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A New Isoflavone Glycoside from the Stems of *Tilia taquetii* Schneider

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Tilia taquetii Schneider is a deciduous tree belonging to a family *Tiliaceae*. The genus *Tilia* comprises six species distributing in Korea and *T. taquetii* is known to be Korean endemic plant.[1] Because its lumber has highly durable properties, this tree has long been used as raw materials for household furniture. The flowers of this tree have been used as the folk medicine for the alleviation of flu symptoms in Korea.[2] However, as far as we know, no report has been published so far on this plant either in biological or phytochemical viewpoint. As our continuing program searching for bioactive compounds from plants collected in Jeju Island,[3] we became interested in the ethanol extract of *T. taquetii* stems in which DPPH radical scavenging activities were observed. This led us to undertake a chemical investigation on the extract to identify the active constituents. As the result, we herein report the isolation and characterization of a new compound, orobol 4'-*O*-*β*-glucopyranoside (**1**) together with four known compounds, 3'-*O*-methylorobol (**2**), cleomiscosin A (**3**), cleomiscosin B (**4**), and oleic acid (**5**) (Figure 1).

Compound **1** was obtained as a viscose liquid. It showed a $[M+Na]^+$ peak at *m/z* 471.0905 (calcd *m/z* 471.0903) in the high resolution FAB-MS indicating its molecular formula $C_{21}H_{20}O_{11}$ (12 unsaturations). This compound showed one methylene, eleven methine and nine quaternary carbon signals in ^{13}C and DEPT NMR spectra. Further inspection of ^{13}C NMR data in combination with HMQC spectrum revealed that the twenty one carbons in **1** are made up of fifteen sp^2 and six sp^3 hybridized ones. As all of six sp^3 carbons appeared to be oxygen-bearing, a hexose sugar unit is suggested in this compound, which is also confirmed by ^1H NMR spectrum. The aglycone unit composed by the remaining fifteen sp^2 carbons was deduced as a flavonoid because the NMR data displayed characteristic subunits of two aromatic rings, one carbonyl and one olefin. By the observation of a down field ^1H singlet (H-2, δ 8.08 ppm) exhibiting HMQC correlation with a C-2 signal (δ 155.4 ppm), this flavonoid appeared to be an isoflavone. The B ring in this isoflavone is suggested to have 1,2,4-trisubtituted structure by the ^1H signals [7.09 (d, J = 2.0 Hz), 7.24 (d, J = 8.3 Hz), 6.95 (dd, J = 8.3, 2.0 Hz)]. Based on these data, the isoflavone was confirmed as 5,7,3',4'-tetrahydroxyisoflavone, known as orobol. The sugar unit was identified as a glucose based on ^1H and ^{13}C NMR data (Table 1). Hydrolysis of **1** and TLC analysis of the aqueous phase also confirmed glucose as a carbohydrate moiety.[4] The *β*-configuration of the glucose was deduced by the large coupling constant (J = 7.6 Hz) of the anomeric proton (H-1"). The connection of the sugar was established by HMBC and NOESY data (Figure 2). As the H-1" has a ^3J correlation with C-4' (δ_C 147.1 ppm), the sugar is assigned at C-4' position. The observation of the NOESY correlations in H-2/H-2', H-2/H-6' and H-5'/H-1" also confirmed these assignments. Therefore, the natural product **1**, orobol 4'-*O*-*β*-glucopyranoside, is elucidated as a new compound. Even though several orobol glycosides have been isolated from plant sources, most of them have sugar units at C-5 or C-7 position.[5] Interestingly, the compound **1** is the first orobol glycoside where the sugar is attached to C-4' position.

By the similar spectroscopic analysis, the compound **2** was also identified as an analogue of isoflavone, 3'-*O*-methylorobol.[6] The compounds **3** and **4** were obtained as white solids. Theses compounds showed NMR spectrum

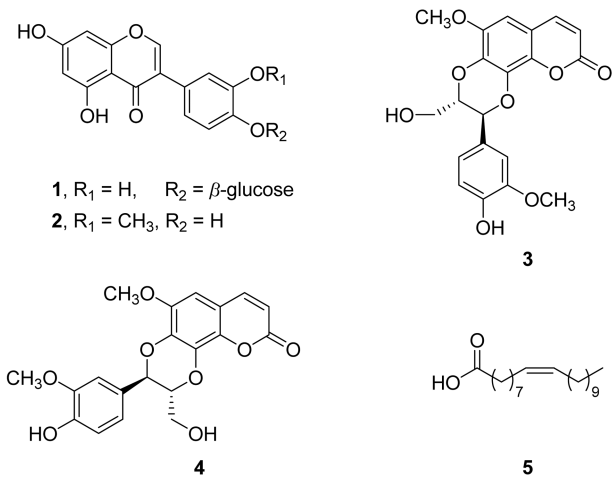

Figure 1. Compounds isolated from the stems of *T. taquetii*.

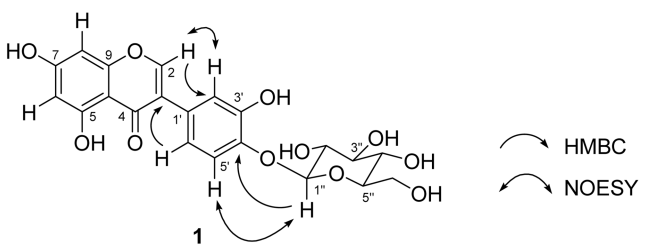

Figure 2. Key HMBC and NOESY correlations in the compound **1**.

patterns as observed in 6,7,8-trioxycoumarine derivatives. In addition, the presence of phenylpropanoid subunit was also suggested in these compounds. By the analysis of 1D and 2D NMR spectra, the compounds **3** and **4** were identified as coumarinolignans, cleomiscosin **A** and **B** respectively.[7] The compound **5** isolated from a nonpolar fraction was oleic acid, whose identification was made by comparing its NMR data to those obtained from authentic sample. Even though the isolates **2-5** were known metabolites from natural sources, this is the first example of their isolations from *T. taquetii*.

The antioxidative activities were determined for compounds **1-5** using DPPH method.[8] The 50% DPPH scavenging concentrations (RS_{50}) for **1** and **2** were respectively 40.0 μg/mL and 77.8 μg/mL showing moderate activity compared to a positive control, ascorbic acid (RS_{50} = 19.3 μg/mL). The compounds **3-5**, however, exhibited no activity on this test with RS_{50} values over 150 μg/mL.

Experimental Section

Reagents and Instruments. All solvents of analytical grade were used without further purification. ^{1}H (400 MHz), ^{13}C (100 MHz) and 2D (HMQC, HMBC) NMR spectra were recorded using JEOL JNM ECX-400 spectrometer. The chemical shift values are reported in ppm relative to the solvent used. Optical rotations were measured on a Jasco P-1030 automatic polarimeter. UV spectra were recorded on a Biochrom Libra S22 spectrophotometer. Column chromatography was performed on silica gel 60 (0.040-0.063 mm) and RP-C_{18} silica gel (230-400 mesh) purchased from Merck. Sephadex LH-20 (25-100 μm, Fluka) was used for the gel chromatography. TLC was performed on silica gel GF_{254} plates (0.50 mm, Merck).

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

No	δ_C (mult)[a]	δ_H (int, mult, *J* in Hz)	HMBC (H→C)
2	155.4 (d)	8.08 (1H, s)	C-3, C-4, C-9, C-1'
3	124.4 (s)		
4	182.1 (s)		
5	164.1 (s)		
6	100.4 (d)	6.21 (1H, d, 2.1)	C-5, C-7, C-8, C-10
7	166.3 (s)		
8	95.0 (d)	6.33 (1H, d, 2.1)	C-6, C-7, C-9, C-10
9	159.8 (s)		
10	106.4 (s)		
1'	127.9 (s)		
2'	118.2 (d)	7.09 (1H, d, 2.0)	C-3, C-4', C-6'
3'	148.3 (s)		
4'	147.1 (s)		
5'	118.5 (d)	7.24 (1H, d, 8.3)	C-1', C-3'
6'	121.8 (d)	6.95 (1H, dd, 8.3, 2.0)	C-3, C-2', C-4'
1"	104.2 (d)	4.81 (1H, d, 7.6)	C-4'
2"	75.0 (d)	3.52 (1H, dd, 9.1, 7.6)	C-1", C-3"
3"	77.7 (d)	3.48 (dd, 9.1, 7.8)	C-4"
4"	71.4 (d)	3.41-3.46 (1H, m)	
5"	78.5 (d)	3.43 (1H, m)	
6"	62.6 (t)	3.73 (1H, dd, 13.0, 4.8) 3.91 (1H, dd, 13.0, 1.6)	C-4"

[a]Determined by DEPT experiments.

Plant Material. *T. taquetii* stems were collected from Jeju Island in September, 2009. A voucher specimen (J-234) is deposited at Natural Product Laboratory, Department of Chemistry, Cheju National University.

Extraction and Isolation. The pulverized stems of *T. taquetii* (0.9 kg) was extracted three times with 70% aqueous ethanol under stirring for 24 hr at room temperature. The combined extract solutions were filtered, and the filtrate was concentrated under reduced pressure to afford a gummy mass (61.9 g). A portion of the extract (35.4 g) was suspended in water (1.0 L) and successively partitioned with *n*-hexane, ethyl acetate (EtOAc) and *n*-butanol. The EtOAc-soluble fraction (7.4 g) was subjected to vacuum liquid chromatography (VLC) over silica gel using step gradient solvents (*n*-hexane-EtOAc-methanol) to give 38 fractions (Frs EA1 to EA38). A nonpolar fraction EA6 (33.8 mg) was further purified using silica gel column chromatography with *n*-hexane-EtOAc (3:1) eluents to afford the compound **5** (8.7 mg). Fraction EA15 (59.4 mg) was purified by recrystallization from methanol to give the compound **4** (3.7 mg). A fraction EA23 (351.5 mg) eluted with *n*-hexane-MeOH (90:10) was subjected to gel filtration chromatography with Sephadex LH-20 using solvent gradients (chloroform-methanol 3:1 to 1:3) to give the compound **1** (11.0 mg). The similar separation procedure was repeated one more time with the remaining ethanol extract (23.0 g). The obtained EtOAc-soluble fraction (4.9 g) was subjected to VLC to afford 26 fractions (Frs EA1'-EA26'). Fraction EA9' was purified by recrystallization from methanol to afford the compound **2** (4.0 mg). Fraction EA15' (95.4 mg) was also purified by recrystallization from methanol to give the compound **3** (7.1 mg).

Hydrolysis of the Compound 1. The compound **1** was treated with 5% HCl (4 mL) at 90 °C for 2 hr. The hydrolyzed solution was concentrated under reduced pressure. To this were added H_2O (1 mL) and $CHCl_3$ (1 mL). The carbohydrate was identified by TLC analysis of the H_2O phase upon comparison of the authentic D-glucose (R_f = 0.17; CH_2Cl_2:MeOH:H_2O = 70:27:3).[4]

Orobol 4'-β-D-glucopyranoside (1). Viscose liquid; UV (CH_3OH): λ_{max} 248 nm; $[\alpha]_D^{20}$ −40.4° (*c* 0.11, MeOH); ^{1}H and ^{13}C NMR data: Table 1; HR-FAB-MS: *m/z* 471.0905 $[M+Na]^+$ (calcd for $C_{21}H_{20}O_{11}Na$ 471.0903, Δ −0.2 mmu).

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