http://dx.doi.org/10.5012/bkcs.2011.32.11.4083

Identification of a New Isomer of Dihydrophaseic Acid 3'-*O*-β-D-Glucopyranoside from *Nelumbo nucifera*

Ui Joung Youn, Jun Lee, Joo Won Nam, Yoo Jin Lee, and Eun-Kyoung Seo*

*The Center for Cell Signaling & Drug Discovery Research, College of Pharmacy, Ewha Womans University, Seoul 120-750, Korea. *E-mail: yuny@ewha.ac.kr*
Received August 8, 2011, Accepted September 14, 2011

Key Words : *Nelumbo nucifera*, Nymphaeaceae, (1'*R*,3'*S*,5'*R*,8'*S*,2*E*,4*E*)-Dihydrophaseic acid 3'-*O*-β-D-Glucopyranoside, Circular Dichroism (CD)

Nelumbo nucifera Gaertner (Nymphaeaceae), commonly known as lotus, is a perennial aquatic plant that is consumed all over the world, especially in India and Southeast Asia.[1] *N. nucifera* has traditionally been used for medicinal purposes as an antidepressant, antipyretic, diuretic, or sedative.[2] There have been phytochemical reports of phenolic compounds[3,4] and a sesquiterpenoid, (2*Z*)-dihydrophaseic acid in lotus seeds.[5] In the present study, a new isomer, (1'*R*,3'*S*,5'*R*,8'*S*,2*E*,4*E*)-dihydrophaseic acid 3'-*O*-β-D-glucopyranoside (**1**), was isolated from the seeds of *N. nucifera* together with a known compound, (1'*R*,3'*S*,5'*R*,8'*S*,2*Z*,4*E*)-dihydrophaseic acid 3'-*O*-β-D-glucopyranoside (**2**),[6] which has not been previously reported from the family Nymphaeaceae. Phaseic acid forms the basic skeleton of **1** and **2** and is biosynthesized by cyclization of 8'-hydroxy abscisic acid[7] which is a derivative of a plant growth hormone, abscisic acid.[8,9] This paper describes the unambiguous structure elucidation of compound **1** using 1D and 2D NMR and CD experiments.

Compound **1** was obtained as a colorless amorphous powder. Its molecular formula was established as $C_{21}H_{32}NaO_{10}$ on the basis of the molecular ion peak at *m/z* 467.1866 $[M + Na]^+$ (calcd for $C_{21}H_{32}NaO_{10}$, 467.1888) in the positive high resolution ESIMS. The ^1H and ^{13}C NMR spectra of **1**

Table 1. ^1H and ^{13}C NMR data of compounds **1** and **2** (CD_3OD)

Position	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1		nd[a]		171.1
2	5.82 (s)	126.9	5.77 (s)	121.3
3		143.3		149.2
4	7.82, *d* (*J* = 15.6 Hz)	131.2	7.90, *d* (*J* = 15.6 Hz)	134.1
5	6.27, *d* (*J* = 15.6 Hz)	132.9	6.43, *d* (*J* = 15.6 Hz)	132.3
6	1.97, *s*	20.8	1.93, *s*	21.2
1'		49.5		49.5
2'$_{ax}$	1.94, *ddd* (*J* = 2.0, 6.8, 13.6 Hz)	43.0	1.96, *ddd* (*J* = 2.0, 6.8, 13.6 Hz)	43.0
2'$_{eq}$	1.81, *m*		1.78, *m*	
3'	4.24, *m*	74.2	4.24, *m*	74.0
4'$_{ax}$	2.16, *ddd* (*J* = 2.0, 6.8, 13.6 Hz)	42.9	2.17, *ddd* (*J* = 2.0, 6.8, 13.6 Hz)	42.9
4'$_{eq}$	1.82, *m*		1.81, *m*	
5'		87.7		87.7
7'$_{exo}$	3.78, *dd* (*J* = 7.2, 2.0 Hz)	77.2	3.79, *dd* (*J* = 7.2, 2.0 Hz)	77.3
7'$_{endo}$	3.75, *d* (*J* = 7.2 Hz)		3.73, *d* (*J* = 7.2 Hz)	
8'		83.4		83.3
CH_3-9'	1.15, *s*	19.9	1.16, *s*	19.8
CH_3-10'	0.92, *s*	16.5	0.92, *s*	16.4
1"	4.36, *d* (*J* = 7.6 Hz)	103.3	4.34, *d* (*J* = 7.6 Hz)	103.2
2"	3.13, *t* (*J* = 8.8 Hz)	75.2	3.13, *t* (*J* = 8.8 Hz)	75.1
3"	3.35, *m*	78.2	3.36, *m*	78.2
4"	3.26, *m*	71.8	3.26, *m*	71.8
5"	3.29, *m*	78.1	3.27, *m*	78.1
6"	3.67-3.85, *m*	62.9	3.64-3.85, *m*	62.9

[a]nd: Not detected.

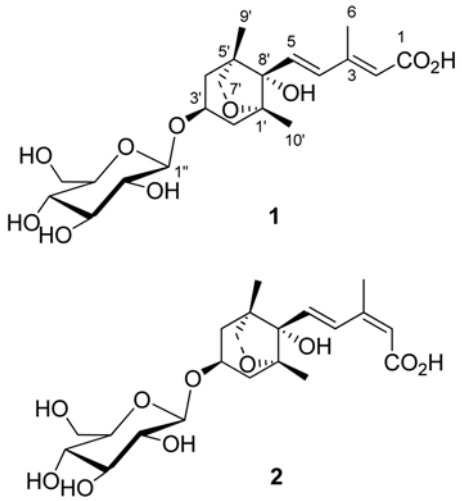

Figure 1. Compounds **1** and **2** isolated from the seeds of *N. nucifera*.

(Table 1) showed a methyl group at δ_H 1.97 (3H, s)/δ_C 20.8 (C-6), olefinic signals at δ_H 6.27 (1H, d, J = 15.6 Hz)/δ_C 132.9 (C-5), 7.82 (1H, d, J = 15.6 Hz)/δ_C 131.2 (C-4), 5.82 (1H, s)/δ_C 126.9 (C-2), and an olefinic quaternary carbon at δ_C 143.3 (C-3), indicated the presence of 3-methyl-penta-2,4-dienoic acid moiety.[6] In addition, the ^{1}H, ^{13}C, and DEPT spectra of **1** showed signals indicating two methyl groups, two methylenes, a secondary oxymethine, an oxymethylene group, two oxygenated quaternary carbons, and a quaternary carbon, which clearly indicated the presence of bicyclohexane ring skeleton.[5] A β-D-glucopyranosyl moiety appeared at δ_H 4.36 (1H, d, J = 7.6 Hz, H-1"), 3.13 (1H, t, J = 8.8 Hz, H-2"), 3.35 (1H, m, H-3"), 3.26 (1H, m, H-4"), 3.29 (1H, m, H-5"), and 3.67-3.85 (2H, m, H-6").[6] The connectivity of a 3-methyl-penta-2,4-dienoic acid moiety with the bicyclohexane ring was demonstrated by the three bond HMBC correlation of H-5 with C-8'. The HMBC correlation of the anomeric proton at δ_H 4.36 (H-1") with δ_C 74.2 (C-3) confirmed the position of the β-D-glucopyranosyl moiety at C-3' of the aglycon (Fig. 2). Accordingly, compound **1**

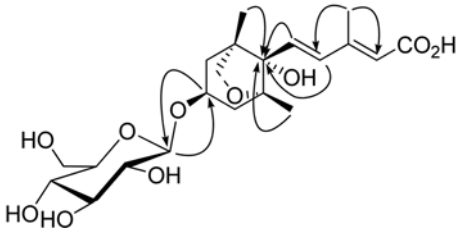

Figure 2. Important HMBC correlations of **1**.

showed similar NMR data with the reported values of dihydrophaseic acid 3'-*O*-β-D-glucopyranoside[6,10] except for two signals of olefinic bridge (C_2–C_3) at δ_C 126.9 (C-2) and δ_C 143.3 (C-3) in the ^{13}C-NMR spectrum. On the other hand, values for compound **2** were the same as those reported for (2*Z*)-dihydrophaseic acid 3'-*O*-β-D-glucopyranoside.[6,10] Therefore, the NMR data of compounds **1** and **2** were compared to determine their stereochemistry. The olefinic double bond (C_2–C_3) in **1** did not show the NOESY correlation between H-2 and CH_3-6, whereas the NOESY data of **2** displayed a correlation between H-2 and CH_3-6. These results provided strong evidence for the presence of a *cis* (*Z*) olefinic double bond (C_2–C_3) in **2** and the presence of a *trans* (*E*) olefinic double bond at C_2–C_3 of **1** (Fig. 3).

To determine the absolute configuration of **1**, a circular dichroism (CD) experiment was performed. Two stereoisomers with C-8' (*S*), that is, (1'*R*,3'*R*,5'*R*,8'*S*)-dihydrophaseic acid 3'-*O*-β-D-glucopyranoside (**2**) (Fig. 4) and (1'*R*,3'*R*,5'*R*, 8'*S*)-*epi*-dihydrophaseic acid 3'-*O*-β-D-glucopyranoside, have been reported to show a negative cotton effect at 235 nm and a positive cotton effect at 272 nm, respectively,[6,10] which is similar to the CD data of **1**. Therefore, **1** was found to have C-8' (*S*) configuration. The relative stereochemistry of C-1', C-3', and C-5' in **1** was characterized through extensive analyses of the coupling patterns of the protons in the cyclohexane ring and the NOESY spectrum (Fig. 3). A small coupling constant (^4J = 2.0 Hz) for the oxymethylene H-7'exo proton on the axially oriented C-1'–O–C-7' bridge was due to long range W-coupling with H-2'ax in dihydrophaseic

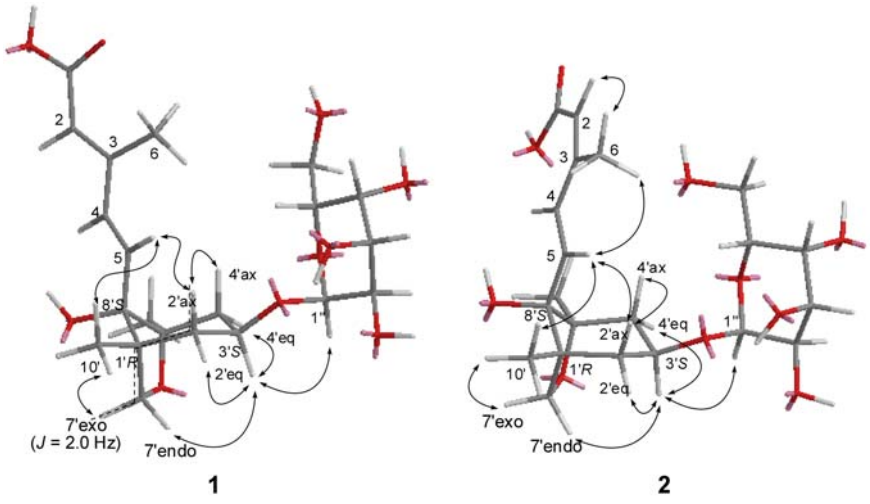

Figure 3. The key NOESY correlations of **1** and **2**.

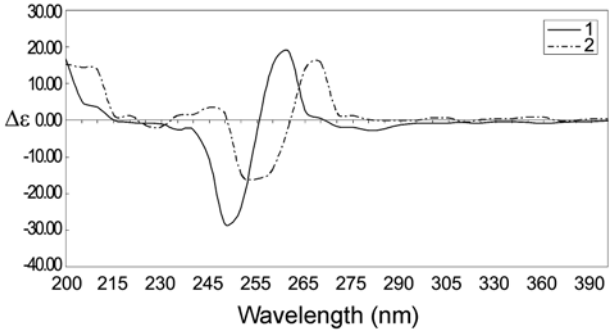

Figure 4. CD spectra of **1** and **2**.

acid. The coupling pattern suggested that the cyclohexane ring took a chair conformation.[11] The NOESY correlations between H-2'ax and H-4'ax/H-5 indicated that these protons were located on the same side (β axial) in the cyclohexane ring. On the other hand, correlations of an oxymethine proton H-3' with H-2'eq/H-4'eq were observed in the NOESY spectrum. Additional NOE correlations between H-3' and H-7'endo/H-1" implied that an oxymethine H-3' proton was occupied on the same side (α face) with an oxymethylene O–C-7' bridge in the cyclohexane ring (Fig. 3). According to the above observations, the stereostructure of **1** was assigned as a new geometric isomer, (1'*R*,3'*S*,5'*R*,8'*S*,2*E*, 4*E*)-dihydrophaseic acid 3'-*O*-β-D-glucopyranoside.

The known compound, (1'*R*,3'*S*,5'*R*,8'*S*,2*Z*,4*E*)-dihydrophaseic acid 3-*O*-β-D-glucopyranoside (**2**)[6] was identified by comparison of the physical and spectral data with published values. To the best of our knowledge, compound **2** was isolated from the family Nymphaeaceae for the first time.

Experimental Section

General Method. UV and IR spectra were recorded on a U-3000 spectrophotometer (Hitachi, Japan) and a FTS 135 FT-IR spectrometer (Bio-Rad, CA), respectively. CD spectra were recorded on a JASCO J-810 polarimeter. 1D and 2D NMR experiments were performed on a UNITY INOVA 400 MHz FT-NMR instrument (Varian, CA) with tetramethylsilane (TMS) as internal standard. Mass spectrometry was carried out with a JEOL JMS-700 Mstation mass spectrometer. Thin-layer chromatography (TLC) was performed on precoated silica gel 60 F254 (0.25 mm, Merck). Silica gel (230-400 mesh, Merck, Germany) and Sephadex LH-20 (Pharmacia Co.) were used for column chromatography. Preparative HPLC was run on an Acme 9000 HPLC (Young Lin, South Korea) using the YMC-pack ODS-A column and the flow rate was 1 mL/min.

Plant Material. The lotus seeds were purchased from the PuriMed Company in Seoul, South Korea, in June 2008. A voucher specimen (No. EAC265) was deposited at the Natural Product Chemistry Laboratory, College of Pharmacy, Ewha Womans University.

Extraction and Isolation. The lotus seeds (20 kg) were extracted with MeOH (25 l × 4) for 48 h by percolation at room temperature. The solvent was evaporated *in vacuo* to give a concentrated MeOH extract (3 kg), which was then diluted with distilled water to afford an aqueous MeOH solution. The MeOH extract (3 kg) was suspended in distilled water and fractionated with *n*-hexane, EtOAc, and *n*-BuOH, successively. The BuOH extract (150 g) was chromatographed over a silica gel (3000 g) column, eluting with a gradient solvent system of $CHCl_3$-MeOH (100:1 to 1:1), to afford twenty five fractions (B1-B15). Fraction B9 (3.0 g) was chromatographed on sephadex LH-20 gel (300 g) column, eluting with H_2O-MeOH (100:0 to 50:50), to afford three subfractions (B9.1 to B9.7). Subfraction B9.2 (0.2 g) was subjected to the prep. HPLC (MeOH-H_2O/0.1% formic acid = 10:90) to yield **1** (4 mg, t_R 56 min) and **2** (3 mg, t_R 58 min).

(1'*R*,3'*S*,5'*R*,8'*S*,2*E*,4*E*)-Dihydrophaseic acid 3'-*O*-β-D-glucopyranoside (1): white amorphous powder; UV (MeOH): λ_{max} (log ε) 268 (3.9) nm; CD: (c = 0.1, MeOH): 246 (–28.3), 263 (+18.8); IR ν_{max} (KBr): 3298, 2918, 1690, 1610, 1454 cm^{-1}; ^{1}H- (400 MHz, CD_3OD) and ^{13}C-NMR (100 MHz, CD_3OD): see Table 1; HRESIMS: *m/z* 467.1866 $[M + Na]^+$ (calcd for $C_{21}H_{32}NaO_{10}$, 467.1888).

(1'*R*,3'*S*,5'*R*,8'*S*,2*Z*,4*E*)-Dihydrophaseic acid 3'-*O*-β-D-glucopyranoside (2): white amorphous powder; UV (MeOH): λ_{max} (log ε) 268 (3.8) nm; CD: (c = 0.1, MeOH): 250 (–14.9), 267 (+15.5); IR ν_{max} (KBr): 3310, 2915, 1690, 1620, 1450 cm^{-1}; ^{1}H- (400 MHz, CD_3OD) and ^{13}C-NMR (100 MHz, CD_3OD): see Table 1; ESIMS: *m/z* 467 $[M + Na]^+$.

Acknowledgments. This research was supported by RP-Grant 2010 of Ewha Womans University.

References

1. Mabberley, D. J. A *Portable Dictionary of the Higher Plants*. In *The Plant Book*; Mabberley, D. J., Ed.; Cambridge of University Press: New York, NY, 1995; p 392.
2. Duke, J. A. *Handbook of Medicinal Herbs*, 2 nd ed.; CRC Press: New York, 2002; p 473.
3. Yen, G.-C.; Duh, P.-D.; Su, H.-J. *Food Chem.* **2005**, *89*, 379.
4. Youn, U. J.; Lee, J.-H.; Lee, Y. J.; Nam, J. W.; Bae, H.; Seo, E.-K. *Chem. Biodivers*. **2010**, *7*, 2296.
5. Seo, J. H.; Choi, Y. H.; Yoo, M. Y.; Hong, K.-S.; Lee, B. H.; Yon, G. H.; Kim, Y. S.; Kim, Y.-K.; Ryu, S. Y. *Saengyak Hakhoechi* **2006**, *37*, 290.
6. Champavier, Y.; Comte, G.; Vercauteren, J.; Allais, D. P.; Chulia, A. J. *Phytochemistry* **1999**, *50*, 1219.
7. Milborrow, B. V. *Chem. Commun*. **1969**, *17*, 966.
8. Zeevaart, J. A. D.; Creelman, R. A. *Annu. Rev. Plant Physiol. Plant Mol. Biol*. **1988**, *39*, 439.
9. Adrian, J. C.; Joan, E. K. *Trends Plant Sci*. **1999**, *4*, 472.
10. del Refugio Ramos, M.; Jerz, G.; Villanueva, S.; Lopez-Dellamary, F.; Waibel, R.; Winterhalter, P. *Phytochemistry* **2004**, *65*, 955.
11. Zaharia, L. I.; Gai, Y.; Nelson, K. M.; Ambrose, S. J.; Abrams, S. R. *Phytochemistry* **2004**, *65*, 3199.