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A NEW γ-BUTENOLIDE GLYCOSIDE FROM THE ROOT OF *STYPHNOLOBIUM JAPONICUM*

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Abstract – A new γ-butenolide glycoside, named styphnoloside A (**1**), was isolated from the root of *Styphnolobium japonicum* (L.) Schott (= *Sophora japonica* L.), together with saikoisoflavonoside A (**2**) and sophoraside A (**3**). The structure of **1** was characterized as puerol B 2”-*O*-neohesperidoside based on one- and two-dimensional (1D and 2D) NMR, MS, and electronic circular dichroism (ECD) spectral data. The absolute configuration of the aglycone moiety of **1** was assigned by comparing its experimental ECD spectrum with the calculated ECD spectrum.

Styphnolobium japonicum (L.) Schott (= *Sophora japonica* L.) is a deciduous tree belonging to the Leguminosae family and is native to China. The bud, fruit and root are used in crude drugs known as "huai-gen" in Chinese. The dried flowers of *S. japonicum* are used in traditional Japanese medicine for anti-hemorrhagic, anti-hemostatic, and analgesic effects.[1,2] There have been few phytochemical investigations of the root of *S. japonicum* to date aside from our previously reported isolation and structural elucidation of several flavonoids[3,4] and butenolides.[5,6] In the current paper, we report the isolation, purification, and structure elucidation of a new γ-butenolide glycoside, styphnoloside A (**1**), together with two known compounds, saikoisoflavonoside A[7] (**2**) and sophoraside A[5,6] (**3**), from the root this plant.

The roots were dried, extracted with methanol (MeOH) under reflux, and the organic solvent was removed by vacuum evaporation. The combined MeOH extract was suspended in water and partitioned with diethyl ether (Et_2O), ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH) successively to yield the corresponding soluble layers. The *n*-BuOH soluble portion was separated by octadecyl-silyl (ODS) column chromatography into 16 fractions (Fr. 1 to 16). Fr. 2 was further subjected to ODS column

chromatography to afford 10 fractions (Fr. 2-A to 2-J). Fraction 2-D was purified by reverse phase HPLC to isolate compounds **1** and **2**. Compound **3** was isolated from Fr. 2-E by HPLC purification using an ODS column (Figure 1).

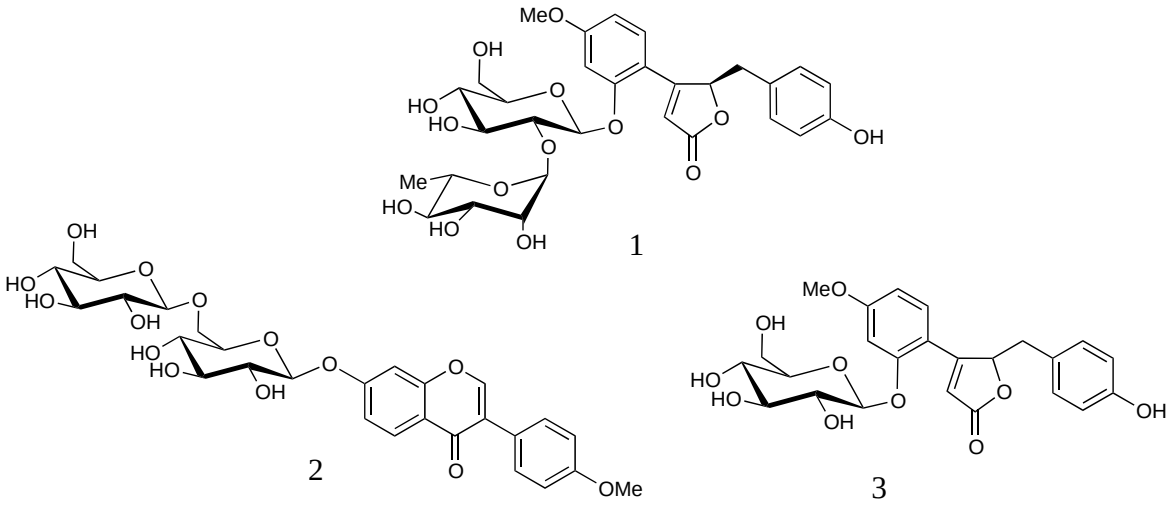

Figure 1. Structure of compounds **1**, **2**, and **3** isolated from the root of *S. japonicum*

Compound **1** was obtained as a pale yellow amorphous, $[\alpha]_D^{25}$ -145.8 (c 0.3, MeOH). The molecular formula of **1** was determined to be $C_{30}H_{36}O_{14}$ by high-resolution-fast atom bombardment mass spectrometry (HR-FAB-MS) at *m/z*: 643.2018 $[M+Na]^+$ (calcd for $C_{30}H_{36}O_{14}Na$: 643.2003).

The ^{1}H-NMR spectrum (Table 1) displayed signals due to a rhamnose methyl at δ_H 1.03 (3H, d, *J*=6.2 Hz), two methylene protons at δ_H 2.84 (1H, dd, *J*=14.5, 5.6 Hz) and δ_H 3.23 (1H, dd, *J*=14.5, 3.8 Hz), an oxygenated sp^3 methine proton at δ_H 6.05 (1H, ddd, *J*=5.6, 3.8, 1.2 Hz), oxygenated glucose methylene protons at δ_H 3.63 (1H, m) and δ_H 3.82 (1H, dd, *J*=11.8, 1.5 Hz), a singlet signal from a methoxy proton at δ_H 3.90 (3H, s), two anomeric protons at δ_H 5.26 (1H, d, *J*=1.8 Hz) and δ_H 5.33 (1H, d, *J*=7.4 Hz), four aromatic protons at δ_H 6.36 (1H, d, *J*=1.2 Hz), δ_H 6.80 (1H, dd, *J*=8.8, 2.4 Hz), δ_H 6.90 (1H, d, *J*=2.4 Hz) and δ_H 7.45 (1H, d, *J*=8.8 Hz), and an AA'BB' type coupling system at δ_H 6.85 (2H, d, *J*=8.6 Hz) and δ_H 6.61 (2H, d, *J*=8.6 Hz).

The ^{13}C-NMR spectrum (Table 1) of **1** showed 29 carbon resonances that were classified by their chemical shift values and heteronuclear single quantum coherence (HSQC) spectrum as: one methoxy carbon (δ_C 56.5), eight sp^2 methine carbons (δ_C 104.7, 110.8, 116.0, 116.1, 132.2, 132.2), six sp^2 quaternary carbons (δ_C 127.4, 157.5, 158.6, 165.2, 167.0, 176.7), an sp^3 methine carbon at δ_C 85.6, and an sp^3 methylene carbon at δ_C 39.5. In addition, a glucopyranosyl and a rhamnopyranosyl were inferred by comparisons of the ^{1}H-NMR and ^{13}C-NMR data with that in the literature[8] (rhamnose: δ_C 18.2, 70.6, 72.4, 72.5, 74.2 and 103.2, and glucose: δ_C 62.8, 71.6, 78.6, 79.0, 81.6 and 102.3). These NMR spectral data

are listed in Table 1. Although the carbon signal attributed to C-1" was not observed in MeOH-d_4, this signal was detected in aqueous acetone-d_6.

The ^{1}H- and ^{13}C-NMR spectroscopic data of **1** were very similar to those of puerol B,[6,9] except for the chemical shift values of the glucose and rhamnose moieties.

The heteronuclear multiple bond connectivity (HMBC) spectrum exhibited correlations between δ_H 2.84 (H-4a, α), δ_H 3.23 (H-4a, β) and δ_C 85.6 (C-4), δ_C 127.4 (C-1') and δ_C 132.2 (C-2'6'). And long-range correlations between δ_H 6.36 (H-2) and δ_C 176.7 (C-1), δ_C 85.6 (C-4) were observed. Furthermore, the HMBC experiments of indicated connections between δ_H 7.45 (H-6"), δ_C 158.6 (C-2"), δ_C 165.2 (C-4") and δ_C 167.0 (C-3). A resonance at δ_H 3.90 (-OMe) also correlated with δ_C 165.2 (C-4") in HMBC experiments. These results of HMBC experiments revealed the presence of a puerol B moiety (the aglycone of **1**).[6,9] In addition, the long-range correlations of two anomeric protons at δ_H 5.26 (H-R1) and δ_H 5.33 (H-G1) with δ_C 81.6 (C-G2) and δ_C 158.6 (C-2"), respectively, confirmed the linkage of these sugars (Figure 2).

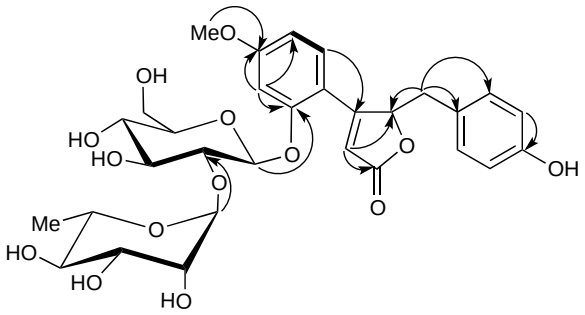

Figure 2. Diagnostically significant long-range correlation of **1**

The absolute configuration of **1** at C-4 was established by comparison of the experimental electronic circular dichroism (ECD) spectrum with that calculated for (-)-puerol B (**4**, the aglycone moiety of **1**).[6,9] Acid hydrolysis of **1** using HCl yielded **4**. The first negative Cotton effect in the experimental ECD spectrum of **4** was at 285 nm and the second positive Cotton effect was at 250 nm, consistent with the spectrum calculated for (4*R*)-puerol B (Figure 3). Therefore, the absolute configuration of C-4 was *R*.

The sugar units liberated by acid hydrolysis of compound **1** were identified as D-glucose and L-rhamnose by HPLC analysis of derivatives prepared by reaction with L-cysteine methyl ester and phenyl isothiocyanate according to a previously reported method.[10] Acid hydrolysis of **1** with 2N-HCl liberated D-glucose and L-rhamnose, and the glucose linkage and chemical shift values of the rhamnose moiety were indicative of α. Based on the NMR and acid hydrolysis data, the structure of **1** was determined to be (4*R*)-3-(2-(α-L-rhamnopyranosyl)-(1→2)-β-D-glucopyranosyloxy-4-merthoxyphenyl)-4-(4-hydroxy-benzyl)but-2-en-4-olide (Figure 1). We named this compound styphnoloside A. Compounds **1**, **2**, and **3**

were tested for tyrosinase inhibitory activity, α-glucosidase inhibitory activity and 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity, *in vitro*. However, none of them showed significant activities up to a concentration of 1.6 mM, respectively.

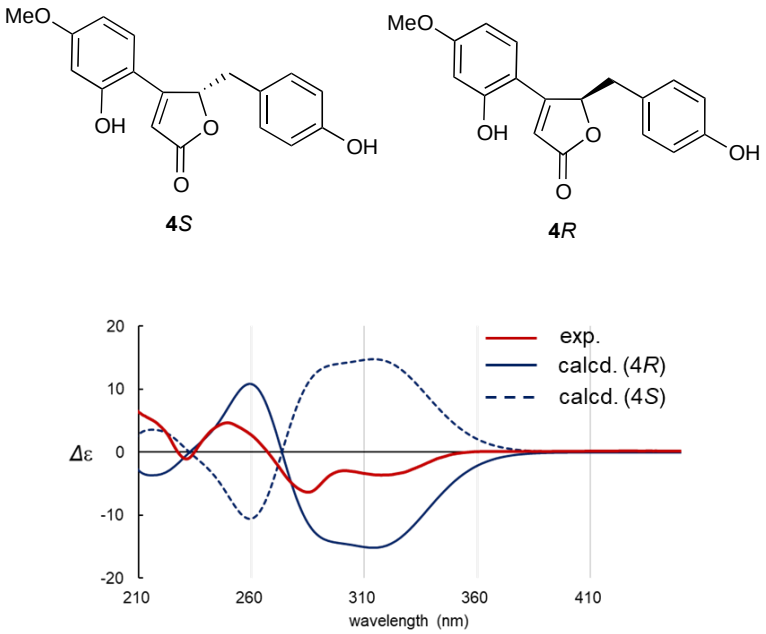

Figure 3. Experimental ECD spectrum of **4** and the calculated ECD spectrum of (3*R*)- and (3*S*)-**4**

Table 1. ^{1}H- and ^{13}C-NMR spectral data of compound **1** in MeOH-d_4

Position	δH Mult. (*J* in Hz)	δc	Position	δH Mult. (*J* in Hz)	δc
1	-	176.7 (s)	Glc 1	5.33 (d, *J*=7.4 Hz, 1H)	102.3 (d)
2	6.36 (d, *J*=1.2 Hz, 1H)	116.0 (d)	2	3.72 (m, 1H)	81.6 (d)
3	-	167.0 (s)	3	3.63 (m, 1H)	79.0 (d)
4	6.05 (ddd, *J*=1.2,3.8,5.6 Hz, 1H)	85.6 (d)	4	3.38 (m, 1H)	71.6 (d)
4a	2.84 (dd, *J*=5.6,14.5 Hz, 1H)	39.5 (t)	5	3.38 (m, 1H)	78.6 (d)
	3.23 (dd, *J*=3.8,14.5 Hz, 1H)		6	3.63 (m, 1H)	62.8 (t)
1'	-	127.4 (s)		3.82 (dd, *J*=1.5,11.8 Hz, 1H)	
2',6'	6.85 (d, *J*=8.6 Hz, 2H)	132.2 (d)	Rha 1	5.26 (d, *J*=1.8 Hz, 1H)	103.2 (d)
3',5'	6.61 (d, *J*=8.6 Hz, 2H)	116.1 (d)	2	4.02 (dd, *J*=1.8,3.3 Hz, 1H)	72.4 (d)
4'	-	157.5 (s)	3	3.70 (m, 1H)	72.5 (d)
1"	-	N.D.*	4	3.35 (m, 1H)	74.2 (d)
2"	-	158.6 (s)	5	3.73 (m, 1H)	70.6 (d)
3"	6.90 (d, *J*=2.4 Hz, 1H)	104.7 (d)	6	1.03 (d, *J*=6.2 Hz, 3H)	18.2 (q)
4"	-	165.2 (s)			
5"	6.80 (dd, *J*=8.8,2.4 Hz, 1H)	110.8 (d)			
6"	7.45 (d, *J*=8.8 Hz, 1H)	132.2 (d)			
OCH_3	3.90 (s, 3H)	56.5 (q)			

The solvent peak was used as an internal standard (δc 49.2, δH 3.31).
*N.D.: not detected This signal was observed in aqueous acetone-d_6 (δc, 115.2 ppm).

EXPERIMENTAL

General Experimental Procedures FAB-MS and HR-FAB-MS were recorded on a JMS-700 (JEOL). ^{1}H-, ^{13}C-, and 2D-NMR spectra were recorded at 400 MHz on an Agilent 400-MR NMR system (400 MHz for ^{1}H and 100 MHz for ^{13}C) at room temperature. NMR chemical shifts were recorded in ppm with solvent signals as internal references (δ_H 3.31, δ_C 49.2 for MeOH-d_4, δ_H 2.50, δ_C 39.5 for DMSO-d_6, and δ_H 2.05, δ_C 29.9 for acetone-d_6). Optical rotations were measured using a P-2300 polarimeter (JASCO, solvent; MeOH). ECD spectra were measured using a J-720WI spectrophotometer (JASCO, solvent; MeOH). UV spectra were obtained on a Shimadzu UV-1280 spectrophotometer.

Plant Material *S. japonicum* (L.) Schott (= *Sophora japonica* L.) roots were collected from the botanical garden of Josai University, Saitama, Japan, in May 2017. The plant was authenticated by Dr. Yoshiaki Shirataki. A voucher specimen was deposited at the Laboratory of Pharmacognosy and Natural Medicines, Josai University, as 2017-05-12.

Extraction and Isolation Dried roots of *S. japonicum* (500 g) were extracted with MeOH (1200 mL) for 3 h under reflux. This extraction was repeated 3 times. The combined MeOH extract (42 g) was suspended in water and partitioned with Et_2O, EtOAc and *n*-BuOH successively to yield the Et_2O layer (6.1 g), EtOAc layer (9.2 g), *n*-BuOH layer (10.8 g) and H_2O layer (14.2 g). The *n*-BuOH layer was chromatographed using ODS and gradient elution with a H_2O:MeOH mixture (H_2O:MeOH = 1:1→ 1:3 → MeOH) to afford 16 fractions (Fr. 1 to 16). A portion of Fr. 2 (1.1 g) was chromatographed using ODS and isocratic elution with a MeCN:H_2O mixture (MeCN:H_2O = 1:3) to afford 10 fractions (Fr. 2-A to 2-J). Compound **1** (13.8 mg) and compound **2** (13.8 mg) were obtained from Fr. 2-D (141.5 mg) using HPLC and a DOCOSIL column (Senshu Pak, ϕ 10 × 150 mm, MeCN:H_2O = 1:4, isocratic). Compound **3** (10.7 mg) was obtained from Fr. 2-E (30.0 mg) using HPLC and an ODS column (Senshu Pak, ϕ 10 × 150 mm, MeCN:H_2O = 1:3, isocratic). Spectroscopic analysis by NMR and MS identified **2** and **3** as saikoisoflavonoside A and sophoraside A, respectively. The spectral data of **2** and **3** were consistent with the literature values.[5-7]

Styphnoloside A (**1**), pale yellow, amorphous; molecular formula: $C_{30}H_{36}O_{14}$; $[\alpha]_D^{25}$ -145.8 (c 0.3, MeOH); UV λmax MeOH nm (ε): 286 (15295), 308 (14909); HR-FAB-MS *m/z*: 643.2018 ($[M+Na]^+$) (calcd for $C_{30}H_{36}O_{14}Na$: 643.2003); ECD (MeOH): 307 nm (Δε -5.5), 287 nm (Δε -7.9), 248 nm (Δε +4.7), 231 nm (Δε -0.7); ^{1}H-NMR (400 MHz, MeOH-d_4) and ^{13}C-NMR (100 MHz, MeOH-d_4) spectral data are listed in Table 1.

Acid Hydrolysis of Compound 1 A solution of **1** (12.1 mg) in 2N-HCl (3 mL) was heated at 100 °C in a screw-capped vial for 6 h. **4** was obtained as a powder (4.2 mg). The mixture was neutralized by addition of Dowex 1×8 (chloride form) and filtered. The reaction mixture was dried in vacuo and

dissolved in 1 mL of pyridine containing L-cysteine methyl ester (5 mg/mL) and reacted at 60 °C for 1 h. Phenyl isothiocyanate (5 mg) was added and the mixture was heated at 60 °C for 1 h, then was directly analyzed by HPLC [Cosmosil 5C18-AR-II (ϕ 4.6 × 250 mm, Nacalai Tesque): 25% MeCN in 50 mM H_3PO_4; flow rate 0.8 mL/min; column temperature 35 °C; detection 250 nm]. The t_R of the peak at 16.00 min coincided with that of D-glucose. The t_R of L-rhamnose was 27.33 min.[10]

(-)-Puerol B (**4**), brown powder; $[\alpha]_D^{21}$ -79.4 (c 0.4, acetone); ECD (MeOH): 317 nm (Δε -3.6), 285 nm (Δε -6.4), 268 nm (Δε -0.1), 250 nm (Δε +4.7), 231 nm (Δε -1.1).

Calculation of ECD Spectra Plausible twenty-four conformations of **4** were manually generated around the dihedral angles C-4–C-3–C-1”–C-2”, O–C-4–C-4a–C-1’ and C-4–C-4a–C-1’–C-2’ and optimized using the semi-empirical PM6[11] method available in the Gaussian 09 program.[12] Re-optimization of DFT at the B3LYP/6-31G (d) level of theory[13-16] in MeOH solvent simulated using the polarizable continuum model (PCM[17]) yielded seven conformers.[18,19] The three low-energy (Boltzmann distribution >1%) conformers contributed 98.2% of the Boltzmann distribution. Further optimization of these conformers at B3LYP/6-31++G (d,p) level and Excitation energies, rotatory strengths, and oscillator strengths for each transition were calculated for the first 40 electronic states at the same level.

ECD spectra were simulated by overlapping Gaussian functions for each transition formulated as:

$$\Delta\varepsilon\,(\tilde{\nu}) = \frac{1}{(2.296\times10^{-39})\sigma\sqrt{\pi}}\sum_{i=1}^{n}\tilde{\nu}_i R_i \exp\left[-\left(\frac{\tilde{\nu}-\tilde{\nu}_i}{\sigma}\right)^2\right]$$

where $\tilde{\nu}$ is the central wavenumber of the Cotton effect, σ is half the bandwidth at 1/e peak height, and $\tilde{\nu}_i$ and R_i are the excitation energy (in wavenumber) and rotatory strength for transition i, respectively. The σ value was evaluated from the corresponding UV spectrum, and a value of 0.32 eV was chosen. The total ECD spectrum was obtained according to the Boltzmann-averaged contribution of each conformer.

Biological activities Assessment Tyrosinase inhibitory activity, α-glucosidase inhibitory activity and DPPH radical scavenging activity were evaluated according to previously reported methods with minor modification.[20-22]

REFERENCES

1. 'Chuyaku Daijiten', 1st edn., Shanghai Science-Technology Publishing Co. and Shogakukan Inc., Vol. 1, Shogakukan Inc., Tokyo, 1985, pp. 195-197.
2. H. Ishida, T. Umino, K. Tsuji, and T. Kosugi, *Chem. Pharm. Bull*., 1989, **37**, 1616.
3. S. Shibata and Y. Nishikawa, *Chem. Pharm. Bull*., 1963, **11**, 167.
4. M. Komatsu, I. Yokoe, and Y. Shirataki, *Yakugaku Zasshi*, 1976, **96**, 254.

5. Y. Shirataki, Y. Tagaya, I. Yokoe, and M. Komatsu, *Chem. Pharm. Bull*., 1987, **35**, 1637.
6. T. Nohara, J. Kinjo, J. Furusawa, Y. Sakai, M. Inoue, Y. Shirataki, Y. Ishibashi, Y. Yokoe, and M. Komatsu, *Phytochemistry*, 1993, **33**, 1207.
7. M. H. Hwang, Y. S. Kwon, and C. M. Kin, *Natural Medicines*, 1998, **52**, 527.
8. S. Kobayashi, T. Miyase, and H. Noguchi, *J. Nat. Prod*., 2002, **65**, 319.
9. J. Kinjo, J. Furusawa, and T. Nohara, *Terahedron Lett*., 1985, **26**, 6101.
10. T. Tanaka, T. Nakashima, T. Ueda, K. Tomii, I. Kouno, and Y. Nishikawa, *Chem. Pharm. Bull*., 2007, **55**, 899.
11. J. J. P. Stewart, *J. Mol. Model*., 2007, **13**, 1173.
12. M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, J. E. Peralta, F. Ogliaro, M. J. Bearpark, J. Heyd, E. N. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. P. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, N. J. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox, Gaussian 09; Gaussian, Inc.: Wallingford, CT, USA, 2013.
13. A. D. Becke, *J. Chem. Phys*., 1993, **98**, 5648.
14. C. Lee, W. Yang, and R. G. Parr, *Phys. Rev. B*, 1988, **37**, 785.
15. B. Miehlich, A. Savin, H. Stoll, and H. Preuss, *Chem. Phys. Lett*., 1989, **157**, 200.
16. P. J. Stephens, F. J. Devlin, C. F. Chabalowski, and M. J. Frisch, *J. Phys. Chem*., 1994, **98**, 11623.
17. S. Miertuš, E. Scrocco, and J. Tomasi, *Chem. Phys*., 1981, **55**, 117.
18. K. L. Bak, A. E. Hansen, K. Ruud, T. Helgaker, J. Olsen, and P. Jorgensen, *Theor. Chim. Acta*, 1995, **90**, 441.
19. J. Autschbach, T. Ziegler, S. J. A. van Gisbergen, and E. J. Baerends, *J. Chem. Phys*., 2002, **116**, 6930.
20. Y. N. Huang, Y. L. Zhao, X. L. Gao, Z. F. Zhao, Z. Jing, W. C. Zeng, R. Yang, R. Peng, T. Tong, L. F. Wang, J. Q. Cen, and H. Gao, *J. Ethnopharmacol*., 2010, **131**, 306.
21. K. Takao, K. Toda, T. Saito, and Y. Sugita, *Chem. Pharm. Bull*., 2017, **65**, 1020.
22. K. Yamauchi, T. Mitsunaga, and I. Batubara, *Nat. Resour*., 2011, **02**, 167.