A New Acyclic Diterpene from *Trigonotis peduncularis*

Myoung-Chong Song, Hye-Joung Yang, Dae-Keun Kim,† and Nam-In Baek*

Graduate School of Biotechnology & Plant Metabolism Research Center, Kyung Hee University, Yongin 446-701, Korea
**E-mail: nibaek@khu.ac.kr*
†Department of Pharmacy, Woosuk University, Jeonju 565-701, Korea
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Trigonotis peduncularis (Boraginaceae) is an annual herb that grows in Korea, Japan and China. It reaches a height of 10-30 cm, has azure flowers, and possesses a fine hairy body.[1] The young leaflets are favorably ingested in the Korean diet[1] and *T. peduncularis* is known to be a diuretic and an emollient.[2,3] It is used not only for the treatment of diarrhea and dysentery, but also to remedy or prevent muscle paralysis, pleurisy, and influenza. However, only limited studies on the pharmacological constituents of the plant have been done.[3] Flavonoids such as astragalin, nicotiflorin, rutin, and isoquercitrin, which exhibit antiatherogenic activity, were previously reported in *T. peduncularis*.[4] To isolate and identify other secondary metabolites from *T. peduncularis*, whole plants were extracted in 80% aqueous methanol (MeOH) and successively fractionated using ethyl acetate (EtOAc), *n*-butanol (*n*-BuOH), and water. Repeated column chromatography using silica gel and octadecyl silica gel (ODS) for EtOAc and *n*-BuOH fractions led to the isolation of three acyclic diterpenoids. From the results of spectroscopic data including NMR, MS, and IR, the chemical structures of the isolates were determined. A new acyclic diterpenoid, 3*S*,6*E*,10*E*,14ξ-tetramethyl-3,14,15-trihydroxyhexadeca-1,6,10-triene 3-*O*-β-D-glucopyranoside, named lyciumoside X (**3**), was discovered along with identification of the known diterpenoids phytol (**1**) and lyciumoside III (**2**).

Experimental Section

Plant Materials. Whole plants of *T. peduncularis* (Boraginaceae) were collected at Kyung Hee University in Suwon, Korea in April 2005 and were identified by Prof. Dae-Keun Kim, Woosuk University, Jeonju, Korea. A voucher specimen (KHU0547) was reserved at the Laboratory of Natural Products Chemistry, Kyung Hee University, Yongin, Korea.

Instruments. For instrumental and general methods, see previous papers.[5,6]

Isolation of Diterpenes. Whole plants of *T. peduncularis* (9.0 kg) were extracted three times for 24 h at room temperature with 80% aqueous MeOH (18 L × 3). The MeOH extracts were successively partitioned with water (2 L), EtOAc (2 L × 2), and *n*-butanol (*n*-BuOH, 2 L × 2). The EtOAc extract (TPE, 29 g) was subjected to silica gel (SiO_2, 300 g, 70-230 mesh, Merck, Darmstadt, Germany) column chromatography (cc) (F 7 × 13 cm), eluted with *n*-hexane-EtOAc (10:1 → 5:1 → 3:1 → 1:1, v/v, 1.0 L each) and chloroform ($CHCl_3$)-MeOH (10:1 → 5:1 → 3:1 → 1:1, v/v, 1.0 L each), and monitored by thin layer chromatography (TLC) to obtain nine fractions (TPE1 to TPE9). TPE3 [675 mg, Ve/Vt (elution volume/total volume) 0.08-0.12] was purified by SiO_2 (100 g) cc (Φ 3 × 12 cm) and eluted with *n*-hexane-EtOAc (6:1, v/v, 2100 mL) to give compound **1** (153 mg, Ve/Vt 0.28-0.33; SiO_2 TLC R_f 0.3, *n*-hexane-EtOAc = 5:1). The *n*-BuOH fraction (TPB, 31 g) was subjected to SiO_2 (300 g) cc (Φ 6 × 10 cm), eluted with $CHCl_3$-MeOH (10:1, v/v, 2.2 L) and $CHCl_3$-MeOH-water (15:3:1 → 10:3:1 → 7:3:1 → 65:35:10 → 6:4:1 → 6:5:1, v/v, lower layer of each 1.5 L) and monitored by TLC to produce eleven fractions (TPB1 to TPB11). TPB8 (6.7 g, Ve/Vt 0.60-0.74) was subjected to SiO_2 (150 g) cc (Φ 4 × 12 cm) and eluted with EtOAc-*n*-BuOH-water (5:4:1, v/v, 2000 mL) to give sixteen fractions (TPB8-1 to TPB8-16). TPB8-5 (2 g, Ve/Vt 0.20-0.32) was subjected to SiO_2 (100 g) cc (Φ 3 × 12 cm) and eluted with $CHCl_3$-MeOH-water (9:3:1, v/v, lower layer, 2600 mL) to obtain compound **2** (46 mg, Ve/Vt 0.45-0.48; SiO_2 TLC R_f 0.3, $CHCl_3$-MeOH-water = 7:4:1). TPB8-5-4 (104 mg, Ve/Vt 0.33-0.34) was purified using ODS (100 g) cc (Φ 3 × 12 cm) and eluted with MeOH-H_2O (1:3) to obtain compound **3** (6 mg, Ve/Vt 0.56-0.66; ODS TLC R_f 0.25, MeOH-H_2O = 1:1) (Figure 1).

Phytol (compound 1): Colorless oil; $[\alpha]_D$ +0.2° (*c* 1.2, $CHCl_3$); EIMS *m/z*: 296 $[M]^+$, 278, 263, 196, 182, 126, 123, 71, 57; IR (KBr window) ν_{max} 3334, 2954, 2923, 2868, 1669 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$, δ_H) 5.37 (1H, tq, *J* = 6.8, 1.4 Hz, H-2), 4.12 (2H, d, *J* = 6.8 Hz, H-1), 1.99 (2H, t, *J* = 7.0 Hz, H-4), 1.66 (3H, br. s, H-20), 1.00-1.66 (methine &

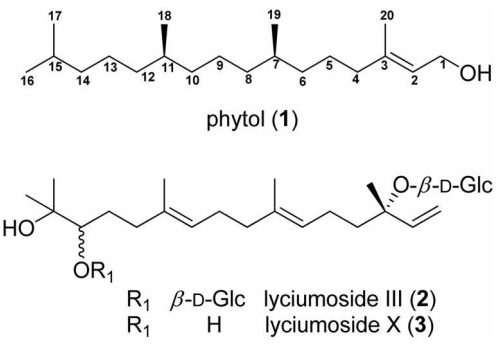

Figure 1. Chemical structures of diterpenoids isolated from whole plants of *Trigonotis peduncularis*.

Table 1. ^{13}C-NMR data (100 MHz, δ_C) of compounds **1-3** from *T. pseudoincisa* in $CDCl_3$ (**1**) and CD_3OD (**2** and **3**)

No. of Carbon	compound 1	compound **2**	compound **3**
1	59.4	115.8	115.8
2	123.0	144.3	144.3
3	140.1	81.3	81.4
4	39.9	42.7	42.5
5	25.2	23.6	25.0
6	36.7	125.9	121.9
7	32.8	135.8	129.1
8	37.5	40.8	42.3
9	24.5	27.4	26.0
10	37.4	125.7	121.8
11	32.7	135.8	128.5
12	37.3	36.8	36.5
13	24.8	30.6	30.7
14	39.4	90.2	78.9
15	28.0	74.8	73.7
16	22.7	23.9	25.0
17	22.8	26.6	25.9
18	19.8	16.1	16.5
19	19.8	16.2	16.7
20	16.2	23.3	23.2
1'		99.5	99.5
2'		75.0	75.1
3'		78.1	78.2
4'		71.2	71.4
5'		77.6	77.6
6'		62.5	62.5
1''		106.3	
2''		75.9	
3''		78.3	
4''		71.6	
5''		77.8	
6''		62.7	

methylene), 0.87 (6H, d, J = 6.4 Hz, H-16,17), 0.85 (3H, d, J = 6.0 Hz, H-18), 0.84 (3H, d, J = 6.8 Hz, H-19); ^{13}C-NMR (100 MHz, $CDCl_3$, δ_C); see Table 1.

Lyciumoside III (compound 2): White powder (MeOH); $[\alpha]_D$ −31.1° (c 0.48, MeOH); negative FABMS m/z: 647 $[M-H]^+$, 485 $[M-H\text{-glc}]^-$; ^{1}H-NMR (400 MHz, CD_3OD, δ_H) 5.84 (1H, dd, J = 11.2, 18.0 Hz, H-2), 5.13 (1H, dd, J = 18.0, 1.2 Hz, H-1a), 5.11 (1H, dd, J = 11.2, 1.2 Hz, H-1b), 5.11 (1H, m, H-10), 5.02 (1H, t, J = 6.6 Hz, H-6), 4.34 (1H, d, J = 7.6 Hz, H-1'), 4.25 (1H, d, J = 7.6 Hz, H-1''), 3.37 (1H, dd, J = 6.4, 3.8 Hz, H-14), 3.05-3.77 (m, β-D-glucopyranosyl), 1.53 (6H, br.s, H-18/H-19), 1.28 (3H, s, H-20), 1.07 (3H, s, H-16), 1.03 (3H, s, H-17); ^{13}C-NMR (100 MHz, CD_3OD, δ_C); see Table 1.

Lyciumoside X (3*S*,6*E*,10*E*,14*ξ*-tetramethyl-3,14,15-trihydroxyhexadeca-1,6,10-triene 3-*O*-*β*-D-glucopyranoside, compound 3): White powder (MeOH); $[\alpha]_D$ −32.0° (c 0.01, MeOH); IR (KBr window) ν_{max} 3420, 1660, 1250, 862, 845, 830 cm^{-1}; positive FABMS m/z 525 $[M+K]^-$, 509 $[M+Na]^+$, 487 $[M+H]^+$; positive HRFABMS m/z: 487.3279 (calcd. 487.3271 for $C_{26}H_{47}O_8$); ^{1}H-NMR (400 MHz, CD_3OD, δ_H) 5.92 (1H, dd, J = 11.2, 17.7 Hz, H-2), 5.25 (1H, dd, J = 11.2, 1.2 Hz, H-1a), 5.20 (1H, dd, J = 17.7, 1.2 Hz, H-1b), 5.18 (1H, m, H-10), 5.15 (1H, t, J = 7.6 Hz, H-6), 4.33 (1H, d, J = 8.0 Hz, H-1'), 3.80 (1H, br d, J = 10.0 Hz, H-6'a), 3.62 (1H, dd, J = 10.0, 5.6 Hz, H-6'b), 3.16-3.26 (m, H-2'-H-5'), 3.13 (1H, t, J = 6.8 Hz, H-14), 1.66 (3H, s, H-19), 1.62 (3H, br. s, H-18), 1.37 (3H, s, H-20), 1.15 (3H, s, H-17), 1.12 (3H, s, H-16); ^{13}C-NMR (100 MHz, CD_3OD, d_C); see Table 1.

Enzymatic hydrolysis of compound 2. β-Glucosidase, from bitter almonds (EC3.2.1.21, 4.5 U/mg, Sigma), was resuspended in 10 mL NaAc/HAc buffer (0.1 M, pH 5.0) and 10 mg of compound **2** was added to a final concentration of 1 mg/mL. The mixture was stirred for 24 h at 37 °C and extracted two times with 10 mL n-butanol. The organic layers were combined and evaporated *in vacuo*. The concentrate was purified by ODS cc using MeOH-H_2O (1:1, v/v) as eluent to give a purified product (3.4 mg).

Low Temperature Extraction and TLC Analysis. Fresh whole plants of *T. peduncularis* (5 g) were extracted three times in 80% aqueous MeOH (100 mL × 2) below 10 °C for 24 h and filtered. The combined filtrates were concentrated under vacuum and the concentrate was dissolved in MeOH (2 mL). TLC analysis was performed on silica gel ($CHCl_3$-MeOH-water = 65:35:10) and ODS (MeOH-water = 3:1). The solutions of sample and compound **3** were applied to the TLC using $CHCl_3$-MeOH-water (65:35:10) for the silica gel and MeOH-water (3:1) for the ODS as developing solvents, and the R_f values of compound **3** were recorded as 0.65 and 0.45, respectively.

Results and Discussion

Fresh whole plants of *T. peduncularis* were extracted with 80% aqueous MeOH, and the concentrated extract was partitioned with EtOAc, n-BuOH, and water. From the EtOAc and n-BuOH fractions, three diterpenoids were isolated through repeated SiO_2 and ODS column chromatography. The two known compounds **1** and **2** were identified as phytol (yield: 1.7 × 10^{-3} %, **1**) and lyciumoside III (yield: 5.1 × 10^{-4} %, **2**), respectively, through the comparison of several spectroscopic data with those of the literature.[7] Compound **2**, lyciumoside III, was previously isolated from *Lycium chinensis* Mill along with lyciumoside I-IX.[7,8] However, this is the first report of isolation of these acyclic diterpenoids (**1** and **2**) from *T. peduncularis*.

Compound **3** (yield: 6.7 × 10^{-5} %), a white powder, showed absorbance bands due to hydroxyl (3420 cm^{-1}) and olefine (1660 cm^{-1}) groups in the IR spectrum. The pseudomolecular ion peaks such as $[M+K]^+$, $[M+Na]^-$, and $[M+H]^-$ were detected at m/z 525, 509, and 487, respectively, in the positive FABMS spectrum and the molecular formula of $C_{26}H_{46}O_8$ was determined from an ion peak, m/z 487.3279, for $C_{26}H_{47}O_8$ ($[M+H]^-$) in the positive HRFABMS. In the ^{1}H-NMR spectrum (400 MHz, CD_3OD), three olefine methine proton signals [δ_H 5.92 (H-2), δ_H 5.18 (H-10), and δ_H 5.15 (H-6)], two exomethylene proton signals [δ_H 5.25 (H-1a) and δ_H 5.20 (H-1b)], a hemiacetal methine proton signal (δ_H 4.33, H-1'), several oxygenated methine and methylene proton signals at δ_H 3.13-3.26, and five singlet methyl proton signals [δ_H 1.66 (H-19), δ_H 1.62 (H-18), δ_H 1.37 (H-20), δ_H 1.15 (H-17), and δ_H 1.12 (H-16)] were

observed. These results suggested that compound **3** is a terpene glycoside with three olefins. The ^{13}C-NMR spectrum (100 MHz, CD_3OD) exhibited twenty six carbon signals including three double bonds, which consisted of two olefine quaternary carbon signals [δc 129.1 (C-7) and δc 128.5 (C-11)], three olefine methine carbon signals [δc 144.3 (C-2), δc 121.9 (C-6), and δc 121.8 (C-10)], an exomethylene carbon signal at δc 115.8 (C-1), two oxygenated quaternary carbon signals [δ_C 81.4 (C-15) and δ_C 78.9 (C-3)], one hemiacetal carbon signal at δc 99.5 (C-1'), five oxygenated methine carbon signals [δc 78.2 (C-3'), δc 77.6 (C-5'), δc 75.1 (C-2'), δc 73.7 (C-14), and δc 71.7 (C-4')], an oxygenated methylene carbon signal [δc 62.5 (C-6')], which indicated the presence of D-glucopyranose, and five methyl carbon signals [δc 25.9 (C-17), δc 25.0 (C-16), δc 23.2 (C-20), δc 16.7 (C-19), and δc 16.5 (C-18)]. The configuration of the D-glucopyranose was determined as β from the coupling constant (J = 8.0 Hz) of the anomeric proton signal at δ_H 4.33 in the ^{1}H-NMR spectrum of compound **3**.[7] We concluded from all the above evidence that compound **3** is an acyclic diterpene glucopyranoside with three hydroxyl groups, three double bonds including an exomethylene, and a β-D-glucopyranoside. Determination of the final structure of compound **3**, including the location of the functional group, was accomplished by 2D NMR experiments such as gradient correlated spectroscopy (gCOSY), gradient heteronuclear single quantum correlation (gHSQC), and gradient heteronuclear multiple bonding connectivity (gHMBC). In the gHMBC spectrum (Figure 2), an olefine methine proton signal at δ_H 5.92 (H-2) showed cross peaks with an exomethylene carbon signal at δc 115.8 (C-1) and an oxygenated quaternary carbon signal at δc 81.4 (C-3) by J_2 correlation, and with a methyl carbon signal at δc 23.2 (C-20) and a methylene carbon signal at δc 42.5 (C-4) by J_3 correlation. Another olefine methine proton signal at δ_H 5.18 (H-6) showed cross peaks with a methylene carbon signal at δc 25.0 (C-5) and an olefine quaternary carbon signal at δc 129.1 (C-7) by J_2 correlation, and with a methyl carbon signal at δc 16.7 (C-19) and two methylene carbon signals at δc 42.3 (C-8) and δc 42.5 (C-4) by J_3 correlation. The other olefine methine proton signal at δ_H 5.18 (H-10) showed cross peaks with a methylene carbon signal at δc 26.0 (C-9) and an olefine quaternary carbon signal at δc 128.5 (C-11) by J_2 correlation, and with a methyl carbon signal at δc 16.5 (C-18) and two methylene carbon signals at δc 36.5 (C-12) and δc 42.3 (C-8) by J_3 correlation. Therefore, three double bonds were determined to be located between C-1 and C-2, between C-6 and C-7, and between C-10 and C-11. An oxygenated methine proton signal at δ_H 3.14 (H-14) showed cross peaks with a methylene carbon signal at δc 30.7 (C-13) and an oxygenated quaternary carbon signal at δc 73.7 (C-15) by J_2 correlation, and with two methyl carbon signals [δc 25.0 (C-16) and δc 25.9 (C-17)] and a methylene carbon signal at δc 36.5 (C-12) by J_3 correlation. The anomeric proton signal of β-D-glucopyranoside at δ_H 4.33 (H-1') showed cross peaks with an oxygenated quaternary carbon at δc 81.4 (C-3) and an oxygenated methine carbon signal at δc 77.6 (C-5') by J_3 correlation, and with an oxygenated methine carbon signal at δc 75.1 (C-2') by J_2. To confirm the stereochemistry of double bond and chiral center of compound **3**, an enzymatic hydrolysis of compound **2** was performed using a β-glucosidase. The hydrolysate was purified and its NMR data and specific rotation ([α]$_D$ −29.3°, *c* 0.12, MeOH) were determined to be same as those of compound **3**. It was reported that the geometric structures of both double bonds at C-6 and C-10 in compound 2 were *E*, and that steric configuration of C-3 was *S*. Thus, compound **3** was identified as (3*S*,6*E*,10*E*,14ξ-tetramethyl-3,14,15-trihydroxyhexadeca-1,6,10-triene 3-*O*-β-D-glucopyranoside, a novel compound which we named lyciumoside X (**3**).

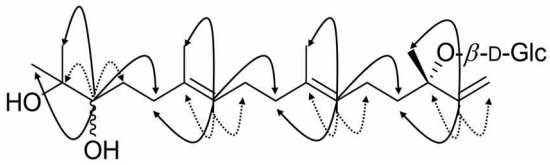

Figure 2. ^{1}H-^{13}C long-range correlations (J_2 and J_3) observed in the gHMBC spectrum of compound **3**. The dotted-line and solid-line arrows indicate the long-range correlations J_2 and J_3, respectively, between proton and carbon signals in the gHMBC spectrum.

TLC experiments were carried out to confirm whether compound **3** naturally occurred in *T. peduncularis* or was artificially produced through hydrolysis of lyciumoside III (**2**) during the isolation process. Fresh whole plants were extracted in aqueous MeOH at low temperature, and the extracts were directly compared with compound **3** using silica gel and ODS TLC. The extracts showed the same R_f values as those of compound **3**. R_f values were 0.65 and 0.45 on silica gel ($CHCl_3$-MeOH-water = 65:35:10) and ODS (MeOH-water = 3:1) TLC, respectively. Accordingly, compound **3** was confirmed as a genuine component of the plant.

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