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Isolation and Structure Determination of a New Tetralone Glucoside from the Roots of *Juglans mandshurica*

Jong-Keun Son

College of Pharmacy, Yeungnam University, Gyongsan, 712-749, Korea

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A new 4,5,8-trihydroxy-α-tetralone-5-O-β-D-glucoside along with 1,4,8-trihydroxynaphthalene-1-O-β-D-glucoside and gallic acid were isolated from the roots of *Juglans mandshurica*. Their structures were elucidated on the basis of spectroscopic studies.

Key words : *Juglans mandshurica,* 5-(4,5,8-trihydroxy)-α-tetralone glucoside, 1-(1,4,8-trihydroxy)-naphthalene glucoside.

INTRODUCTION

The roots of *Juglans mandshurica* has been used as a folk medicine for treatment of cancer in Korea. Several naphthoquinones and naphthalenyl glucosides from Juglans species have been reported (Binder *et al.,* 1989, Gupta *et al.,* 1972, Hedin *et al.,* 1980, Hirakawa *et al.,* 1986, Muller and Leistner, 1987, Pardhasaradhi and Hari, 1978, Talpatra *et al.*, 1988). In the course of isolating possible cytotoxic compounds from the roots of this plant, we have isolated 1,4,8-trihydroxynaphthalene-1-O-β-D-[6'-O-(3",4",5"-trihydroxybenzoyl)]-glucopyranoside and 1,4,8-trihydroxynaphthalene-1-O-β-D-[6'-O-(3",5"-dimethoxy-4"-hydroxybenzoyl)]-glucopyranoside (The manuscript for structure determination of the compounds was submitted to *Journal of Natural Products.*). In this paper we present isolation and structure determination of a new compound, 4,5,8-trihydroxy-α-tetralone-5-O-β-D-glucopyranoside (1), along with 1,4,8-trihydroxynaphthalene-1-O-β-D-glucopyranoside (2) and gallic acid on the basis of spectroscopic studies.

MATERIALS AND METHODS

General experimental procedures

The nmr spectra were recorded on a Bruker 300 MHz (ARX 300) and a Bruker 500 MHz (AMX 500) spectrometers. Samples dissolved in acetone-d_6 were reported in ppm downfield from TMS. The 2D nmr spectra were recorded by using Bruker's standard pulse program. The fab mass spectra were measured by VG TRIO 2A mass spectrometer. Silica gel 60 (70-230 and 270-400 mesh) and TLC plate (Si-gel 60 F 254) were purchased from EM Scientific (Darmstadt, F. R. Germany). Gallic acid and sugar standards were purchased from Wako Pure Chemical Industry (Osaka, Japan) and Sigma Chemical Company, LTD (St. Louis, MO, USA) respectively. All other chemicals and solvents were analytical grade and used without further purification.

Extraction and isolation

Roots of *Juglans mandshurica* were collected September of 1993 at a mountain area of Pyongchanggoon, Gangwon-do, Korea, and dried at room temperature for two weeks. A voucher specimen is preserved at the College of Pharmacy, Yeungnam University. The roots of *Juglans mandshurica* (3 kg) were extracted twice with MeOH by reflux for 12 hours. The MeOH solution was evaporated to dryness (300g). The MeOH extract of the roots of *Juglans mandshurica* was partitioned between H_2O (3,000 ml) and hexane (3,000 ml). The resulting H_2O layer was extracted with $CHCl_3$, EtOAc. and *n*-BuOH, successively. The EtOAc extract (58.9 g) was loaded on a silica gel column. The column was eluted with $CHCl_3$-MeOH-H_2O (7:2:0.1) and 50-ml fractions were collected. Fraction 11 and 12 were combined and the solution was taken to dryness *in vacuo* (5.8 g). The residue was chromatographed on a silica gel column and eluted by stepwise gradient of hexane and EtOAc. Fractions were selected on the basis of TLC and pooled, which afforded **2** (200 mg) and gallic acid (70 mg) as dark brown powders. The *n*-butanol extract (28 g) was chromatographed on silica gel column in a manner of stepwise gradient with $CHCl_3$ and MeOH.

Correspondence to: Jong-Keun Son, College of pharmacy, Yeungnam University, Gyongsan, 712-749, Korea.

The elution started from 99%-$CHCl_3$. Among 12 fractions collected, fraction 8 (700 mg) was loaded on a Sephadex LH-20 column and the column was eluted with MeOH. The major fraction from the column was dried under *vacuo,* which gave **1** as white powder.

Compound 1: mp 115-120°C, UV λ_{max} (ε_{max} in methanol) nm 206 (29,700), 256 (12,400), 348 (4,910), IR ν max (KBr) cm^{-1} 3410, 2923, 1646, 1075, positive fab-MS (%) m/z [M+1] 357.3 (12, calculated for $C_{16}H_{20}O_9$; 356.11), 339 (2), 277 (8), 207 (1)

Compound 2: mp 170 -175°C, UV λ_{max} (ε_{max} in methanol) nm 224 (84,300), 306 (13,200), 328 (11, 900), 342 (11,830), IR ν_{max} (KBr) cm^{-1} 3390,, 2937, 1699, 1613, 1521, 1259, 1071; positive fab-MS (%) m/z $[M+Na]^+$ 361 (75), $[M+1]^+$ 339 (25, calculated for $C_{16}H_{18}O_8$; 338.10), 279 (32), 255 (43), 239 (41), 221 (41), 215(50), 207 (68), 177 (53), 176 (100), 115 (55).

Gallic acid: ^{13}C-nmr δ (acetone-d_6) 109.9 (C3 and C5), 122.0 (C2), 138.6 (C5), 146.0 (C4 and C6), 168.3 (C1), ^{1}H-nmr δ (acetone-d_6) 7.15 (s, H3 and H5,)

Hydrolysis of 1 and 2.

Each compound (20 mg) was dissolved in 4N-HCl-dioxane (1:1, 10 ml) and the solution was refluxed for 2 hrs. The resulting mixture was partitioned with ethyl ether (10 ml x 3). The water layer was neutralized with $AgNO_3$, filtered and the filterate was used to detect sugar with standard sugars on both cellulose TLC (pyridine-EtOAc-AcOH-H_2O=36:36:7:21) and silica TLC ($CHCl_3$-MeOH-H_2O=26:14:5).

RESULTS AND DISCUSSION

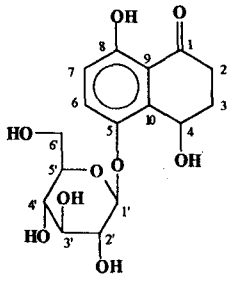
The MeOH extract of the roots of *Juglans mandshurica* was partitioned between H_2O and hexane. The resulting H_2O layer was extracted with $CHCl_3$, EtOAc and *n*-BuOH successively. The EtOAc extract was chromatographed twice on silica gel column, which afforded **2** and gallic acid. The BuOH extract was eluted through a silica gel column and a major fraction from the column was purified further by a Sephadex LH-20 column to give **1** as white powder. The positive fab mass spectrum of **1** showed M^++1 peak at m/z 357.3. After acid hydrolysis of **1**, glucose was identified on TLC plates with authentic sample. The ir band at 1640 cm^{-1} (hydrogen bonded C=O) and λ_{max} (ε_{max}) on the uv spectrum at 206 nm (29,700), 256 nm (12,400) and 348 nm (4910) strongly suggest the presence of an ortho-hydroxy-acetophenone type moiety in the molecule (Talpatra *et al.,* 1988). Noise decoupled ^{13}C-nmr and DEPT spectra of 1 showed total 16 carbon peaks (Table I) including eight methine, five quaternary and three methylene carbon peaks. Characteristic peaks of the spectra include a peak (206.3 ppm) due to the ketonic carbon, six peaks in the aromatic region (157.6, 148.1, 134.0, 127.8,118.2 and 115.4 ppm), six peaks due to the glucose carbons (103.3, 77.3, 76.7, 74.3, 70.2, and 61.6 ppm) and three peaks due to the C-2, C-3 and C-4 carbon (29.3, 33.0 and 60.2 ppm). The carbon peaks due to the glucose moiety were easily assigned by comparison of the chemical shifts with other glucosides (agrawal, 1989). The peak due to

Table I. NMR Data for Compound **1** and **2**.

	1		2	
1	δ_c	δ_H (multiplicity, J in Hz)	δ_c	δ_H (multiplicity, Jin Hz
2	206.3		148.3	6.77 (d, 8.3)
	33.0	3.01 (ddd, 17.5, 12.8, 5.8)	108.0	
3	28.3	2.46 (ddd, 17.5, 3.3, 3.4)	112.3	7.24 (d, 8.3)
4	60.20	2.18 (m)	149.7	
5	148.1	5.33 (dd, 3.0, 3.0)	113.9	7.69 (dd, 8.2, 0.6)
6	127.8		126.9	7.32 (dd, 8.2, 7.7)
7	118.2	7.57 (d, 9.0)	111.8	6.82 (dd, 7.7, 0.6)
8	157.6	6.83 (d 9.0)	154.8	
9	115.4		117.3	
10	134.0		128.1	
1'	103.3	4.74 (d, 7.4)	104.6	5.06 (d, 7.3)
2'	74.3	3.52 (m)[a]	74.7	3.5 (m)[c]
3'	76.7[d]	3.43 (m)[b]	77.9[e]	3.5 (m)[c]
4'	70.2	3.43 (m)[b]	71.1	3.5 (m)[c]
5'	77.3[d]	3.52 (m)[a]	78.2[e]	3.5 (m)[c]
6'	61.6	3.82 (dd, 12.0, 1.5)	62.4	3.78 (dd, 11.3, 4.6)
		3.66 (dd, 4.9, 12.0)		3.96 (brs, 11.3)
4-OH		3.25 (s)		8.85 (brs)
8-OH		12.08 (s)		9.30 (s)

[a,b,c]Chemical shift values and multiplicities are obscure due to the overlapped signal. [d,e]The assignments may be reversed.

the C-1 carbon, the ketone conjugated with the adjacent aromatic ring, appears unusually downfield at 206.3 ppm, because this ketone has a strong intramolecular hydrogen bond with the OH group on C-8-carbon (Atta-ur-Rahman, 1986). Three peaks of C-2, C-3 and C-4 carbon could be deduced to be those at 33.02, 29.8 and 60.2 ppm, respectively. Between the two peaks (157.6 and 148.1 ppm) due to the OH-attached quaternary carbons in aromatic region, the one at 157.6 ppm could be assigned to the C-8 carbon peak because the aromatic carbon peaks located at the ortho position form ketone group appear at further upfield than the one at the meta position. ^{1}H-nmr spectrum of 1 showed one phenolic OH peak at 12.08 ppm (OH at C-8) and one aliphatic OH peak at 3.25 ppm (OH at C-4). Therefore, the location of glucosidic linkage on the tetralone ring must be at C-5 position. The configuration of anomeric proton of the sugar part was proposed to be b form on the basis of coupling constant of the proton peak (7.4 Hz at 4.82 ppm). The ^{1}H, ^{1}H-COSY spectrum of **1** showed three set of spin-spin coupling systems among protons; the first between H-6 and H-7 (7.57 and 6.83 ppm), the second among five protons of H-2a, H-2b, H-3a, H-3b and H-4 (2.46, 3.01, 2.18 and 5.33 ppm) and the third among protons on glucose moiety. Based on the above experimental results, the structure of **1** was proposed (Fig. 1). Compound **2** showed M^++1 peak at m/z 339 and an intense fragment ion peak due to the naphthalene ring moiety at m/z 176. Glucose was identified on a TLC plate with standard sample from the acid hydrolysate of **2**. In the aromatic region of ^{1}H-nmr spectrum of **2**, two sets of spin-spin coupling systems due to five protons were recognized; one between H-2 and H-3 (J = 8.3 Hz at 6.77 ppm and J = 8.3 Hz at 7.24 ppm), and the other one among H-5, H-6 and H-7 (J = 8.3, 0.6 Hz at 7.69 ppm, J = 8.3, 7.7 Hz at 7.32 ppm and J = 7.7, 0.6 Hz at 6.82 ppm). Two broad peaks due to phenolic OHs at 8.75 ppm and 9.30 ppm were also shown. In ^{13}C- and DEPT nmr spectra of **2**, six peaks due to glucose moiety (104.6, 78.2, 74.7, 71.1, 77.9 and 62.4 ppm) and ten peaks due to naphthalene ring including three peaks of carbons which are adjacent to OH group (148.3, 149.7 and 154.8 ppm) were shown. The chemical shift values of **2** were essentially identical to those of naphthalene glucosyl part of 1,4,8-trihydroxynaphthalene-1-O-β-D-[6'-O-(3",4",5"-trihydroxybenzoyl)]-glucopyranoside. Assignment of chemical shifts of **2** was based on comparison with those of above compound. The compound **2** was already reported from *Juglans nigra* (Muller and Leistner, 1987). Gallic acid was identified by comparison of ^{13}C-nmr spectrum and TLC with those of standard meterial.



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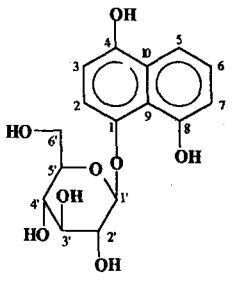

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