Prunioside A: A New Terpene Glycoside from *Spiraea prunifolia*

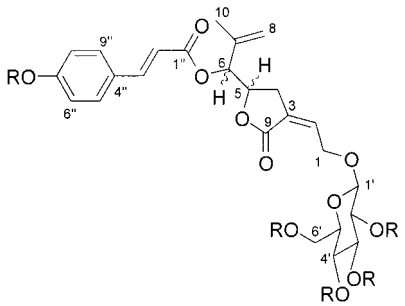
Hyuncheol Oh,[†] Gi-Su Oh,[‡] Won-Gil Seo,[‡] Hyun-Ock Pae,[‡] Kyu-Yun Chai,[‡] Tae-Oh Kwon,[‡] Young-Hang Lee,[‡] Hun-Taeg Chung,[‡] and Ho-Sub Lee*[,†]

Professional Graduate School of Oriental Medicine, and Medicinal Resources Research Center, Wonkwang University, Iksan, Chonbuk 570-749, Republic of Korea

Received November 30, 2000

Prunioside A (**1**) has been isolated from an EtOAc-soluble extract of *Spiraea prunifolia* var. *simpliciflora* by a combination of chromatographic techniques. The structure was determined primarily by extensive NMR experiments. Compound **1** is a unique terpene glycoside. Its acetylated derivative (**1a**) inhibited nitric oxide production in murine macrophage-like RAW 264.7 cells in a dose-dependent manner.

Plants in the genus *Spiraea* are known to produce various alkaloids (spiradines, spiramines, spirasines), benzaldehyde, flavonoids (e.g., spiracin), terpenoids, and terpenoid glycosides.[1−4] *Spiraea prunifolia* var. *simpliciflora* Nakai (Rosaceae) is a deciduous, latifoliate shrub that can be found in most parts of Korea. The roots of this plant have been used traditionally for the treatment of malaria, fever, and emetic conditions.[2,3] However, little is known concerning the chemical constituents of *S. prunifolia*.[2,3] Recently, a methanol extract of *S. prunifolia* was found to show a suppressive effect on the synthesis of nitric oxide in murine macrophage-like RAW 264.7 cells stimulated with interferon-γ (IFN-γ) plus lipopolysaccharide (LPS).[5] Since the overproduction of nitric oxide (NO) catalyzed by inducible nitric oxide synthase (iNOS) is known to be responsible for some inflammatory diseases, an iNOS inhibitor could have potential for therapeutic use.[6] On the basis of the above considerations, a methanol extract of *S. prunifolia* was investigated, and a new secondary metabolite, named prunioside A (**1**), was encountered as a major constituent of the EtOAc-soluble fraction. Details of the isolation and structure determination of this compound are presented here. Compound **1** and its acetate (**1a**) were evaluated for their inhibiting effects on nitric oxide production in RAW 264.7 cells.



1: R = H
1a: R = Ac

Compound **1** has the molecular formula $C_{25}H_{30}O_{11}$, as deduced from the ^{13}C NMR and HRFABMS data, which indicated 11 degrees of unsaturation. DEPT data indicated that 25 of the protons are bound to carbon atoms, and comparison of DEPT results and the molecular formula indicated the presence of five hydroxyl groups. The presence of five hydroxyl groups was confirmed by formation of a pentaacetate (**1a**) upon treatment of **1** with acetic anhydride. The ^1H, ^{13}C, and DEPT data for **1** included signals characteristic of a *p*-coumaroyl[7,8] and β-glucopyranosyl groups (Table 1). The chemical shift changes in the ^1H spectrum (Table 1) upon acetylation were consistent with five hydroxyl groups accounted for by the above two structural units. The NMR data also indicated that the remainder of the molecule contained an ester carbonyl group, terminal and trisubstituted olefin units, two oxymethines, an oxymethylene, a vinyl methyl group, and one additional methylene unit.

Analysis of the 2D NMR data (COSY, HMQC, and HMBC) confirmed the presence of the *p*-coumaroyl group and a glucopyranosyl group. The *J* values for the latter group were instrumental in identifying it as a glucopyranosyl unit, and the coupling constant for the anomeric proton H-1′ (7.4 Hz) indicated that this moiety is connected to the aglycon via a β-linkage. The remainder of the structure of the molecule was proposed by analysis of COSY, HMQC, and HMBC data. COSY correlations enabled establishment of the spin system from H-6 to H_2-4. The terminal olefinic group (C-7−C-8) was connected to C-6 on the basis of HMBC cross-peaks between H-6/C-7, H-6/C-8, and H-8/C-6. HMBC correlations of the methyl signal at δ 1.82 (H_3-10) with C-6, C-7, and C-8 located the vinyl methyl group at C-7. The connectivity from C-4 to C-1 was established by observation of a COSY correlation between H_2-1 and H-2 and by HMBC cross-peaks observed between H_2-1/C-3, H-2/C-4, and H_2-4/C-2. The connection of C-3 and C-9 was evidenced by HMBC correlations of the carboxylic carbon signal at δ 169.7 with H_2-4 and H-2. The γ-butyrolactone ring moiety was established based on chemical shift considerations (C-9 at δ 169.7 and C-5 at δ 77.7), along with an HMBC cross-peak between H-5 and C-9. C-1″ of the *p*-coumaroyl group was found to acylate the oxygen at C-6 of the terpene unit by virtue of an HMBC correlation of H-6 with C-1″. Finally, mutual HMBC correlations of H_2-1 with C-1′ and of H-1′ with C-1 provided key evidence for connection of the glucosyl moiety to C-1 via a glycoside linkage, thus completing the planar structure of prunioside A as shown in **1**.

The partial relative stereochemistry shown for **1** was deduced by analysis of coupling constants and NOESY data for acetylated compound **1a**. The geometry of the C-2″−C-3″ double bond was assigned as *E* on the basis of the coupling constant between H-2″ and H-3″ (16.1 Hz).[7] The C-2−C-3 double bond was assigned the *Z*-geometry on the

* To whom correspondence should be addressed. Tel: +82-63-850-5191. Fax: +82-63-850-7324. E-mail: host@wonkwang.ac.kr.
[†] Professional Graduate School of Oriental Medicine.
[‡] Medicinal Resources Research Center.

10.1021/np0005513 CCC: $20.00
Published on Web 06/13/2001

Table 1. NMR Data[a] of Prunioside A (**1**) and Its Acetate (**1a**)[b]

	compound **1**			compound **1a**	
C/H	δ_C	δ_H (int., mult., *J* in Hz)	HMBC[b] (^{13}C no.)	δ_C	δ_H (int., mult., *J* in Hz)
1	66.5	4.87 (1H, dddd, 15.8, 5.7, 2.8, 2.8)	2, 3, 1′	66.7	4.84 (1H, m)
		4.70 (1H, dddd, 15.8, 5.7, 2.8, 2.8)	2, 3, 1′		4.72 (1H, m)
2	140.2	6.35 (m)	4, 9	138.6	6.25 (1H, m)
3	126.2			132.9	
4	31.7	3.01 (1H, dddd, 16.7, 8.2, 5.5, 2.8)	2, 3, 6, 9	31.7	3.12 (1H, m)
		2.77 (1H, dddd, 16.7, 5.5, 2.8, 2.8)	2, 3, 6, 9		2.79 (1H, m)
5	77.7	4.93 (1H, ddd, 8.3, 5.5, 4.9)	4, 6,[c] 7c, 9	77.7	4.95 (1H, m)
6	78.1	5.40 (1H, d, 4.9)	4, 5, 7, 8, 10, 1″	78.5	5.44 (1H, d, 5.1)
7	141.4			141.7	
8	115.2	5.09 (1H, s)	6, 7, 10	115.7	5.12 (1H, s)
		5.04 (1H, s)	6, 7, 10		5.06 (1H, s)
9	169.7				
10	19.3	1.82 (1H, s)	6, 7, 8	19.3	1.83 (3H, s)
1′	104.2	4.27 (1H, d, 7.4)	1, 5′[c]	101.3	4.73 (1H, d, 8.3)
2′	74.8	3.17 (1H, dd, 9.2, 7.4)	1′, 3′	72.2	4.88 (1H, dd, 9.6, 8.3)
3′	77.9	3.38 (1H, dd, 9.2, 9.2)	1′, 2′, 4′	73.6	5.22 (dd, 9.9, 9.6)
4′	71.6	3.33 (1H, dd, 9.2, 8.7)	3′, 5′, 6′	69.5	4.99 (1H, dd, 10.1, 9.9)
5′	77.5	3.26 (1H, ddd, 8.7, 5.5, 2.8)	1′, 4′	72.5	3.88 (1H, m)
6′	62.9	3.65 (1H, dd, 11.9 5.5)	4′, 5′	62.7	4.24 (1H, dd, 12.0, 5.1)
		3.80 (1H, dd, 11.9, 5.5)	4′, 5′		4.09 (1H, dd, 12.0, 2.3)
1″	166.5			166.04	
2″	114.6	6.35 (1H, d, 16.1)	1″, 4″	118.6	6.58 (1H, d, 16.3)
3″	146.6	7.61 (1H, d, 16.1)	1″, 2″, 4″, 5″, 9″	145.3	7.70 (1H, d, 16.3)
4″	126.5			127.1	
5″	131.3	7.55 (2H, d, 8.7)	3″, 6″, 7″	130.5	7.76 (2H, d, 8.7)
6″	117.1	6.89 (2H, d, 8.7)	4″, 5″, 7″, 9″	123.5	7.22 (2H, d, 8.7)
7″	161.6			153.7	
8″	117.1	6.89 (2H, d, 8.7)	4″, 5″, 7″, 9″	123.5	7.22 (2H, d, 8.7)
9″	131.3	7.55 (2H, d, 8.7)	3″, 6″, 7″	130.5	7.76 (2H, d, 8.7)

[a] Data were recorded using an acetone-d_6 solution at 500 MHz (^{1}H) and 125 MHz (^{13}C). [b] Signals corresponding to acetyl groups were observed at δ 21.1, 170.79, 20.72, 170.35, 20.67, 170.09, 20.65, 169.8, 20.60, 169.55 in the ^{13}C NMR spectrum and at δ 2.27 (3H,s) 2.01 (3H, s) 1.98 (3H, s), 1.933 (3H, s), 1.928 (3H, s) in the ^{1}H NMR spectrum. [c] Observed only in decoupled-HMBC.[11]

basis of a NOESY correlation of H-2 with H_2-4 in **1a**. The relative configurations of C-5 and C-6 could not be established from the NMR data, and exhaustive efforts to obtain crystals of **1** or **1a** were unsuccessful.

Prunioside A (**1**), 2-(2-*O*-*β*-D-glucopyranosylethylene)-4-(1-*O*-*p*-coumaroyl-2-isobutenyl)-*γ*-butyrolactone, appears to be a unique, highly oxidized monoterpene glycoside. Although no close structural analogues of **1** have been reported, some simple oxidized geraniol derivatives[4] and α-methylene-*γ*-butyrolactones[9,10] have been described from *Spiraea* species.

Compound **1** did not show any inhibitory effect at 200 *μ*g/mL on nitric oxide production by the murine macrophage-like RAW 264.7 cells stimulated by INF-*γ* and LPS for 18 h. On the other hand, compound **1a** inhibited the production of NO by the stimulated cells in a dose-dependent manner (Figure 1A). The mechanism for the inhibition of NO production by compound **1a** was suggested to be the suppression of iNOS mRNA, as shown by reverse transcriptase-polymerase chain reaction (RT-PCR) analysis (Figure 1B).

Experimental Section

General Experimental Procedures. The optical rotation was recorded on an Optical Activity AA-10 automatic polarimeter. The UV spectrum was recorded on a Hewlett-Packard HP 8453 spectrophotometer. The IR spectrum was recorded on a JASCO FT/IR-5300. NMR spectra were recorded in acetone-d_6 using a JEOL Eclipse-500 MHz spectrometer, and chemical shifts were referenced relative to the corresponding residual solvents signals (δ 2.04/29.9, respectively). HMQC and HMBC data were optimized for $^1J_{CH} = 140$ Hz and $^nJ_{CH} = 8$ Hz, respectively. Decoupled HMBC data were obtained using an HMQC pulse sequence optimized for $^nJ_{CH} = 8$ Hz.[11] FABMS data were obtained on a JEOL JMS HX-110 spectrometer using 3-nitrobenzyl alcohol as a matrix. HPLC separations were performed on an Alltech HS Hyperprep 100 BDS C_{18} column (1.0 × 25 cm; 8-*μ*m particle size) with a flow rate of 2 mL/min. Compounds were detected by UV absorption at 254 nm.

Plant Material. The roots of *S. prunifolia* were collected from the Iksan City area in Chonbuk Province, Korea, in May 1998. The roots were identified and authenticated by Prof. Tae-Oh Kwon, Life and Natural Science College, Wonkwang University. Fresh roots were dried in a well-ventilated darkroom, and a voucher specimen (No. VMRRC 980202) was deposited in the Herbarium of the Medicinal Resources Research Center, Wonkwang University.

Extraction and Isolation. The air-dried roots (500 g) were extracted with MeOH for 24 h. The MeOH extract was concentrated, suspended in H_2O, and sequentially partitioned with *n*-hexane, CH_2Cl_2, and EtOAc. The EtOAc-soluble fraction (2.3 g) was subjected to C_{18} flash column chromatography with a stepwise gradient of 40 to 100% (v/v) MeOH in H_2O. A portion (60 mg) of the fraction eluted at 60% MeOH in H_2O (542 mg) was subjected to semipreparative reversed-phase

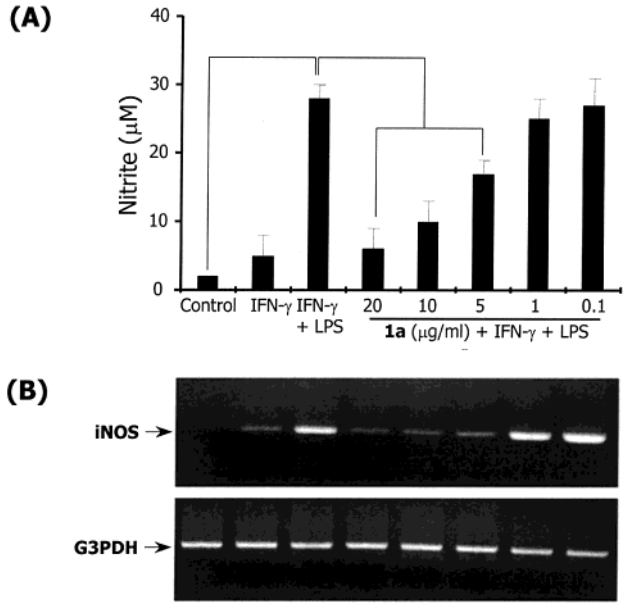


Figure 1. Dose-dependent effect of compound **1a** on NO production as well as iNOS mRNA expression in LPS and IFN-γ stimulated RAW 264.7 cells. (A) The cells were cultured with various concentrations of compound **1a** for 18 h. NO released was measured by using the Griess reagent and expressed as the means ± SD of three independent experiments. (B) The inhibition of iNOS mRNA expression was analyzed by RT-PCR. The figure shows the agarose-gel electrophoresis of the RT-PCR products for iNOS and G3PDH (control).

HPLC using a gradient from 20 to 30% CH_3CN in H_2O over 50 min, then 100% CH_3CN for 10 min to yield **1** (35 mg).

Prunioside A (1): white oily gum; $[\alpha]^{25}_D$ +42° (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ϵ) 213 (3.92), 227 (3.97), 312 (4.01) nm; IR (Nujol) ν_{max} 3387, 1753, 1707, 1604, 1514, 1442, 1371, 1165 cm^{-1}; ^1H, ^{13}C, and 2D NMR data, Table 1; FABMS *m/z* 529 $[M + Na]^+$; HRFABMS *m/z* 529.1680 (calcd for $C_{25}H_{30}O_{11}Na$, 529.1684).

Acetylation of Prunioside A (1). A solution of **1** (10 mg) in CH_3CN (2 mL) was combined with acetic anhydride (0.5 mL) and triethylamine (2 mL), and the resulting solution was stirred at room temperature for 24 h. The solvent was then evaporated under N_2. The residue was redissolved in 1.5 mL of CH_2Cl_2 and extracted with H_2O (2 × 2 mL). The organic phase was dried and subjected to reversed-phase HPLC using a gradient from 45 to 65% CH_3CN in H_2O over 40 min, then 100% CH_3CN for 10 min to afford pentaacetate **1a** (8 mg, 56% yield).

Compound 1a: $[\alpha]^{25}_D$ +28° (*c* 0.25, MeOH); IR film ν_{max} 2961, 1758 (broad), 1636, 1515, 1436, 1372, 1221, 1165 cm^{-1}; ^1H and ^{13}C NMR data, Table 1; FABMS *m/z* 739 $[M + Na]^+$.

Macrophage Cell Line Culture. The murine macrophage cell line RAW264.7 was obtained from the American Tissue Culture Collection (Rockville, MD). The cells were maintained in complete RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 1% l-glutamine, 1% nonessential amino acids, 1% antibiotic/antimycotic (100 U/mL of penicillin, 25 μg/mL of amphotericin D, and 100 μg/mL of streptomycin), 1.5% sodium bicarbonate, and 1% minimal essential vitamins at 37 °C in a humidified 5% CO_2 atmosphere.

Measurement of Nitrite Concentration. Experiments were undertaken on cells grown in the presence of various concentrations of **1** or **1a** dissolved in DMSO with IFN-γ (5 U/mL) and LPS (10 ng/mL) for 18 h. The final concentration of DMSO in culture media was 0.1%. Supernatants in cultured macrophages were collected and mixed with an equal volume of the Griess reagent [1% sulfanilamide, 0.1% *N*-(1-naphthyl)-ethylenediamine dihydrochloride in 2.5% phosphoric acid solution] and incubated for 10 min at room temperature. Nitrite concentration was determined by measuring the absorbance at 540 using an ELISA plate reader. The level of nitrite reflects nitric oxide synthesis. Sodium nitrite was used as a standard. The cell-free medium contained 5−8 μM of nitrite, and this value was determined in each experiment and subtracted from the value obtained with cells.

Analysis of mRNA Levels For iNOS and Glyceraldehyde-3-phosphate Dehydrogenase (G3PDH). Total RNA was extracted from the cells by the acid-guanidinium isothiocyanate phenol chloroform (AGPC) method.[12] RT-PCR was performed according to the method described previously.[13]

Acknowledgment. This work was supported by the Brain Korea 21 project, and the Medicinal Resources Research Center of Wonkwang University sponsored by KOSEF and the Chonbuk provincial government. We thank Prof. James B. Gloer, University of Iowa, for helpful discussions.

References and Notes

(1) *Dictionary of Natural Products on CD-ROM*; Chapman & Hall/CRC Press: London, 2000; Version 8:2.
(2) Ro, J. S. *Kor. J. Pharmacol.* **1982**, *13*, 39−42.
(3) Woo, M. H.; Lee, E. H.; Chung, S. O.; Kim. C. W. *Kor. J. Pharmacol.* **1996**, *27*, 389−396.
(4) Takeda, Y.; Fukumoto, K.; Tachibana, M.; Shingu, T.; Fujita, T.; Ichihara, T. *Phytochemistry* **1990**, *29*, 1591−1593.
(5) So, H.-S.; Park, R.; Oh, H.-M.; Pae, H.-O.; Lee, J.-H.; Chai, K.-Y.; Chung, S.-Y.; Chung, H.-T. *J. Ethnopharmacol.* **1999**, *68*, 209−217.
(6) Niwa, N.; Nakamura, N.; Kitajima, K.; Ueda, M.; Tsutsumishita, Y.; Futaki, S.; Takaiahi, Y. *Biochem. Biophys. Res. Commun.* **1997**, *239*, 367−371.
(7) Asakawa, Y.; Toyota, M.; Ueda, A.; Tori, M.; Fukazawa, Y. *Phytochemistry* **1991**, *30*, 3037−3040.
(8) Ihrahim, R.; Barron, D. In *Methods in Plant Biochemistry: Plant Phenolics;* Harborne, J. B., Ed.; Academic Press: London, 1989; Vol. 1, pp 127−129.
(9) Kim, C.-S.; Hara, T.; Datta, P. K.; Itoh, E.; Horike, M. *Biosci., Biotechnol., Biochem.* **1998**, *62*, 1546−1549.
(10) Kim, C.-S.; Datta, P. K.; Hara, T.; Itoh, E.; Horike, M. *Biosci.. Biotechnol., Biochem.* **1999**, *63*, 152−154.
(11) Furihata, K.; Seto H. *Tetrahedron Lett.* **1995**, *36*, 2817−2820.
(12) Chomczynski, P.; Sacchi, N. *Ann. Biochem.* **1987**, *162*, 156−159.
(13) Niwa, M.; Tsutsumishishita, Y.; Kawai, Y.; Takahara, N.; Nakamura, N.; Futaki, S.; Takaishi, Y.; Kondoh, W.; Moritoki, H. *Biochem. Biophys. Res. Commun.* **1996**, *224*, 579−585.

NP0005513