Isolation of a New Secolignan Compound from *Quercus glauca*

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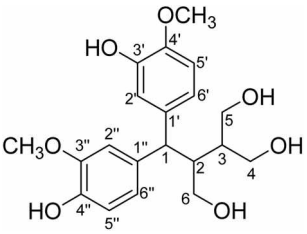
Quercus glauca Thunb (Fagaceae), distributed in the southern regions of Korea, is a big tree traditionally being used for household furnitures. In our continuing search for bioactive compounds from plants endemic to Jeju Island,[1] we have found that the ethanol extract of *Q. glauca* leaves exhibited significant antioxidative activity. Previous chemical investigation on this plant indicated the existence of flavonoids, triterpenoids and gallates.[2] In the present study on this plant, we have isolated and identified a secolignan type new compound **1**, 4-(3'-hydroxy-4'-methoxyphenyl)-4-(4"-hydroxy-3"-methoxyphenyl)-2,3-di(hydroxymethyl)-butan-1-ol.

The ethanol extract was prepared from the leaves of *Q. glauca*, collected in Jeju Island. The extract was successively partitioned into hexane, ethyl acetate and *n*-butanol. The purification of the ethyl acetate fraction by chromatography over celite, reversed-phase SiO_2 and Sephadex LH-20 led to the isolation of compound **1** as an amorphous powder.



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The molecular formular of **1** was determined as $C_{20}H_{26}O_7$ (eight unsaturations) based on the high resolution FABMS and NMR data. Analysis of ^{13}C NMR data indicated the presence of 12 carbons corresponding to two aromatic rings and additional eight aliphatic carbons. Further careful analysis of NMR spectral data suggested that two aromatic rings are likely to have very similar substitution patterns. For example, aromatic NMR signals of ^1H (six signals at δ_H 6.95 and 6.94, 6.83 and 6.83, 6.70 and 6.69) and ^{13}C (12 signals at δ_C 112-149 ppm, see Table 1) were showed up with close duplication. It was evident that each aromatic ring is 1,3,5-trisubstituted benzene moiety based on ^1H-^1H coupling analysis. For example, each benzene ring has three signals whose splittings are a doublet (J = 8.0 Hz), a doublet (J = 2.0 Hz) and a doublet of doublet (J = 2.0 and 8.0 Hz). DEPT analysis of aliphatic carbons suggested the presence of three methines, three *O*-methylenes and two *O*-methyls. The connectivity of aliphatic carbons such as ^1CH-^2CH-^3CH-$^4CH_2O$ skeleton was accomplished by COSY and HMBC analysis (Figure 1). The linkage of C-2/C-6 and C-3/C-5 was also confirmed by ^1H-^1H COSY correlation data.

Table 1. 1D and 2D NMR data for **1** in CD_3OD

no[a]	δ_C (mult)[b]	δ_H (int, mult, J in Hz)	HMBC (H → C)
1	52.2 (d)	3.98 (1H, d, 14.0)	C-2, C-3, C-6, C-1', C-2', C-6', C-1", C-2", C-6"
2	45.1 (d)	2.64 (1H, m)	C-2, C-3, C-4, C-5
3	44.0 (d)	1.96 (1H, m)	C-1, C-5, C-6
4	63.7 (t)	3.69 (1H, m)	C-2, C-3, C-5
		3.61 (1H, dd, 11.5, 4.4)	
5	59.8 (t)	3.70-3.72 (2H, m)	C-3
6	60.3 (t)	3.53 (1H, dd, 11.5, 2.2)	C-2, C-3
		3.38 (1H, dd, 11.5, 5.5)	
1'	137.9 (s)	–	–
2'	113.1 (d)	6.95 (1H, d, 2.0)	C-1', C-3', C-4', C-6'
3'	145.9 (s)	–	–
4'	149.2 (s)	–	–
5'	116.4 (d)	6.70 (1H, d, 8.0)	C-1', C-3', C-4'
6'	121.8 (d)	6.83 (1H, dd, 8.0, 2.0)	C-2', C-4'
1"	137.3 (s)	–	–
2"	112.9 (d)	6.94 (1H, d, 2.0)	C-1", C-3", C-4", C-6"
3"	148.9 (s)	–	–
4"	145.8 (s)	–	–
5"	121.4 (d)	6.69 (1H, d, 8.0)	C-1", C-3", C-4"
6"	116.2 (d)	6.83 (1H, dd, 8.0, 2.0)	C-2", C-4"
4'-OCH_3	56.6 (q)	3.83 (3H, s)	C-4'
3"-OCH_3	56.5 (q)	3.82 (3H, s)	C-3"

[a]Each position for 1'-6' is interchangeable with 1"-6" respectively. [b]Determined by DEPT experiments.

The analysis HMBC data indicated that the aromatic rings A and B are both attached to C-1. For example, H-1 showed $^2J_{CH}$ correlation with C-1' and C-1" as well as $^3J_{CH}$ correlation with C-2', C-2", C-6', and C-6" in the benzene rings. The structures of the rings A and B are different only in the positions of -OH and -OCH_3 groups. In the ring A, the attachment of methoxy group to C-4' was confirmed by HMBC correlation of the methoxy signal with C-4'. The

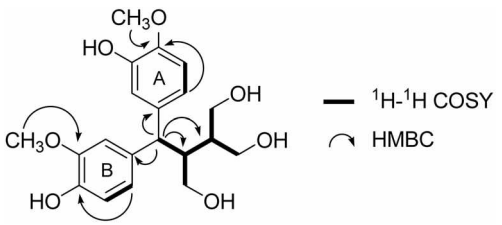
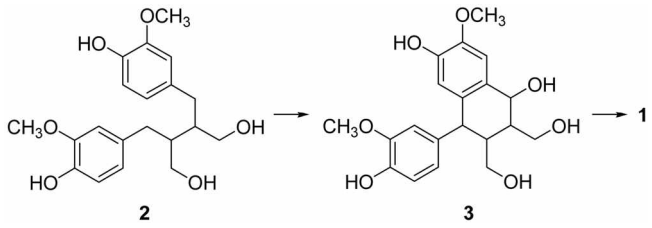


Figure 1. Key COSY and HMBC correlations in compound **1**.



Scheme 1. Proposed biosynthetic pathway to the secolignan **1**.

position of C-4' is also confirmed to be *meta* to C-6' by HMBC correlation of H-6' with C-4'. On the other hand, in the ring B, the methoxy group was attached to C-3" on the basis of HMBC correlation of the methoxy signal with C-3". Since the chemical shifts of NMR signals (δ_H and δ_C) of each position for the rings A and B were very close, it was difficult to obtain clear assignment of each set of NMR data. Therefore, the NMR data of each position for the aromatic rings A and B in Table 1 are interchangeable.

Since the aromatic rings attached to C-1 are bulky, the compound **1** appeared to have relatively rigid conformation around C-1 and C-2. To minimize steric congestion, H-1 and H-2 are positioned to have antiperiplannar conformation, which was evident by its ^{1}H-^{1}H coupling constant ($J_{H\text{-}1/H\text{-}2}$ = 14.0 Hz). This type of large coupling constant is reported for the other secolignan compounds, which shares structural similarity at C-1 with the compound **1**.[3a] The rigid conformation of **1** was also verified by the appearance of different δ_C (59.8 *vs*. 63.7 ppm) for the non-equivalent carbons, C-4 and C-5. The identification of stereochemistry at C-1 and C-2 could not be accomplished.

The compound **1** belongs to secolignans, of which only a few similar compounds have been previously reported in the literature.[3] It could be interesting to suggest the biosynthesis of compound **1** from a typical lignan derivative **2** as shown in Scheme 1. The lignan **2** should be transformed to the cyclic intermediate **3** by the process of ring closure and benzylic oxidation. The following C-C bond cleavage in **3** would lead to the ring opened secolignan derivative **1** (Scheme 1).

The compound **1** was examined for antioxidation activity using DPPH radical scavenging test.[4] Its radical scavenging activity (RS_{50} = 15.7 μg/mL) was slightly lower than that of catechin (RS_{50} = 8.1 μg/mL), a natural antioxidant.

Experiment Section

Reagent and Equipment. Thin layer chromatography was performed on Merck prepared plates (silica gel 60 F-254 on aluminum). Column chromatography was performed using Merck silica gel 60 (230-400 mesh). Sephadex LH-20 (25-100 μm) for Gel filtration chromatography (GFC) was obtained from Fluka. The UV absorbance was performed with a Biochrom Libra S22 UV-visible spectrophotometer. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a JNM-LA 400 (JEOL) instrument, with chemical shift data reported in ppm relative to the solvent used. 2D NMR spectra were recorded on the same instrument using field gradient FG2 (inverse) probe. Melting point was recorded on Fisher Scientific 12-132T. Optical rotation was recorded using a polarimeter, Jasco P-1030. High resolution MS spectral data was obtained from the Korea Basic Science Institute (Seoul).

Isolation of the Compound 1. The plant, *Q. glauca* was collected from Halla Botanical Garden in Jeju Island, and air dried and cut into small pieces. The voucher specimen (J-36) was prepared and deposited in the laboratory of natural product, department of chemistry, Cheju National University.

The dried *Q. glauca* leaves powder (300 g) was suspended in 6.0 L of 70% EtOH and mechanically stirred for 24 h at room temperature. The solution was filtered and the filtrate was concentrated under reduced pressure to give the oily extract (64.8 g). After the extract was suspended on 1 L of distilled water, successive solvent fractionation was performed using hexane, ethyl acetate and *n*-butanol. The EtOAc fraction (11.4 g) was chromatographed over celite with *n*-hexane, CH_2Cl_2, EtOAc and MeOH successively. The obtained ethyl acetate subfraction was chromatographed over reversed-phase silica gel with gradient solvents (H_2O-MeOH) system to provide eight fractions. The second fraction was further purified using Sephadex LH-20 with $CHCl_3$/MeOH (1/1 to 1/2) to give the compound **1** (46 mg).

4-(3'-Hydroxy-4'-methoxyphenyl)-4-(4"-hydroxy-3"-methoxyphenyl)-2,3-di(hydroxymethyl)butan-1-ol (1). Amorphous powder; UV (CH_3OH) 280.6; Melting point 122 °C; $[\alpha]_D^{20}$ = −11.0 (*c* 0.001, MeOH); HR-FABMS data [*m/z* 378.1692 $[M]^+$, calcd for $C_{20}H_{26}O_7$ 378.1679, Δ −1.3 mmu]. For ^{1}H and ^{13}C NMR data, see Table 1.

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