Steroidal constituents from the leaves of *Hosta longipes* and their inhibitory effects on nitric oxide production

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ABSTRACT

Hosta longipes (FR. et SAV.) MATSUMURA (Liliaceae) is an edible vegetable in Korea. This study was conducted with the aim of evaluating the potential of *H. longipes* as a functional food for the treatment of neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. In this respect, the study resulted in the identification of three new steroidal compounds, longipenane (**1**), longipenane 26-*O*-β-D-glucopyranoside (**2**) and neogitogenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→4)]-β-D-galactopyranoside (**3**), along with two known steroidal saponins (**4** and **5**). The identification and structural elucidation of these compounds were based on 1D and 2D NMR measurements, high-resolution FAB mass spectroscopy (HR-FAB-MS), and chemical methods. A proinflammatory mediator, nitric oxide (NO), in murine microglial BV-2 cells was used to assess the anti-neuroinflammatory effect of the isolated compounds from *H. longipes*. Among them, compounds **4** and **5** showed strong inhibitory effects on NO production without high cell toxicity in lipopolysaccharide-activated BV-2 cells (IC_{50} = 17.66 and 13.16 μM, respectively).

Neuroinflammation is a defense mechanism in the central nervous system against various harmful infections and injuries. However, substantial evidence supports the idea that neuroinflammation by activated microglia is strongly involved in the pathogenesis and progression of neurodegenerative diseases such as multiple sclerosis, Alzheimer's disease, and Parkinson's disease.[1] Microglia cells are the resident immune cells of the central nervous system. Under normal conditions, these cells play a role in homeostasis regulation and defense against injury.[2] However, in response to abnormal stimuli including environment toxins and neurotoxins, microglia are activated and release various proinflammatory factors including nitric oxide (NO),[3] leading to possible exacerbation of neuronal cell death resulting in neurodegenerative diseases.[4]

Hosta longipes (FR. et SAV.) MATSUMURA (Liliaceae) is an edible vegetable in Korea and widely distributed throughout Korea, China, and Japan.[5] It is called "Bi-Bi-Chu" in Korea, and its young leaves are consumed raw as a salad. This plant has been used as a Korean traditional medicine for the treatment of swelling, inflammation and snake bites.[5,6] In previous phytochemical and biological activity studies, its EtOH extract showed antioxidant activity[7] and steroidal saponins isolated from this plant showed

cytotoxic activity towards HeLa cells.[8] However, little phytochemical and biological investigations on *H. longipes* have been conducted. In our screening procedures, the MeOH extract of leaves of *H. longipes* showed an inhibitory effect on NO production in lipopolysaccharide (LPS)-activated BV-2 cells, a murine microglial cell line. Therefore, as part of a continuing search for bioactive constituents from Korean medicinal plant sources, we attempted to investigate the active constituents of this herb.

The MeOH extract from the leaves of *H. longipes* was suspended in distilled water and then partitioned with *n*-hexane, CHCl₃, EtOAc and BuOH. The CHCl₃ layer showed a considerable inhibitory effect on nitric oxide (NO) production in LPS-activated BV-2 cells, and EtOAc and BuOH layers displayed moderate inhibitory effects in our screening procedures. Chemical investigation of these layers using successive column chromatography over silica gel, Sephadex LH-20, and preparative HPLC resulted in the isolation and identification of three new steroidal compounds **1**-**3** and two known steroidal saponins **4** and **5** (Fig. 1).

Compound **1** was obtained as a colorless gum. The molecular formula was determined to be $C_{27}H_{44}O_5$ from the molecular ion peak $[M+H]^+$ at *m/z* 449.3266 (calcd for 449.3267) in the positive-ion HR-FAB-MS. The IR spectrum displayed strong OH vibrations at 3382 cm⁻¹ and a carbonyl band at 1660 cm⁻¹. The ¹H NMR spectrum showed the presence of four methyl groups [δ_H 1.08, 0.93 (each 3H, d, *J* = 7.0 Hz), 1.24 and 1.09 (each 3H, s)], three
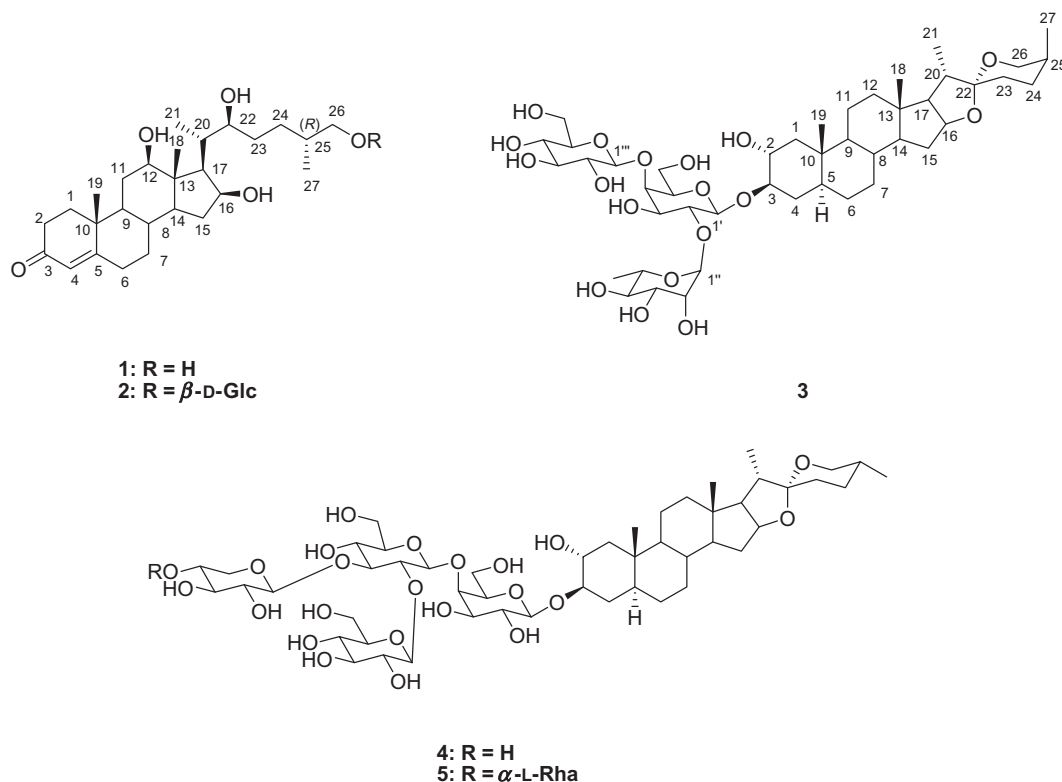
Figure 1. Structures of **1–5** from the leaves of *H. longipes*.

oxygenated methine [δ_H 4.34 (1H, dt, J = 7.0, 4.0 Hz), 4.04 (1H, t, J = 6.0 Hz) and 3.35 (1H, m)], one oxygenated methylene [δ_H 3.44 (1H, dd, J = 5.5, 10.5 Hz), and 3.34 (1H, m)], and one olefinic proton [δ_H 5.71 (1H, s)] signals (Table 1). The ^{13}C NMR spectrum indicated 27 carbon resonances, which were classified by DEPT and HMQC experiments as one carbonyl carbon (δ_C 201.0), one trisubstituted double bond (δ_C 173.4 and 123.1), three oxygenated methines (δ_C 78.1, 72.9 and 70.9), one oxygenated methylene (δ_C 67.2), four methyls, eight methylenes, six methines and two quaternary carbons. These NMR data were very similar to those of (25S)-16(S), 22(S), 26-trihydroxycholest-4-en-3-one,[9] except for the absence of one methylene proton and carbon resonances and the presence of one oxygenated methine [δ_H 3.35 (1H, m); δ_C 78.1]. The HMBC correlation of H-18/C-12 and NOE correlations of H-12/H-9 and H-14 (Fig. 2) confirmed the presence of an OH group at C-12. The relative stereochemistry was assumed to be the same as that of (25S)-16(S),22(S),26-trihydroxycholest-4-en-3-one[9] based on the J values and confirmed by the NOE correlations of H-8/H-18 and H-19, H-9/H-12 and H-14, H-16/H-14, H-17 and H-21, and H-18/H-20 (Fig. 2). The β-orientation of the OH group at C-12 was confirmed by the NOE correlations of H-12/H-9 and H-14. The relative configuration of the OH group at C-22 was anti to the Me group at C-20 by comparing the J value of H-22 (t, J = 6.5 Hz) with that of (22S)-bethosides B and C [t, J = 6.9 Hz for H-22 (22β); ddd, J = 11.1, 3.3, 2.1 Hz for H-22 (22α)].[10] The absolute configuration at C-25 was confirmed as R by examination of the Mosher esters of **1**; R ester **1a** showed a larger difference ($\Delta\delta$ 0.211) between two H-26 signals than that of S ester **1b** ($\Delta\delta$ 0.081 ppm).[11] Thus, compound **1** was characterized as (25R)-12β,16β,22β,26-tetrahydroxycholest-4-en-3-one and named longipenane.

Compound **2** was obtained as a colorless gum. The molecular formula was determined to be $C_{33}H_{54}O_{10}$ from the molecular ion peak [M+H]$^+$ at m/z 611.3795 (calcd for 611.3795) in the positive-ion HR-FAB-MS. The IR spectrum displayed strong OH vibrations at 3382 cm^{-1} and a carbonyl band at 1656 cm^{-1}. The ^{1}H NMR spectrum of **2** showed an anomeric proton at δ_H 4.23 (1H, d, J = 8.0 Hz, H-1′), which is a characteristic signal for a β-glucopyranosyl unit, and six oxygenated protons attributed to a sugar at δ_H 3.87 (1H, dd, J = 12.0, 2.0 Hz, H-6′a), 3.66 (1H, dd, J = 12.0, 10.5 Hz, H-6′b), 3.34 (1H, m, H-3′), 3.29 (1H, m, H-4′), 3.27 (1H, m, H-5′) and 3.18 (1H, dd, J = 9.0, 8.0 Hz, H-2′). The ^{13}C NMR spectrum of **2** showed signals for a β-glucopyranosyl unit (δ_C 103.4, 76.8, 76.6, 73.9, 70.5 and 61.5) and the other signals in the ^{1}H and ^{13}C NMR spectra were fairly similar to those of **1**. The chemical shifts of C-26 in **2** [δ_H 3.74, 3.38 (each 1H, dd J = 10.5, 6.5 Hz); δ_C 74.9] were downfield shifted compared with those of C-26 in **1**, indicating the location of the β-glucopyranosyl unit at C-26. This was confirmed by the HMBC correlation of H-1′/C-26 (Fig. 3). The enzymatic hydrolysis of **2** with β-glucosidase (Almonds) yielded the aglycone **2a**, whose ^{1}H NMR spectrum was in good agreement with that of **1**, and D-glucose {[α]$_D^{25}$ +62.2 (c = 0.05, H$_2$O)}. Thus, compound **2** was characterized as (25R)-12β,16β,22β,26-(β-D-glucopyranosyl)-tetrahydroxycholest-4-en-3-one and named longipenane 26-O-β-D-glucopyranoside.

Compound **3** was obtained as a white powder. The molecular formula was determined to be $C_{45}H_{74}O_{18}$ from the molecular ion peak [M+H]$^+$ at m/z 903.4954 (calcd for 903.4953) in the positive-ion HR-FAB-MS. The ^{1}H NMR spectrum indicated the presence of two tertiary methyl groups [δ_H 0.93 and 0.81 (each 3H, s)], two secondary methyl groups [δ_H 1.14 and 1.08 (each 3H, d, J = 7.0 Hz)], typical steroid methyls, and three anomeric proton [δ_H 6.27 (1H, br s), 5.20 and 4.97 (each 1H, d, J = 7.5 Hz)] signals showing HSQC correlations with three anomeric carbon signals [δ_C 102.8, 107.6 and 101.4, respectively] (Table 2). Comparison of the signals from the aglycone part of **3** in the ^{1}H and ^{13}C NMR spectra with those from the aglycone part of cistocardin[12] showed that the aglycone part of **3** was the same as that of cistocardin, and this was confirmed