectivity (HMBC) spectrum of 1, long-range correlations were observed between the anomeric proton (H-1) of Glc$^\beta$/H11032 at $\delta_H$ 5.65 and C-3 of the aglycone at $\delta_C$ 148.50, H-1 of Glc$^\beta$ at $\delta_H$ 5.60 and C-4 of the aglycone at $\delta_C$ 146.93, H-1 of Glc$^{\beta}$m$^\beta$ at $\delta_H$ 5.64 and C-3 of the aglycone at $\delta_C$ 148.63, and between H-1 of Glc$^{\beta}$m$^\beta$ at $\delta_H$ 5.59 and C-4 of the aglycone at $\delta_C$ 147.02. Accordingly, the structure of larrealignan A (1) was elucidated as shown in Chart 1.

Larrealignan B (2) was found to have a molecular formula

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Table 1. $^1$H- and $^{13}$C-NMR Chemical Shift Assignments of 1 and 2 in C$_5$D$_5$N

a, b) Assignments may be interchangeable.
of C$_{37}$H$_{54}$O$_{19}$ as determined by HR-ESI-TOF-MS analysis (m/z 825.3144 [M + Na$^+$]). The 1H-NMR spectrum of 2 contained signals for three anomic protons at δ 5.68 (d, J = 7.5 Hz), 5.66 (d, J = 7.6 Hz), and 5.58 (d, J = 7.6 Hz), as well as signals for two 1,3,4-trisubstituted aromatic rings at δ 7.62 (1H, d, J = 8.5 Hz), 7.58 (1H, d, J = 1.5 Hz), and 6.89 (1H, dd, J = 8.5, 1.5 Hz); 7.56 (1H, d, J = 8.0 Hz, 6.91 (1H, d, J = 1.5 Hz), and 6.77 (1H, dd, J = 8.0, 1.5 Hz), two methyl groups at δ 0.77 (3H, d, J = 7.0 Hz) and 0.77 (3H, d, J = 6.5 Hz), and one methoxy group at δ 3.82 (3H, s). Enzymatic hydrolysis of 2 with β-D-glucosidase yielded D-glucose and an NDGA derivative (2a)\(^6\), which gave meso-tetra-O-methyl-NDGA (2b, [α]$_D^0$ +0.0) by treatment of 2a with trimethylsilyldiazomethane (TMS-CH$_2$N$_2$). The above data suggest that 2 is a trigloscide of 2a. Using the same procedures as described for 1, all the 13C-NMR signals for the glucosyl moieties of 2 were assigned to the three terminal β-D-glucopyranosyl units (Glc' , Glc", and Glc"\(^\prime\)'). In the HMBC spectrum of 2, long-range correlations were observed between H-1 of Glc' at δ$_{H}$ 5.66 and C-3 of the aglycone at δ$_C$ 148.99, H-1 of Glc' at δ$_{H}$ 4.58 and C-4 of the aglycone at δ$_C$ 147.46, and between H-1 of Glc" at δ$_{H}$ 5.68 and C-4' of the aglycone at δ$_C$ 146.13. Thus, the structure of larrealignan B (2) was characterized as shown in Chart 1 or its antipode in regard to the C-8 and C-8' configurations.

A number of lignan glycosides have been isolated from the plant kingdom. Although lignan bisdesmosides have been reported from *Eucommia ulmoides*\(^1\) (Eucommiaceae), *Sedum sarmentosum*\(^1\) (Crassulaceae), and *Daphne pseudomezereum*\(^2\) and *D. feddei*\(^3\) (Thymelaeaceae), *larrealignan A* (1a) and *B* (2) are believed to be the first representatives of lignan tetra- and tridesmosides, respectively.

The isolated compounds (1—3) and the aglycones (1a, 2a) of 1 and 2 were evaluated for their cytotoxic activities against HL-60 cells. Compounds 1—3 did not exhibit cytotoxicity even at a sample concentration of 20 μM. However, 1a and 2a showed moderate cytotoxicity with respective IC$_{50}$ values of 7.0 and 4.9 μM, whereas etoposide used as a positive control gave an IC$_{50}$ value of 0.33 μM.

**Experimental**

Optical rotations were measured using a JASCO P-1030 (Tokyo, Japan) automatic digital polarimeter. IR spectra were recorded on a JASCO Fourier transform (FT-IR) 620 spectrophotometer. UV spectra on a JASCO V-630 spectrophotometer, and circular dichroism (CD) spectra on a JASCO J-720 instrument, respectively. NMR spectra were recorded on a Bruker DPX-500 spectrometer (500 MHz for 1H-NMR, Karlsruhe, Germany) or a Bruker DRX-600 spectrometer (600 MHz for 1H-NMR) using standard Bruker pulse programs. Chemical shifts are given as δ values with reference to tetramethylsilane (TMS) as an internal standard. HR-ESI-TOF-MS data was recorded on a Waters-Micromass LCT mass spectrometer (Manchester, U.K.).

Methylation of 2a (14.5 mg). The CD spectrum showed no Cotton effect in the 600—200 nm region. HPLC analysis of the sugar fractions under the same conditions as in the case of 1 showed the presence of n-glucose, t$_{R}$ (min): 16.45 (n-glucose, positive optical rotation).

**Enzymatic Hydrolysis of 1**

Compound 1 (4.1 mg) was treated with β-D-glucosidase (EC 3.2.1.21, Sigma, 31.4 mg) in HOAc/NaOAc buffer (pH 5.0, 5 ml) at room temperature for 44 h. The reaction mixture was diluted with H$_2$O (5 ml) and extracted with EtOAc (10 ml×3). After concentration of the EtOAc soluble phase, it was chromatographed on silica gel eluted with CHCl$_3$–MeOH–H$_2$O (3:2:1) to afford meso-NDGA (1a; 0.9 mg). The H$_2$O soluble phase was chromatographed on silica gel eluted with CHCl$_3$–MeOH–H$_2$O (3: 2: 7) to afford 3 (11.2 mg).

The isolated compounds (1—3) and the aglycones (1a, 2a) of 1 and 2 were evaluated for their cytotoxic activities against HL-60 cells. Compounds 1—3 did not exhibit cytotoxicity even at a sample concentration of 20 μM. However, 1a and 2a showed moderate cytotoxicity with respective IC$_{50}$ values of 7.0 and 4.9 μM, whereas etoposide used as a positive control gave an IC$_{50}$ value of 0.33 μM.
methyl-NDGA (2b, 2.0 mg).9)

Cell Culture and Assay for Cytotoxic Activity against HL-60 Cells
The cell growth was measured with an MTT reduction assay as described in a previous paper.14) Briefly, HL-60 cells were maintained in RPMI 1640 medium containing heat-inactivated 10% (v/v) FBS supplemented with l-glutamine, 100 unit/ml penicillin G sodium salt, and 100 μg/ml streptomycin sulfate. The cells (4×10^4 cells/ml) were continuously treated with each compound for 72 h, and cell growth was measured with an MTT reduction assay procedure. A dose–response curve was plotted for 1a and 2a, and the concentration giving 50% growth inhibition (IC_{50}) was calculated.

References