DOI 10.5012/bkcs.2010.31.01.199

Anti-inflammatory Constituents from *Solanum nigrum*

Xing-Fu Cai, Young-Won Chin, Sei-Ryang Oh, Ok-Kyung Kwon, Kyung-Seop Ahn, and Hyeong-Kyu Lee*

Immune Modulator Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-806, Korea
**E-mail: hykylee@kribb.re.kr*
Received October 14, 2009, Accepted November 6, 2009

Key Words: *Solanum nigrum*, Spirostane, Spirost-5-ene-3β,12β-diol, Phenylpropanoids, Leukotriene C_4

The whole plant of *Solanum nigrum* L. (Solanaceae) has been used as a folk medicine in Asian countries for the treatment of inflammation, edema, and mastitis.[1,2] Chemical constituents of this plant have been reported to comprise alkaloids, glycoproteins, flavonoids, polyphenols, and triterpenoids.[3,4] According to the literatures, a wide range of bioactivities including anti-inflammatory,[5] antioxidant,[6] antinociceptive,[5] antipyretic,[5] antitumor,[7,8] antiulcerogenic,[9] cancer chemopreventive,[10] hepatoprotective,[11] and immunomodulatory[8] effects were observed while the active constituents responsible for these activities were not fully resolved so far. In the present study, the structure elucidation of a new compound along with 14 known compounds and their inhibitory activities on leukotriene C_4 release are described.

The 50% EtOH extract of the fruits of *S. nigrum* was partitioned successively into ethyl acetate and water. The ethyl acetate-soluble fraction was purified by silica gel, reversed-phase C18 gel column chromatography yielded one new compound **1** together with 14 known compounds, *N-trans*-feruloyltyramine (**2**),[12] (*R*)-3-(4-hydroxy-3-methoxyphenyl)-*N*-[2-(4-hydroxyphenyl)-2-methoxyethyl]acrylamide (**3**),[13] (*E*)-ethyl caffeate (**4**),[14] ethyl 4-hydroxy-3-methoxycinnamate (**5**),[15] guaiacylglycerol-β-ferulic acid ether (**6**),[16] 3-*O*-acetylbetulinic acid (**7**),[17] chlorogenic acid (**8**),[18] caffeic acid (**9**),[19] methylsinapate (**10**),[20] β-sitosterol (**11**),[21] drummondol (**12**),[22,23] 2α,9-dihydroxy-1,8-cineole (**13**),[24] tryptophol acetate (**14**),[25] and 4-amino-3-methoxyphenol (**15**)[26] (Figure 1).

Compound **1** was obtained as an amorphous powder and its molecular formula was assigned as $C_{26}H_{40}O_4$, based on the $[M-H]^-$ peak at *m/z* 415.2859 (calcd for $C_{26}H_{39}O_4$ 415.2848) in the HRESIMS. The ^{1}H NMR spectrum showed two tertiary methyl groups at δ_H 0.78 (3H, s, H-18) and 1.06 (3H, s, H-19), one secondary methyl at δ_H 1.03 (3H, d, J = 6.4 Hz, H-21), an olefinic proton at δ_H 5.35 (1H, d, J = 5.1 Hz, H-6), assignable to a spirostane moiety.[27] The ^{13}C and DEPT NMR spectroscopic data showed three methyls at δ_C 11.6, 14.0, and 19.9, two olefinic carbons at δ_C 122.4 and 142.3, three oxygenated methines at δ_C 72.4, 80.4, and 82.3, one oxygenated methylene at δ_C 63.3, and one spiroketal carbon at δ_C 110.6. From ^{1}H, ^{13}C, and DEPT NMR data, it was observed that a methyl group corresponding to C-27 in F ring was absent in this molecule when compared with the typical spirostane-type compound.[27] In the HMBC experiment, the presence of a double bond ($\Delta^{5,6}$) in the B ring was determined by the observation of long range correlations between the methyl proton at δ_H 1.06 (Me-19) and the olefinic carbon at δ_C 142.3 (C-5), as well as the olefinic proton at δ_H 5.35 (H-6) and δ_C 38.0 (C-10) as shown in Figure 2. HMBC correlations of H-18 (δ_H 0.78) to both of C-12 and C-13 were able to locate a hydroxy group on C-12. The relative configuration of the hydroxy group at C-12 was assigned as β orientation, based on the observed ROESY correlations of H-12 to H-14 and H-17, and H-17 to H-12, H-14 and H_3-21. The ROESY correlations of H_2-26 to H-16, and H-16 to H-17, and H-17 to H-12 confirmed the relative configuration of the C-22 position as *R**. By comparing the coupling constant of H-3 ($W_{1/2}$ = 24 Hz) with the published value ($W_{1/2}$ = 24 Hz),[28] the hydroxy group on C-3 was found to be β-oriented. Thus, the structure of **1** was determined to be a spirost-5-ene-3β,12β-diol.

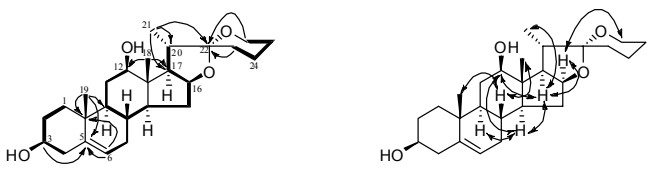

Figure 2. Key ^{1}H-^{1}H COSY (▬), HMBC (H ⟶ C) and ROESY (⟷) correlations of compound **1**.

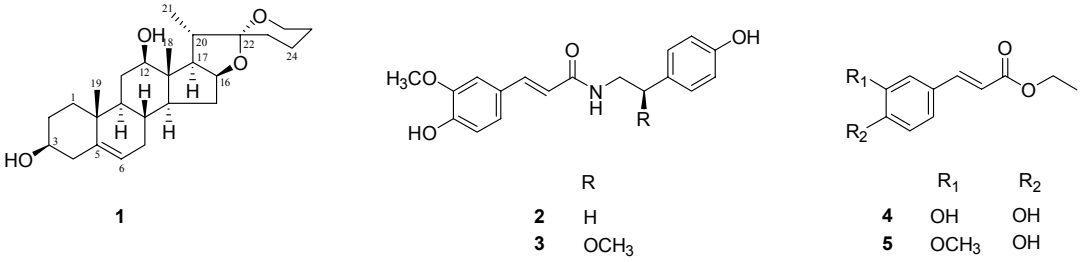

Figure 1. Structures of **1-5**.

Table 1. ^{1}H-(400 MHz), and ^{13}C-NMR (100 MHz) Chemical Shifts of Compound **1** in MeOH-d_4

position	**1**	
	δ_C	δ_H (J in Hz)
1	38.6 t	1.08, 1.86 (m)
2	32.3 t	1.53, 1.81 (m)
3	72.4 d	3.39 (m $W_{1/2}$ 24)
4	43.0 t	2.24 (m)
5	142.3 s	-
6	122.4 d	5.35 (d, 5.1)
7	31.8 t	1.62 (m)
8	31.9 d	1.46 (m)
9	51.4 d	1.08 (m)
10	38.0 s	-
11	32.4 t	1.39 (m)
12	80.4 d	3.28 (dd, 6.7, 4.5)
13	47.0 s	-
14	56.5 d	1.10 (m)
15	33.0 t	2.01 (m)
16	82.3 d	4.41 (q, 7.3, 7.3, 7.3)
17	63.3 d	1.90 (m)
18	11.6 q	0.78 (s)
19	19.9 q	1.06 (s)
20	43.8 d	1.89 (m)
21	14.0 q	1.03 (d, 6.4)
22	110.6 s	-
23	31.7 t	1.71 (m)
24	31.7 t	1.71 (m)
25	24.9 t	1.92 (m)
26	63.0 t	3.76 (m)

Assignments were based on ^{1}H, ^{13}C, DEPT, ^{1}H-^{1}H COSY, HMQC and HMBC experiments.

Table 2. Inhibition of LTC_4-Release by Compounds **1-14** Obtained from the Fruits of *S. nigrum*[a,b]

compound	IC_{50} (μM)
	RBL-1
1	22.4 ± 0.1
2	16.6 ± 1.2
3	22.6 ± 1.3
4	2.2 ± 1.2
5	10.3 ± 1.3
zileuton[c]	0.75 ± 0.03

[a]The results are means ± standard deviation of three independent replicates. [b]Compounds (**6-14**) were found to be inactive (IC_{50} 25 > μM). [c]A positive control.

Spirostane-type compounds with C_{26} have not been reported from natural sources so far while a few steroids with C_{26} have been found in the marine organisms.[29,30]

All the compounds obtained from the fruits of *S. nigrum* were evaluated for their inhibitory activities on LTC_4 release, and the results were summarized in Table 2. Of the compounds tested, compound **4** exhibited the most potent inhibition of LTC_4 release with an IC_{50} value of 2.2 μM (the positive control, zileuton, 0.75 μM). Compound **5**, an analog of **4**, also was found to inhibit LTC_4-release (IC_{50} 10.3 μM), consistent with the literature.[31] The new compound (**1**) displayed weak inhibition of LTC_4 release (IC_{50} 22.4 μM). Leukotrienes including LTC_4 are lipid mediators found elevated in various inflammatory diseases such as allergic rhinitis, asthma, and atopic dermitis. Currently, antileukotrienes are being prescribed for the treatment of asthma and have been approved for the treatment of allergic rhinitis.[32] In the present study, compound **4** was found to possess the inhibitory activity and seemed to be a potential therapeutic effect on the aforementioned inflammatory diseases.

Experimental Section

General Procedures. Melting points were determined on a Kofler micro-ho tstage (uncorrected). Optical rotations were measured on a JASCO P-1020 polarimeter. UV spectra were measured on a Shimadzu UV-1601 UV-visible. The NMR spectra were recorded on a Varian Unity 400 FT-NMR spectrometers with the tetramethylsilane as an internal standard. Chemical shifts are presented in ppm. HRESIMS were measured on a Waters Q-Tof Premier mass spectrometer. Column chromatography (CC) was performed on silica gel (70 - 230 and 230 - 400 mesh, Merck), reverse-phase C18 gel (40 μm, Nacalai, USA, Inc.). Thin layer chromatography (TLC) was performed on Kieselgel 60 F_{254} (Merck) or RP-18 F_{254s} (Merck) plates. Spots were visualized by spraying 10% aqueous H_2SO_4 solution on the plates and heating them for 5 min.

Plant Material. The fruits of *Solanum nigrum* were collected at a farm of Ggamajoong Korea Co. (Jeungpyeong, Korea) in July 2007. The plant material was identified by Dr. Joongku Lee (KRIBB), and a voucher specimen (01283) has been deposited at the Plant Extract Bank, Korea Research Institute of Bioscience and Biotechnology, Korea.

Extraction and Isolation. The fresh fruits of *S. nigrum* (15.0 kg) was extracted with aqueous EtOH (50%) at room temperature (20 L × 3) to obtain 2 kg of the solid extract. The EtOH extract was suspended in H_2O and extracted with EtOAc (3 L × 3) to give the EtOAc-soluble fractions (97.0 g). The EtOAc-soluble fraction (97.0 g) was chromatographed on a silica gel column eluted with a stepwise gradient of hexane and EtOAc to yield six fractions (Fr. A-F: 0.9 g, 2.4 g, 12.0 g, 18.7 g, 8.5 g, 41.0 g each). Fr. B (2.4 g) was subjected to a silica gel column chromatography eluted with a $CHCl_3$-MeOH (100 : 1) to give compounds **5** (28.0 mg), **7** (3.0 mg), **11** (5.0 mg) and **14** (3.0 mg). Compounds **4** (3.3 g) and **15** (84.0 mg) were purified from Fr. C (12.0 g) by a reversed-phase C-18 column chromatography using an isocratic solvent system MeOH-H_2O (1 : 2). Fr. D (18.7 g) was applied to a reversed-phase C-18 column chromatography MeOH-H_2O (3 : 1) and gave compound **9** (1.7 g). Fr. E (8.5 g) was chromatographed on a silica gel column, using $CHCl_3$-MeOH-H_2O (70 : 30 : 4) as eluting solvent, to yield seven sub-fractions (Fr. E1-E7: 0.5 g, 0.9 g, 1.1 g, 2.7 g, 0.4 g, 0.25 g, 2.0 g each). Fr. E3 (1.1 g) was further chromatographed on a reversed-phase C18 column MeOH-H_2O (1 : 2) to give compounds **1** (8.5 mg), **12** (5.0 mg) and **13** (3.0 mg). Fr. E4 (2.7 g) was chromatographed on a reversed-phase C18 column using MeOH-H_2O (1 : 3) to give compounds **3** (15.0 mg), **6** (5.8 mg), and **10** (6.0 mg). Compound **2** (17.0 mg) was isolated

from Fr. E6 (0.25 g) by a RP C-18 column eluted with MeOH-H_2O (1 : 2). Compound **8** (5.9 g) was obtained from Fr. F (41.0 g) by a silica gel column chromatography eluted with a solvent mixture of $CHCl_3$-MeOH-H_2O (70 : 30 : 4).

Spirost-5-ene-3*β*,12*β*-diol (1): White amorphous powder. mp 283 - 284 °C. $[\alpha]_D^{19}$ −66.0° (*c* = 0.1, MeOH). UV (MeOH) λ_{max} (log ε): 203 (3.80) nm. HR-negative ESI-MS *m/z*: 415.2859 $[M-H]^-$ (calcd. for $C_{26}H_{39}O_4$, 415.2848). ^{1}H- and ^{13}C-NMR data see Table 1.

LTC$_4$ Release Assay. This assay was performed according to the literature.[33] Rat basophilic leukemia (RBL-1) cells were plated in 96-well plates at a density of 1×10^5 cells/well. The test compounds were added and then incubated for 10 mins. Later, calcium ionophore A23187 was added and incubated further for 15 minutes. After incubation period, plates were centrifuged at 3000 rpm and 4 °C for 10 min. LTC_4 in the supernatant of each well was measured using an ELISA kit (Cayman Chemical Co., USA) according to the manufacturer's recommended procedures. In brief, 50 μL of supernatant, LTC_4 acetylcholinetransferase, and LTC_4 antiserum were added to each EIA well. Following incubation for 18 h at room temperature, the well was emptied and washed five times with washing buffer. Ellman's reagent (200 μL) was added to each well, and the plate was placed on an orbital shaker for 1 h in the dark. The absorbance was measured at a wavelength of 405 nm. Zileuton (Sequoia Research. Products Ltd, Oxen, UK) was used for a positive control.

Acknowledgments. This research was supported by a grant (PDM0500930) from the Plant Diversity Research Center of the 21st Century Frontier Research Program funded by the Ministry of Science and Technology of the Korean government and a grant of KRIBB Research Initiative Program (KGS2210 911).

References and Notes

1. Sultana, S.; Perwaiz, S.; Iqbal, M.; Athar, M. *J. Ethnopharmacol.* **1995**, *45*, 189.
2. Prashanth, K.; Shashidhara, S.; Kumar, M.; Sridhara, B. *Fitoterapia.* **2001**, *72*, 481.
3. An, H. J.; Kwon, K. B.; Cho, H. I.; Seo, E. A.; Ryu, D. G.; Hwang, W. J.; Yoo, S. J.; Kim, Y. K.; Hong, S. H.; Kim, H. M. *Eur. J. Cancer Prev.* **2005**, *14*, 345.
4. Lee, S. J.; Lim, K. T. *Toxicol. In Vitro.* **2006**, *20*, 1088.
5. Zakaria, Z. A.; Sulaiman, M. R.; Morsid, N. A.; Aris, A.; Zainal, H.; Pojan, N. H.; Kumar, G. H. *Methods Find Exp Clin Pharmacol.* **2009**, *31*, 81.
6. Heo, K. S.; Lim, K. T. *J. Med. Food* **2004**, *7*, 349.
7. Li, J.; Li, Q. W.; Gao, D. W.; Han, Z. S.; Li, K. *Pharmazie* **2008**, *63*, 534.
8. Li, J.; Li, Q. W.; Gao, D. W.; Han, Z. S.; Lu, W. Z. *Phytother. Res.* **2009**, DOI: 10.1002/ptr.2769
9. Jainu, M.; Devi, C. S. *J. Ethnopharmacol.* **2006**, *104*, 156.
10. Jeong, J. B.; Jeong, H. J.; Park, J. H.; Lee, S. H.; Lee, J. R.; Lee, H. K.; Chung, G. Y.; Choi, J. D.; Lumen, B. O. *J. Agric. Food Chem.* **2007**, *55*, 10707.
11. Lin, H. M.; Tseng, H. C.; Wang, C. J.; Lin, J. J.; Lo, C. W.; Chou, F. P. *Chem. Biol. Interact*. **2008**, *171*, 283.
12. Sarker, S. D.; Bartholomew, B.; Nash, R. J. *Fitoterapia* **2000**, *71*, 328.
13. Xing, X. C.; Ho, P.; Bourquin, G.; Yeh, L. A.; Cuny, G. D. *Tetrahedron* **2003**, *59*, 9961.
14. Baderschneider, B.; Winterhalter, P. *J. Agric. Food Chem.* **2001**, *49*, 2788.
15. Hu, K.; Jeong, J. H. *Arch. Pharm. Res.* **2006**, *29*, 563.
16. Ichikawa, M.; Ryu, K.; Yoshida, J.; Ide, N.; Kodera, Y.; Sasaoka, T.; Rosen, R. T. *J. Agric. Food Chem.* **2003**, *51*, 7313.
17. Ma, Z. Z.; Hano, Y.; Nomura, T.; Chen, Y. J. *J. Nat. Prod.* **2000**, *63*, 390.
18. Tamura, Y.; Hattori, M.; Konno, K.; Kono, Y.; Honda, H.; Ono, H.; Yoshida, M. *Chemoecology* **2004**, *14*, 113.
19. Yokozawa, T.; Zhou, J. J.; Sun, X. L.; Hattori, M.; Namba, T.; Tanaka, T.; Nishioka, I.; Oura, H. *Natural Medicines* **1994**, *48*, 330.
20. Dall'Acqua, S.; Innocenti, G.; Viola, G.; Piovan, A.; Caniato, R.; Cappelletti, E. M. *Chem. Pharm. Bull.* **2002**, *50*, 1499.
21. Chang, I. M.; Yun, H. S.; Yamasaki, K. *Kor. J. Pharmacog.* **1981**, *12*, 12.
22. Calis, I.; Kuruuzum, Uz. A.; Lorenzetto, P. A.; Ruedi, P. *Phytochemistry* **2002**, *59*, 451.
23. Cheng, Y. X.; Zhou, J.; Deng, S. M.; Tan, N. H. *Planta Med.* **2002**, *68*, 91.
24. Southwell, I. A.; Russell, M. F.; Maddox, C. D.; Wheeler, G. S. *J. Chem. Ecol.* **2003**, *29*, 83.
25. Fenn, P.; Durbin, R. D.; Kuntz, J. E. *Phytochemistry* **1977**, *16*, 899.
26. Heidelberger, M.; Jacobs, W. A. *J. Am. Chem. Soc.* **1919**, *41*, 1450.
27. Zhou, X.; He, X.; Wang, G.; Gao, H.; Zhou, G.; Ye, W.; Yao, X. *J. Nat. Prod.* **2006**, *69*, 1158.
28. Ori, K.; Mimaki, Y.; Mito, K.; Sashida, Y.; Nikaido, T.; Ohmoto, T.; Masuko, A. *Phytochemistry* **1992**, *31*, 2767.
29. Kingston, J. F.; Fallis, A. G. *Can. J. Chem*. **1982**, *60*, 820.
30. Kurata, K.; Taniguchi, K.; Shiraishi, K.; Suzuki, M. *Phytochemistry* **1990**, *29*, 3678.
31. Wakabayashi, T.; Kumonaka, Y.; Ichikawa, H.; Murota, S. J. P 1985/60152454.
32. Duroudier, N. P.; Tulah, A. S.; Sayers, I. *Allergy* **2009**, *64*, 823.
33. Bickel, D.; Roder, T.; Bestmann, H. J.; Brune, K. *Planta Med.* **1994**, *60*, 318.